American Association of Equine Practitioners

60TH ANNUAL CONVENTION PROCEEDINGS

December 6 - 10, 2014
Salt Lake City, Utah

Proceedings of the 60th Annual Convention of the American Association of Equine Practitioners

Salt Lake City, Utah
December 6–10, 2014

Program Chair: G. Kent Carter, DVM

ACKNOWLEDGMENTS
Katherine S. Garrett, DVM, DACVS, Educational Programs Committee Chair
Carey M. Ross, Scientific Publications Coordinator

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Policy Statement

The primary purpose of publishing the Proceedings is to provide documentation of the scientific presentations in abstract form, available at the AAEP annual convention. Its further purpose is to offer easily accessible information that will assist the AAEP membership, and others in the equine industry, in the daily responsibility of providing the best possible care for the horse.

Mission Statement

To improve the health and welfare of the horse, to further the professional development of its members, and to provide resources and leadership for the benefit of the equine industry.

Future AAEP CE Dates

2015	Resort Symposium	Big Island, Hawaii	January 28–30
2015	360° on Diagnosing, Imaging, and Treating From the Foot to the Fetlock: Everything You Need or Want to Know	Fort Collins, Colorado	June 28-July 1
2015	360° on Ophthalmology	Gainesville, Florida	July 8–11
2015	Focus on Ambulatory	Minneapolis, Minnesota	July 26–28
2015	Focus on Poor Performance	Lexington, Kentucky	September 10–12
2015	61st Annual Convention	Las Vegas, Nevada	December 5–9
2016	62nd Annual Convention	Orlando, Florida	December 3–7
2017	63rd Annual Convention	San Antonio, Texas	November 17–21
2018	64th Annual Convention	San Diego, California	December 1–5
2019	65th Annual Convention	Denver, Colorado	December 7–11

The AAEP and IVIS (International Veterinary Information Service) have joined their resources to disseminate more widely the scientific content of the AAEP Conventions. The papers in this proceedings book are searchable on the IVIS website at http://www.ivis.org.

From Your President

American Association of Equine Practitioners
4075 Iron Works Parkway • Lexington, KY 40511
859.233.0147
fax: 859.233.1968
www.aaep.org

Dear AAEP Colleagues and Guests,

It is my distinct honor and privilege to welcome you to Salt Lake City and the 60th Annual AAEP Convention. This beautiful Rocky Mountain region is the host for this year's premiere continuing education experience for AAEP members, and I genuinely hope you have an outstanding convention while in Salt Lake.

As you have come to expect, the program this year is exceptional in content and diversity. Program Chair and President-Elect Dr. Kent Carter has done a masterful job organizing an extraordinary continuing education agenda. The fruits of his labor are evident in the strength of the scientific program; thank you Dr. Carter! A special thank also goes out to Dr. Ed Kanara, Dr. Katie Garrett and Dr. Eric Mueller, Chair and Co-Chair of the Educational Programs Committee, the entire Educational Programs Committee, and especially, the entire AAEP staff. The year round tireless efforts and commitment of these folks are instrumental in delivering the highest quality continuing education to AAEP members.

This year, our very own Dr. Marvin Beeman, a highly respected practitioner, and the epitome of the AAEP, will open up the convention as the Keynote Speaker. Dr. John Madigan, one of our profession's most talented individuals, will deliver the Milne Lecture. In addition, the Kester News Hour promises to continue the tradition of delivering state of the art continuing education. The Business Sessions, with Mr. Stephen M.R. Covey set to deliver a Business Keynote, are filled with tangible information for members. This is the second consecutive year that a student program will be included at the convention. Take a moment to introduce yourself to some of these aspiring veterinarians. You will be glad you did.

The In-Depth, How To, and Abstract Sessions contain peer-reviewed relevant scientific information and practice tips beneficial to virtually every equine practitioner. You may notice a reduction in the number of table topics this year. This shift is partly due to the strength of the scientific program, and the desire to focus more on the scientific subject matter within the program. Our trade show is expansive as always. While visiting the trade show, please take a moment to thank all of our educational partners for their efforts on behalf of AAEP and its members.

As the year winds down to a close, it is gratifying to inform you that AAEP represents you, the member, on many fronts. Your association has been active and instrumental in many aspects of the equine industry and veterinary medicine: veterinary legislative issues, the carriage horse industry, drug compounding, horse racing, and the performance horse industry, to mention only a few. AAEP and its members have a respected voice regarding the health and welfare of the horse, and that is something that every one of you can be proud of.

The abundance of quality CE opportunities available at this meeting is certain to keep you busy. That said, I sincerely hope you take time to see some old friends and foster new relationships. Thank you for being a part of AAEP, and have a great convention!

All the Best,

Jeff A. Blea, DVM

Jeff A. Blea, DVM
2014 AAEP President

Raising the Standard in Horse Health

From Your President-Elect and Program Chair

® American Association of Equine Practitioners
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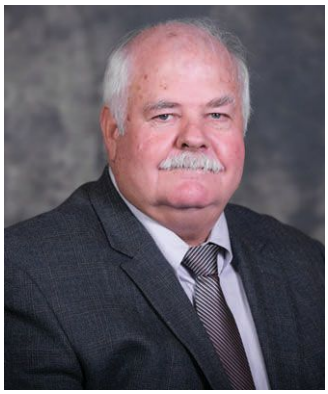

Dear AAEP Members and Colleagues:

As this year's Program Chair, I am pleased to welcome you to Salt Lake City and introduce you to the 2014 convention program. This year is the 60th anniversary of the AAEP's Annual Convention and I am confident you will find the program educational and entertaining.

The annual convention takes years of planning and countless hours of volunteers' participation. I wish to personally express my gratitude to the 49 members of the Educational Programs Committee for their efforts in this process and special recognition goes to the Chair, Dr. Katie Garrett, Vice-Chair, Dr. Eric Mueller, and Mrs. Carey Ross for their stewardship in the process. The whole AAEP staff provides countless hours of dedication and hard work to make this meeting a success.

The opening session will feature our keynote speaker, Dr. Marvin Beeman, a well-known equine veterinarian and AAEP member for over 55 years. Dr. Beeman will stimulate the audience and encourage us all. Following will be the always popular Kester News Hour which will provide updates in equine medicine, surgery, and reproduction. This year's Kester News Hour will be presented by Dr. Carol Clark, Dr. Lisa Fortier, and Dr. Terry Blanchard.

The Frank J. Milne State of the Art Lecture will be given by Dr. John Madigan. Dr. Madigan will be presenting his body of work in equine infectious disease and neonatology and is certain to entertain the audience while sharing his work.

The business program will feature an all-star lineup of speakers including the keynote, Stephen M. R. Covey, an internationally known speaker. This year's business program will provide outstanding continuing education to the participants.

The meeting will also feature a mixture of in-depth and how-to sessions that will cover all aspects of equine medicine, surgery, and reproduction. In addition, there will be sessions on biosecurity and pharmacology. The always popular table topics encouraging small group interactive sessions will be offered throughout the program.

Dr. David Frisbie will once again offer the interactive case-based lameness session. This session offers a unique opportunity for the audience to participate in the session.

I encouraged the Educational Programs Committee to offer a session on the American Mustang. It was my thought that the AAEP membership should be more knowledgeable about the mustang on public lands and their management.

In response to numerous requests, we are offering a session on horse handling skills. Dr. Mark Fitch has sought participation from several horse handling expert veterinarians to share how they manage difficult patients. There will be something useful to everyone attending this session.

We will have a huge trade show and time will be set aside in the program for attendees to visit exhibitors. Please make an effort to thank our educational partners for helping the AAEP bring this meeting to you.

As a native of Utah I encourage you to take advantage of any free time you have and get out and see Salt Lake City and the surrounding area. The mountains less than 1 hour from your hotel offer world class winter sports and spectacular scenery.

Enjoy the Convention!

Sincerely,

G. Kent Carter, DVM, MS, Diplomate ACVIM

Raising the Standard in Horse Health

2014 AAEP Board of Directors

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2014 AAEP Awards

Distinguished Life Member – Terry D. Swanson, DVM
The AAEP Distinguished Life Member designation is awarded in recognition and appreciation of dedication and meritorious service to the veterinary profession and the advancement of equine medicine.

Distinguished Service Award – Larry R. Bramlage, DVM, MS, DACVS and C. Wayne McIlwraith, BVCs, PhD, DSc, FRCVS, DACVS, DECVS, DACVSMR
Awarded to an individual who has provided exemplary service to the AAEP or a similar organization to the benefit of the horse, horse industry, or the profession of equine veterinary medicine.

Distinguished Educator Award (Academia) – Terry L. Blanchard, DVM, MS, DACT
Awarded to an individual educator who by their actions and commitment has demonstrated a significant impact on the development and training of equine practitioners.

Research Award – Don P. Knowles, DVM
The AAEP Research Award was established to highlight the value of equine research and give recognition to an individual who has recently completed research that has or will make a significant impact on the diagnosis, treatment or prevention of equine disease.

The Lavin Cup – Ms. Robbie Timmons
Named for AAEP past president, A. Gary Lavin, VMD, this award is presented to a non-veterinary individual or organization that has demonstrated exceptional compassion or developed and enforced rules and guidelines for the welfare of the horse.

George Stubbs Award – Ms. Dell Hancock
This award recognizes outstanding contributions made to equine veterinary medicine by an individual other than a veterinarian.

General Instructions for Authors
61st AAEP Convention
Las Vegas, Nevada
December 5–9, 2015

To submit a paper, go to http://aaep2015.abstractcentral.com

ALL papers must be submitted online by March 16, 2015, 3:00 p.m. ET.

The AAEP Proceedings is protected by copyright and information submitted and accepted becomes the property of AAEP. However, requests for copies or reprints will be honored by AAEP only with the cooperative permission of the presenting author, who by his or her presentation represents all authors. AAEP reserves the right not to accept any submission without further recourse.

Presentations for the AAEP Convention will be selected directly from the review-ready submissions to the AAEP. Submissions may include case series with follow-up data, or the results of experimental or observational studies as scientific papers, as well as "How to" and review papers. Selection will be made by the Educational Programs Committee. The quality of the submission will determine the selection. Missing data or proposed, but not completed procedures, will exclude the submission from consideration. AAEP invites information dealing with any subject germane to equine practice, but special consideration will be given to submissions by practitioners and material with practical content or new information. At least one author of a report describing diagnosis, treatment, or the interpretation of medical information should be a veterinarian.

All submissions should strictly adhere to the Instructions for Authors. Submissions will be ranked using the AAEP Scoring Criteria (found at the end of this document) and the highest-ranking papers will be selected for the available time.

Authors are expected to acknowledge all sources of funding or support for the work described and to disclose to the Educational Programs Committee any financial interest (including ownership, employment, consultancy arrangements, or service as an officer or board member) they have with companies that manufacture or sell products that figure prominently in the paper or with companies that manufacture or sell competing products. Such an interest will not necessarily influence the decision to accept or reject a submission for the program, but must be included in the Acknowledgments section for the convention proceedings.

Guidelines:

Failure to adhere to the following format will result in nonacceptance. It is the author's responsibility to convince the Educational Programs Committee of the value of the submission, as well as to portray to the reader the contents of the presentation. **Specific instructions for Scientific papers, "How to" papers, Review papers, ≤ 250 word abstracts, and Business papers, and can be found in their respective sections.**

Headings should include (but are not limited to) the following:

1. Take Home Message
2. Introduction
3. Materials and Methods
4. Results
5. Discussion
6. References

Title:

The title should be 15 words or fewer, at the top and on the first page.

Example:
Upper Respiratory Dysfunction in Horses During High Speed Exercise

Take Home Message:

This should be a short, concise summarization of the main conclusion and should be no longer than two or three sentences (approximately 50 words). "How to" papers do not require a take-home message.

Example:
Local anesthetic injected into the coffin joint is not selective for only this joint. Such injections will desensitize much of the navicular bone and its suspensory ligaments.

Introduction:

The rationale for the submission should be given briefly and significant published work acknowledged here. The clinical significance should also be included, as well as a clear statement of the objective or purpose of the submission. The statement of objectives is usually found in the last sentence of the Introduction.

Materials and Methods:

This section should describe experimental methodology in the case of a didactic study or, in the case of a clinical study, should include a description of the population from which the animals were selected and how they were selected for inclusion in the report.

Data obtained and how they were obtained must be described. A description of the statistical methods used to summarize data, test hypotheses, and characterize the significance of results should also be included. For weights and measures, metric units should be used. Dosages should be expressed entirely in metric units and with specific time intervals.

Example:
22 mg/kg, q 12 h, IV (not 10mg/lb, BID, IV)

Results:

Actual results with numbers and data must be presented. When possible, quantify findings (mean, median, proportion) and present them with appropriate estimates of measurement error or uncertainty (such as standard deviation (SD), standard error (SE) or confidence interval) in addition to the results of hypothesis testing. If the data can be well represented with a graph or figure, these *are encouraged* if subsequent publication is not anticipated. If numbers and data

are not presented due to concerns regarding publication in a refereed journal, indications of relative differences between groups such as odds ratios, % change, and significant differences must be included in the submission to be considered acceptable. In these instances, the authors should submit the data in the form of means, standard deviations, or other descriptions of comparisons among groups in an appendix, which will not be published and only used for review purposes.

Discussion:

Important findings documented in the results of the study should be stated. Results should be related to other work which has been done and how the results differ or agree with previously published work and why any differences may have occurred should be discussed. The practical take home message for the equine practitioner should be clearly defined and stated in the summarizing final statement. This statement may be longer, but should be similar in content to the take home message at the beginning of the paper.

References:

All submissions must include references. References to published works should be limited to what is relevant and necessary. Number references in the text with superscript numbers consecutively in the order in which they are first cited. Under references, list all authors when there are three or fewer; list only the first three and add "et al." when there are four or more. The author is responsible for the formatting and accuracy of all reference citations. Since readers frequently depend upon the reference citations to guide them in further reading, it is imperative that the citations are correct so that libraries can locate the papers a reader may wish to obtain.

Examples:

Journal article:

Auer JA, Martens RJ, Williams EH. Periosteal deformities in foals. *Am J Vet Res* 1982;181:459–466.

Murphy CJ, Lavoie JP, Groff J, et al. Bilateral eyelid swelling attributable to lymphosarcoma in a horse. *J Am Vet Med Assoc* 1989;194:939–942.

Some common journal abbreviations include: *Acta Vet Scand, Am J Vet Res, Can J Vet Res, Can Vet J, Cornell Vet, Compend Contin Educ Pract, Equine Vet J, Equine Vet J Suppl, J Am Vet Med Assoc, J Vet Diagn Invest, J Vet Intern Med, Prev Vet Med, Vet Clin North Am Equine Pract, Vet Radiol, Vet Rec, Vet Surg.* Other journal names should be abbreviated in accordance with the National Library of Medicine and *Index Medicus*.

Book:

Turner AS, McIlwraith CW. *Techniques in large animal surgery*. Philadelphia: Lea and Febiger, 1982;186–191.

Banks P, Bartley W, Birt LM. *The biochemistry of the tissues 2nd ed*. London: John Wiley & Sons, 1968;24.

Devlin TM, ed. *Textbook of biochemistry with clinical correlations*. New York: John Wiley & Sons, 1982;14–36.

Chapter in a book:

Axelrod B. Glycolysis. In: Greenberg DM, ed. *Metabolic pathways, vol 1. 3rd ed.* New York: Academic Press, 1967; 112–145.

Kainer RA. Functional anatomy of equine locomotor organs. In: Stashak TS, ed. *Adams' lameness in horses 4th ed*. Philadelphia: Lea and Febiger, 1987;12-18.

Proceedings:

Divers TJ. Acute renal failure in horses and cattle, in *Proceedings*. 3rd Am Coll Vet Int Med Forum 1985;93–95.

Lamb CR, Koblik PD, O'Callaghan MW, et al. Comparison of bone scintigraphy and radiography as aids in the evaluation of equine lameness: Retrospective analysis of 275 cases, in *Proceedings*. Am Assoc Equine Pract 1989;35: 359–368.

Footnotes:

References to dissertations, theses, abstracts, personal communications and papers submitted but not yet accepted for publication should be footnoted:

Jones CD. The selective advantage of the ABO blood groups [thesis]. Ithaca, NY: Cornell University; 1990.

Bramlage LR. Lexington, KY. (personal communication) 1996.

Smith AB. Unpublished data. January 1990.

Evans LH. Entrapment of the epiglottis. Am Assoc Equine Pract. In Press 1981.

Products and equipment should be identified by chemical or generic names or descriptions.

All products should be footnoted, along with the manufacturer's full address. A trade name may be included in a lettered footnote along with the name and location (full mailing address including zip code) of the manufacturer when the product or equipment was essential to the outcome of the experiment or treatment.

Example:
All horses were sedated with a combination of detomidine HCL[a] (10–20 mg/kg IV) and butorphanol tartrate[b] (0.01–0.02 mg/kg IV).
a Dormosedan® Orion Corporation, Espoo, Finland.
b Torbugesic®, Fort Dodge Animal Health, Fort Dodge, IA 50501.

Figures:

- The resolution should be at least 300 dpi.
- Figures should be cited in the text in parentheses (Fig. 1) consecutively in the order of which they are first mentioned.
- The figure itself should also be numbered to correspond to the citation in the text.
- Figures must include captions, 40 words or fewer.

Tables:

Tables should be self explanatory and should supplement the text. Provide a concise, descriptive title for each table.

Acknowledgments:

Acknowledgments should include financial and material support for research (e.g. Grayson Jockey Club Research Foundation, AQHA Foundation) and technical support for work performed. Authors are expected to disclose the nature of any financial interests (including ownership, employment, consultancy arrangements, or service as an officer or board member) they have with companies that manufacture or sell products that figure prominently in the submission or with companies that manufacture or sell competing products.

Permissions:

If you wish to use previously published material, including text, photographs, or drawings, you must acknowledge the original source and submit written permission from the copyright holders (author and publisher) to reproduce the material. Provide this permission when you submit your original manuscript.

Conflicts of Interest:

Summary

1. Any and all authors listed on the paper must disclose any actual or potential conflicts of interest
2. Any and all authors listed on the paper must disclose if no conflict exists
3. The nature of the conflict (actual or potential) needs to be described

At the point of submission, the American Association of Equine Practitioner (AAEP)'s policy requires that authors must disclose and describe the nature of any actual or potential financial and/or personal relationships they have with companies that manufacture or sell products that figure prominently in the submission or with companies that manufacture or sell competing products. (This includes ownership, employment, consultancy arrangements, or service as an officer or board member.) When considering whether a conflicting interest or connection should be declared, the author is asked to answer the following: Is there any arrangement that would embarrass you or any of your co-authors if it was to emerge after publication and you had not declared it?

As an integral part of the online submission process, Submitting authors are required to confirm whether they or their co-authors have any actual or potential conflicts of interest to declare, and to provide details of these. It is the Submitting author's responsibility to ensure that all authors adhere to this policy.

If the paper is published, the conflict of interest information will be communicated in a statement in the proceedings.

If the authors have no conflict of interest to declare, they must also state this at submission.

Conflict of Interest in Industry Sponsored Research:

Authors whose papers are submitted for publication must declare all relevant sources of funding in support of the preparation of a paper. The AAEP requires full disclosure of financial support as to whether it is from the industry, the pharmaceutical or any other industry, government agencies, or any other source. This information should be included in the Conflict of Interest section of the published paper.

Authors are required to specify sources of funding for the study and to indicate whether or not the text was reviewed by the sponsor prior to submission, i.e., whether the study was written with full investigator access to all relevant data and whether the sponsor exerted editorial influence over the written text. In addition to disclosure of direct financial support to the authors or their laboratory and prior sponsor-review of the paper, submitting authors are asked to disclose all relevant consultancies prior to submission. This information should be included in the Conflict of Interest section at the end of paper.

All authors are requested to disclose any actual or potential conflict of interest including any financial, personal or other relationships with other people or organizations within three years of beginning the submitted work that could inappropriately influence, or be perceived to influence, their work.

Example of COI Statement

Conflict of Interest: Dr. John Doe has no conflict of interest. Dr. Jane Doe has served as a paid technology analyst for the venture capitalists that initiated the formation of Company ABC and served as a member of the Board of Directors of Company ABC from its inception until 2008. Company ABC is currently commercializing the use of Product XYZ. Dr. Jane Doe has also served as a paid consultant and continues to serve on the Company ABC Advisory Board.

Ethical Considerations:

1. Authors are expected to disclose the nature of any financial interests they have with companies that manufacture or sell products that figure prominently in the submission or with companies that manufacture or sell competing products. (This includes ownership, employment, consultancy arrangements, or service as an officer or board member.)
2. If your paper or presentation references the use of a compounded pharmaceutical, please be certain that you are familiar with the FDA guidelines on the use of compounded pharmaceuticals and that the product you reference is in compliance. See section below regarding papers using compounded medications or medical devices.
3. All submissions should cite levels of evidence-based medicine.

You should plan to include any ethical considerations as part of your oral presentation if your paper is accepted.

IACUC Approval:

AAEP is dedicated to the humane use of animals in scientific research in accordance with the Institutional Animal Care and Use Committee (IACUC). AAEP recognizes the difficulty practitioners may have when attempting to obtain IACUC approval therefore the Educational Programs Committee has compiled a list of liaisons that practitioners can use as a resource. This list can be found on the AAEP website (www.aaep.org).

Compounded Medications or Medical Devices:

To be considered for selection in the Annual Convention program, abstracts that include the use of compounded drugs must adhere to the tenets described in the AAEP Equine Veterinary Compounding Guidelines (2005). Specifically, compounded drug or medical devices cannot be used in lieu of a FDA approved product if the approved product has a label indication for the purpose or condition being evaluated or described in the abstract.

An exception to this policy will be made for abstracts reporting clinical trials conducted in fulfillment of the requirements for the approval of a new drug (FDA) or biologic (USDA).

Submitted papers that use compounded drugs or medical devices will be reviewed by at least two individuals with

expertise in this area selected by the CE Steering Committee. The individuals will then make a recommendation to the EPC about the suitability of the submission for potential inclusion in the program.

Standard of Care:

The AAEP is sensitized to having people use the term "Standard of Care" from the podium. If you plan to do this please include this in your abstract or written submitted material so the EPC can confirm its agreement with your statement.

1. A diagnostic and treatment process that a clinician should follow for a certain type of patient, illness, or clinical circumstance. Adjuvant chemotherapy for lung cancer is "a new standard of care, but not necessarily the only standard of care." (New England Journal of Medicine, 2004).
2. In legal terms, the level at which the average, prudent provider in a given community would practice. It is how similarly qualified practitioners would have managed the patient's care under the same or similar circumstances. The medical malpractice plaintiff must establish the appropriate standard of care and demonstrate that the standard of care has been breached.

Deadline:

ALL papers must be submitted online by March 16, 2015, 3:00 p.m. E.T.; under no circumstances will submissions received after the deadline be considered or reviewed. ALL deadlines must be adhered to in order to have the published Proceedings available at the meeting.

Review Process:

To respect the integrity of the Annual Convention program and ensure the fairness of the review process, AAEP has adopted blind reviewing in which the identity of the authors and reviewers are not known to each other. Papers will be reviewed, scored, and selected by the Educational Programs Committee. Please follow the blinding guidelines below.

Blinding Guidelines:

- The title page and/or frontmatter of the blinded version of a paper should contain no references to any author or to his/her affiliation.
- All unpublished works by an author of the submitted manuscript should be blinded.
- When referring to an author's publication, the form of third person should be used.
- Any acknowledgments section should be removed from the blinded version. Also, please delete any notes that indicate affiliation, conference presentations, grants, author or departmental web sites, etc.
- Do not use author name or affiliation in the names of the submitted files.

Scoring Criteria:

One goal of the Educational Programs Committee (EPC) in choosing submissions for the AAEP annual meeting is to combine the best available clinical research with clinical experience and expertise to meet the needs of our patients. The AAEP Scoring Criteria can be found at www.aaep.org or request a copy from cross@aaep.org

Pre-Press Approval:

Authors will have final approval at the page proof stage. Changes/updates in numbers, dosages or inappropriate grammar may be made within one week of receiving page proofs. Final grammatical changes will be the decision of the editors. Substantial changes or removal of any data will result in forfeiture of complimentary registration and travel, and exclusion from the program.

Reimbursement:

Presenting authors will receive one complimentary registration and a reimbursement of $550 to help support travel.

Mentors for Authors:

Paper submissions by private practitioners and first time authors are highly encouraged. The AAEP website (www.aaep.org) features a list of members in various areas of expertise that have agreed to volunteer their time to mentor an author who needs guidance.

Continuing Education Approval Process:

AAEP obtains continuing education units (CEU) from the Registry of Approved Continuing Education (RACE). RACE requires criteria to be met by every speaker before approving AAEP's program for CEU. These criteria are below.

Qualified presenters for *Scientific/Clinical* Programs for Veterinarians shall as a minimum:

1. Be board-certified in the subject matter (i.e. Interns, residents, or an individual in training do **not** qualify), OR
2. Have an advanced degree(s) (e.g. PhD from a suitably accredited institution) in the subject matter, OR
3. Be a member of the faculty of an American Veterinary Medical Association (AVMA) or Canadian Veterinary Medical Association (CVMA)-accredited college of veterinary medicine, OR
4. Provide evidence of special knowledge in the subject area being presented which shall include one or more publications in a peer-reviewed, scientific journal within the last five (5) years, OR
5. Be a veterinarian employed by USDA, CDC, or other governmental regulatory agency.
6. Provide other evidence of special knowledge approved at the discretion of two (2) RACE Reviewers.

Presenters who do not meet the minimum standard will be required to submit reference letters from such individuals who can support/attest to speaker's/presenter's ability to serve as subject matter expert for the topic under consideration. Additional information can be found at http://www.aavsb.org/RACE/Standards/

Scientific Papers: Guidelines for Authors
61st AAEP Convention
Las Vegas, Nevada
December 5–9, 2015

To submit a paper, go to
http://aaep2015.abstractcentral.com

ALL papers must be submitted online by March 16, 2015, 3:00 p.m. ET.

Authors who do ***not*** intend to publish in a refereed journal are welcome to submit a scientific paper.

Scientific Paper selection will be made by the Educational Programs Committee. The quality of the Scientific Paper will determine the selection. Missing data or proposed, but not completed procedures, will exclude the Scientific Paper or other paper from consideration. AAEP invites information dealing with any subject germane to equine practice, but special consideration will be given to presentations by practitioners and material with practical content or new information. At least one author of a report describing diagnosis,

treatment, or the interpretation of medical information should be a veterinarian.

Scientific papers should be formatted as described in the General Instructions for Authors. Scientific papers should be no fewer than 600 words. No upper word limit.

The "How to" Paper: Guidelines for Authors 61st AAEP Convention Las Vegas, Nevada December 5–9, 2015

To submit a paper, go to http://aaep2015.abstractcentral.com

ALL papers must be submitted online by March 16, 2015, 3:00 p.m. ET.

"How to" papers are presented to describe and explain a technique or procedure used in equine veterinary medicine or the equine industry. The technique should be relatively new or not widely understood or used in practice. The goal of the "How to" paper is to give the equine veterinarians the information they need to critically evaluate the pros and cons of the technique and implement it in their practice if they choose.

"How to" papers can be patterned after a modification of the style for a Scientific Paper supporting a scientific presentation. Refer to General Instructions for Authors as you prepare your submission.

The title should begin with "How to . . ." and clearly identify the technique or procedure that will be presented. A "Take Home Message" is not required for "How to" papers. The Introduction should include why you use the technique. If there is a problem with the traditional methods or the currently used method can be improved, this should be explained.

The Materials and Methods section should explain exactly how the technique is performed so that another veterinarian familiar with the subject area could follow your example. You may use a step-by-step method for the paper and the presentation. All medications, supplies, and equipment used should be described using generic names. Trade names and addresses of commercial products critical to the technique can be included in footnotes.

The Results section should include a summary of what happens when you use this technique. The number of horses treated in this manner and an assessment of the outcome should be included. You may use personal assertions or data to assert its value, but you must explain how you determined that the technique works.

In the Discussion section you can give your personal views as to why you think the technique works. Discuss the pros and cons of your approach. Explain how the technique has helped you in your practice and why this should be important to your colleagues. The end of the discussion should contain a summary of the technique and its advantages in the take home message. Case selection, case study number, and case follow-up should all be included.

Review Paper: Guidelines for Authors 61st AAEP Convention Las Vegas, Nevada December 5–9, 2015

To submit a paper, go to http://aaep2015.abstractcentral.com

ALL papers must be submitted online by March 16, 2015, 3:00 p.m. ET.

Review papers are presented for the purpose of updating the membership on a new subject or for gathering information that may be conflicting. The aim of the paper is to help the membership put the information in perspective, and to make judgments on conflicting information. A review paper will not principally present original data. The goal is to clarify existing knowledge on a subject and help the membership better use the information in their day to day practice.

Review papers should generally be formatted as described in the "Instructions for Authors of Manuscripts" except where otherwise noted here. The paper should be titled "Review of Some Subject." The content of review articles should be organized with headings and subheadings that provide a logical flow to the material presented. A "Take Home Message" is required for a Review Paper. The Introduction should define the subject matter and put it in context, explaining why the review is necessary. The purpose of the review paper should be clearly stated in the Introduction.

Agreement and disagreement within the subject matter should be identified along with the strengths and limitations of the information sources. Reference should be made to the authors who generally support the opinions stated. The author's perspective, including his/her own interpretation of the information if it is different from previously published opinions, should be included. The end of the discussion should contain a summary and the conclusion that the author has drawn for the audience, based upon the reviewed data. As with a Scientific Paper, a "Take Home Message" should be provided by the author that summarizes the practical application of the information for the practitioner.

An appropriately complete reference list should be included. The format for references is the same as that described in "General Instructions for Authors."

Illustrations should be provided in the format described in "General Instructions for Authors". If previously published material is submitted, including text, photographs or drawings, the author must acknowledge the original source and submit written permission from the copyright holders (author and publisher) to reproduce the material. This permission must accompany the original manuscript at the time of submission.

Abstracts ≤ 250 Words: Guidelines for Authors

For those who intend to publish in a refereed journal

61st AAEP Convention
Las Vegas, Nevada
December 5–9, 2015

To submit a paper, go to
http://aaep2015.abstractcentral.com

ALL papers must be submitted online by March 16, 2015, 3:00 p.m. ET.

In order to encourage submission of the newest scientific information for inclusion in the AAEP Annual Convention program and simultaneously not jeopardize future publication of this material in a refereed journal, the following criteria have been developed for these submissions of Scientific Papers that will be published in the AAEP Proceedings. In such instances, the published abstract can be ≤ 250 words. However, these "abbreviated abstracts" should follow a structured format with the same subheadings (Take Home Message, Introduction, Materials & Methods, Results and Discussion) as the full-length scientific paper. Please be aware that the Take Home Message is included in the total word count. The abbreviated abstract does not need references but appropriate acknowledgments should be included. Note that this abbreviated abstract format does not apply to Review, How To, or In-Depth Papers. **A full paper** conforming to the General Instructions for Authors must also be submitted to allow the reviewers to assess the experimental design, materials and methods, statistical analyses, results (with graphs, tables, charts, etc.) and a discussion of the results as it pertains to interpretation and conclusions (**see specific guidelines below for full papers**). The submitting author must include a statement that only the short abstract can be published in the AAEP Convention Proceedings/DVD. It remains the authors' responsibility to preserve their right to publish in a refereed journal by contacting the respective journal to discuss their prior-publication criteria, so that an accepted abbreviated abstract will not jeopardize publication in the refereed journal. These submitted abbreviated abstracts should be identified with the words "RESEARCH ABSTRACT" at the end of the title.

Guidelines for Full Papers

- No more than 4 single-spaced pages. This does not include tables, figures, and references
- 12 point font
- 1″ margins
- When submitting online, please put both papers in one document; the 250 word abstract should be first, followed by the full-length scientific paper.

A full paper must be included with all 250 word abstracts in order for the abstract to be considered for the program.

Business of Practice Papers: Guidelines for Authors

61st AAEP Convention
Las Vegas, Nevada
December 5-9, 2015

To submit a paper, go to
http://aaep2015.abstractcentral.com

ALL papers must be submitted online by March 16, 2015, 3:00 p.m. ET.

The theme for 2015 Business of Practice sessions will be on "Implementing Strategy into Your Business" with the intent of bringing forward the idea of how strategy affects all aspects of business. Areas to consider for presentation include, though are not limited to:

- Pricing for your strategy
- Strategy & how it can affect long term practice valuation
- How to finance
- Discovering available/leveraging free local resources for strategic planning
- Strategy & how it affects culture & HR (hiring, firing, compensation, getting & keeping right people on the bus)
- Work/life integration & strategy
- Mentorship as a strategy
- Medical records (efficiency/legal aspects/types)
- Marketing to your strategy/value proposition
- Female perspectives on tactics to improve work/life integration
- Translating strategic planning to implementation
- Measurables to assess success

These suggestions are purely intended to spark ideas. We welcome paper submissions on any topic pertaining to the Business of Practice.

The AAEP Proceedings is protected by copyright and information submitted and accepted becomes the property of AAEP. However, requests for copies or reprints will be honored by AAEP only with the cooperative permission of the presenting author, who by his or her presentation represents all authors. AAEP reserves the right not to accept any submission without further recourse. All submissions should strictly adhere to these Instructions for Authors.

Guidelines

Failure to adhere to the following format will result in nonacceptance. It is the author's responsibility to convince the Educational Programs Committee of the value of the submission, as well as to portray to the reader the contents of the presentation. You may request examples of previously accepted Business papers from cross@aaep.org.

Headings may include (but are not limited to) the following:

1. Take Home Message (not required for "How to" papers. See section at the end of these instructions for 'How to" paper guidelines).
2. Introduction
3. Solution
4. Results
5. Discussion
6. References

Title:

The title should be 15 words or fewer, at the top and on the first page.

> Example:
> Breaking the Silence: Disclosing Medical Errors

Take Home Message:

This should be a short, concise summarization of the main conclusion and should be no longer than two or three sentences (approximately 50 words). "How to" papers do not require a take-home message.

> Example:
> In circumstances where a medical error results in an adverse outcome, a thoughtful response on the part of the veterinarian, staff, and practice is required. This paper will review communication techniques for constructively responding to these difficult situations.

Introduction:

Significant published work should be acknowledged here. A clear statement of the business challenge, or the objective or purpose of the submission should be included. The statement of objectives is usually found in the last sentence of the Introduction.

Solution:

A description of a single or numerous business solutions are explained in detail.

Results:

Any results should be presented in this section. If the data can be well represented with a table or figures, these *are encouraged.*

Discussion:

Important findings documented in the solution or results of the study should be stated. Solutions or results can be related to other work that has been done and how the results differ. The practical take home message for the equine practitioner should be clearly defined and stated in the summarizing final statement. This statement may be longer, but should be similar in content to the take home message at the beginning of the paper.

References:

Submissions may include references. References to published works should be limited to what is relevant and necessary. Number references in the text with superscript numbers consecutively in the order in which they are first cited. Under references, list all authors when there are three or fewer; list only the first three and add "et al." when there are four or more. The author is responsible for the formatting and accuracy of all reference citations. Since readers frequently depend upon the reference citations to guide them in further reading, it is imperative that the citations are correct so that libraries can locate the papers a reader may wish to obtain. Reference examples can be found in the General Instructions for Authors.

Footnotes:

References to personal communications and papers submitted but not yet accepted for publication should also be footnoted:

Figures:

- The resolution should be at least 300 dpi.
- Figures should be cited in the text in parentheses (Fig. 1) consecutively in the order of which they are first mentioned.
- The figure itself should also be numbered to correspond to the citation in the text.
- Figures must include captions, 40 words or fewer.

Tables:

Tables should be self-explanatory and should supplement the text. Provide a concise, descriptive title for each table.

Acknowledgments:

Acknowledgments should include financial and material support for research and technical support for work performed. Authors are expected to disclose the nature of any financial interests (including ownership, employment, consultancy arrangements, or service as an officer or board member) they have with companies that manufacture or sell products that figure prominently in the submission or with companies that manufacture or sell competing products.

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Conflicts of Interest:

1. Any and all authors listed on the paper must disclose any actual or potential conflicts of interest
2. Any and all authors listed on the paper must disclose if no conflict exists
3. The nature of the conflict (actual or potential) needs to be described

Ethical Considerations:

1. Authors are expected to disclose the nature of any financial interests they have with companies that manufacture or sell products that figure prominently in the submission or with companies that manufacture or sell competing products. (This includes ownership, employment, consultancy arrangements, or service as an officer or board member.)
2. If your paper or presentation references the use of a compounded pharmaceutical, please be certain that you are familiar with the FDA guidelines on the use of compounded pharmaceuticals and that the product you reference is in compliance.

Deadline:

ALL papers must be submitted online by March 16, 2015, 3:00 p.m. E.T.; under no circumstances will submissions received after the deadline be considered or reviewed. ALL deadlines must be adhered to in order to have the published Proceedings available at the meeting.

Review Process:

Papers will be reviewed, scored, and selected by the Educational Programs Committee. Since the presentation ability of business speakers is crucial, the review for these papers requires a two-step process: 1. Initial acceptance of the paper while the author is blinded. 2. The process becomes unblinded before final selections are made.

This two-step review process was implemented to protect the association from selecting speakers whose presentations may have a strong commercial bend.

Blinding Guidelines:

- The title page and/or frontmatter of the blinded version of a paper should contain no references to any author or to his/her affiliation.

- Any acknowledgments section should be removed from the blinded version. Also, please delete any notes that indicate affiliation, conference presentations, author or departmental web sites, etc.
- Do not use author name or affiliation in the names of the submitted files.

Scoring Criteria:

The subject matter is relevant to the business operations of a veterinary business. How-to Cases should be based upon personal experience in a veterinary business. Papers describing a business process should be applicable to an equine veterinary business and should be supported by references from business publications.

Pre-Press Approval:

Authors will have final approval at the page proof stage. Changes/updates may be made within one week of receiving page proofs. Final grammatical changes will be the decision of the editors. Substantial changes or removal of any data will result in forfeiture of complimentary registration and travel, and exclusion from the program.

Reimbursement:

Presenting authors will receive one complimentary registration and a reimbursement of $550 to help support travel.

Mentors for Authors:

Paper submissions by private practitioners and first time authors are highly encouraged. Please email Carey Ross (cross@aaep.org) to request a list of members in various areas of expertise that have agreed to volunteer their time to mentor an author who needs guidance.

"How to" Paper Submissions for Business Papers:

"How to" papers are presented to describe and explain a technique or procedure used in practice. The goal of these papers is to give the equine veterinarians the information they need to critically evaluate the pros and cons of the technique and implement it in their practice if they choose.

"How to" Papers should follow the same guidelines in this document, except where otherwise noted below.

The Title should begin with "How to . . ." and clearly identify the technique or procedure that will be presented.

A "Take Home Message" is not required for "How to" papers.

The Introduction should include why you use the technique. If there is a problem with the traditional methods or the currently used method can be improved, this should be explained.

The Materials and Methods section should explain exactly how the technique is performed so that another veterinarian familiar with the subject area could follow your example. You may use a step-by-step method for the paper and the presentation.

The Results section should include a summary of what happens when you use this technique. You may use personal assertions or data to assert its value, but you must explain how you determined that the technique works.

In the Discussion section you can give your personal views as to why you think the technique works. Discuss the pros and cons of your approach. Explain how the technique is helpful and why this should be important to your colleagues. The end of the discussion should contain a summary of the technique and its advantages in the take home message. Case selection, case study number, and case follow-up should all be included.

Want to know how your AAEP Annual Convention program came together?

The Educational Programs Committee (EPC) is charged with creating and reviewing educational content to produce high-quality CE for the AAEP. The committee is composed of AAEP member volunteers from both small and large private practices as well as academia and industry. Members include both general practitioners and specialists.

The Salt Lake City scientific program includes invited papers for the "In-depth" and "How to" sessions as well as sessions comprised of papers that independent authors submitted for consideration. Topic choices for the invited "In Depth" and "How To" sessions are based on member feedback from the 2010 AAEP CE Needs Analysis survey. Topic session leaders are selected by the Program Chair, and then these session leaders invite a slate of speakers to prepare the papers that become an "In Depth" overview or a series of related "How To" talks. Although invited, these papers undergo a rigorous peer review process by the EPC.

Papers submitted by independent authors are each assigned 3 reviewers from the EPC. The reviewers do not know the names of the authors. Content is scored using the criteria of Study Design, Study Quality, Innovation and Impact, Practicality, and Manuscript Quality. Once papers are scored they are discussed by the section facilitators and reviewers. The highest ranking papers are included on the program to accommodate the number of slots available. This year 193 papers were submitted for the 73 available slots on the program.

Non-scientific sessions addressing business, welfare, ethical and industry concerns are also planned as the scientific program materializes. Speakers who are invited to participate in these sessions prepare papers that are also reviewed by members of the EPC for inclusion in the Proceedings.

The peer review process for the AAEP Proceedings is rigorous. It requires an enormous effort by every one of the 49 members of the EPC member to create the best possible program for the AAEP membership. Several thousand volunteer hours were spent putting together the Salt Lake City program, so please thank them for all their hard work creating this program for you.

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How to Use a Smartphone Camera for Ocular Photography in the Horse

Dennis E. Brooks, DVM, PhD, DACVO

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1. Introduction

Smartphones are becoming quite ubiquitous and all have digital photographic and video capability (Fig. 1). They are always with us. The imaging technologies of these smartphones are increasingly quite impressive. Utilizing these capabilities will allow rapid telemedicine consults and are accessible for owners for use in monitoring eye conditions. An unintended consequence of this photographic capability is it can allow veterinarians to view and image the adnexa, cornea, iris, lens, retina, and optic disc of the horse.[1–4] The technique mimics the distant direct technique of the ophthalmoscope. Adaptors, macro lenses, and other accessories also allow slit lamp photography and imaging of cytologic specimens. Software apps are already available to aid photography of the horse globe to levels that are, with a little practice, astonishing! The views with some smartphones are often superior to that of the direct ophthalmoscope.

One major problem with ocular photography is how to properly illuminate the eye so that the camera autofocus can discriminate the tissue that is to be imaged (Figs. 2 and 3). Software apps are available to utilize the "flash" of the camera as an illumination beam to view the cornea, lens, and fundus.

Fig. 1. Smartphone cameras can be utilized to photograph the eyelids, cornea, iris, lens, and fundus of the horse.

NOTES

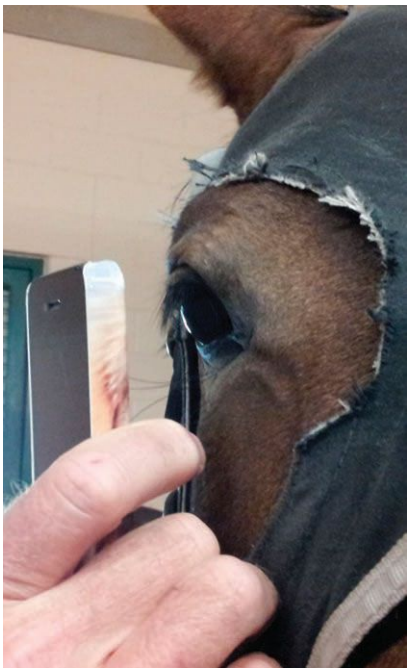

Fig. 2. Smartphone is held close to the eye of the horse similar to a direct ophthalmoscope.

These light-emitting diodes (LEDs) are unfortunately very bright such that adjunctive methods are needed in some cases to reduce the illumination intensity.

Fig. 3. The retina and optic disc in this horse were imaged with an iPhone 4s smartphone and the Camera Awesome app in video setting. The pupil was dilated with tropicamide and the room lights dimmed. The illumination light was partially blocked with a small piece of elasticon tape, the camera moved to within two centimeters of the cornea, and the video capture started. I emailed the video to my computer and used Microsoft Live Movie Maker to capture the image.

Fig. 4. The camera aperture is to the left and the light for the flash and illumination to the right.

2. The "Camera Awesome" App

The "Camera Awesome" app[a] is compatible with iPhone[b] and iPad[c] and recently released a version for Android phones. It is simple to use and allows the veterinarian to obtain both photographic images and videos of the horse eye. Some selective editing is possible. It does not allow dimming of the illumination beam such that a diffuser must be utilized (Figs. 4 and 5). Camera Awesome is easy to use, and this starts with the main interface. Most of the screen is given to image space with the vitals hidden away, easily accessed with a single touch.

Unlike the default camera app that comes with the iPhone, Camera Awesome allows for separate focus and metering points (Figs. 6–8). This is similar to using the exposure or focus lock on a digital single lens reflex camera and recomposing, without the need to recompose. Tapping the screen will recombine the focus and metering points. If you want to return the focus and metering points to the middle of the screen, a double tap anywhere will do.

On the video side, the controls are fairly simplistic and the Awesomize feature is not available. But the camera does have the ability to prerecord for five seconds before you hit the record button. This is useful if you are trying to get a horse to stand still. The video also allows for splitting focus and light metering and will adjust these during recording,

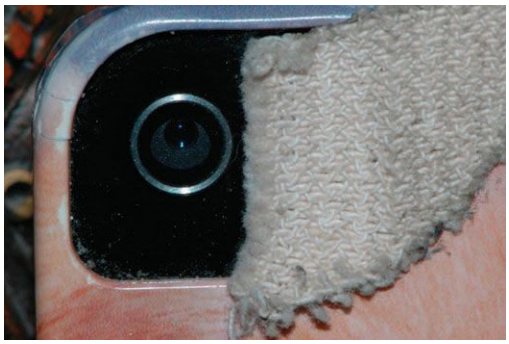

Fig. 5. A piece of bandage tape acts as a diffuser to lower the brightness.

Fig. 6. The drop down menu allows access to a number of functions including still images and video. The flashlight icon in the upper row turns the flash on in Camera Awesome. Hitting the triangle to the left of the shutter release (button in the center, currently selected for single flash camera) brings up vital shooting options such as single shot, image stabilization, big button (it makes the entire surface of the iPhone screen into a shutter release button), slow burst, fast burst, self-timer, and interval shooting. Image stabilization works well. The icons in the lower right switch from camera to video.

enabling simplistic racking functions. The flash can also be turned on or off while shooting video.

3. Camera+ App

The Camera+ app[d] allows for photographic images only. It does not have video capability. It does have a rheostat for adjusting light intensity! Several drop down menus are available in Camera+ (Figs. 9–12).

Fig. 7. The light flash icon is to the right in the bar at the upper right.

Fig. 8. Camera Awesome has separate moveable aids to increase exposure and focus when taking still photographs and videos.

The exposure can be adjusted separately from focus to more easily control the darkness or lightness of the images. The specific area to be imaged can be delimited with the focus box. The stabilizer mode achieves the sharpest photos. Rapid streams of shots in Burst mode can help get a focused image in the moving target.

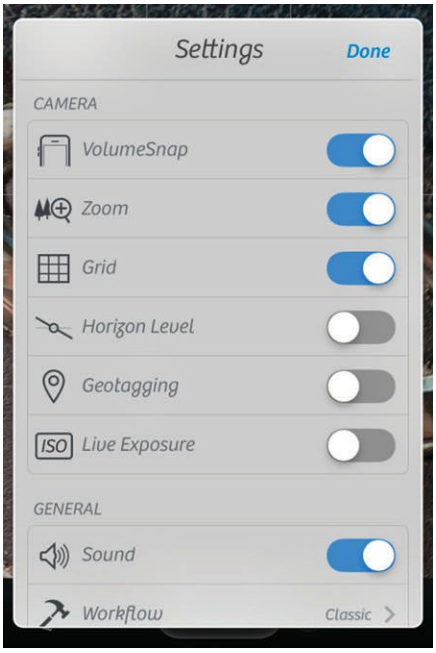

Fig. 9. A menu bar provides the ability to adjust several features in Camera+.

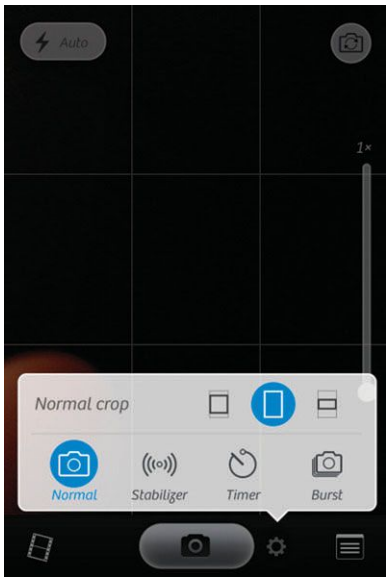

Fig. 10. Limited editing and photographic aids are available in menus with Camera+.

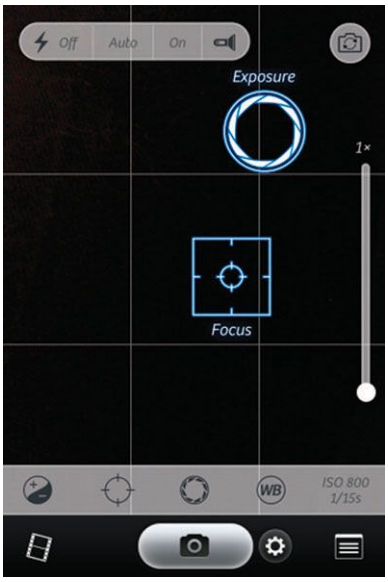

Fig. 12. Separate focus and exposure boxes aid smartphone imaging in Camera+.

The iPhone's flash can be used as a continuous fill light to improve photo quality for macro shots. Up to 6× zoom with advanced digital processing is also available in the Camera+ app. Multiple images can be "stitched" together with software such as Panorama's AutoStitch[e] (Figs. 13–15).

4. Other Uses for the Smartphone and Ocular Photography

The camera of the smartphone is also capable of photographing cornea, lens, and lid problems in horses and can be used to photograph microscope specimens (Figs. 16 and 17).

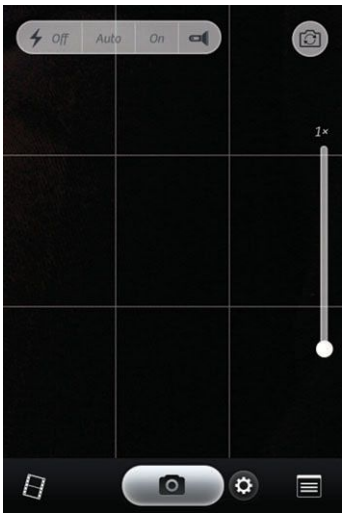

Fig. 11. Sliding the rheostat on the right allows altering the illumination light intensity in Camera+.

5. Tips for Smartphone Photography of the Horse Eye

1. Use your camera's flash as a light. It is important to get as much light as you possibly can on the tissue to be imaged but NOT too much as bright light really hurts.
2. Clean your lens. It may seem silly, but give your lens a wipe down before you start snapping photos with your phone. While most people are pretty good about keeping their

Fig. 13. Several software programs are available to automatically combine or "stitch" several images into a larger panoramic image.

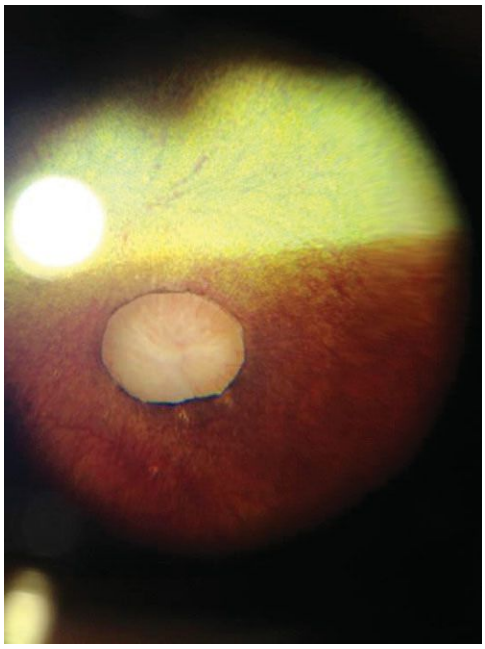

Fig. 14. Equine fundus image with a smartphone provided courtesy of Tim Knott and Rob Lowe, UK.

Fig. 16. Corneal edema imaged with a smartphone camera.

grubby fingers away from camera lenses, it's not as easily done with smartphones.

3. Check your resolution. Use the largest resolution and highest image quality possible.
4. Videography may be simpler than still photography in the moving horse eye. The shutter lag inherent in digital cameras is often too slow to get the image you wanted. Videos can be saved and images easily captured from the video using software such as Windows Live Movie Maker[f] or any Apple computer[g].
5. Equine phoneoscopy was done initially with the iPhone 4 or 4s as the distance between the camera aperture and flash was close. The iPhone 5 and other cameras may have too much space between camera and flash.
6. It is easiest to image lens, vitreous, and retinal regions if the pupil is dilated.
7. The camera must be held close to the cornea to get good images.

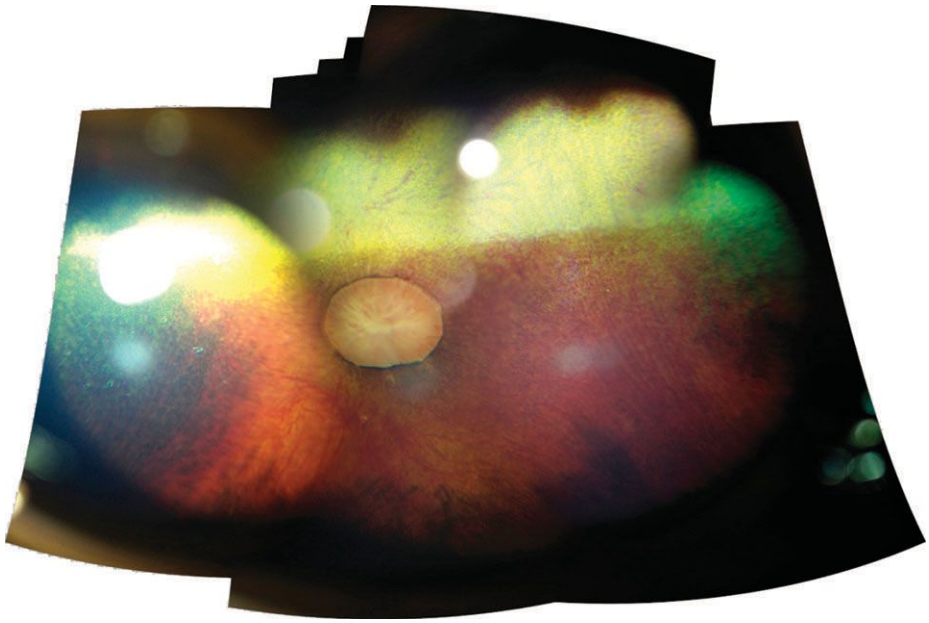

Fig. 15. Stitched image courtesy of Tim Knott and Rob Lowe, UK.

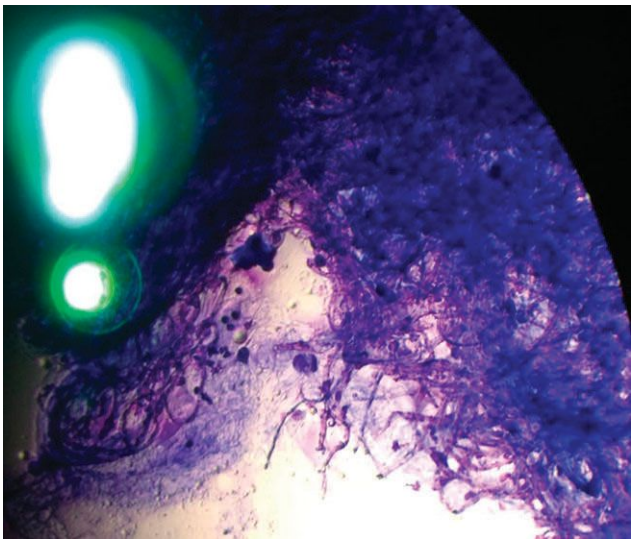

Fig. 17. Fungal hyphae imaged through the ocular of a microscope with a smartphone camera (image courtesy of Dr. Sarah Czerwinski, University of Florida).

8. The flash should be reduced with sticky tape to reduce photophobia and blepharospasm. The Camera+ app has a rheostat to reduce the light intensity.
9. Software is available for automatic stitching of multiple photographs of the same subject (Panorama's AutoStitch app).
10. The iPhone light is great for "dazzle reflex" testing.
11. Practice photography on enucleated eyes or anesthetized animals.

Acknowledgments

Tim Knott from the UK is the true leader in equine phoneoscopy and deserves credit for seeing the potential of the smartphone for helping horses with eye problems. Brian Patterson and Rob Lowe from the UK also piqued my interest.

Conflict of Interest

The Author declares no conflicts of interest.

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[b]iPhone, Apple Inc., Cupertino, CA 95014.
[c]iPad, Apple Inc., Cupertino, CA 95014.
[d]Camera+ app, taptaptap, Tasty Bits for Your iPhone, http://taptaptap.com.
[e]Panorama's AutoStitch, Cloudburst Research, Vancouver, BC, V6E 2E9, Canada.
[f]Windows Live Movie Maker, Microsoft, Redmond, WA 98052.
[g]Apple Computer, Apple Inc., Cupertino, CA 95014.

How to Use Ophthalmoscopes

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1. Introduction

The posterior segment or fundus (the internal structures of the globe behind the lens) consists of the vitreous, retina, and optic nerve and is examined using low magnification indirect ophthalmoscopy followed by high magnification direct ophthalmoscopy. The two methods are complementary rather than exclusive.[1] The results of an examination of the horse fundus are always more easily achieved in a dark environment with a dilated pupil.

2. Methods

Examination in the Dark

Indirect and Direct Ophthalmoscopy

The normal appearance of the equine fundus requires considerable practice for correct interpretation, because there is much normal variation. Most pathologic lesions of the fundus are identified near and below the optic nerve head and typically involve hyperpigmentation or depigmentation.

Indirect Ophthalmoscopy

This is a useful technique for screening the ocular fundus and can be performed most simply using a bright pen light or transilluminator and a condensing lens. The system produces a low magnified reversed inverted virtual image, such that a large field of view is produced (Figs. 1–4). Mydriasis, a bright light source, and darkness are essential for a detailed fundic examination.

3. Results and Discussion

How to Do Indirect

A condensing lens is held some 2–8 cm from the horse's eye and the light source is held level with the bridge of the observer's nose (Figs. 1, 3, and 4). The aim is that the observer's eye, the light source, the lens, and the patient's pupil should all lie in the same axis. The plane of the lens must be parallel to

Fig. 1. Indirect ophthalmoscopy is best used for low magnification screening of the horse fundus for lesions.

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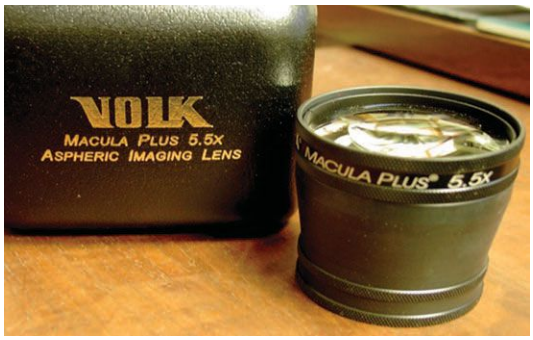

Fig. 2. A 5.5 D lens is very good for magnifying lesions of the horse fundus.

that of the horse's iris and pupil. The light is directed into the horse's eye so that the tapetal reflection is obtained and the lens is moved to and fro until a sharp clear image is produced. The observer-patient distance is approximately 50–75 cm.

In horses a +20 diopter (D) condensing lens is the most versatile in use, although the image is minified. A 20 D lens minifies the fundic view with 0.79× and 0.84× magnification laterally and axially, respectively. The 20 D lens provides a nice panoramic, screening view of the equine fundus, but it is not satisfactory for detailed highly magnified observations. Indirect ophthalmoscopy with a 14 D lens provides a magnified view of 1.18× lateral magnification and 1.86× axial magnification. Indirect ophthalmoscopy with a 5.5 D lens provides 3.88× lateral magnification and 20.10× axial magnification in the horse.

Direct Ophthalmoscopy

The use of a standard direct ophthalmoscope produces an upright image of greater magnification than is possible with the indirect ophthalmoscope when used close to the patient's eye (Fig. 5). However, viewing the fundus directly along a beam of light necessarily restricts the field of view. The direct ophthalmoscope provides the most magnified view of the fundus in the horse, with a lateral magnification of 7.9× and an axial magnification of 8.4×. Both distant direct ophthalmoscopy and close direct ophthalmoscopy should form part of a direct ophthalmoscopic examination.

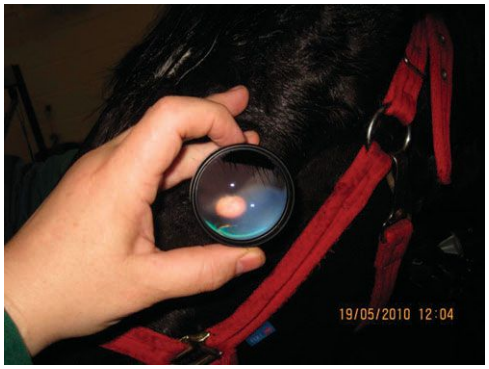

Fig. 3. The image with the indirect ophthalmoscope is upside down and reversed.

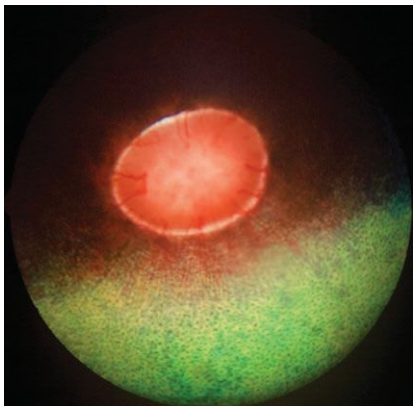

Fig. 4. This is the image of a horse fundus observed with the indirect ophthalmoscopy technique.

Fig. 5. The direct ophthalmoscope is the basic instrument used to view the horse retina and optic disc.

Fig. 6. The direct ophthalmoscope has a large aperture for large pupils.

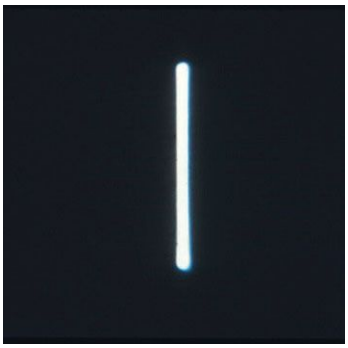

Fig. 7. The direct ophthalmoscope has a slit aperture. This slit is 2.2 mm wide when focused on the cornea and iris, which makes it too wide and bright to detect subtle aqueous flare.

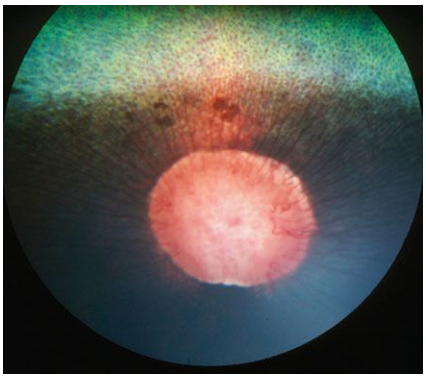

Fig. 9. The image with a direct ophthalmoscope is upright and true in orientation.

There are also controls on the head of the ophthalmoscope for spot size (use the largest diameter, Fig. 6) and brightness (use as bright as the animal will tolerate). There may be controls for a red-free filter (rarely used) and a cobalt blue filter (use to look for corneal ulcers).

Red is seen as black with a "red-free" or green filter. The "red-free" (don't call it "green") filter is useful for enhancing the appearance of blood vessels and hemorrhages by making blood show up black. The "red-free" filter in the ophthalmoscope is used to differentiate between small retinal melanomas, or nevi, and a choroidal nevi. The retinal blood supply and its retinal pigment epithelium act like a red filter. Therefore, a melanoma located in the choroid will not be seen when viewed with the "red-free" filter since red and green cancel each other out. On the contrary, a melanoma located on or in the retina will still be seen with the "red-free" filter in place.

The slit on the ophthalmoscope is designed to look at the optic disc and is too wide at 2.2 mm for detecting trace aqueous flare (Fig. 7). This is especially true if there is corneal edema. In attempting to look for aqueous flare or evaluate the anterior chamber depth, the slit should be aimed from the front and the examiner look from the side to get the best view. If the scope has a cobalt blue filter (Fig. 8) this can be used to look for subtle corneal abrasions and corneal ulcers following the use of topical fluorescein dye.

How to Do Direct

Distant Direct Ophthalmoscopy

This technique uses the tapetal fundus as a means of retro-illuminating the structures anterior to it. The ophthalmoscope is set to 0 diopter (no magnification) and directed to find the tapetal reflex in the pupil at an observer-patient distance of 25–40 cm (Figs. 9 and 10). It is a useful way of assessing whether there are any opacities between the observer and the fundus and is usually used as a quick screening method prior to a more detailed assessment. Any opacities present in the ocular media (cornea, aqueous, lens, vitreous) will appear as black forms against the fundus reflex. An assessment of comparative pupil sizes may also be made using this technique.

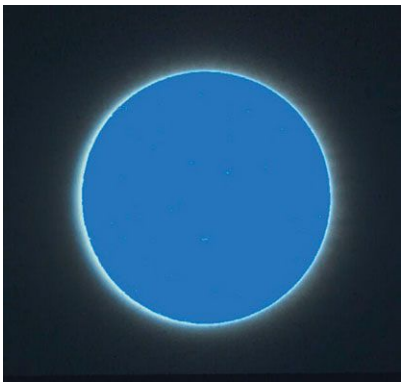

Fig. 8. The direct ophthalmoscope has a cobalt blue filter for looking at corneal abrasions and ulcers.

Fig. 10. The distant direct ophthalmoscopic technique is seen here.

Fig. 11. The close direct ophthalmoscopic technique is demonstrated.

Close Direct Ophthalmoscopy

Direct ophthalmoscopy can be performed with direct ophthalmoscopes. Direct ophthalmoscopy can be used to examine all aspects of the eye and adnexa but is most commonly done to examine the retina and optic nerve head. The aperture of the instrument must be held as close as possible to the observer's eye and the subject's eye (Figs. 11 and 12). As the examiner reduces the strength of the plus lenses, the focus of observation gradually extends posteriorly, so that magnified details of the lids, cornea, angle, aqueous, iris, lens, and vitreous are successively visualized until the features of the fundus are brought into focus. For a detailed examination, darkness is essential and mydriasis is helpful.

For examination of the external eye and adnexa a setting of +20 to +15 D (green numbers are plus) is required. The iris and angle may be examined with a setting of +15 to +12 D (Fig. 13). For the lens, the setting will be about +12 to +8 D, depending on whether the anterior or posterior parts are being examined. Intermediate settings will be required for the aqueous and vitreous (Fig. 14). Close examination of the fundus is usually performed with the ophthalmoscope placed some 2 cm from the eye and a setting between +2 D or −2D (red numbers are minus; usually 0) is required. The examination is often made easier and safer if the hand holding the ophthalmoscope is rested lightly against the horse's head, so that sudden movements do not damage the eyes of the horse or the examiner.

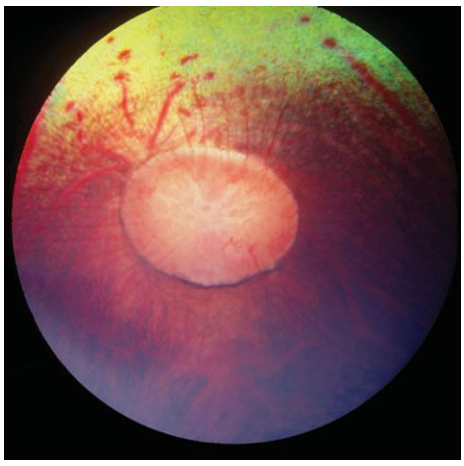

Fig. 12. The view of the horse fundus with a direct ophthalmoscope.

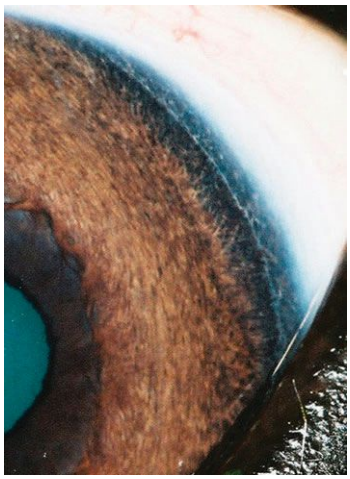

Fig. 13. The view of the horse drainage angle with a +15 direct ophthalmoscopic setting.

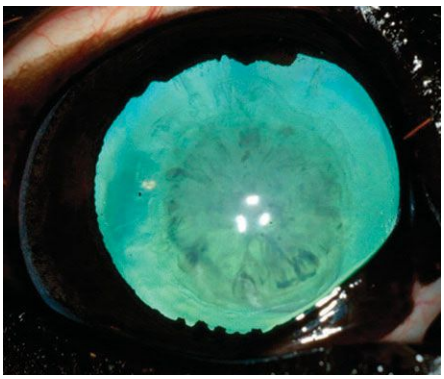

Fig. 14. The view of the horse lens (with cataract) with a +8 direct ophthalmoscopic setting.

Acknowledgments

Conflict of Interest

The Author declares no conflicts of interest.

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How to Reach the Medical Standards of Care for Ulcerative and Non-Ulcerative Equine Keratopathies

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1. Introduction

Keratopathies may be ulcerative or nonulcerative in the horse. Ulcerative keratopathies may be infectious or sterile but are always associated with increased tear film protease activity. Ulcerative infectious keratopathies in horses may be caused by bacteria, fungi, and possibly viruses. Sterile ulcers may be caused by foreign bodies, tear film problems, corneal denervation, or basement membrane corneal dystrophies.

Nonulcerative keratopathies may also be infectious or sterile and range from cellular invasions of the stroma by inflammatory or neoplastic cells to persistent corneal edema. Infectious stromal abscesses, glaucoma-induced stria, uveitis-associated endothelial edema, eosinophilic keratitis, calcific band keratopathy, neoplasia, and immune mediated keratitis (IMMK) are major concerns.[1,2]

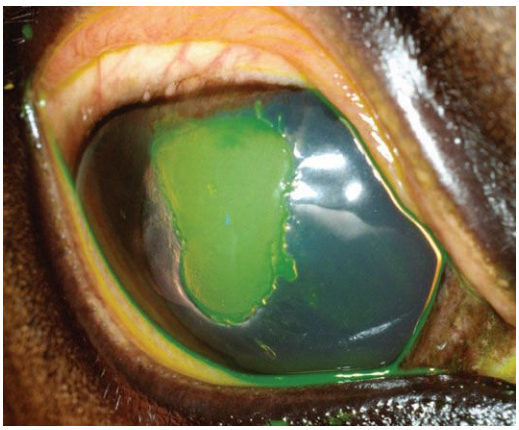

Fig. 1. Fluorescein staining indicates corneal epithelium is missing to cause a corneal ulcer.

2. Medical Standards of Care

Ulcers

The current medical standard of care of treatment of ulcerative keratitis (Fig. 1) in the horse is a topical broad spectrum, nonirritating antibiotic, careful utilization of a mydriatic/cycloplegic, and an antiprotease compound such as serum. A systemically administered nonsteroidal drug is also critical to quick healing.

Ulcer Expectations

If the ulcer does not diminish in size at a rate of ~1 mm/day, the cornea does not vascularize at a rate of

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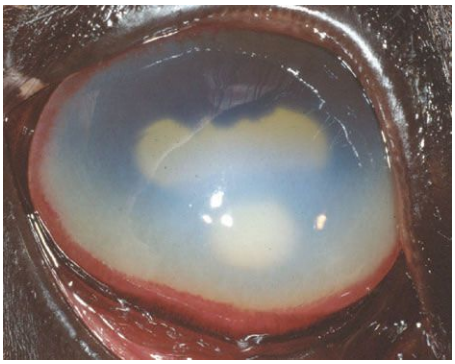

Fig. 2. A deep stromal abscess is present with corneal edema and vascularization.

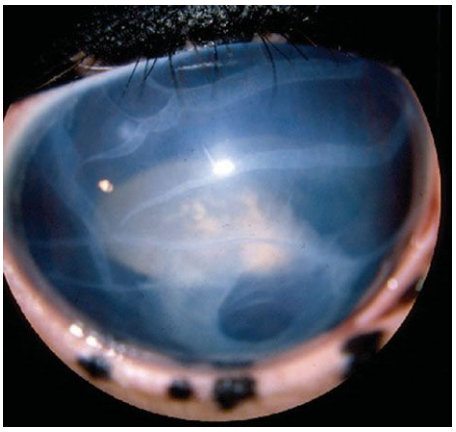

Fig. 4. Multiple band opacities from glaucoma are called stria.

1 mm/day, and/or the signs of uveitis do not improve when therapy is initiated, then consider culture and/or cytology to document a change in the infection to decide if different antimicrobials are indicated. Additional antiproteases may also be beneficial. A suspicion of fungal involvement should always exist in ulcers that do not vascularize or heal. If the ulcer deepens, then amnion, conjunctival, or corneal grafting surgery is recommended. Contact lenses, chemical cautery with phenol or trichloroacetic acid, debridement of loose epithelium, gridding, and/or burring can be used for superficial noninfected ulcers.

Stromal Abscesses (SA)

The current medical standard of care of treatment of superficial and deep stromal abscesses (Fig. 2) is topical antimicrobials (including antifungals), atropine, and systemic antimicrobials and NSAIDs.

SA Expectation

Stromal abscesses must vascularize to heal. Therapy may take weeks. Vascularization and a reduction in the associated uveitis are signs of improvement. As the SA resolves, the blood supply leaves and the lesion will appear pale and then white. Superficial SAs tend to respond better to medical therapy than deep SAs. Intrastromal voriconazole may have some use in specific cases of SA. Surgery may be necessary to remove the abscess if medical therapy fails to resolve the inflammation in 2 to 4 weeks.

Linear Keratopathy or Stria

Broad white linear opacities can follow blunt globe trauma or immune mediated endotheliitis in the horse. These are single and nonprogressive (Fig. 3). Stria are breaks in Descemet's membrane following elevated IOP in glaucoma. Stria in glaucoma are multiple and branching in nature (Fig. 4).

Linear Keratopathy and Stria Expectations

Intraocular pressure measurement is important. Careful monitoring over months to years is recommended. Therapy is generally not available or helpful, but if edema is present topical steroids are indicated.

Corneal Neoplasia

The current medical standard of care of treatment of epithelial dysplasia (Fig. 5) is topical 1% 5FU or 0.04 mitomycin C. Corneal squamous cell carcinoma (SCC) requires keratectomy and cryotherapy or ra-

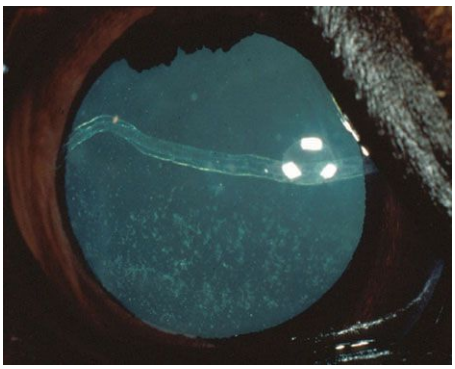

Fig. 3. A single band opacity is present. Intraocular pressure was normal.

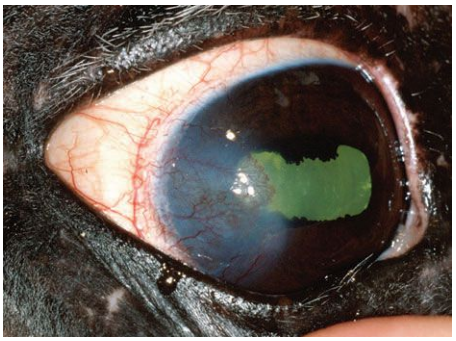

Fig. 5. Epithelial dysplasia is a precursor to SCC and benefits from topical therapy.

Fig. 6. Eosinophilic keratitis is classically near the nasal limbus.

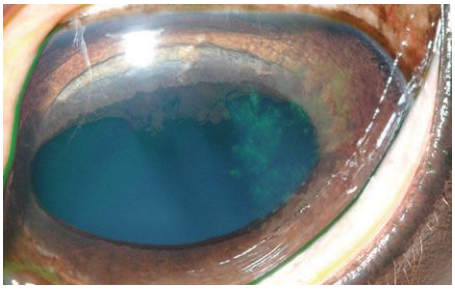

Fig. 8. Subepithelial keratomycosis is actually quite common in many geographic regions. It manifests as faint subtle fluorescein staining, as shown in this eye.

diotherapy. Preferably, the keratectomy should be covered with an amnion graft, but conjunctiva can be used.

Neoplasia Expectations

Scarring of the cornea may be difficult to differentiate from tumor recurrence. Keratectomy and adjunctive therapies are needed for carcinoma *in situ* and SCC. Rapidly progressive and invasive SCC may necessitate enucleation.

Eosinophilic Keratitis (EK)

Eosinophilic keratitis is an immune-mediated corneal condition that is probably related to systemic parasites. It can be ulcerated or nonulcerated and infected with bacteria and fungi in some cases. The current medical standard of care of treatment of eosinophilic keratitis (Figs. 6 and 7) is topical steroids and antimicrobials. Ulcers can complicate the treatment.

EK Expectations

These lesions are typically slow to heal. Scarring of the cornea occurs. Cyclosporine A (2%), in combination with systemic nonsteroidal anti-inflammatory drugs (NSAIDS), are indicated. Topical cromolyn sodium (4.0% TID) or lodoxamide (0.1% TID) can also aid healing. Systemic corticosteroids may be necessary. Horses should be dewormed twice with ivermectin 10 days apart. Switching to moxidectin may also be beneficial. Superficial lamellar keratectomy to remove plaques speeds corneal healing.

Subepithelial Keratomycosis (SEK)

Fungal infections in horses may begin as faint corneal opacities that are slightly painful. Fluorescein retention may be faint (Fig. 8) or absent. Rose bengal retention may be present. Slitlamp examination finds opacification beneath the epithelium. Cytology and culture following corneal scraping are diagnostic. Horses with the diagnosis of SEK are treated with topical antifungals.

SEK Expectations

Resolution of SEK will take several weeks. Immune-mediated keratitis should be considered in the differential diagnosis.

Immune Mediated Keratitis (IMMK)

Epithelial, subepithelial, stromal, and endothelial forms are possible. Immune mediated keratitis is a diagnosis made by appearance, biopsy and cytology, and response to therapy (Fig. 9). The current medical standard of care of treatment of IMMK is topical corticosteroids, nonsteroidals, or cyclosporine A.

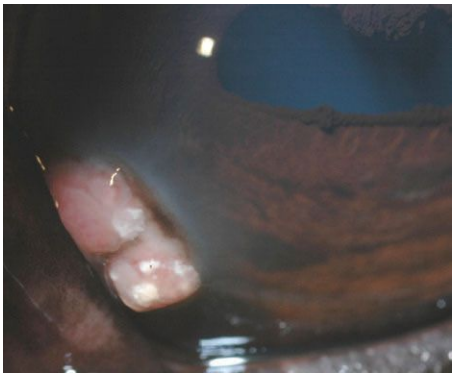

Fig. 7. Some horses with EK have yellowish substances near the limbus.

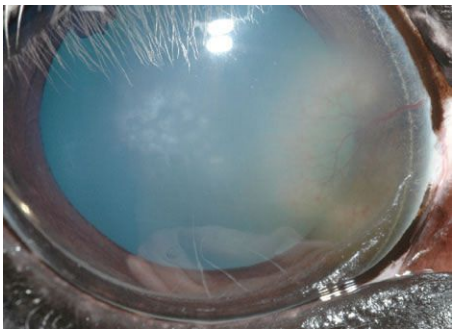

Fig. 9. A yellow brown discoloration of the stroma is found in some eyes with IMMK.

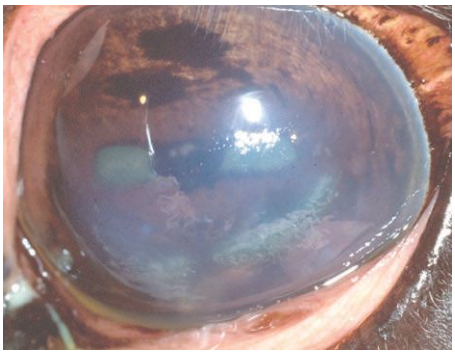

Fig. 10. Calcific band keratopathy is painful due to epithelial calcium deposition.

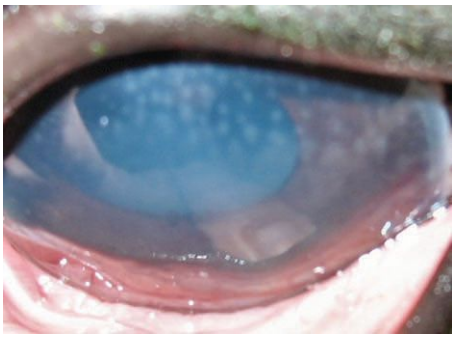

Fig. 11. Punctate opacities characterize some eyes with herpes keratitis.

IMMK Expectations

Topical dexamethasone rapidly resolves many of these eyes, but co-infection is always a concern. Consider adding topical cyclosporine, and erythromycin or doxycycline, and systemic steroids and/or doxycycline to difficult cases. Corneal biopsy is recommended for recalcitrant eyes. Topical NSAIDS such as diclofenac or flurbiprofen are also indicated for IMMK eyes that recur.

Calcific Band Keratopathy (CBK)

Corneal degeneration from uveitis or chronic ulcers can result in calcium deposition into the diseased cornea. It can be a very irritating and painful problem. The current medical standard of care of treatment of calcific band keratopathy (Fig. 10) is topical 0.2% EDTA and debridement. Any accompanying ulcers are also treated with antimicrobials and antiproteases. Many of these horses also have equine recurrent uveitis (ERU) and the topical steroids may have induced the calcium deposition.

CBK Expectations

The calcium is generally chelated in a manner of days, but superficial keratectomy may be necessary if the calcium deposition is deeper in the stroma. Debridement, keratectomy, and/or burring may be necessary for resolution.

Herpes and Viral Keratitis

Multiple, superficial, white, punctate, or linear opacities of the cornea, with or without fluorescein dye retention, are found associated with equine herpes virus 2 and perhaps other viruses. The focal punctate corneal opacities (Fig. 11) may be found at the end of superficial corneal vessels and may retain rose bengal stain. Varying amounts of ocular pain, conjunctivitis, and iridocyclitis are present. Multiple foals in a herd may be affected. The current medical standard of care of treatment of suspected herpes keratitis is topically administered 0.1% idoxuridine, 1.0% trifluorothymidine, or 3.0% acyclovir.

Herpes Expectations

Viral keratitis is overdiagnosed, in the author's view, but still exists in nature. The response to therapy can be quite variable and the clinician may become frustrated. Further diagnostic workups and cultures of ulcerated lesions can be helpful. Topical NSAIDs for treatment of equine herpes ulcers may have as much benefit as antivirals.

Keratoconjunctivitis Sicca (KCS)

The horse more commonly develops a qualitative KCS rather than the quantitative KCS in dogs. Diagnosis is based on Schirmer tear tests, rose bengal retention, and increased tear film breakup time. Equine KCS can be found with fungi and equine herpes, but can also be found with corneal edema and corneal fibrosis.

KCS Expectations

Qualitative KCS may be temporary and associated with corneal abrasions. Topical antimicrobials, serum, and a fly mask are indicated. Careful monitoring is indicated.

Traumatic Bullous Keratopathy

Blunt trauma to the globe can result in generalized and dramatic corneal edema (Fig. 12). Uveitis may

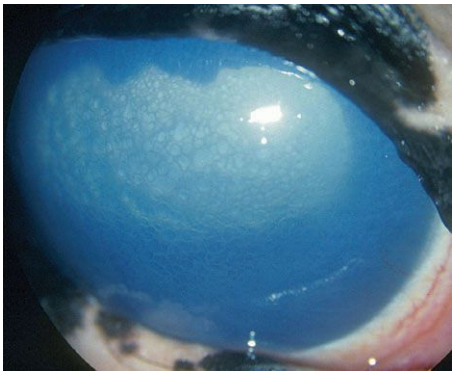

Fig. 12. Generalized edema following blunt ocular trauma is termed bullous keratopathy.

or may not be present. The edema can triple the corneal thickness. The hyperosmotic, Muro 128 (5% NaCl), is indicated TID for these eyes.

Traumatic Bullous Keratopathy Expectations

Traumatic bullous keratopathy should resolve, beginning in the periphery first, over 3 to 6 weeks. Thermal keratoplasty or a Gundersen flap may help recalcitrant eyes. The edema is permanent if Descemet's membrane detaches.

Acknowledgments

Conflict of Interest

The Author declares no conflicts of interest.

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How to Diagnose and Treat Immune-Mediated Keratitis of the Horse

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1. Introduction

The pathogenesis of many nonulcerative keratopathies of horses is believed to be mediated by a dramatic corneal immune response to a foreign protein, microbial antigen, or a self-antigen.[1,2] Such suspected immune-mediated keratitis (IMMK) of horses has been classified primarily according to the apparent depth of the inflammatory response.[3] Geographic differences in primary corneal insult, antigen exposure, and in the duration of the IMMK prior to its recognition have led to some discrepancy in the clinical presentations and responses to therapy. It does seem that the more chronic the duration of IMMK, the poorer response to medical therapy. The diagnosis of IMMK is based on clinical signs and response to therapy.

2. Epithelial IMMK

This is a unilateral disease affecting horses of any age. Vascularization is not a prominent feature of the disease. There is a diffuse central superficial corneal opacity usually associated with very slight blepharospasm and discomfort. There may be some slight associated conjunctival hyperemia or chemosis. The superficial opacity represents irregular coalescing clumps or islands of thickened epithelium with no underlying stromal edema. The unaffected areas of cornea appear normal. Fluorescein is weakly and transiently retained in the interstices between the islands of abnormal epithelium Fig. 1. Topical dexamethasone results in rapid corneal clearing in most cases and the disease has not been observed to recur after successful treatment.

A Note of Caution

Subepithelial keratomycosis has recently been identified in Florida, Canada, Brazil, Mexico, Denmark, and Germany. It looks identical to some cases of the epithelial keratitis form of IMMK. Biopsy and response to topical antifungal therapy are factors in making the diagnosis of subepithelial keratomycosis. It does seem capable of self-resolution in a few cases which is quite confounding!!

3. Chronic Superficial Stromal IMMK

This disease is characterized by an insidious onset with affected eyes showing only slight to moderate discomfort. The lesions appear to be initially restricted to the area under the upper lid (Fig. 2) and, less frequently, the third eyelid and lower lid. The paracentral cornea is commonly involved in the U.S. There is prominent subepithelial arborizing vascularization from the limbus, perivascular epithelial edema, and a superficial yellow-white stromal cell

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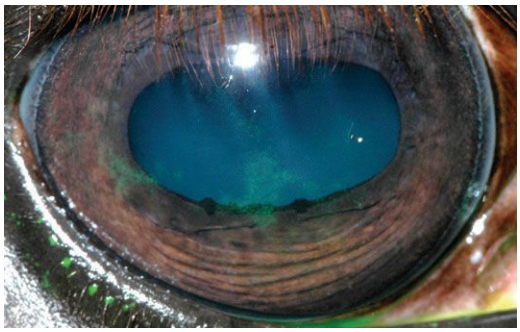

Fig. 1. Fluorescein is weakly and transiently retained in the interstices between the islands of abnormal epithelium in this eye with epithelial IMMK.

infiltrate. Tear production is normal and no fluorescein uptake occurs. The apposing palpebral conjunctiva is moderately hyperemic.

The disease is initially unilateral but the contralateral eye may become affected with time. Topical treatment with cyclosporine A twice daily usually results in clearing of the cornea in 7 to 10 days in cases of short duration. Long-standing cases may be refractory to CsA. Most cases of chronic superficial keratitis are not responsive to topical corticosteroids. Subconjunctival CsA implants are helpful for this condition. Successful resolution of refractory cases of chronic superficial keratitis in the U.S. required superficial keratectomy and conjunctival grafting.

4. Chronic Deep Stromal IMMK

This is an episodic keratitis recurring at irregular intervals of up to several years. There is frequently history of initiating ocular trauma and the disease may derive from a local adaptive response to autoantigen in an immunocompetent cornea.

In the acute or active phase of the disease there is an extensive and dense, deep, stromal edema, white cellular infiltrate, and fibrovascular response with isolated blood vessels encroaching on the affected stroma at various levels. The intensity of the stromal changes varies between cases and between

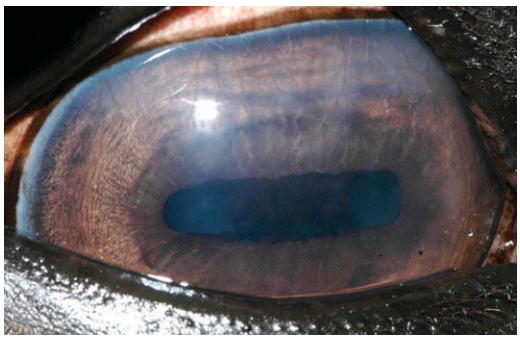

Fig. 2. The lesions appear to be initially restricted to the area under the upper lid in stromal IMMK.

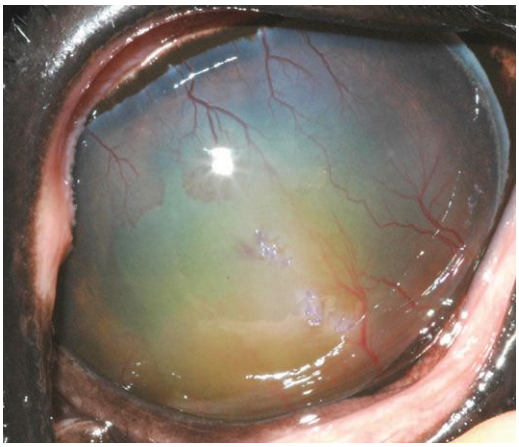

Fig. 3. In some eyes with stromal IMMK, a yellow-green tinged coloration may appear within the midstromal central and paracentral cornea. Some sutures are present following corneal biopsy.

episodes. Despite the dramatic appearance of affected eyes, the disease is associated with no ocular pain. Subepithelial bullae may form and rupture, however, to create fluorescein positive superficial erosions that are associated with transient ocular discomfort. In some eyes, a yellow-green tinged coloration may appear within the midstromal central and paracentral cornea (Fig. 3). The ventral paracentral cornea is most commonly affected. Subepithelial calcium deposition may occur in some eyes. In the quiescent or inactive phase of the disease there is a modest diffuse stromal fibrosis with some superficial vascularization.

The therapeutic benefit of topical corticosteroids is very limited in acute episodes of chronic deep keratitis although they may slowly accelerate clearing of the cornea. Topical cyclosporine A twice daily results in significant suppression of the acute corneal reaction and clearing of the cornea within 10 to 14 days in eyes with chronic deep keratitis. However, treatment may need to be maintained for long periods to prevent recrudescence of the disease. Spontaneous clearing of the cornea can also occur. Topical and/or systemic doxycycline aids healing of chronic deep keratitis with the yellow-green stroma. Topical NSAIDs are also often beneficial. Superficial keratectomy and conjunctival grafting are necessary for healing of cases of chronic deep keratitis in a few cases.

5. Endotheliitis

This type of IMMK is characterized by acute, uni- or bilateral, central edema, and deep vascularization. Endothelial immunoreactivity to a persistent, possibly viral, heteroantigen may be involved in the pathogenesis. Affected eyes are nonpainful with no evidence of anterior uveitis in the UK, but can be very painful in horses in North America. There is a deep diffuse fibrocellular infiltrate, opacification,

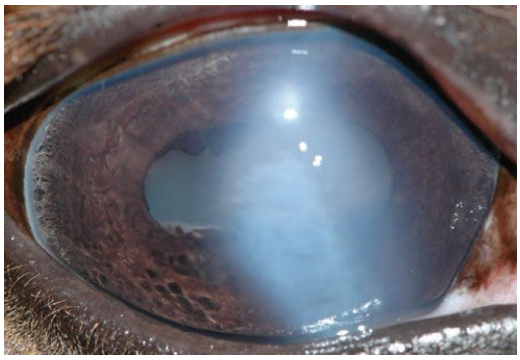

Fig. 4. There is a deep diffuse stromal edema in the central cornea of this eye with immune mediated endotheliitis.

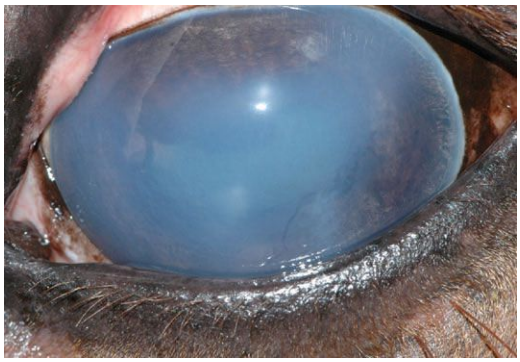

Fig. 5. The other eye in the horse in Fig. 4 has more diffuse edema.

and stromal edema in the central cornea which may evolve rapidly into bullous keratopathy (Figs. 4 and 5). Isolated arborizing blood vessels encroach upon the affected area at the level of Descemet's membrane and/or endothelium. In some cases, dense clumps of cells may be evident adherent to the endothelium in the region of the terminal blood vessels. In long-standing cases stromal mineralization can occur. Rapid clearing of the cornea and regression of the blood vessels using topical dexamethasone occurs in many cases of endotheliitis in the UK. It is a difficult disease in control in North America. Endotheliitis may be a precursor to glaucoma in the horse. Treatment should continue for a weeks following any corneal clearing. Recurrence of the disease is possible in a small number of long-standing cases. A successful outcome following penetrating keratoplasty has been reported in the U.S.

Acknowledgments

Conflict of Interest

The Author declares no conflicts of interest.

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How to Use the Clinical Examination to Determine the Significance of Abnormalities of the Horse Cornea and Adnexa

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1. Introduction

The normal tear film is continuous over the entire surface of the horse cornea and conjunctiva. The tear film "breaks up" from instability and discontinuity of the ocular surface if the cornea is scarred, infected or edematous, and if blinking does not occur often enough.[1,2]

Precorneal Tear Film

The precorneal tear film is a trilayered mucin dominated gel produced by the meibomian glands (outer oily layer), the lacrimal and nictitans glands (aqueous layer), and the conjunctival goblet cells (inner mucin layer). The tear film serves as an extracellular matrix to the cornea, which provides an optically smooth surface and aids nutrition to the cornea. It contains vitamin A, proteinases, proteinase inhibitors, growth factors, and cytokines that affect corneal health. The tear film is replaced every 7 min in the horse![3]

Third Eyelid

The nictitans or third eyelid (TE) of the horse is located at the medial canthus. It displays rapid, near horizontal movement as it protects the cornea and distributes the tear film. The TE conjunctiva may be completely pigmented or may contain no melanin. A large gland produces part of the tear film and is found at the base of a T-shaped piece of supportive TE cartilage.

Conjunctiva

Conjunctiva is a mucous membrane that is pigmented near the limbus in some horses. The conjunctival epithelium covering the globe (bulbar conjunctiva) begins at the corneoscleral junction or limbus, lies superficial to the sclera, and reflects forward at the fornix where it becomes palpebral conjunctival epithelium. The palpebral conjunctival epithelium lines the inner eyelid surface and continues to the mucocutaneous junction at the lid margin. The ventromedial portion of the palpebral conjunctiva covers the nictitating membrane. Components of the ocular immune system and tear producing cells are present in the conjunctiva.

2. The Clinical Examination: Observations

The Cornea Looks "Dry"

Keratoconjunctivitis Sicca

"Dry eye" or keratoconjunctivitis sicca (KCS) is an abnormality of the tear film and may be commonly

NOTES

Fig. 1. The corneal surface is not shiny due to the absence of tears in this eye with KCS.

due to qualitative changes in the tear film or, rarely, due to reduced quantity of tears in the horse. Environmental, immunologic, traumatic, and infectious causes of KCS are reported. Mild to severe ulcerative keratitis and conjunctivitis can be present. The corneal surface is not shiny in KCS (Fig. 1). Treatment with a fly mask, topical serum, and cyclosporine A can be beneficial. This may not be a permanent condition.

The Conjunctiva is "Red"

Conjunctivitis

Because the eye has limited ways to react to injury, the conjunctiva gets red (Fig. 2) or becomes inflamed in nearly all types of eye disease. Conjunctivitis is a nonspecific finding that indicates ocular inflammation, but may also be seen in systemic diseases. Infectious and noninfectious diseases of the eyelids, cornea, sclera, anterior uvea, nasolacrimal system, and orbit can result in conjunctivitis in the horse.

Habronemiasis is a parasitic disease resulting in conjunctival and ocular granulomas. *Thelazia lacrymalis* is a commensal parasite of the conjunctival fornices and nasolacrimal ducts of horses, which can incite conjunctivitis, superficial keratitis, dacryocystitis, and mild eyelid swelling.

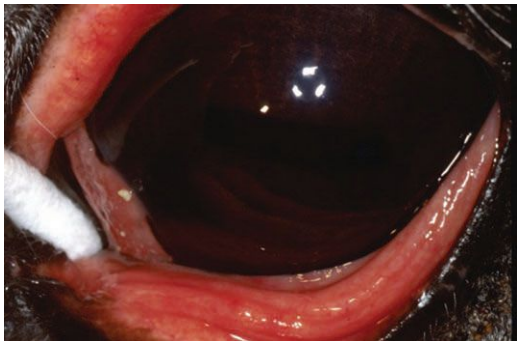

Fig. 2. A red eye means conjunctivitis.

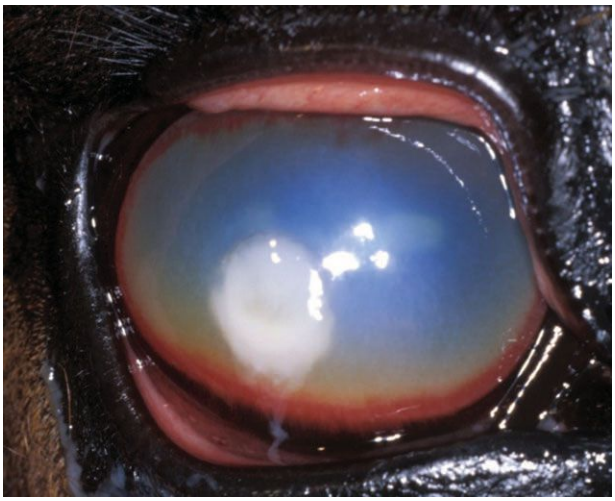

Fig. 3. The cornea changes color to red when blood vessels move into the cornea, to blue if edema is present, and to white/yellow if neutrophils migrate into the cornea. Conjunctivitis is also pronounced in this eye.

Foal conjunctivitis is associated with neonatal maladjustment syndrome, septicemia, immune-mediated hemolytic anemia, environmental allergens and irritants, dermoids, and subconjunctival or episcleral hemorrhages secondary to birth trauma.

Allergic conjunctivitis due to exposure to environmental antigens is treated with topical corticosteroids and is often difficult to eliminate completely due to the nature of the horse's environment.

Conjunctival neoplasia may be focal or diffuse and includes squamous cell carcinoma, hemangiosarcoma, and lymphoma. Tumors of the conjunctiva may resemble conjunctivitis in the early stages. The eyelid, globe conjunctiva, and third eyelid conjunctiva are susceptible to neoplasia.

3. Cornea

The horse cornea should normally be clear, smooth and shiny.

The Cornea is "Red"

Vascularization of the cornea will result in the entire or focal regions of the cornea being red (Fig. 3). The position of the redness can indicate the location of inflammation. The higher the intensity of redness, the more the incitatory activity there is for the neovascularization at the corneal site.

The Cornea is Hazy or "Blue"

Edema causes a blue color of the cornea if severe and more subtle haziness if the edema is slight (Figs. 3 and 4). Severe edema can result in bullae or "bubbles" in the epithelium if very severe. Corneal edema may surround a focal ulcer, foreign body or abscess, or involve the entire cornea in uveitis and glaucoma.

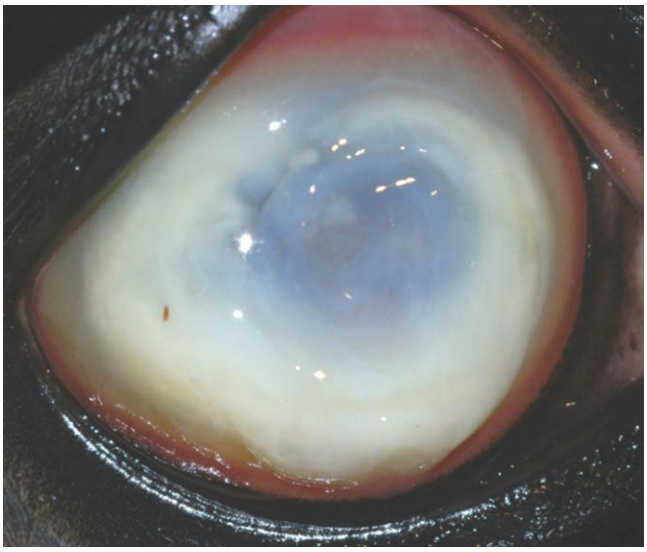

Fig. 4. A prominent white cellular infiltrate with a darker thin center is present.

The Cornea is "White or Yellow"

Neutrophil and macrophage invasion of the cornea causes a yellow/white opacity (Figs. 3 and 4).

The Cornea is "Shiny" or "Dark"

A very shiny area of the cornea indicates that the cornea is thin (Fig. 2). A dark area can also be from a thin area of cornea (Figs. 5–8), or can be from rupture of the cornea and iris prolapse. Fibrin covering an iris prolapse can be red to pink in color.

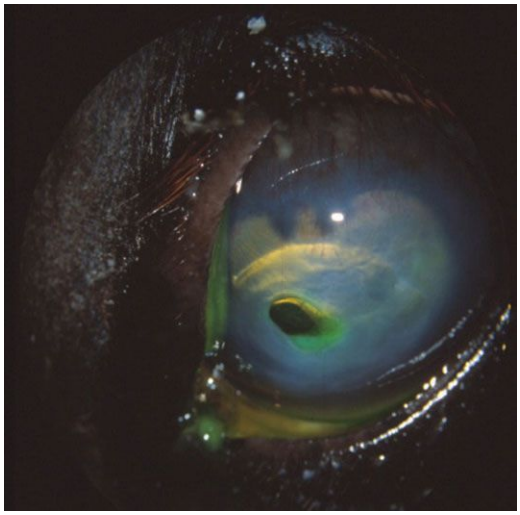

Fig. 5. Deep corneal injuries may allow the observation of a darker corneal area if the tapetal reflection is not detectable as in this descemetocele. Fluorescein dye is retained ventral to the deep lesion.

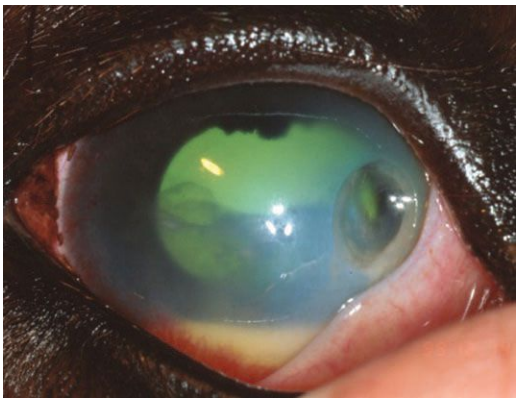

Fig. 6. A thin (shiny) corneal ulcer with edema and hypopyon are present.

The Cornea is "Soft and Gelatinous"

Excessive tear film proteinase activity is termed "melting" and results in a liquefied grayish-gelatinous appearance to the stroma of the ulcer (Fig. 9).

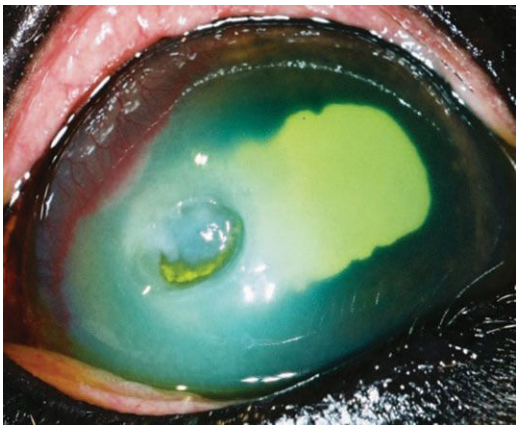

Fig. 7. A cellular infiltrate, central melting, and a thin cornea are present. The tapetal reflection is very apparent through the thinned cornea in the ulcer.

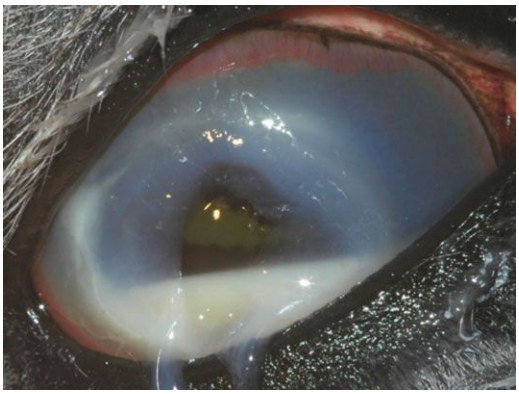

Fig. 8. Corneal cellular infiltrate with thinning of the center and hypopyon are present.

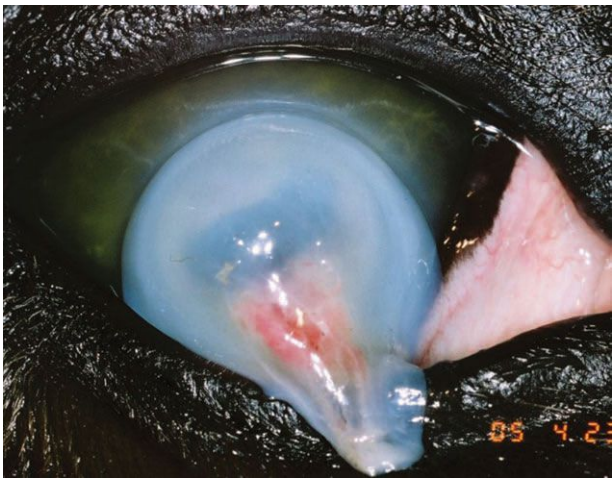

Fig. 9. Severe melting from excessive tear film enzyme activity.

The Cornea is "Dry"

The cornea will appear dry if there is a deficiency of the precorneal tear film.

Corneal Ulcers

Equine corneal ulceration is very common in horses and is a sight-threatening disease requiring early clinical diagnosis and appropriate medical and surgical therapy.

Ulcers can range from simple superficial breaks or abrasions in the corneal epithelium (Figs. 10–12) to full-thickness corneal perforations with iris prolapse. Both bacterial and fungal keratitis in horses may present with a mild early clinical course, but require prompt therapy if serious ocular complications are to be avoided.

Many severe cases of equine ulcerative keratitis present initially as minor corneal epithelial ulcers or infiltrates with slight pain, blepharospasm, epiphora, and photophobia. At first, anterior uveitis and corneal vascularization may not be clinically pronounced. Slight droopiness of the eyelashes of the upper eyelid may be an early, yet subtle, sign of corneal ulceration.

Fig. 10. Dry cornea associated with fungi.

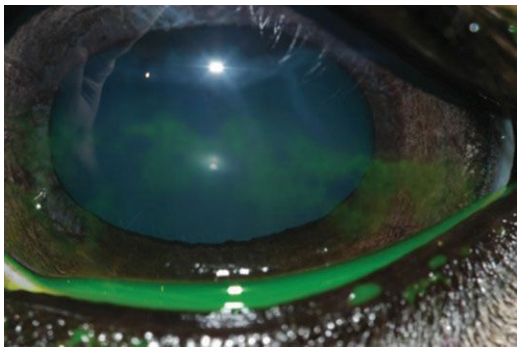

Fig. 11. Corneal abrasion indicated by faint fluorescein staining occurs when some but not all of the epithelium is missing.

Fluorescein dye retention is diagnostic of a corneal ulcer. Faint fluorescein dye retention may indicate an abrasion or partial epithelial cell layer defect due to infiltration of fluorescein dye between inflamed epithelial cell junctions. Cobalt blue filters can enhance detection of abrasions (Fig. 13). Fluorescein dye retention is bright in color when the entire corneal epithelium is missing to expose the stroma.

Corneal destruction results from the excessive production and release of proteinases and other en-

Fig. 12. Iris prolapse is dark with blue edema.

Fig. 13. Cobalt blue filters aid abrasion detection.

Fig. 14. Eyelashes of the upper eyelid are normally perpendicular to the cornea but may droop if a painful eye problem is present. The eyelashes return to the normal position when the pain is reduced.

Fig. 16. Habronema has caused blepharitis.

zymes from microbes, tear film leukocytes, and keratocytes. These proteinases can lead to rapid degeneration of collagen and other components of the stroma, potentially inducing keratomalacia or corneal "melting."

Corneal ulcers in horses should be aggressively treated no matter how small or superficial they may be. Corneal infection and iridocyclitis are always major concerns for even the slightest corneal ulcerations. Iridocyclitis or uveitis is present in all types of corneal ulcers and must be treated in order to preserve vision. Some fungi may produce antiangiogenic compounds that inhibit vascularization.

The healing time of a superficial, noninfected corneal ulcer is about 1 mm/day, i.e., a 5 mm diameter ulcer should take around 5 days to re-epithelialize or heal.

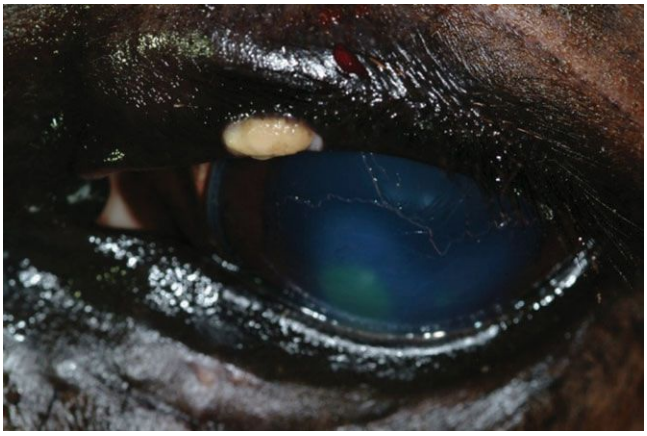

Fig. 15. Traumatic blepharitis with swelling is present.

4. Eyelids

Which Lid is Affected?

The eyelids are thin and highly vascular and have several important functions. They protect the eye, produce and distribute the precorneal tear film, prevent corneal drying, pump tears into the lacrimal puncta, and help control the amount of light entering the eye. Eyelid blinking maintains the continuity of the tear film. The tear film "breaks up" to cause discontinuity of the ocular surface if blinking does not occur often enough.

Upper eyelid disease is more significant than lower lid disease as most (75%) of the eyelid movement is in the upper eyelid.

Nasal canthal disease is more significant than lateral canthal disease due to the location of the tear drainage apparatus in the nasal region. The eyelashes of the upper lid are normally perpendicular to the cornea. If the conjunctiva, cornea, or iris become inflamed, the horse will pull the eyeball posteriorly such that the eyelashes begin to droop (Fig. 14).

The Eyelids are Swollen

Blepharitis or inflammation of the eyelids can be caused by infection, parasites, allergy, immune disease and trauma in the horse (Figs. 15–17). Traumatic blepharitis can develop into eyelid abscesses and may be associated with orbital fractures, penetrating and lacerating trauma, subpalpebral lavage system irritation, and bony sequestra. Demodex infestation may lead to lid alopecia, meibomianitis, and blepharitis. *Thelazia lacrimalis* is a spirurid nematode and a commensal parasite of the equine conjunctival fomices and nasolacrimal ducts. This parasite can cause diffuse blepharitis. Habronemiasis, a common cause of granulomatous blepharitis,

Fig. 17. Brush fire blepharitis with hair loss is present in this horse.

occurs mainly in the summer months when house and stable flies serve as vectors. Dying microfilaria in the eyelids, conjunctiva, lacrimal caruncle, medial canthus, and nictitans are thought to incite an immune-mediated hypersensitivity. Fly-bite blepharitis, dermatophilus, and staphylococcal folliculitis are other causes of blepharitis, especially in young foals.

Eyelid Lacerations Need Repair

Eyelid lacerations should be carefully repaired with a two layer closure (Figs. 18 and 19). Extra care should be directed at repositioning the eyelid margin. Removal of a torn eyelid may have serious consequences for the eye and life of the animal.

Eyelid Masses are Most Commonly Tumors

Eyelid melanomas are found in grey horses, with Arabians and Percherons also at increased risk. Melanomas may be single or multiple. Treatment is surgical excision and cryotherapy.

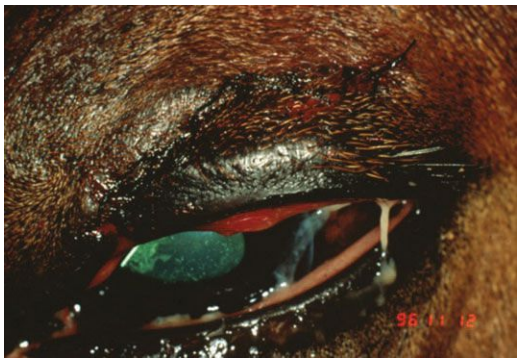

Fig. 19. The laceration in Fig. 18 is surgically repaired.

Squamous cell carcinoma (SCC) is the most common tumor of the eye and adnexa in horses (Fig. 20). The etiopathogenesis may be related to the ultraviolet (UV) component of solar radiation, periocular pigmentation, and an increased susceptibility to carcinogenesis. The UV component is the most plausible carcinogenic agent associated with SCC, as it targets the tumor suppressor gene p53, which is altered in equine SCC.

The prevalence in horses increases with age with the mean age at diagnosis 11.1 ± 0.4 years in one report. Belgians, Clydesdales, and other draft horses have a high prevalence of ocular SCC, followed by Haflingers, Appaloosas, and Paints. Squamous cell carcinoma is least prevalent in Arabians, Thoroughbreds, and Quarter Horses. Cryotherapy, immunotherapy, irradiation, radiofrequency hyperthermia, CO_2 laser ablation, or intralesional chemotherapy should follow surgical excision of equine ocular SCC.

Sarcoids are solitary or multiple tumors of the eyelids and periocular region of the horse (Fig. 21). Retroviruses and papilloma viruses may be involved in the etiology. It is suspected that flies may be able to transfer sarcoid cells from one horse to trau-

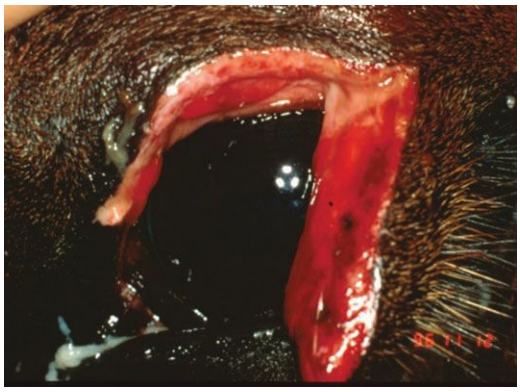

Fig. 18. A large laceration of the upper eyelid is present.

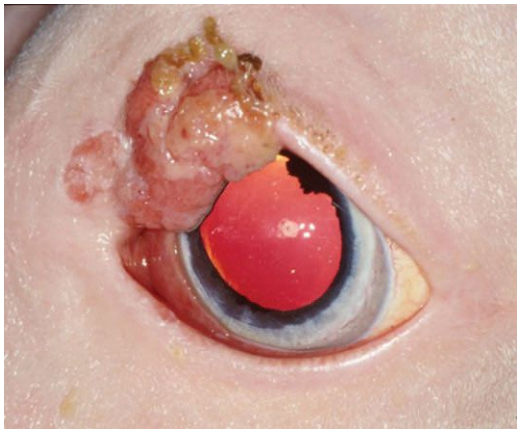

Fig. 20. An upper lid squamous cell carcinoma is present.

Fig. 21. An upper eyelid sarcoid is present in this horse.

matic skin lesions in other horses. There are geographic differences in the aggressiveness of the sarcoid in horses. Mules appear to suffer from an aggressive form of sarcoid. Immunotherapy for sarcoids includes autogenous vaccines and immunomodulators of mycobacterial products.

Acknowledgments

Conflict of Interest

The Author declares no conflicts of interest.

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How to Diagnose and Treat Common Ophthalmic Diseases in the Neonatal Foal

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1. Introduction

Examination of the neonate typically occurs at 12–24 hours for healthy foals with a normal foaling. The ocular portion of the exam includes a thorough evaluation of the ocular and periocular structures. The globe and adnexa of the normal foal are fully developed at birth, but there are several normal anatomic and physiologic differences between the neonate and adult eye. Ocular diseases in foals can be classified as congenital, inherited, or acquired. It is important for the veterinarian to be able to recognize these conditions so that any therapy can be instituted and so the owner can be informed about conditions which may impact the foal's future life.

2. Materials and Methods

A quiet dark stall is the ideal place in which to perform the ophthalmic examination. The mare should be restrained by a handler using a halter and lead rope. The exam is typically well-tolerated by the foal; a hand stabilizing the ventral mandible while an assistant supports the chest and tail is often sufficient.

The veterinarian attending to neonatal foals should carry the equipment required to perform an ophthalmic examination in order to identify and treat common ocular conditions.

Equipment for Performing the Examination

- direct ophthalmoscope or smartphone camera
- transilluminator
- 14 or 20 D lens
- sterile fluorescein strips
- balanced saline solution
- tropicamide 1% ophthalmic solution for mydriasis
- lidocaine 2% for auriculopalpebral nerve block
- 3 cc syringe and 25 g needle to administer topical medications
- topical anesthestic (tetracaine or proparacaine)
- culturettes
- sterile cotton swabs
- scalpel for cytology
- microscope slides

Equipment for Placing Tacking Sutures for Entropion

- sedation—diazepam/butorphanol
- lidocaine cream or 2% injectable for local anesthesia
- needle drivers, small forceps, scissors
- 4–0 nonabsorbable suture (nylon or silk on a P-3 or similar needle)

NOTES

Normal Ocular Characteristics of the Equine Neonate

The cornea of a normal foal should be bright and glossy. Foals with systemic illness have decreased corneal sensitivity compared to adults, which may predispose them to injury.[1] Downward orientation of the eyelashes is a subtle indicator of ocular pain in horses.

The iris is slightly gray in color and the pupil slightly circular compared to an adult, becoming horizontal over the first month of life.[2,3]

The lens should also be clear, although the anterior and posterior lens sutures may be visible.[4] These should be differentiated from larger cataracts.

Examination of the fundus reveals tapetal color that relates to the coat color. The optic disc is round and pale to deep pink in color. Pale grey streaks may be visualized radiating from the optic nerve and are bundles of axons traveling from the retina to the nerve.[5–7]

Assessment of Vision and Reflexes

The menace response is learned; thus, it is absent until about 2 to 3 weeks of age.[5] The dazzle reflex and observing ambulation are beneficial in determining the visual status of the foal. It is important to use a very bright light source to assess the dazzle reflex. The pupillary light reflexes are often sluggish for the first several days of life.[4] The palpebral reflex may yield lagophthalmos in the young foal.[8] Physiologic rotational strabismus results in a mild ventronasal rotation of the globe, giving the appearance that the foal is gazing downwards.[9] This typically resolves after 2 to 4 weeks.

Fig. 2. Microphthalmos in a foal. Note the flattened orbit and prolapsed nictitans.

3. Results

Congenital or Inherited Diseases

Cataracts

Cataracts are opacities in the lens and are a common ocular congenital abnormality in foals. Cataracts are inherited in Thoroughbreds and Quarter Horses. Rocky Mountain Horses also have inherited cataracts, but often in conjunction with other signs of anterior segment dysgenesis, such as microphthalmia, persistent pupillary membranes, and aniridia.[10]

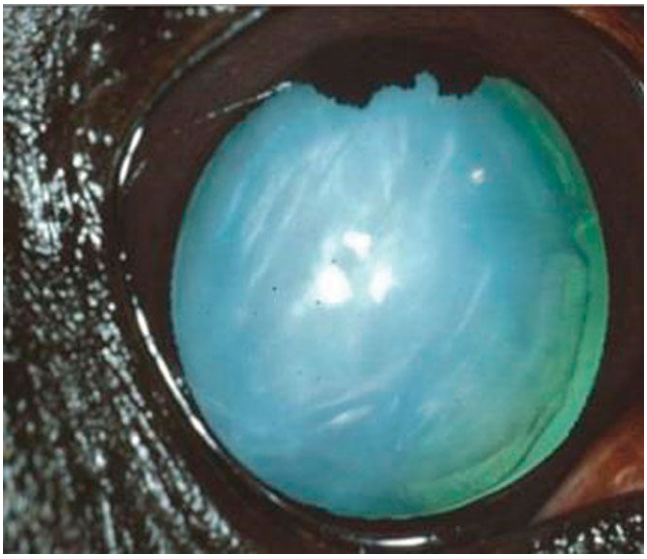

Fig. 1. Immature cataract in a foal. Note the clear area of lens nasally. Nonetheless, this cataract causes significant visual impairment.

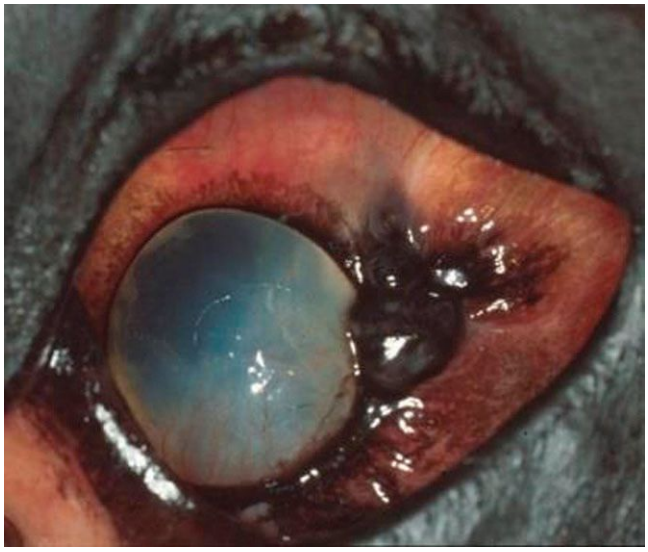

Fig. 3. Microphthalmos in a foal. Note small cornea and globe and conjunctival exposure.

It is important to assess all foals with cataracts for these other abnormalities.

The location and extent of the cataract within the lens is important for prognosis (Fig. 1). A mature cataract occupies the entire lens, thus is blinding. Cataracts within the nucleus of the lens typically don't progress and become smaller relative to the rest of the lens with growth.[10] Perinuclear cataracts occur soon after birth and may progress. The Morgan horse is predisposed to inherited nuclear or perinuclear cataracts. Cataracts may also occur as an acquired condition secondary to uveitis.

Phacoemulsification cataract surgery is a treatment option for foals with cataracts. It has a high initial success rate in foals; however, the prognosis for long term vision in horses, regardless of age and etiology, following cataract surgery is guarded; in a recent study approximately 30% horses were visual two years after phacoemulsification.[11] An electroretinogram and ocular ultrasound are performed pre-operatively to ensure normal retinal structure and function.

Microphthalmia

Microphthalmia is characterized by an abnormally small globe. The globe may be normally proportioned and visual, or present in conjunction with other abnormalities causing blindness. The palpebral fissure is usually small and the third eyelid prominent (Figs. 2 and 3). Entropion is a possible sequela, leading to chronic irritation. The affected orbit is also abnormally flat and small; this becomes more prominent as the animal ages.[10]

Dermoids

Dermoids are plaques of epidermal tissue in an abnormal location, such as on the cornea, conjunctiva, or eyelid.[8] They most often occur in the dorsotemporal limbus. They are pigmented and may contain hair follicles, which can cause irritation and ulceration. Surgical removal via keratectomy or blepharoplasty is the recommended treatment for corneal and eyelid dermoids, respectively.

Persistent Pupillary Membranes (PPMs)

Persistent pupillary membranes are strands of iris connecting to the iris, cornea, or lens. Focal opacities may be present in the lens or cornea, usually without affecting vision.[12] Surgical transection of the membranes is not necessary.

Persistent Hyaloid Artery

The hyaloid artery is responsible for vascular supply of the lens in utero. It typically regresses with age, sometimes leaving a translucent band running from the posterior lens capsule to the optic nerve.[4] A persistent hyaloid artery remnant was noted in about 85% of foals, often bilaterally.[13]

Aniridia

Aniridia is a partial or total absence of the iris. It is a rare condition and has been reported in Belgians[6] and Quarter Horses.[14] Aniridia in conjunction with congenital cataracts has been reported in Thoroughbreds.[15] The ciliary processes and lens equator are visible in these horses (Fig. 4). These foals will not tolerate bright light and exhibit photophobia and blepharospasm.

Fig. 4. Aniridia in a foal. Note prominent ciliary processes.

Strabismus

Dorsal strabismus is observed in Appaloosa foals with congenital stationary night blindness, although the relationship is unclear.[6] These foals have a "stargazing" appearance (Fig. 5). This should be differentiated from physiologic rotational strabismus that resolves 2 to 4 weeks postnatally.

Congenital Glaucoma

Congenital glaucoma is uncommon, resulting in rapid enlargement of the globe (buphthalmos).[8] Management is challenging and blindness is inevitable.

Fig. 5. Dorsal strabismus in a foal with congenital stationary night blindness.

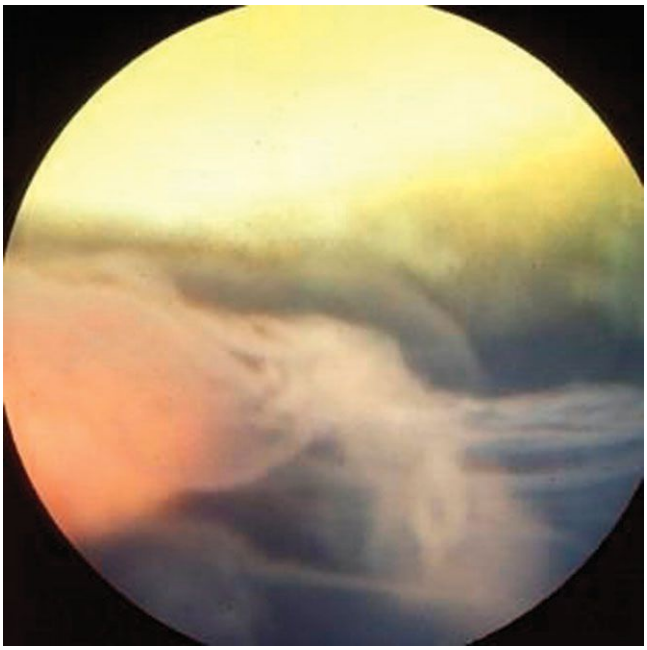

Fig. 6. Complete retinal detachment in a foal.

Congenital Retinal Detachments

Congenital retinal detachments are another uncommon disease with a poor prognosis for vision and no therapeutic options (Fig. 6).[8]

Multiple Congenital Ocular Anomalies

Signs of multiple congenital ocular anomalies include congenital miosis, iris hypoplasia, cataracts, increased corneal curvature (cornea globosa), uveal cysts, and retinal dysplasia.[16]

This disease is linked to an autosomal dominant gene.[17] These abnormalities are associated with the silver coat color, common in the Rocky Mountain Horse, the breed in which these abnormalities were first described.

Acquired Diseases

Entropion

Entropion is a common ocular abnormality in foals and requires immediate therapy. It most commonly involves the lower eyelid (Fig. 7); however, both the upper and lower lids may be affected. Enophthalmos results from dehydration secondary to systemic illness or maladjustment. Other causes include loss of orbital fat from cachexia, trauma, scarring, or congenital microphthalmos.[10]

When the globe is displaced caudally in the orbit, the eyelids are no longer supported by the globe and roll inwards. The inversion of the eyelids means that the hairs from the lids are contacting the cornea, often causing ulceration. This painful irritation causes the globe to retract further (spastic entropion), worsening the degree of entropion.

Fig. 7. Lower lid entropion in a foal.

Treatment for entropion involves correcting the eyelid position with tacking sutures or a tarsorrhaphy while the foal is treated for systemic disease and any secondary corneal ulcerations heal. This can be performed with mild sedation and topical anesthesia using lidocaine. A nonabsorbable suture such as 4–0 silk or nylon is preferred in a vertical mattress pattern (Fig. 8). The space between the bites of skin correlates to the amount of eyelid rolling in. The suture end closest to the cornea should be cut short to avoid subsequent ulceration from contacting the cornea. These sutures may be left in place for 2 to 4 weeks, but should be monitored daily to make sure that they have not become loose.

If the entropion is suspected to be a primary anatomic problem, permanent surgical correction of

Fig. 8. Tacking for entropion using suture.

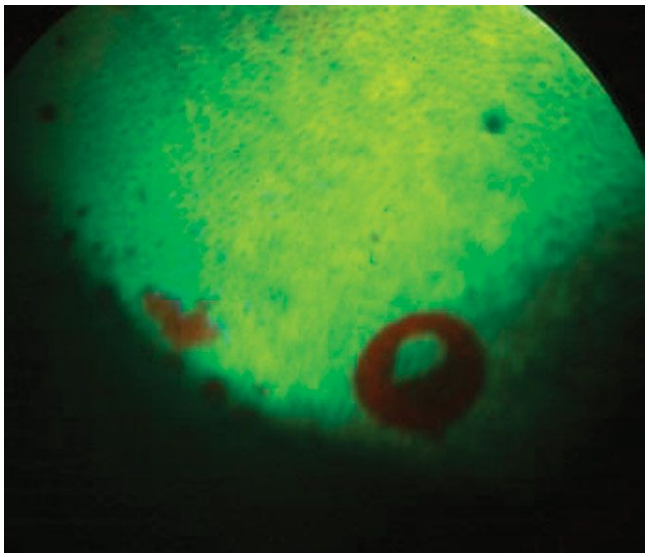

Fig. 9. Retinal hemorrhages in the tapetal fundus of a foal.

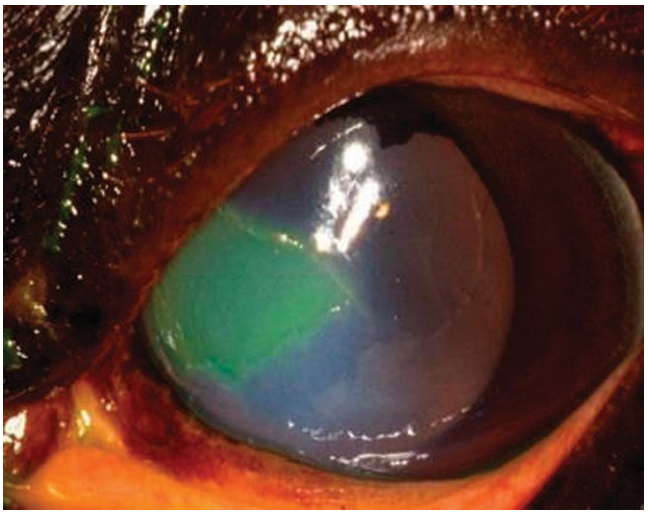

Fig. 10. Uncomplicated, sterile corneal ulcer in a foal.

the eyelid inversion is advised. Because the foal will continue to grow, it is important to delay this procedure as long as possible to avoid overcorrection and potential ectropion and poor cosmesis.[18]

Ocular Hemorrhage

Subconjunctival and episcleral hemorrhage are frequently observed following dystocia. It is self-limiting and resolves within several days.[6] Episcleral hemorrhage may be seen secondary to systemic illness, such as neonatal maladjustment syndrome.[14] Retinal hemorrhages are typically visualized in the tapetal fundus (Fig. 9), ranging from 16 to 29% in two studies.[4,19] These were self-limiting and not related to ophthalmic or neurologic disease.[19]

Corneal Ulceration

Foals, especially systemically ill foals, have decreased corneal sensitivity.[1] This, combined with an absent menace response for the first couple of weeks of life, may predispose the neonatal foal to corneal trauma. It also means that the clinical signs of corneal ulceration may be more difficult to detect than in an adult; the level of discomfort may be mild despite serious corneal disease.

Uncomplicated corneal ulcers are superficial, noninfected, and associated with minimal uveitis (Fig. 10). Close monitoring is essential as these ulcers do have the potential to melt. Therapy includes topical serum, prophylactic antimicrobials (usually triple antibiotic ointment), and atropine for underlying uveitis. Systemic nonsteroidal anti-inflammatory drugs (NSAIDs) provide analgesia and minimize uveitis.

Complicated ulcers may be melting, deep, or infected. Targeted therapy based on results from cytology and bacterial culture and sensitivity results is prudent. Topical atropine and systemic NSAIDs are required. Aggressive therapeutic regimes may be necessary with administration of serum and selected antimicrobials every 1 to 4 hours. Surgery is recommended for corneal ulcers >50% of stromal depth.

Uveitis

Iridocyclitis is inflammation of the anterior uvea. It may be unilateral or bilateral. In foals this occurs most often secondary to sepsis associated with *Salmonella*, *E. coli*, and *Streptococcus*. One study found that uveitis was a negative prognostic indicator in septic foals.[20] Other organisms associated with uveitis in horses include *Actinobacillus equuli*, adenovirus, and equine viral arteritis.[6] Clinical signs include blepharospasm, epiphora, conjunctival and scleral hyperemia, miosis, variable corneal edema, aqueous flare, fibrin in the anterior chamber, hypopyon, and hyphema (Fig. 11).

Aggressive treatment is essential as uveitis has the potential to cause blindness. Therapy for uveitis involves systemic antibiotics and NSAIDs and topical corticosteroids (such as prednisolone acetate 1% or dexamethasone 0.1%). Topical steroids should be administered as frequently as q 4 h, depending on the severity of the uveitis. Topical atropine reduces painful ciliary muscle spasm and treats uveitis by stabilizing the blood-aqueous barrier and is administered q 24 to q 12 h. Gastrointestinal motility must be closely monitored as atropine is a parasympatholytic and can be absorbed into the systemic circulation through the eye. In some cases, tissue plasminogen activator can be injected into the anterior chamber to dissolve fibrin.[10]

4. Discussion

The ophthalmic examination is an essential part of the neonatal examination. It is important for the equine practitioner to be able to recognize normal

Fig. 11. Foal with uveitis. Note fibrin in anterior chamber.

features of the foal's globe, in addition to common diseases. Ophthalmic disease is common in foals admitted to the University of Florida's ICU; 21% have corneal ulcers and 19% have entropion. The practitioner should be proficient at managing common ocular disease in the neonate including entropion, corneal ulceration, and uveitis.

Acknowledgments

Conflict of Interest

The Authors declare no conflicts of interest.

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How to Navigate Topical Antifungal Ophthalmic Products

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1. Introduction

Keratomycosis is a common and dangerous disease of the equine eye. Case presentation and disease course is variable. Cytology gives the best stall-side identification of etiologic organism. A sample with good yield enables the practitioner to determine bacteria from fungi and additionally should provide fungal differentiation of multicellular hyphae from unicellular yeast. However, occasionally cytology cannot be performed due to the nature of the lesion (intact epithelium, deep lesion). Furthermore, culture or polymerase chain reaction (PCR) is required to obtain actual species identification. Since fungi are typically slower growing than other organisms, it may be weeks into treatment before a culture result is produced. Once a positive culture has been obtained, additional testing must be requested if sensitivities are desired. Therefore, instead of therapeutic treatment to target an organism, antifungal treatment is often empirical.

Empiric antifungal therapy can vary based on region. Retrospective studies collecting data on fungal species involved in keratomycosis have been performed in multiple regions within the United States.[1–4] Regional isolates associated with fungal keratitis vary. Fungi appear in three main forms—yeast (single cell, budding), mold (hyphae, branching), and dimorphic populations. Regardless of the region, *Aspergillus,* the filamentous multicellular hyphae, appears to be the most common organism implicated in the disease. In the southeastern U.S., most studies point to primarily *Aspergillus* and another filamentous organism *Fusarium.*[1,5] Conversely, in the northeast, the yeast *Candida* is more frequently isolated.[2] Finally, a recent study originating from the west, specifically California, confirmed *Aspergillus* in almost all of the keratomycosis cases that cultured positive.[3]

Because of their structural commonalities with mammalian cells, specific pharmacologic targeting is more difficult. Most antifungals act on the fungal cell wall. Topical antifungal therapy offers increased concentrations at the site of disease, decreased systemic side effects, improved economics, and improved ease of administration. However, topical antifungal medications are fairly new, with only one product[a] gaining FDA approval. They vary substantially in cost, formulation, and availability. The purpose of this paper is to illustrate the antifungal options currently available and provide a basis behind choosing them to treat keratomycosis in the horse.

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Table 1. Quick Reference of Topical Ophthalmic Antifungal Options

Name	Formulation	Cost	Availability	Penetration	Spectrum
Amphotericin B	0.15% to 3%	$$$	Less common compound	Limited	Both
Natamycin*	5% suspension*; 3.33%	$$$	Common	Some Corneal	Filamentous, Most yeast
Ketoconazole	1% to 5%	$$	Less common compound	Corneal	Poor filamentous
Miconazole	1% ophthalmic	$$	Commonly compounded	Corneal	Filamentous
	2% derm, 2% vaginal	$	Over the counter	Limited	Filamentous
Fluconazole	2 mg/ml	$$	Less common compounded	Intraocular	Yeast
Itraconazole	1 % (± in 30% DMSO)	$$	Commonly compounded	Corneal	Filamentous (not *Fusarium*)
Voriconazole	0.5%, 1%, 3%	$$$	Less commonly compounded	Intraocular	Both
SSD	1% cream	$	Widely	Limited	Both
Povidone-Iodine	0.2% (1:50) solution to 5%	$	Widely	Limited	Both

Note: * denotes FDA approval.

2. Materials and Methods

Formulation for Topical Medications

Topical medications are formulated as a solution, a suspension, or an ointment. These medications are deposited into the precorneal tear film where they follow one of three routes. The two nontherapeutic routes are nasolacrimal drainage and into the systemic circulation via the conjunctiva and nasopharynx. Ocular absorption is the third desired route for therapy. This is through both the cornea and conjunctiva/sclera. Corneal absorption is limited by the hydrophilic or phobic affinity of the drug and the integrity of the cornea. The epithelium is the rate-limiting barrier for absorption of hydrophilic drugs, while the stroma is the rate-limiting barrier for lipophilic drugs. Therefore, drug penetration can improve in the presence of a corneal ulcer. For example, it has been demonstrated that when 25% to 50% of the corneal epithelium is removed drug penetration into the cornea and anterior chamber can be increased over ninefold.[6] Other considerations that can significantly impact concentration of drug in the ocular tissues include lacrimation, blink rate, and protein content of tears—all of which can be substantially altered in horses with keratomycosis.

Indications for Topical Antifungal

Indications for use of a topical antifungal can be either empiric or therapeutic. A positive cytology or culture necessitates the use of a topical antifungal as a therapeutic. However, indications for empiric therapy are less well defined. Specific factors such as appearance of the lesion, geographic location, season, and clinician preference can all guide empiric use.

Appearance

Fungal keratitis can vary widely in presentation. Superficial keratomycosis can manifest as a gritty or dull lesion with minimal vascular response. Midstromal lesions may form central plaques or areas of malacia surrounded by a deeper furrow. Deep lesions may be epithelialized in the form of a deep stromal abscess.

Geographic Location

Horses everywhere are at risk. However, while fungal keratitis has been reported to occur all across the United States, the most frequent reports emerge from the southeastern U.S. The clinical manifestations may vary by region.

Season

Similarly to the increased prevalence in the southeast, hot and humid summer months are thought to yield a higher prevalence of keratomycosis in horses.

Categories of Antifungals

The following is not an exhaustive list of antifungal therapies available. Instead they are the most common antifungals for ophthalmic topical use. Please refer to Table 1 for a quick comparison of their benefits and drawbacks.

Polyenes

Polyenes target ergosterol in the cell membrane of fungi. **Amphotericin B** has been validated for use topically.[7] However, its penetration is limited when the corneal epithelium is intact (e.g., deep stromal abscess). **Natamycin** is another polyene. This is the only FDA-approved topical antifungal agent and is formulated as a 5% suspension. However, this formulation is irritating to healthy corneal cells, and therefore it is often compounded to a 3.33% suspension. It has better coverage of filamentous organisms than amphotericin, often making it a better choice in equine keratomycosis.

Azoles

Azoles also target ergosterol, but instead they inhibit its synthesis through the cytochrome P450 system. A concurrent action is inhibition of mitochondrial oxidative and peroxidative enzymes.

The first sub-group within the azoles is the imidazoles (named based on parent ring structure).

Ketoconazole is an imidazole with fairly limited spectrum but good corneal and intraocular penetration.[4,5]

Miconazole has variable reported activity against the filamentous fungi.[2,4] It comes as a 1% compounded suspension; however, the over-the-counter 2% dermatologic and 2% vaginal cream preparations have also been used as an ophthalmic treatment. Please see the below discussion for additional details on these formulations.

The second group of azoles is the triazoles, again named for their parent ring structure.

Despite being well absorbed, **fluconazole** primarily targets yeast, which limits its usefulness in equine keratomycosis.

Itraconazole is a triazole with a broader spectrum but has a reduced penetration relative to fluconazole. This penetration is believed to be improved by the addition of 30% dimethyl sulfoxide (DMSO) and is commonly available. This formulation has been seen to penetrate into the corneal stroma but has not been found in measurable levels within the aqueous humor.[8] In a study by Mathes et al, itraconazole and DMSO were found to have the least keratocyte damage relative to miconazole and natamycin.[9]

With excellent penetration and good efficacy against yeast and hyphae, voriconazole has emerged as a good option for antifungal therapy. Clode et al tested three concentrations (0.5%, 1%, and 3%) and found 1% delivered the best combination of penetration and mitigation of side effects.[10] Voriconazole was found to outperform natamycin, fluconazole, itraconazole, ketoconazole, and miconazole against in vitro isolates in a study by Pearce et al.[4]

Betadine (Povidone-Iodine) Solution

Betadine solution is an antiseptic with both antibacterial and antifungal properties. Widely used as a surgical preparation, there is also some who advocate its use as a therapy. Concentrations from 0.5% to 1.0% betadine have been validated to disinfect the surgical site or donor cornea as a protocol for preoperative antisepsis.[11] The use of a 2% dilute betadine solution every 24 hours has been suggested in the treatment of fungal keratitis.[12] However, this should be limited to adjunctive therapy only. A large metastudy concluded that 5% betadine solution did not reduce the bacterial load of human corneal ulcers significantly. This was theorized to be due to the lack of penetration into the corneal stroma.[13]

Silver Sulfadiazine

Silver sulfadiazine (SSD) is a 1% dermatologic cream with broad antibacterial and antifungal activity historically used to treat burns. Use as a corneal antifungal with topical application every 12 to 24 hours has been suggested.[12] There has been limited in vitro data in horses[14] that it is fungicidal when used as an ophthalmic topical. A large meta study[15] investigated topical drug trials for fungal keratitis in people. This study showed that based on the trials included, there was no evidence that any particular drug was more effective in the management of fungal keratitis. One specific comparison was topical SSD vs miconazole. However, the study noted that the trials included in this review were of variable quality and were generally underpowered. Therefore, the evidence behind use of SSD as an antifungal ophthalmic remains inconclusive.

Dosage: Frequency and Duration

Dosage of ophthalmic drugs is significantly different from other pharmacokinetics. Most topical drugs follow first-order kinetics (maximum concentration then exponential decline). Unlike systemic drugs where usually >75% is bioavailable, after topical application of an ophthalmic solution less than 1% and no more than 10% of the dose enters the eye. Furthermore, the lacrimal lake limits the volume that can be administered, and the concentration is limited by what is tolerable to the corneal surface. Therefore, frequent application of low-volume, dilute solution is a mainstay to equine topical treatment. For example, a dosing regime may use topical antifungals every 2 to 4 hours initially and only reducing to six-times a day once clinical improvement is seen.

Ointments are a good option for decreasing the frequency of application. Rate of dissipation from the precorneal tear film of drugs administered in an ointment is significantly slower than a liquid. This is because less of the drug is immediately lost to nasolacrimal drainage, increasing bioavailability. Eye ointments can be dosed anywhere from every 4 to 12 hours. Disadvantages include danger if corneal perforation is imminent, imprecise dosing, and a creation of a film on the cornea significantly distorting vision.

Regardless of topical of choice, a positive cytology or culture of fungus should necessitate a continued course of topical antifungal therapy. Recrudescence is common. Durations from 3 to 8 weeks depending on response have been reported. Preparing clients for a minimum of 6 weeks of topical therapy is usually realistic.

Specific Questions

Is vaginal and athlete's foot preparation of miconazole acceptable to apply topically to the horse eye?

Two percent miconazole is available as a dermatologic and vaginal cream over-the-counter (OTC) preparation. These have been used as topical medications in horses. While this is an economic option, these formulations are not designed for the ocular surface. There are anecdotal reports of ophthalmic problems resulting from its use in horses and, therefore, use is with risk. The primary inactive ingredient in vaginal miconazole is benzoic acid.

Benzoic acid causes moderate eye irritation in the form of chemosis, hyperemia, and keratitis. This medication is the fourth most common ingredient in the dermatologic preparation. Furthermore, the pH and osmolality have not been investigated with regards to ophthalmic tolerance. At this time neither formulation is recommended. However, it should be noted that miconazole ophthalmic ointment is readily available from compounding pharmacies that ship and is reasonably priced (approximately $25 for a tube vs $10 for nonophthalmic OTC formulations).

Should I use an empiric antifungal on every case of corneal ulceration?

When evaluating a painful eye, efforts should be made to obtain a diagnosis—specifically through at minimum, cytology. More severe cases additionally warrant a culture. However, in the face of an open diagnosis, empiric therapy may sometimes still be warranted. *A simple case of corneal ulceration is unlikely to necessitate empiric or prophylactic treatment with an antifungal.* Simple corneal ulcers are superficial, uninfected, and heal within 3 to 5 days. A wound or lesion deeper than superficial epithelium, the presence of cellular infiltrate or corneal melting or malacia, secondary uveitis, or an appearance typical of fungal keratitis all indicate that fungal infection may be present.

While we have discussed the use of empiric and treatment antifungals, no studies have been performed on the use of *prophylactic* topical antifungal therapy. Prophylactic systemic antifungals are used in human patients at risk of infection. Therefore, in areas where fungal keratitis is endemic or during high-risk seasons (summer), prophylactic therapy may be warranted. However, the overall *rate of keratomycosis seen at the field level is low*. Based on retrospective studies comprised of referral institutions, the rate of equine keratomycosis was anywhere from 5.7% of all horses presenting to the ophthalmology service[1] to 25% cases of severe ulcerate keratitis.[16] These numbers may even be overrepresented, as pretreatment with antibiotics and topical steroids has been shown to be predisposing factors in fungal keratitis.

3. Results

Resolution of fungal keratitis is characterized by an increase in comfort, decrease in uveitis, decrease in the severity of corneal infiltrate and edema, and epithelization (if applicable). In a recent retrospective study on keratomycosis at a referral institution, 73% of horses were treated with medical therapy alone.[3] Of the investigated cases, 77% retained their globes and 53% retained vision. However, this prognosis is likely significantly worse than what would be encountered on a primary field case due to the severity of the cases.

Lack of resolution may be due to inadequate duration of therapy, lack of susceptibility of the organism, lack of penetration of the medication to the sight of infection, infrequent medication administration, continued stimulus (thick plaque, foreign body), or other factors. Steps in reevaluation of a nonhealing ulcer include repeat detailed examination, repeat cytology, obtain or repeat culture, and potentially referral. Remember that owner compliance can be challenging, and often placement of a subpalpebral lavage system will enhance results due to consistent topical application.

4. Discussion

In conclusion, there are a wide variety of topical antifungals for the equine practitioner currently available. No perfect treatment exists and very little prospective literature is available on their use as a topical medication. Since only one topical ophthalmic antifungal is currently FDA-approved, compounding is a necessity. Knowledge about geographic trends in isolates, drug properties, and cost/availability all play a part in choice. By better understanding the options, client money is better spent and results are better achieved.

Acknowledgments

Conflict of Interest

The Authors declare no conflicts of interest.

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[a]Natacyn (natamycin) 5% Ophthalmic Suspension, Alcon Laboratories, Irvine, CA 92618.

Stability of Voriconazole by Constant-Rate Infusion for Ocular Delivery in Horses

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Voriconazole 1% solution is not compatible with constant-rate infusion delivery and the use of other aqueous-based medications may have similar results. Voriconazole 1% solution is stable when stored in the commercial glass vial at controlled temperatures between 23 and 40ºC up to 30 days. Authors' addresses: Department of Veterinary Clinical Sciences, Center for Veterinary Health Sciences (Smith, Gilmour); Department of Physiological Sciences, Center for Veterinary Health Sciences (Maxwell); Department of Veterinary Pathobiology, Center for Veterinary Health Sciences (Gull); and Department of Statistics (Payton), Oklahoma State University, Stillwater, OK 74078; e-mail: kathryn.m.smith@okstate.edu. *Corresponding and presenting author. © 2014 AAEP.

1. Introduction

The study objective was to establish the effect of storage in a constant-rate infusion (CRI) pump on the sterility and stability of voriconazole 1% solution.

2. Materials and Methods

Nine vials of voriconazole 1% solution[a] were prepared. Each vial was used to prime a commercially-available CRI pump and attached subpalpebral lavage system (CRI/SPL unit), with the remaining solution stored in the commercial glass vial. Three CRI/SPL units and their 3 corresponding vials were stored at 23°C, 33°C, and 40°C. Fungal and aerobic bacterial cultures were performed on all solutions on the first and last storage day. Samples obtained at regular intervals were analyzed for voriconazole concentration using high-performance liquid chromatography.

3. Results

No bacterial or fungal contamination was identified in any solution. All solutions stored in the glass vial remained stable throughout the study. Voriconazole concentration significantly increased (by 35–134%) after passage through the SPL and several units became blocked with precipitate ($p < 0.05$).

4. Discussion

Voriconazole 1% solution was not compatible with CRI/SPL delivery but remained stable when stored in the commercial glass vial.

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Conflict of Interest

The Authors declare no conflicts of interest.

Footnote

[a] Vfend I.V., Pfizer Pharmaceuticals, New York, NY 10017.

Research Abstract

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Safety of Subconjunctival Injection of Allogeneic Mesenchymal Stem Cells in Horses

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Clinical trials should be performed to assess subconjunctival injection of allogeneic mesenchymal stem cells (MSCs) in the treatment of equine eye disorders. Authors' addresses: Large Animal Clinical Sciences, (Joswig, Hardy, Easterwood, and Watts); and Veterinary Integrative Biosciences (Cummings), Texas A&M University, College Station TX 77843; e-mail: AWatts@cvm.tamu.edu. *Corresponding and presenting author. © 2014 AAEP.

1. Introduction

Subconjunctival injection of allogeneic mesenchymal stem cells (MSCs) has the potential to become a new therapeutic in equine ophthalmology. Our objective was to evaluate the safety of allogeneic MSC subconjunctival injection. We hypothesized there would be no difference in adverse events after subconjunctival MSC injection versus control.

2. Methods

Upper-lid subconjunctival injection of 3 million MSCs in 0.5 mL was performed in a randomly assigned eye (n = 14 horses). The opposite eye was injected with cell-free medium (0.5 mL). Serial evaluations of both eyes were performed by investigators blinded to treatment assignment. The injections were repeated to the same eye 3 weeks later. Flow cytometry was used to determine major histocompatibility complex (MHC) II expression of MSCs.

3. Results

There were no adverse events or complications in either the MSC or control injected eyes. Redness and chemosis were significantly different (higher scores in the MSC treated eyes) on days 1, 22, and 28, but not at other time points. Blepharospasm, blepharoedema, and miosis were absent throughout the study. Eyes that had epiphora on day 0 continued to have epiphora throughout the trial and there were no significant differences in epiphora between groups. The MSCs were negative for MHC II.

4. Discussion

In preclinical models, subconjunctival injection of MSCs speeds healing after chemical keratitis. This may be due to the regulation of inflammation and healing. In the horse, subconjunctival administration of two injections of 3 million allogeneic MSCs appears to be safe.

Research Abstract

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Conflict of Interest

The Authors declare no conflicts of interest.

Incidence of Colic in Ophthalmic Versus Orthopedic Patients in a Hospital Environment

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The incidence of colic in patients hospitalized with an ocular complaint was not significantly different from the incidence in those hospitalized with a nonocular complaint. Authors' address: New Bolton Center, University of Pennsylvania School of Veterinary Medicine, Kennett Square, PA 19348; e-mail: scherrer@vet.upenn.edu. *Corresponding and presenting author. © 2014 AAEP.

1. Introduction

Ophthalmic patients have been suggested as an "at risk" group due to chronic pain, long-term hospitalization, use of atropine, and frequent sedation.

2. Materials and Methods

Records of ophthalmic ($n = 105$) and orthopedic ($n = 197$) horses admitted from July 1, 2011–June 30, 2012 were used to determine patient breed, age, and sex, presenting complaint, medical therapy, and surgical intervention for all included horses. An episode of abdominal discomfort was identified if the animal showed behavior that warranted abdominal palpation per rectum or fluid therapy administration (enteral or intravenous).

3. Results

Horses with ocular disease significantly differed from orthopedic cases in median age (10.7 vs 3.8 years), sex (3% vs 30% intact males), breed (28% vs 62% Thoroughbreds), likelihood of having general anesthesia (65% vs 80%), and median length of hospital stay (3 vs 2 days), but not in incidence of colic (8% vs 5%), respectively. A logistic regression model using disease category (ocular or orthopedic), general anesthesia (yes or no), age in years, and length of hospitalization in days as predictors of colic showed marginally good fit (log likelihood ratio = 7.69, $P = 0.1$). Only the length of hospitalization had significant predictive power (log odds = 1.09, $P < 0.01$). Amongst horses that experienced episodes of colic, 16/17 resolved without surgery, with no difference between ocular and orthopedic cases in median colic duration (1 day for both groups), median hospitalization time (7 vs 3 days), or duration of systemic nonsteroidal anti-inflammatory drug use (7 vs 5 days).

4. Discussion

Horses hospitalized with ocular disease were not at an increased risk of colic relative to orthopedic cases and episodes of colic in both groups were resolved with medical management in all but one case.

Acknowledgments

Conflict of Interest

The Authors declare no conflicts of interest.

Research Abstract

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Review of Techniques for Prediction of Ovulation in the Mare

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A combination of growth pattern and size of the dominant follicle, increase in follicular wall thickness, development of an irregular shape, softening of the follicle, and a decrease in endometrial edema is suggestive of impending ovulation. Authors' address: Colorado State University, Department of Clinical Sciences, Fort Collins, CO 80523; e-mail: Patrick.McCue@ColoState.edu. *Corresponding and presenting author. © 2014 AAEP.

1. Introduction

A decision on when to breed a mare by live cover or artificial insemination is usually dependent on an accurate prediction of impending ovulation. Prediction of the potential interval to ovulation is accomplished in clinical practice by interpretation and integration of multiple factors on an individual mare (Table 1). The goal of this paper is to review the parameters that can be used in clinical practice to predict impending ovulation in the mare.

2. Reproductive History

An individual mare will often ovulate a follicle of approximately the same diameter each cycle.[1] Consequently data from previous cycles can often be used to predict follicle size at ovulation of subsequent cycles. Unfortunately, some mares will ovulate a dominant follicle during one estrous cycle that is of a very different size, either markedly smaller or larger, than follicles of other cycles. Prediction of ovulation is difficult in these mares and more than one breeding may be required so the cycle is not missed.

Table 1. Parameters Used to Predict Ovulation in Mares

Parameter
Reproductive history
Growth pattern of follicle
Diameter of follicle
Shape of follicle
Changes in follicular wall
Tone of follicle
Vascularity using Doppler ultrasound
Echogenic particles in follicular fluid
Endocrine markers
Uterine edema pattern
Degree of cervical relaxation
Periovulatory ovarian pain on palpation
Number of days in estrus
Interval from prostaglandin administration and follicle size at PGF
Interval from hCG or deslorelin administration

3. Growth Pattern of Follicle

A dominant follicle will typically increase in diameter by 2.7 to 3 mm per day during estrus, reach a

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maximum diameter, and subsequently remain the same size for approximately 2 days prior to ovulation.[2–4] The diameter of the follicle may decrease by 2 to 3 mm in the 12 hours preceding ovulation.[3,5] The growth pattern of the dominant follicle is disrupted if a mare receives human chorionic gonadotropin (hCG) or deslorelin acetate to induce ovulation.[1] The ovulation induction agent is generally administered when the mare is in behavioral estrus or has endometrial edema visible on ultrasound and the dominant follicle first reaches a size that is appropriate (often ≥35 mm in diameter for light horse breeds). Ovulation will typically occur 36 to 40 hours following administration of hCG or deslorelin, respectively. The induced follicle often ovulates before it attains its maximum potential diameter.[1,6]

4. Diameter of Follicle

The potential diameter of the preovulatory follicle prior to ovulation can often be predicted based on mare size and breed. In addition, mares typically ovulate follicles that are similar in size in consecutive cycles.[1] In general, mares of smaller light horse breeds will ovulate a follicle that is smaller in diameter than mares of larger breeds. Friesian mares are notorious for developing very large follicles (ie, 50 mm or greater) that remain present for several days prior to ovulation (Stout, personal communication). It has been noted that the diameter of a preovulatory follicle is 5 to 8 mm larger early in the ovulatory season than later in the season and is also greater in single-ovulating mares than mares with unilateral or bilateral double ovulations.[7] It has been suggested that the diameter of the dominant follicle may be the most reliable single criterion in the prediction of ovulation,[3,7] but follicle diameter alone is not useful in predicting ovulation in the 48 hours preceding spontaneous ovulation.[8]

5. Shape of Follicle

A developing dominant follicle is typically spherical in shape during most of the estrous period. It has been reported that 84% to 89% of follicles change shape from spherical to nonspherical (pear-shaped or conical) prior to ovulation.[2–4] A "cone" or "point" or "stigma" may develop as the follicle tunnels toward the ovulation fossa in the hours preceding ovulation. The follicle may become more flattened or irregular in the 3 hours prior to ovulation.

6. Changes in the Follicular Wall

A developing dominant follicle has a relatively thin follicular wall during early to mid-estrus. Follicular wall thickness increases progressively as the interval to ovulation decreases.[4] The echogenicity is potentially due to an increase in the number of granulosa cells, accumulation of mucosubstances between cells[9] or preovulatory luteinization of granulosa cells. Unfortunately, the thickening generally occurs too early to be useful in predicting ovulation.[10] An increase in the echogenicity of the follicular wall[5] and an increase in prominence of an anechoic layer beneath the granulosa[10] have been noted as ovulation approaches. The anechoic layer has been suggested to be due to hypervascularity and edema of the theca interna. A rent or tear in the follicular wall was observed in 77% of mares prior to ovulation.[5] The duration of ovulation, defined as a rapid decrease in follicular size, has been reported to occur in an average of 42 seconds (range 5–90 seconds).[5] Serrations of the inner surface and/or outer periphery of the granulosa cell layer, as indicated by an irregular or notched appearance, was noted in 37% of mares 24 hours prior to ovulation and 59% of mares within 12 hours of ovulation.[11]

7. Tone of Follicle

A developing dominant follicle has a firm tone during the early and middle part of the normal growth phase. The dominant follicle may become noticeably softer as detected by manual palpation within the 12 to 24 hour period prior to ovulation.[3,12,13] In one study, a perceptible softening of the follicle was noted in 28% of mares prior to ovulation, and some follicles softened and then became turgid again prior to ovulation.[14] In another study, it was reported that 90% of follicles were softer at 12 hours before ovulation than they had been at 72 hours prior to ovulation.[14]

8. Vascularity with Doppler Ultrasound

Examination of the dominant follicle in color Doppler-mode revealed that the percentage of the follicle with color display and the prominence of the signals increased between 36 and 12 hours prior to ovulation but decreased during the last 4 hours prior to ovulation.[11] In addition, a loss of color Doppler signals was evident at the apex of follicles that had lost their spherical shape.

9. Evaluation of Follicular Fluid

Echogenic particles within the follicular fluid, presumed to be clusters of granulosa cells, have been noted in 50% to 54% of equine preovulatory follicles[5,15] but is not common enough or consistent enough to be predictive of impending ovulation.[10]

10. Endocrine Markers

Measurement of estrogen concentration, either estradiol 17β, total estrogens, or conjugated estrogens, have been used in an attempt to predict ovulation in mares.[3,16,17] In general, estrogen levels increase in relation to the increase in follicle diameter during estrus, peak approximately 2 days prior to ovulation, and decline near the day of ovulation.[18,19] Unfortunately, the pattern of estrogen secretion is not consistent to be a useful diagnostic marker for prediction of ovulation. Luteinizing hormone concentrations increase gradually during estrus in the mare and reach peak levels near the day of ovulation.[19,20] The presence of luteinizing hormone (LH)

was recently evaluated as a potential predictor of ovulation in mares using a commercial LH kit.[21] LH was first detectable beginning 2.5 ± 1.2 days prior to ovulation and continued to be detectable in all mares out to 2 days after ovulation. Consequently, a single positive test was not a reliable indicator of impending ovulation.

11. Uterine Edema Pattern

Endometrial edema develops in response to the presence of estradiol and the absence of progesterone. In most mares, a predictable pattern of edema development and regression occurs during a heat period,[22,23] which is generally consistent from cycle to cycle in a given mare.[1] The amount of edema peaks approximately 2 days prior to ovulation. Ovulation typically occurs when edema is in a stage of decline or is absent.

12. Degree of Cervical Relaxation

The physical characteristics of the cervix change throughout the estrous cycle based on the relative presence of estrogen and progesterone. A mare in heat, in the presence of estradiol 17β and an absence of progesterone will have a cervix that is relaxed and draped onto the floor of the vagina, pink in color, and moist. In contrast, a mare in diestrus will have a cervix that is high on the cranial vaginal wall, closed tight, dry, and pale in color. These characteristics can be visualized on speculum examination and tone can be detected by manual palpation *per rectum*. The greatest degree of change in cervical color and tone occur near ovulation but are not accurate enough alone to indicate time of ovulation.[14]

13. Periovulatory Ovarian Pain on Palpation

It has been suggested that some mares exhibit ovarian pain associated with follicular development.[10,24] It is more common that mares exhibit discomfort when the site of a fresh ovulation is palpated *per rectum*. Consequently, expression of pain or discomfort on transrectal palpation of the ovaries cannot reliably be used as a predictor of impending ovulation.

14. Number of Days in Estrus

The average length of the equine estrous cycle was reported to be 20.6 days,[14] and the average duration of estrus was 5.7 days. It was further noted that 46%, 32%, and 12% of mares ovulated within 24 hours, 48 hours, or 72 hours, respectively, prior to the end of estrus, while 10% of mares were out of estrus before ovulation occurred.

15. Interval from Prostaglandin Administration

The average interval from prostaglandin administration to the next spontaneous ovulation is approximately 7 to 12 days.[2,25] Assessment of follicle diameter at the time of prostaglandin administration can be used to estimate the interval to subsequent ovulation. The interval from PGF administration to subsequent spontaneous ovulation is inversely proportional to the diameter of the largest follicle at the time of treatment. In general, mares with small follicles at the time of prostaglandin administration take longer to develop a dominant follicle and ovulate than mares with a larger follicle (Table 2).

Table 2. Interval to Subsequent Ovulation Based on Follicle Size at Time of PGF Administration

Follicle Size (mm)	Number of Cycles	Average day to ovulation
10–14	113	9.6 ± 0.2[a]
15–19	84	8.6 ± 0.2[b]
20–24	88	7.6 ± 0.2[c]
25–29	30	6.7 ± 0.4[d]
30–34	15	4.9 ± 0.4[e]

Note: Data within column with different superscripts are significantly different ($p < 0.05$).

Administration of prostaglandin to a mare with a large diestrous follicle (≥35 mm) is associated with one of 3 outcomes: (1) ovulation within 48 hours in the absence of uterine edema (14.5%), (2) ovulation after 48 hours accompanied by uterine edema (75.4%), or (3) regression without ovulation followed by emergence of a new follicular wave (10.1%) (unpublished data).

16. Interval from hCG or Deslorelin Administration

Administration of an ovulation inducing agent is common in broodmare practice. Typically a follicle that has reached a minimum critical size that is characteristic for the type or breed of mare (ie, ≥35 mm in diameter for most light horse breeds) will respond to administration of hCG or deslorelin acetate and ovulate within 24 to 48 hours if the mare had been in heat for 2 to 3 days.[26] If ovulation occurs prior to 24 hours, it is likely that the follicle was already primed to ovulate even before the agent was administered. hCG and deslorelin acetate will usually induce ovulation in approximately 36 and 40 hours, respectively. If used appropriately, it is generally expected that 85% to 95% of mares should ovulate between 24 and 48 hours after administration of hCG or deslorelin acetate.

17. Other Comments

It has been suggested that a majority of mares ovulate at night.[27] However, several studies have indicated that ovulations are equally distributed throughout the day in mares.[1,3,28]

18. Summary

No single morphologic criterion is consistently useful to predict interval to ovulation or impending ovulation. In clinical practice, a combination of growth pattern and size of the dominant follicle, in conjunction with an increase in follicular wall thickness, development of an irregular shape, softening of

the follicle, and a decrease in endometrial edema is suggestive of impending ovulation. Administration of hCG or deslorelin acetate at the appropriate time of the estrous cycle can greatly enhance prediction of ovulation.

Acknowledgments

Conflict of Interest

The Authors declare no conflicts of interest.

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Relationship Between Ultrasonographic Findings at the Time of Breeding and Pregnancy Outcome

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Grade 3 and 4 uterine edema at ovulation did not adversely affect fertility of mares. Authors' addresses: Gluck Equine Research Center, Department of Veterinary Science, University of Kentucky, Lexington, KY 40546 (Squires, Ball, Troedsson); and Rood and Riddle Equine Hospital, PO Box 12070, Lexington, KY 40580-2070 (Bradecamp, Schnobrich, Riddle); e-mail: edward.squires@uky.edu. *Corresponding author; †Presenting author. © 2014 AAEP.

1. Introduction

It is a routine practice to examine the mare's uterus and ovaries during estrus in order to assess follicular development of the ovaries and edema in the uterus as an aid in scheduling breeding. Several reports have described an increase in uterine edema in response to the secretion of estrogen from the pre-ovulatory follicle.[1–3] The peak in circulating estrogen occurs about 24 h prior to ovulation. Thus, the degree of uterine edema increases as the mare develops a pre-ovulatory follicle and approaches ovulation. Near the time of ovulation, the amount of edema decreases and when the mare is in diestrus, edema in the uterus should be minimal.[4] Based on ultrasound examination of the uterus, the uterine edema is usually graded on a scale of 0 to 4 or 0 to 5: 0 = no edema, 3 = maximum normal edema, and greater than or equal to 3.5 to 4 = excessive edema. Assessing the degree of edema has been used to determine the appropriate time to administer human chorionic gonadotropin (HCG) or gonadotropin-releasing hormone (GnRH);[4] whether the mare is in estrus; if the follicle in the transitional mare is competent;[3] diagnosis of inflammation of the uterus; and if the corpus luteum is regressing.

Clinically there are mares that, for unknown reasons, continue to have a high edema score even at ovulation or after ovulation. Some have suggested that maximum or excessive edema at ovulation may be due to subclinical endometritis, excessive estrogen secretion, or failure of progesterone to increase.[3,5] Regardless, the practitioners must decide whether to ignore the presence of excess edema or treat the uterus. Apparently, there have been no studies to determine if high edema scores near ovulation adversely affect fertility or the incidence of early embryonic loss. This study tested the hypothesis that maximum or excessive uterine edema near ovulation lowered pregnancy rates and increased early embryonic loss in a large group of Thoroughbred mares.

2. Materials and Methods

Nine hundred and twenty Thoroughbred mares were examined with ultrasound for a total of 1127

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cycles during the 2013 breeding season on day 2 (day prior to breeding and day ovulating inducing agent was usually given) and day 0 in relation to ovulation (day of OV = 0). Uterine edema was given a score of 0 to 4 with 0 = no edema, 3 = maximum, and 4 = excessive edema. Uterine fluid was also graded based on the vertical measurement of intrauterine fluid in cm. Other data included antibiotics (Y/N), uterine lavage (Y/N), and pregnancy at 14 and 40 to 50 days. Mares were classified in 3 age groups: young (2–9 years), middle-aged (10–16 years), and old (>16 years) and 3 reproductive statuses at the time of breeding: foaling, maiden or barren. Since there were only 4 mare cycles with grade 4 edema at the time of ovulation, the edema scores of 3 and 4 were combined for analyses

Risk factors for pregnancy status were identified in univariable analysis with an X^2 test to compare categorical data between groups. Multivariable logistic regression with the dependent variable of pregnancy rate/cycle was used to develop a model that included independent variables with a $P \leq 0.20$ in a stepwise backward method using SAS ProcJMP[a]; a priori values of ≤ 0.05 were considered to be significant in the final model.

3. Results

The parameters that had significant effects in the logistic model on pregnancy rates per cycle and early embryonic losses were age ($P < 0.03$), reproductive status ($P < 0.01$), uterine lavage ($P < 0.04$), and age × status $P < 0.001$). The presence of uterine fluid greater than 1 cm tended ($P < 0.09$) to affect pregnancy rate/cycle. Edema scores of 3 and 4 prior to ovulation and at ovulation did not significantly affect the pregnancy rate or embryonic loss. Whether mares were given antibiotics also did not affect the outcome parameters (pregnancy rate/cycle or embryonic loss). Based on the X^2 analysis, the pregnancy rate per cycle was less for old mares (28/74, 38%) versus middle-aged mares (237/379, 63%) or young mares (480/673, 71%). Barren mares had greater $P < 0.05$) early embryonic losses between 14 to 50 days (29/217, 13%) than those for foaling (63/727, 8.7%) and maiden (10/182, 5.5%), but pregnancy rates were similar ($P > 0.05$) among mares of various reproductive statuses (66%, 144/217, 65%, 472/727, or 71%, 129/182 for barren, foaling, or maiden mares, respectively). When age was examined as a continuous variable and not categorical, there was an age × status interaction. Mares over 11 years old and barren had a lower pregnancy rate than those barren and less than 11 years. The presence of greater than 1 cm of uterine fluid at the time of ovulation increased ($P < 0.02$) embryonic loss (19/113, 17%) compared to those mares with <1 cm of uterine fluid (60/668, 8%) but the pregnancy rates were similar (58 vs 67%). This effect was primarily due to the presence of fluid in older mares (age × fluid). Based on the X^2 analysis, the presence of grade 3 or 4 edema at ovulation did not ($P = 0.94$) affect the pregnancy rate or embryonic loss. However, those mares that were lavaged had a greater embryonic loss (50/384, 13%) than those not lavaged (52/741, 7%).

4. Discussion

Edema was not a parameter in the logistic model that affected pregnancy rate or embryonic loss. Furthermore, if we examined the pregnancy rates and embryonic losses in mares with minimal edema (0 to 2) compared to those with maximal or excessive uterine edema (3–4, there was no effect on pregnancy rate or early embryonic loss so this could be a reflection of the relatively small number of cycles [8] in which mares had excessive edema [grade 4]). Thus, we concluded that the presence of edema scores of 3 and 4 prior to and after ovulation had no apparent effect on the pregnancy rate per cycle. The overall incidence of mares with grades 3 and 4 uterine edema at ovulation was 73/1126 (6.5%). These mares also had a higher incidence of uterine fluid >1 cm (18%) than those mares with edema scores of 0 to 2 (9.5%). The fact that excessive edema did not lower pregnancy rates or increase embryonic losses was unexpected. Others have suggested that excessive edema at ovulation or after ovulation may be an indication of subclinical endometritis or perhaps an endocrine imbalance.[5] Unfortunately, we were not able to determine the cause of excessive edema around the time of ovulation under the conditions of this study. In contrast, the presence of fluid > 1 cm did affect pregnancy rate and embryonic loss. Others have reported that the fluid during estrus decreases fertility, depending upon the amount of fluid and grade of fluid.[6,7] The echogenicity of uterine fluid was not available in this group of mares. Mares that had uterine lavage during estrus also had a higher embryonic loss rate. This is probably not a direct result of the lavage procedure but an indication that mares that needed a lavage were more than likely those with fluid, older mares, or those with some other reproductive problem.

It was not surprising that reproductive status and age affected pregnancy rates and the incidence of early embryonic loss. Others have reported lower fertility and higher embryonic loss for aged mares and for barren mares compared to younger maiden or foaling mares.[8] There was, in fact, an interaction of age with reproductive status and age and the presence of uterine fluid. Mares over 11 years of age had a higher incidence of having moderate to large amounts of fluid and more barren mares were represented in the aged category than the other two age groups.

In summary, maximum or excessive uterine edema near the time of ovulation did not adversely affect pregnancy rates or the incidence of early embryonic loss.

Acknowledgments

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Conflict of Interest

The Authors declare no conflicts of interest.

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Anti-Müllerian Hormone Predicts Follicular Reserve in Aged Mares

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Follicular reserve (measured as antral follicle count; AFC) declines with mare age. Determination of serum anti-Müllerian hormone (AMH) concentrations is highly correlated with follicular reserve in older mares, and AMH determination may be useful to assess the reproductive longevity of older mares. Authors' addresses: Gluck Equine Research Center, Department of Veterinary Science, University of Kentucky, Lexington, KY 40546-0099 (Claes, Ball, Scoggin, Esteller-Vico, Kalmar, Squires, Troedsson); and Department of Population Health and Reproduction, School of Veterinary Medicine, University of California, Davis, CA 95616 (Conley); email: b.a.ball@uky.edu. *Corresponding and presenting author. © 2014 AAEP.

1. Introduction

The number of follicles remaining in the ovary (follicular reserve; AFC) declines with age in mares until reproductive senescence is reached but at variable ages. Anti-Müllerian hormone is produced by small follicles, and circulating concentrations of AMH have been used to measure follicular reserve in other species. The objective of this study was to examine the relationship between AMH and AFC in mares of different ages.

2. Materials and Methods

Young (3–8 years; n = 10), middle-aged (9–18 years; n = 16), and old (>18 years; n = 19) mares were examined by transrectal ultrasonography, and all antral follicles were counted (AFC) across multiple estrous cycles. Plasma AMH concentrations were measured by ELISA across multiple estrous cycles. Data were analyzed by nonparametric correlation.

3. Results and Discussion

AFC was lower in old compared to middle-aged or young mares. The repeatability of AFC and AMH was high within and across estrous cycles. Overall, AFC was positively associated with plasma AMH concentrations although this relationship was influenced by age. The relationship between AFC and AMH for aged mares ($\rho = 0.86$; $P < 0.0001$) and middle-aged mares ($\rho = 0.60$; $P = 0.01$) was greater than the correlation between AFC and AMH in young mares ($\rho = 0.40$; ns). Plasma AMH concentrations are predictive of antral follicle counts in older mares and may be useful to assess follicular reserve as well as reproductive longevity.

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Acknowledgments

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Conflict of Interest

Dr. Troedsson has a professional affiliation with MOFA™, Minitube of America.

Suppression of Estrogen Biosynthesis During Late Pregnancy in Mares

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Reduction in maternal estrogen concentrations by up to 90% during late gestation did not affect gestation length or neonatal viability although birth weights were reduced. These findings call in to question the value of supplementing estrogens for pregnancy maintenance during late gestation. Authors' address: Gluck Equine Research Center, University of Kentucky, Lexington, KY 40546; e-mail: b.a.ball@uky.edu. *Corresponding and presenting author. © 2014 AAEP.

1. Introduction

Estrogen concentrations during pregnancy in the mare are markedly elevated, and some studies have suggested a potential relationship between low circulating estrogen concentrations in pregnant mares and pregnancy loss. The role of elevated estrogens in equine pregnancy is largely unknown, and the aim of this study was to evaluate the effects of an inhibitor of estrogen synthesis, letrozole, on equine pregnancy during the last trimester.

2. Materials and Methods

Mares were treated with letrozole (n = 6; 500 mg every four days) or untreated as control (n = 6) beginning at 240 days of gestation until foaling. Mares were bled weekly, and concentrations of estrogens, androgens, and progestins were analyzed by ELISA. Gestational length, neonatal viability, and foal birth weights were determined.

3. Results

Letrozole reduced peripheral estrogens during late pregnancy by approximately 90% with a concomitant increase in androgens and no change in progestins. Gestational length was unchanged, neonatal viability was normal, and birth weights of foals born to letrozole treated mares was reduced by approximately 14%.

4. Discussion

Inhibition of estrogen synthesis during late gestation did not disrupt pregnancy or adversely affect neonatal viability although birth weights of foals born to letrozole-treated mares were reduced.

Acknowledgments

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Conflict of Interest

The Authors declare no conflicts of interest.

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Pharmacokinetics of Ceftiofur Sodium in Equine Pregnancy

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Ceftiofur sodium had poor penetration into fetal membranes and compartments after administration to normal, pregnant mares. This drug is not recommended for treating mares with placentitis. Authors' addresses: Large Animal Clinical Sciences (Macpherson, Pozor, Benson, Runcan, Hatzel, Larson, van den Berg, Kelleman, Sanchez), and Physiological Sciences (Vickroy), College of Veterinary Medicine, University of Florida, Gainesville, FL 32610; Large Animal Medicine, College of Veterinary Medicine, University of Georgia, Athens, GA 30602 (Giguère); The Gluck Center, University of Kentucky, Lexington, KY 40546 (Troedsson); and Rood and Riddle Equine Hospital, PO Box 12070, Lexington, KY 40580-2070 (LeBlanc); e-mail: macphersonm@ufl.edu. *Corresponding and presenting author. © 2014 AAEP.

1. Introduction

The pharmacokinetics and drug disposition of ceftiofur sodium, after administration to pregnant mares, was evaluated.

2. Methods

Eleven pregnant pony mares (D270–326) were administered ceftiofur sodium intramuscularly at 2.2 mg/kg (low dose; n = 6) or 4.4 mg/kg (high dose; n = 5). Plasma was obtained at T = 0, 0.5, 1, 2, 4, 8, 12, and 24 h after ceftiofur administration. Eight pony mares were re-enrolled in the study ≥ 3 days from foaling to ensure steady state concentrations of the drug at the time of foaling. Mares were administered ceftiofur sodium (4.4 mg/kg, IM) daily until foaling. Parturition was induced one hour after ceftiofur sodium administration. Allantoic and amniotic fluid, plasma (mare and foal) colostrum, and placental samples were collected at delivery and for 24 h. Samples were analyzed for desfuroylceftiofur acetamide (DCA) concentrations by high performance liquid chromatography (HPLC).

3. Results and Discussion

Mean (± SD) peak concentrations of DCA were 3.97 ± 0.50 mcg/mL (low dose) and 7.45 ± 1.05 mcg/mL (high dose). Terminal half-life was shorter after administration of the low dose (2.91 ± 0.59 h) than after the high dose (4.10 ± 0.72 h). The mean plasma concentration of DCA from mares at foaling

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was 7.96 ± 1.39 mcg/mL and colostrum was 1.39 ± 0.70 mcg/mL. The DCA concentrations in allantoic and amniotic fluid, placental tissues, and foal plasma were nearly undetectable. These results infer incomplete passage of DCA across fetal membranes after administration of ceftiofur sodium to normal pony mares.

Acknowledgments

Conflict of Interest

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Exploration of Safety of a Gastrointestinal Mucosal Cytoprotectant, Oral Misoprostol Regimen in Early-Gestation Mares

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Fourteen of 15 early-gestation pregnancies appeared unaffected by a gastrointestinal mucosal cytoprotectant oral misoprostol regimen (GMCOMR). Eight mares allowed to carry to term delivered normal foals. Authors' address: School of Veterinary Medicine, University of Pennsylvania, New Bolton Center, 382 West Street Road, Kennett Square, PA 19348; email: jlinton@vet.upenn. edu. *Corresponding and presenting author. © 2014 AAEP.

1. Introduction

Misoprostol, a prostaglandin E1 analog, has both gastrointestinal cytoprotectant and abortigenic properties, with increased risk for neurologic, musculoskeletal, and cognitive defects following human fetal exposure. In previous work in mares, 11 mid-gestation pregnancies appeared unaffected by administration of a gastrointestinal mucosal cytoprotectant oral misoprostol regimen (GMCOMR).[1] Here we explored the safety of the same GMCOMR in early-gestation pregnancies and resulting neonates.

2. Materials and Methods

Eleven healthy pony mares and four healthy horse mares in early gestation (37–85 days) were administered a GMCOMR (3.5–5 mcg/kg, Q12, PO for 5 consecutive days). Serum samples for progesterone assay were obtained daily during treatment and for five days after treatment had ended. Pregnancies were monitored by transrectal palpation and ultrasonography. Four horse and two pony pregnancies were purposely terminated two ($n = 1$) to five ($n = 5$) weeks after treatment had ended. Eight pony pregnancies were allowed to continue to term.

3. Results

Fourteen of the 15 pregnancies appeared unaffected. One pony mare whose pregnancy appeared unaffected at four days after treatment had ended was found to be not pregnant six days later. Serum progesterone concentrations all remained within the range of normal for early gestation. The mare's baseline progesterone concentration was lower than any other mare and significantly lower than the mean of five other pony mares whose gestation was within six days (5.48 vs. mean of 16.01±4.1SD, $P < 0.01$). The eight mares allowed to carry to term delivered without complications. All neonatal musculoskeletal, neurologic, and cognitive measures were within normal limits.

4. Discussion

These results suggest a relatively low risk of disruption of early-gestation pregnancy or adverse effects

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on foals following GMCOMR and not different ($P > 0.10$) from gender-matched control foals during the same time frame within the herd.

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Conflict of Interest

The Authors declare no conflicts of interest.

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Effects of Intrauterine Infusion of a Water-Based Suspension of Enrofloxacin on Mare Endometrium

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Daily infusion of an alcohol-free water-based enrofloxacin suspension for 3 days does not appear to cause any permanent harmful changes to the endometrium and may be useful for treatment of endometritis due to enrofloxacin-sensitive bacteria. Authors' addresses: Rood and Riddle Equine Hospital, PO Box 12070, Lexington, KY 40580-2070 (Schnobrich, Barber, Bradecamp); and Comparative Theriogenology, Department of Veterinary Clinical Sciences, College of Veterinary Medicine, Washington State University, Pullman, WA 99164 (Pearson, Tibary); e-mail: mschnobrich@roodandriddle.com. *Corresponding author; †Presenting author. © 2014 AAEP.

1. Introduction

Bacterial endometritis is a common cause of low pregnancy and foaling rates. Many isolates are sensitive to enrofloxacin only. Intrauterine infusion of a commercial enrofloxacin preparation was associated with severe hemorrhagic inflammation and fibrosis in the endometrium. This study examines the effect of an alcohol-free water suspension of enrofloxacin[a] after intrauterine administration.

2. Materials and Methods

Eight light-breed mares were used in the experiment. Each mare was administered a water-based suspension of enrofloxacin (2.5 mg/kg in 50 mL), in utero, daily for 3 days during estrus. The effects of the infusion on the reproductive tract were clinically evaluated (transrectal ultrasonography, vaginoscopy) before treatment, during treatment, and 19 days after treatment. Endometrial biopsies were taken before treatment, after the 3rd treatment, and 19 days after the last infusion.

3. Results

Intrauterine infusion of a water-based enrofloxacin suspension was associated with a transient, but not statistically significant, inflammatory response. There was a significant transient increase in intrauterine fluid and its echogenicity. Endometrial biopsy grade showed a significant increase ($p < 0.05$) following the 3-day course of infusion but returned to pretreatment level on the next cycle.

4. Discussion

These findings suggest that a water-based enrofloxacin suspension may be useful for treatment of bacterial endometritis sensitive to enrofloxacin,

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without the deleterious effects seen with administration of the commercial product.

Acknowledgments

Conflict of Interest

The compounded formulation used in this study was donated by Rood and Riddle Pharmacy. The compounded product was used because the FDA-approved product is not appropriate for intra-uterine use. The agent for the compounding pharmacy did not review this paper and was not part of the study design or data collection or data analysis. Knowledge of the contents of the compounded formulation were made available to the primary investigators. Three authors (Schnobrich, Bradecamp, Barber) work for Rood and Riddle Equine Hospital, which is affiliated with the Rood and Riddle Veterinary Pharmacy. Dr. Lisa K. Pearson has no conflict of interest, Dr. Ahmed Tibary has no conflict of interest.

Footnote

[a]Compounded solution contains Xanthan gum, Polysorbate 80, sterile water, and USP grade Enrofloxacin powder.

Relationship Between Sperm Quality and the Embryo Recovery Rate of Cooled-Shipped Stallion Sperm

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This study describes sperm quality following cooled-storage and the relationship to embryo recovery rate. Cutoff points are reported for sperm quality features that identify differences between cooled-shipped samples of high and average fertility that allows the practitioner to evaluate the stallion's potential as a cooled-shipped semen candidate. Authors' addresses: Department of Large Animal Clinical Sciences, College of Veterinary Medicine and Biomedical Sciences, Texas A&M University, College Station, TX 77843-4475 (Love); Noble Equine Veterinary Service, Purcell, OK 73080-4712 (Noble); 2314 Stone Court, Katy, TX 77493 (Standridge); and Department of Animal Science, College of Agriculture and Life Sciences, Texas A&M University, College Station, TX 77843-4475 (Bearden, Cavinder); email: clove@cvm.tamu.edu. *Corresponding and presenting author. © 2014 AAEP.

1. Introduction

The clinician is limited by the lack of information regarding the level of sperm quality that should be expected for adequate fertility in samples received for breeding. The objective of this study was to determine the relationship of sperm quality after cooled-storage to fertility based on embryo recovery rate.

Materials and Methods

Ejaculates (n = 488) from Quarter Horse and Paint stallions (n = 135) were collected and shipped to an embryo transfer facility for artificial insemination. Samples were evaluated for total sperm motility, morphology, viability, semen concentration and volume, and DNA quality using the Sperm Chromatin Structure Assay. Fertility was measured using embryo recovery rate.

Results

A total of 204 mares were bred 492 estrus cycles. Cutoff values that separate average and high fertility groups were identified for total sperm motility (≥ 65%), progressive sperm motility (≥ 45%), extended sperm concentration (≥ 31.8 million sperm/mL), total sperm number in the inseminate (≥ 1.14 billion sperm), viable sperm (≥ 71%), sperm with abnormal DNA (≤ 26.8%), and morphologically normal sperm (≥ 47%).

Discussion

In this study, guideline values for cooled-stored stallion sperm were identified that describe differ-

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ences in fertility. These guidelines will allow the clinician to more critically interpret the results of sperm quality analysis from cooled-shipped semen.

Acknowledgments

Conflict of Interest

The Authors declare no conflicts of interest.

How to Use Manipulative Tests to Diagnose and Manage Equine Foot Pain

Tracy A. Turner, DVM, MS, DACVS, DACVSMR

A thorough examination and assessment of the equine foot forms an essential part of any lameness examination. Manipulative tests can give the examiner incredible insight into the pain the horse is feeling. This provides the examiner with the critical information necessary for the next diagnostic step, not only diagnostic but therapeutic information for relief of pain. Author's address: 16445 70th Street, NE Elk River, MN 55330; e-mail: turner@anokaequine.com. © 2014 AAEP.

1. Introduction

The equine foot is the most common site for lameness to develop. It is therefore of utmost importance to perform a thorough examination of the foot in order to identify problems or predisposing factors that may lead to lameness. The recent use of magnetic resonance imaging (MRI) in equine lameness diagnostics has markedly improved our understanding of foot-related lameness. Unfortunately, MRI examinations frequently reveal more than one area of pathology. It is then imperative to rely on the clinical examination to determine a hierarchy of importance of the MRI identified lesions.

There are numerous causes of foot pain in the horse. These causes can be categorized as (1) conditions of the hoof wall and horn producing tissues, (2) conditions of the third phalanx, and (3) conditions of the podotrochlear region. Hoof problems would include: hoof wall defects, such as cracks that involve the sensitive tissue; laminitis, laminar tearing (local, due to hoof imbalance), separation or inflammation of the sensitive laminae from the insensitive laminae; abscess formation; contusions of the hoof causing bruising or corn formation; neoplasia, and pododermatitis (thrush or canker). Third phalanx problems include: fractures of the coffin bone (types I–VII), deep digital flexor insertional tenopathy, pedal osteitis (generalized or localized inflammation of the bone), desmopathy of the collateral ligaments, cyst-like lesion formation, and remodeling disease. Conditions of the podotrochlear region have been reported to include distal interphalangeal synovitis/capsulitis, deep digital flexor tendinitis, desmitis of the impar (distal navicular ligament) or collateral sesamoidean ligaments, navicular osteitis or osteopathy, vascular disease of the navicular arteries, and navicular fractures. The common denominator of all of these conditions is that they are characterized by pain that can be localized to the foot.

The examination requires comprehensive evaluation of the external hoof, evaluation for deep pain, evaluation of "hoof balance," and evaluation of radiographs or other imaging modalities. The evaluation of the horse's foot, like all examinations, requires a thorough medical, performance, and shoeing history. The purpose of this paper is to describe the value of manipulative tests for the diagnosis of

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foot problems and to describe how the results of these tests can be used to help manage the foot pain.

2. Manipulative Tests

Diagnostic tests that should be performed are hoof tester examination, distal limb flexion, hoof extension wedge test, palmar hoof wedge test, and lateral medial wall wedge tests. Positive response to any of these tests is important but a negative response is equivocal and does not rule out any problem.

Hoof tester examination should be performed systematically; how you perform the exam is unimportant but get used to a routine.[1,3] I like to begin at the heel on my left side and work around the hoof in a clockwise fashion. Begin with the bar, move to the heel, to quarter and then toe, and then back toward the heel on my right. Space the tester's progress at approximately one-inch intervals. Be sure to include each exit point of the shoeing nails. Next, place the testers in each of the collateral sulci and across the hoof to the opposite hoof wall (I like to progressively move the hoof tester along the hoof wall caudal to cranial to check for alterations in the pain response; then place the testers in the central sulcus to the hoof wall at the toe and then across the heels). Remember that the closer the ends of the hoof testers are, the more accurate the exam is in localizing pain. A positive response should be repeatable and, in the frog region, the pain response should be uniform over those areas and must be evaluated in relation to examination of the remaining foot.[1] That is, a positive response in the heels and quarters of the sole would also be expected to cause a positive response across the distal sesamoidean region in the same area of the foot. Pain is noted as a distinct withdrawal of the foot at the time pressure is applied.

A distal limb flexion test may exacerbate lameness if any of the three distal joints of the leg are affected by synovitis or osteoarthritis.[1,8] A positive response could also be expected by any condition that causes induration of the tissues of the distal limb. The distal limb flexion test is performed by flexing the distal limb, holding the limb in that position, and trotting the horse away after 30 seconds. In the author's opinion, the time is not critical but the examiner should always be consistent so as to develop a feel for the effect of the test. A positive test is noted if the manipulation causes lameness or lameness is exacerbated.

The hoof extension test is performed by elevating the toe with a block, holding up the opposite limb, and trotting the horse away after 60 seconds.[1] The block the author uses is an old hoof knife wrapped in tape as protection from the blade. The palmar hoof wedge test is performed in a similar fashion except that the block is placed under the palmar two-thirds of the frog and forces the horse to stand on that foot. The opposite limb is held up for 60 seconds and the horse is trotted off. The test can be further modified so that the wedge can be placed under either the medial or lateral wall to determine if the pressure or hoof imbalance caused by the wedge exacerbates the lameness. As before, the opposite limb is held up for 60 seconds before the horse is trotted in hand. A positive test is once again noted if the manipulation causes lameness or lameness is exacerbated.

3. Diagnostic Significance

The significance of the hoof tester examination is obvious; it is a simple assessment of pain, with the painful area between the tips of the testers. Abscesses or fractures usually show the most reaction when using hoof testers. This is important so that further diagnostics, specifically radiography, can be centered on the exact point of pain. This improves the likelihood of finding a lesion. Less painful injuries would show less pain. Traditionally, pain over the frog upon hoof tester examination was indicative of navicular bone pain; however, one study showed that hoof testers only had a 50% predictive value for navicular pain.[3] The author believes hoof testers are very accurate for assessment of pain in the sole or hoof wall but over the frog hoof tester pain only represents nonspecific deep pain in the foot.

Flexion tests have been shown to exacerbate 90% of foot lameness.[4] However, there has not been shown to be any specificity. One lameness diagnostician suggests that if the limb is flexed under the torso pain is more likely from the fetlock; whereas if the limb is flexed by pulling it forward, this is more likely to exacerbate coffin joint lameness.[5] This has not been confirmed but the author always flexes the limb by pulling the leg forward. If the test is positive the author will follow this test with medial lateral wedge tests.

The medial to lateral wedge will apply pressure to the wall under the wedge and stretch the capsule and collateral ligaments of the coffin joint opposite the wedge.[6,7] The test appears to be more specific for collateral ligaments.[7] The author reviewed their last 50 cases where the wedge test was positive; over 90% were positive with the wedge placed laterally, and 33 of the 50 showed a distal interphalangeal collateral ligament lesion upon ultrasonographic examination.

The frog wedge test has an 85% positive predictive value for navicular pain.[3] This makes it the best single test for that condition. Whenever this test is positive, the author recommends a navicular bursogram as part of the imaging assessment. The toe wedge test is the most commonly recommended test after flexion and hoof tester examination.[7] However, the test has only a 50% predictive value for navicular pain and is only positive in about 50% of the cases.[4] Despite those poor predictive values, the author uses a positive response as an indicator of deep flexor pain and performs sonography of the deep flexor in the pastern and foot. However, only a small percentage have had a deep flexor lesion.

4. Treatment Significance

Hoof tester examination pinpoints the region where, in the case of an abscess, decompression needs to be performed. Furthermore, the information can indicate the areas of the foot that need to be protected.

Flexion tests indicate to the author potential joint injection as part of the therapeutic options. Which joint depends on the response to diagnostic analgesics and radiography. An exception is a positive medial/lateral wedge test or sonographic finding of distal interphalageal (DIP) collateral desmopathy. The author believes that the risk of making the injury worse is too great if the DIP joint is injected.

A positive frog wedge test is an indicator for treatment of the navicular bursa. The author uses this single test as a positive indicator for the diagnosis and treatment of navicular pain. A positive toe wedge test, in the author's opinion, is an indicator to relieve pressure on the navicular bursa. An important point is that a positive frog wedge indicates that any frog pressure applied to the foot may cause more pain. This is important to know when deciding therapeutic options for shoeing. Positive lateral/medial wedge tests indicate DIP collateral ligament or joint capsule stress. These are cases that need rest if there is a lesion in the collateral ligament. In the absence of collateral lesions, the author recommends shoeing the horse with an asymmetric shoe with the wide branch under the opposite wall of the positive test. This prevents that wall from sinking into the ground and helps prevent the stress from being applied to the ligament during normal movement.

5. Conclusions

Manipulative tests of the foot provide unique information to the examiner that is both beneficial in determining further diagnostics but also provides information important in developing treatment strategies for the lameness.

Acknowledgments

Conflict of Interest

The Author declares no conflicts of interest.

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How to Manage the Club Foot—Birth to Maturity

Stephen E. O'Grady, DVM, MRCVS

A club foot results from a flexural deformity of the distal interphalangeal joint that is characterized by a shortening of the deep digital flexor tendon musculotendinous unit. Flexural deformities are a problem not only in foals but are also responsible for the club foot conformation seen in adult horses. Treatment is most successful when the cause is investigated and therapy is initiated as early as possible and when the biomechanical properties of the foot are thoroughly understood. Flexural deformities in foals to mature horses are addressed through appropriate farriery, often combined with surgery. Author's address: Northern Virginia Equine, PO Box 746, Marshall, VA 20116; e-mail: sogrady@look.net. © 2014 AAEP.

1. Introduction

A club foot can be defined as an upright conformation of the foot associated with a flexural deformity of the distal interphalangeal joint (DIPJ).[1] Grossly, the dorsal hoof wall angle is upright or steep accompanied by a broken forward foot-pastern axis. A flexural deformity of the DIPJ can be defined as a shortening of the musculotendinous unit of the deep digital flexor tendon (DDFT) that results in hyper flexion of the joint.[2–8] The mechanism of this shortening of the musculotendinous unit is not well understood, and the initiating cause is often undetermined but may be related to lameness, nutrition, or genetic predisposition. Flexural deformities are not only observed in foals but are also responsible for the club foot conformation seen in mature horses. The deformity may be congenital or acquired and in many instances may have a genetic basis.[2–4] Treatment is most successful when the cause is investigated, therapy is initiated as early as possible, and when the biomechanical properties of the foot are thoroughly understood. Furthermore, knowledge of the anatomical changes that occur in a club foot and biomechanical principles will enhance interaction with the farrier whose input will be necessary for a successful outcome. Flexural deformities in foals to mature horses are addressed through appropriate farriery, which is often combined with surgery. Flexural deformities have been traditionally referred to as "contracted tendons"; however, the primary defect appears to be a shortening of the musculotendinous unit rather than a shortening of just the tendon structure, thus making "flexural deformity" the preferred descriptive term.[2,8] Shortening of the musculotendinous unit produces a structure of insufficient length to allow normal alignment of the distal phalanx (P3) relative to the middle phalanx, resulting in variable clinical signs ranging from an upright hoof angle to a club foot. As it seems many of the club feet seen in mature horses result from inappropriate management in the first year of life, this paper will discuss the management of club feet (flexural deformities) from birth to the adult horse. As a true club foot is synonymous with a flexural deformity, the terms will be used interchangeably throughout this paper.

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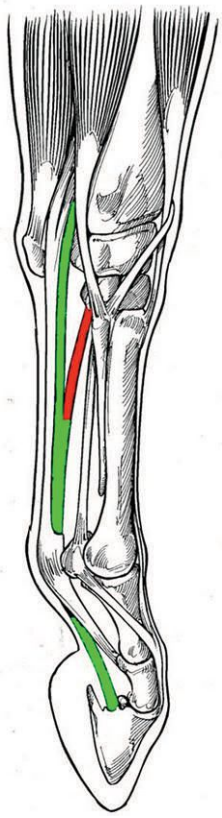

Fig. 1. Illustration shows the structures involved in a flexural deformity of the DIPJ. Note the close association between the AL-DDFT (red line) and the DDFT (green line).

2. Anatomy Review

In the antebrachium, the muscle bellies of the DDFT lie directly on the caudal aspect of the radius and are covered by the muscle bellies of the superficial digital flexor tendon (SDFT) and the flexors of the carpus. The deep digital flexor muscle consists of 3 muscle bellies (the humeral head, the inconsistent radial head, and ulnar head), which form a common tendon proximal to the carpus. This tendon, along with the SDFT, passes through the carpal canal and continues down the palmar aspect of the third metacarpal bone. Below the fetlock, at the level of the middle phalanx, the DDFT passes between the medial and lateral branches of the SDFT, continues distally, and inserts on the flexor surface of the distal phalanx (P3). A strong tendinous band known as the accessory ligament of the DDFT (AL-DDFT) originates from the deep palmer carpal ligament and fuses with the DDFT at the middle of the metacarpus (Fig. 1). The design and function of the anatomical structures is such that any prolonged shortening of the musculotendinous unit affects the position of the DIPJ. This palmar surface of the distal phalanx is pulled palmarly by this shortened musculotendinous unit, placing the DIPJ in a flexed position. The alignment of the bone within the hoof capsule remains constant while the hoof capsule is pulled with the distal phalanx. The flexed position of the DIPJ combined with the altered load on the foot leads to a rapid distortion of the hoof capsule and thus the club foot conformation. It can also be noted from the anatomy that transecting the AL-DDFT lengthens the musculotendinous unit either functionally or by allowing relaxation of the proximal muscle belly associated with the DDFT.

3. Classification of Club Feet

Traditionally, club feet or flexural deformities have been classified as type 1 where the hoof-ground angle is 90° or less and type 2 where the hoof-ground angle is greater than 90°.[3] A recent method of classifying club feet using a grading system (grade 1–4) has been proposed.[2,9] It would appear beneficial to classify the severity of the flexural deformity to devise an appropriate treatment plan and monitor the response to a given therapy. A grading system would also enhance record keeping as well as improve communication between the veterinarian, farrier, and owner with regard to treatment strategies and follow-up. A grade 1 club foot has a hoof angle 3° to 5° greater than the contralateral foot and a characteristic fullness present at the coronet. The hoof-pastern axis generally remains aligned rather than being broken forward. A grade 2 club foot has a hoof angle 5° to 8° greater than the contralateral foot, the angle of the hoof-pastern axis is steep and slightly broken forward, growth rings are wider at the heel than at the toe, and the heel may not touch the ground when excess hoof wall is trimmed from the heel. A grade 3 club foot has a broken-forward hoof-pastern axis, often a concavity in the dorsal aspect of the hoof wall, and the growth rings at the heels are twice as wide as those at the toe. A grade 4 club foot has a hoof angle of 80° or greater, a marked concavity in the dorsal aspect of the hoof wall, a severe broken-forward hoof-pastern axis, and the coronary band from the toe to the heel has lost all slope and is horizontal with the ground (Fig. 2). For simplicity, the author uses a grading system based on the severity or degree of flexion noted in the DIPJ on a well-positioned weight bearing lateral radiographic projection to classify flexural deformities. Any marked flexural deformity should be considered significant and treated accordingly.

4. Club Feet in the Young Horse

Club feet or flexural deformities in foals can be divided into congenital or acquired deformities. As such, congenital deformities are noted at birth, and acquired deformities generally occur from 2 to 8 months of age as the foal grows and develops.[2–4,10]

Congenital Flexure Deformities

Congenital flexural deformities are present at birth, may involve a combination of joints (e.g., carpus, metacarpophalangeal, and distal interphalangeal joints), and are characterized by abnormal flexion of these joints and the inability of these joints to ex-

Fig. 2. Grade 4 club foot. Note the broken forward hoof-pastern axis, fullness of the coronet, the disparity between hoof wall growth at the toe and the heel, the concavity in the dorsal hoof wall, and the poor hoof wall consistency at the ground surface of the capsule.

tend. Proposed etiologies of congenital flexural deformities include malpositioning of the fetus in utero, nutritional mismanagement of the mare during gestation, teratogens in various forages ingested by the mare, maternal exposure to influenza virus, or the deformities could be genetic in origin.[2,6,8,10] The affected foal tends to walk on the toe of the hoof capsule, is unable to place the heel on the ground, and assumes a so-called "ballerina" stance. Treatment of foals with a congenital flexural deformity varies with the severity of the deformity. A mild to moderate flexural deformity in which the foal can readily stand, nurse, and ambulate is generally self-limiting and resolves without treatment. Brief intervals of exercise once or twice daily in a small paddock or on firm footing for the first few days of life may be all that is necessary for the deformity to resolve. If the condition is severe or has not improved by the third day post-foaling, every-other-day intravenous administration of oxytetracycline (2–3 g q 24 hrs) is frequently beneficial.[2,10,11] A variety of bandaging techniques, often combined with splints, can be used to fatigue the muscle portion of the musculotendinous unit. Physical therapy to "stretch" the involved area may hasten recovery. Foals with bilateral congenital flexural deformities usually don't have just 1 isolated structure or joint that is responsible for the deformity, therefore, in the author's opinion, the use of a toe extension is not indicated. In the author's experience, a toe extension will often impede movement and cause the foal to stumble when it attempts to ambulate.

Acquired Flexural Deformities

Acquired flexural deformities generally develop when the foal is between 2 and 8 months old and generally involves the DIPJ initially. It is commonly a unilateral condition but occasionally affects both limbs. The etiology of this deformity is unknown, but speculated causes include genetic predisposition, improper nutrition (i.e., overfeeding, excessive carbohydrate [energy] intake, unbalanced minerals in the diet), and excessive exercise.[2,10] A recent study looking at grazing patterns in a small number of foals showed that foals with long legs and short necks had a tendency to graze with the same limb protracted.[12] Fifty percent of the foals in this study developed uneven feet with a higher heel on the protracted limb leading researchers to feel there may be a possible correlation between conformational traits and an acquired flexural deformity. It is the author's opinion that a large contributing factor to this syndrome is contraction of the muscular portion of the musculotendinous unit caused by a pain response, the source of which could be discomfort anywhere along the limb, physeal dysplasia, or trauma from foals exercising on hard ground. Discomfort may follow aggressive hoof trimming where excessive sole is removed, thus rendering the immature structures within the hoof capsule void of protection. The foal then becomes unwilling to bear full weight and is susceptible to trauma and bruising. Any discomfort or pain in the foot or lower portion of the limb coupled with reduced weight-bearing on the affected limb appears to initiate a flexor withdrawal reflex; this causes the flexor muscles proximal to the tendon to contract, leading to a shortened musculotendinous unit and an altered position of the DIPJ. This shortening of the musculotendinous unit shifts weight-bearing to the dorsal section of the foot causing a decrease in sole depth, bruising of the sole, reduced growth of the dorsal aspect of the hoof wall, and excessive hoof wall growth at the heel. As the flexural deformity may be secondary to pain in these cases, it is essential that a possible source of pain should be carefully evaluated and located by physical examination and, if necessary, by regional analgesia and diagnostic imaging. A genetic component must also be considered for acquired flexure deformities, as some mares consistently produce foals that develop a flexural deformity in the same limb of the dam or grand dam in which a similar deformity is present.[10] However, at present, there is no conclusive scientific evidence to substantiate a genetic basis.

5. Mild Acquired Flexural Deformities (Initial Stage of a Club Foot)

Clinical Signs

The initial clinical sign of a club foot may only be abnormal wear of the hoof at the toe, which is often discovered by the farrier during routine hoof care. Closer or subsequent investigation may reveal that the dorsal hoof wall angle is increased and that after the heels of the hoof capsule have been trimmed to a normal length, the heels may no longer contact the

Fig. 3. A mild flexural deformity before trimming (A) and after trimming (B). Note the change in breakover created under the dorsal hoof in B.

ground. A prominent coronary band may be present at this stage. Most foals affected to this degree may already have a mildly broken forward hoof-pastern axis. Increased palpable digital pulse, heat in the affected foot, and signs of pain when a small hoof tester is applied to the solar aspect of the toe dorsal to the frog are not uncommon clinical findings. Hoof tester pain is generally the result of trauma or excessive weight-bearing on the toe.

Treatment

Conservative treatment such as restricting exercise to reduce further trauma is paramount. Correcting the nutritional status of the foal (i.e., weaning the foal to avoid possible excessive nutrition from the mare and/or decreasing carbohydrates), administering an anti-inflammatory agent (NSAID) to relieve pain, administering oxytetracycline to facilitate muscle relaxation, and carefully trimming the hoof are, in the author's opinion, a good starting point. The NSAIDs should be administered short-term and judiciously in foals due to the potential side effects, such as gastroduodenal irritation and nephrotoxicity. For analgesia, the author will administer firocoxib (0.1 mg/kg bwt q 24 hr) or flunixin meglumine (1.1 mg/kg bwt q 24 hr) combined with a gastric protectant. Hoof trimming is directed toward improving the hoof angle by lightly trimming the heels from the middle of the foot palmarly until the hoof wall at the heels and the frog are on the same plane. The bars can be thinned or removed to possibly improve heel expansion, and the heels adjacent to the sulci should be angled to 45° to promote spreading. Breakover is moved palmarly by creating a mild bevel with a rasp, which begins just dorsal to the apex of the frog and extends to the perimeter of the dorsal aspect of the hoof wall (Fig. 3A and 3B). If improvement is noted, this trimming regimen is optimally performed at 2 week intervals. If the toe is constantly being bruised or undergoing abscessation, a hoof composite[a,b] can be applied to the dorsal aspect of the sole and the distal dorsal aspect of the hoof wall to form a toe "cap" to provide protection. The acrylic composite-impregnated fiberglass or urethane composite used to form the toe cap covers the solar surface to the apex of the frog, protecting that area from further damage. A bevel toward the toe can be created in the composite with a rasp or Dremel® tool to facilitate breakover. If there is adequate integrity of the dorsal section of the hoof wall, the author believes that application of a toe extension to be unwarranted and actually contraindicated. Farriers have traditionally applied toe extensions to create a lever arm using a shoe or a composite, but they only exacerbate wall separation and delay breakover. Furthermore, extensions may contribute to lameness due to excessive stresses on the DDFT when the foal puts full weight on its foot. The above treatment can be temporary, appears to work best when initiated at the first sign of foot deformity before a marked flexural deformity is noted and, when possible, following elimination of any possible inciting causes. The farriery should always be combined with restricted exercise. If the affected foot continues to improve or does not digress, conservative treatment is continued. If a mild flexural deformity progresses in severity to the stage where a marked radiographic flexural deformity is noted, the foal becomes a surgical candidate.

6. Severe Acquired Flexural Deformities (Club Foot)

Clinical Signs

A mild acquired flexural deformity may progress in severity despite conservative treatment, or a severe acquired flexural deformity may be acute in onset. A severe acquired flexural deformity is characterized by a foot with a hoof angle greater than 80°, a prominent fullness at the coronary band, a broken forward pastern-axis, disparity in the length of the

Fig. 4. Severe flexural deformity. Note the prominent coronet, the steep angle of the dorsal hoof wall, the load being placed on the toe, and the heels of the hoof capsule off the ground.

heel relative to the toe of the hoof, and heels that fail to contact the ground (Fig. 4). If the flexural deformity is allowed to persist, the foot eventually assumes a boxy, tubular shape due to the overgrowth of the heels to accommodate the lack of ground contact; heel length will approach the length of the toe. Increased stress on the toe will eventually cause a concavity along the dorsal surface of the hoof wall. Stress exerted on the sole-wall junction in the toe area will cause it to widen, allowing separations to occur.

The diagnosis is straightforward and based on the characteristic foot and limb conformation described above. Radiographs should be used to confirm the diagnosis and assess changes in the joint. The author will administer mild sedation (half the recommended dose of xylazine [0.33–0.44 mg/kg, IV] combined with butorphanol [0.022–0.066 mg/kg] IV) and place each of the foal's feet on separate wooden blocks of equal height, which allows normal loading of both forefeet. Lateral-to-medial weight bearing images of both forefeet should be obtained. The degree of flexion of the DIPJ, the angle of the dorsal hoof wall, and abnormalities at the margin of the distal phalanx should be assessed (Fig. 5).

Treatment

When a marked flexural deformity is present and confirmed by radiographic examination of the feet, conservative treatment and hoof trimming alone are generally unsuccessful in resolving the problem. Elevating the heels has been advocated to reduce tension in the DDFT and to promote weight-bearing on the palmar section of the hoof. However, although elevating the heels improves the hoof-pastern axis and makes the foal more comfortable initially, the author has not been able to subsequently lower the heel or remove the wedge and establish a normal hoof angle with the heel on the ground. Once a marked flexural deformity of the DIPJ and distortion of the hoof capsule is apparent or progressing, the author recommends transection of the AL-DDFT combined with the appropriate farriery.

7. Desmotomy of the AL-DDFT

The author has been consistently successful in treating foals with severe flexural deformities with desmotomy of the AL-DDFT combined with the appropriate farriery. A toe extension is not used at the time of surgery, but an acrylic composite is applied to the solar aspect of the toe to create a reverse wedge. The wedge affords protection for the toe region and appears to redistribute the load to the palmar aspect of the foot, thus mildly increasing the stresses on the DDFT, and restores the concavity to the sole. The farriery is generally performed prior to the surgery either before or while the foal is

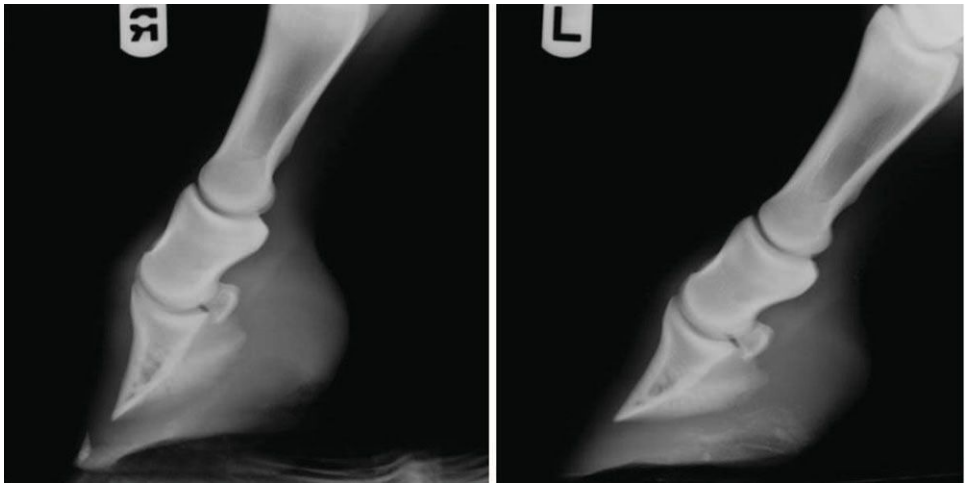

Fig. 5. Radiographic view of the right front (RF) foot shows a broken forward hoof-pastern axis when compared to the left front (LF) foot which shows normal alignment of the digit.

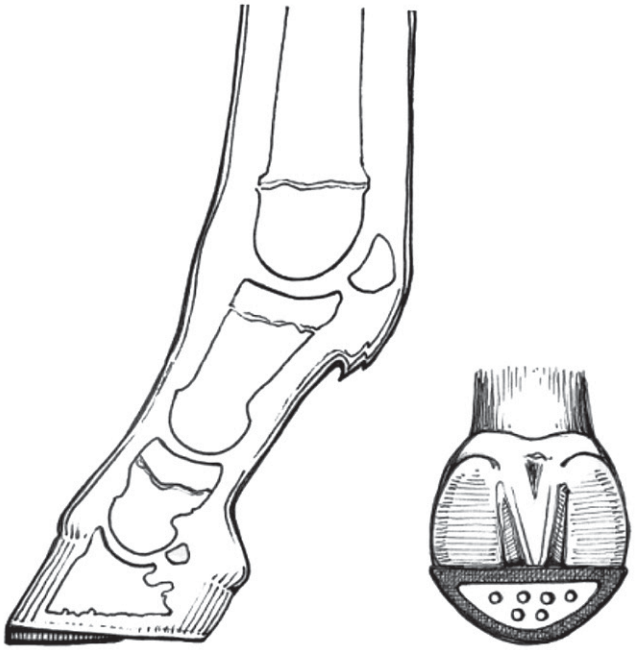

Fig. 6. Illustration of the lateral side of the foot shows the placement of a reverse wedge after the foot has been trimmed. Illustration of the ground surface of the foot shows the composite wedge reinforced with an aluminum plate.

anaesthetized to prevent manipulating the limb and handling the surgical site following the procedure. The heels are lowered from the point of the frog palmarly, until the sole adjoining the hoof wall (sole plane) at the heels becomes solid. Any concavity in the dorsal aspect of the hoof wall is removed with a rasp. The ground surface of the foot dorsal to the frog and the perimeter of the dorsal hoof wall are prepared for a composite using a rasp or Dremel® tool. Deep hoof wall separations in the sole-wall junction at the toe are explored and filled with clay, if necessary, to prevent infection beneath the composite. Foals undergoing this procedure are usually between 3 and 5 months old, and so, because of their size and weight, reinforcing the composite with fiberglass is necessary. A small section of fiberglass is separated into strands and mixed with the composite. The composite is applied to the solar surface of the foot beginning at the apex of the frog and extending to the perimeter of the hoof wall where a thin lip is formed. The composite is molded into a wedge starting at 0° at the apex of the frog and extending to 2° to 3° at the toe[2,13] (Fig. 6). If desired, a piece of 1/8-inch aluminum can be cut out in the shape of the dorsal aspect of the sole. Multiple holes are drilled in the plate, and it is gently placed into the composite. The aluminum is pushed down so that the composite material extrudes through the holes, and the aluminum plate is then covered with additional composite. This additional reinforcement allows the older foals to be walked daily or turned out in a small paddock without the composite wearing out. The foal is placed under general anesthesia, and the surgery is performed in a routine manner as described elsewhere.[8,10,14–17]

8. Aftercare

The surgical aftercare is at the discretion of the attending clinician. Controlled exercise in the form of daily walking or turn-out in a small paddock with firm footing such as a round pen is essential. There is the potential for pain with the initiation of exercise, requiring close monitoring of the foal, and exercise should be increased sequentially. The foal is trimmed at roughly 2 week intervals, based on the amount of hoof growth at the heels with the objective of establishing normal hoof capsule conformation. The composite wedge is removed 1 month after the surgery. At subsequent trimmings, the heels are lowered as necessary from the middle of the foot palmarly, and hoof wall at the toe is trimmed from the dorsal aspect of the hoof wall until the desired conformation is attained. No sole dorsal to the frog is removed. When the desired conformation is reached, the foot is trimmed in a routine manner on a monthly basis. It is important to emphasize that when the hoof capsule returns to an acceptable conformation, only that portion of the sole that is shedding should be removed. This avoids causing discomfort in the dorsal solar area that can result in the horse redeveloping, to some degree, the original deformity.

9. Flexural Deformities in the Mature Horse

Club Feet

There is minimal information in the veterinary literature regarding the management of a mature horse with a club foot. An upright conformation of the foot associated with a flexural deformity of the DIPJ is defined as a club foot[2,10,18–21] (Fig. 7). A flexural deformity is generally diagnosed and treated while the horse is immature but often a mild flexural deformity is ignored or the foal is treated inappropriately. When the horse enters training, the existing flexural deformity may become exacerbated by the type and amount of exercise, inadequate farrier care, such as inappropriate or infrequent trimming and shoeing, or some type of underlying disease. When a club foot conformation is acquired in the adult horse, it is almost always secondary to an underlying cause or disease, such as an injury that results in a non-weight bearing lameness, excessive trimming of the toe resulting in solar pain, chronic low-grade laminitis, or chronic heel pain. Furthermore, flexural deformities have been reported as a cause of decreased athletic performance and chronic, low-grade lameness in the mature horse.[18–21] The altered biomechanics of the foot result in an increased load (i.e., weight-bearing) being placed on the dorsal section of the foot leading

Fig. 7. A grade 3 club foot on a mature horse. Note the broken forward hoof-pastern axis, the concavity in the dorsal hoof wall, the disparity in hoof wall growth between the toe and the heel, and the poor hoof wall consistency distally.

to decreased sole growth, sole bruising, a shortened stride on the affected limb, and various degrees of lameness and poor performance. The majority of horses with a club foot maintain soundness and ability to perform often with minimal therapeutic farriery, yet the club foot conformation and the altered load on the foot may be responsible for poor performance or lameness.

The Hoof Capsule Distortion

To apply the appropriate farriery, understanding the proposed mechanism leading to the club foot conformation is helpful. When a flexural deformity is present, the musculotendinous unit is shortened, the degree of which is dependent on the amount of flexion in the DIPJ. This causes a disparity of hoof wall growth, with more growth at the heel than at the toe to compensate for the decreased length of the musculotendinous structures. The frog generally recedes below the hoof wall due to the excessive hoof wall growth at the heels so that the energy of impact is assumed entirely by the hoof wall, bypassing the soft tissue structures and transferring the load directly onto the bones of digit through the laminar interface. The flexural deformity, combined with the excess hoof wall growth at the heels, places the DIPJ in flexion and the distal phalanx in an abnormal alignment relative to the digit; this promotes toe-first landing and, therefore, excessive load on the dorsal section of the joint and hoof capsule. Hoof abnormalities associated with a club foot conformation are thin flat soles, poor hoof wall consistency, especially at the toe, toe cracks, hoof wall separation, and "white line disease".[22] Injuries associated with a high hoof angle are thought to include inflammation of the DIPJ due to abnormal loading of the joint, sole bruising, and increased strain on the suspensory ligaments of the navicular bone.[19,23]

Radiography

Good quality radiographs, consisting of lateral to medial and a weight bearing (horizontal 0°) dorsopalmar views, are necessary for the clinician and farrier to evaluate the condition and apply the appropriate farriery for the club foot. Good soft-tissue detail allows distortion of the hoof capsule to be accessed.[9] A lateral to medial radiograph reveals the weight-bearing properties of the foot, allows assessment of the hoof capsule, the position of the distal phalanx within the hoof capsule, solar depth, length of the heels, the osseous integrity of the perimeter of the distal phalanx, and the severity of the flexural deformity of the DIPJ. The degree of flexion indicates the amount of shortening of the musculotendinous unit. The radiographs are used to diagnose any pathology present, determine treatment options, and can be used as a template for farriery (Fig. 8).

10. Therapeutic Farriery

Therapeutic farriery forms the mainstay of treatment for club feet. Farriery should be based on principles rather than a particular method, and the principles remain the same regardless of the severity of the flexural deformity.[18,19,22,24] The principles are to achieve normal alignment between the first, middle, and distal phalanges and thus normal orientation and loading of the distal phalanx relative to the ground. Trimming and shoeing is aimed at removing weight-bearing from the toe and dorsal aspect of the distal phalanx and reestablishing weight-bearing to the entire solar surface of the distal phalanx and the corresponding hoof capsule.

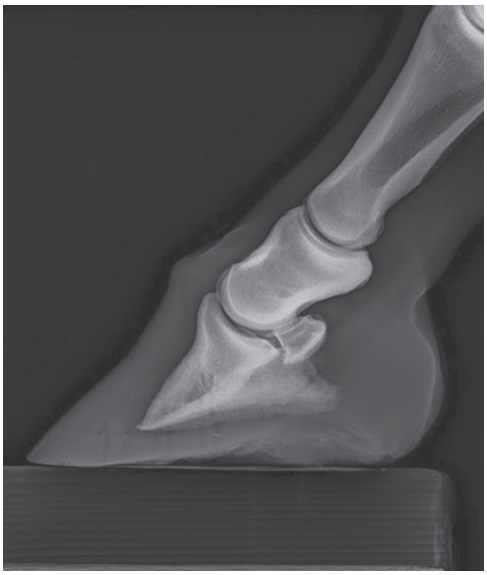

Fig. 8. Radiograph shows a moderate flexural deformity involving the DIPJ in a horse with a club foot. Note the ideal soft tissue parameters of the hoof capsule that can be assessed on this radiograph.

Historically, farriers have been taught to trim (lower) the heels to correct the distorted hoof capsule and promote weight-bearing in the heel area, but this type of trimming comes with a price. As the severity of the flexural deformity increases, so too does the shortening of the musculotendinous unit; therefore, lowering the heels directly increases the tension within the musculotendinous unit, and these stresses may lead to irresolvable tearing of the dorsal lamellae, widening of the sole-wall junction similar to that seen in the chronic laminitic hoof, and increased pain.[18,19,25] The increased forces placed on the DDFT from this type of trimming also promote hoof capsule distortion and abnormal loading. Furthermore, if there is pathology present in the soft tissue structures of the palmar foot, decreasing the height of the heels is likely to place more strain in this section of the foot.

Fig. 9. A wedge pad can be placed under the toe of a horse with a club foot, which will test the response when tension is exerted on the DDFT. The farrier can then determine the amount of hoof wall that can be safely removed from the heels of the hoof capsule.

11. Farriery

Distinguishing between a foot with steep hoof angle and a true club foot is important. High hoof angles without phalangeal misalignment or with mild phalangeal misalignment can generally be managed by adhering to good farriery guidelines for trimming such as using the hoof-pastern axis, the center of rotation, and trimming the heels of the hoof capsule to include the frog. It may be necessary with a high hoof angle or mild phalangeal alignment to gradually trim the heels in a tapered fashion from the apex of the frog to the heels. This increases the ground surface of the foot and attempts to reestablish weight-bearing on the entire solar surface of the foot. Breakover is moved palmarly at the same time to compensate for any increased tension in the DDFT created by lowering the heels. This can be accomplished by rolling, rockering, or grinding breakover into the toe of the shoe. If improvement is noted, the horse should be trimmed/shod at 4 week intervals.

Farriery to correct a high hoof angle accompanied by a flexural deformity (club foot) becomes more of a challenge. Again, the object of farriery is to load the heels, compensate for the shortening of the DDFT, and improve the hoof-pastern axis. To accomplish these objectives, farriery is directed at trimming the heels of the hoof capsule, but the amount of heel to remove can be difficult to determine. In mild to moderate club feet, an estimate of how much heel to remove can be made by placing the thick end of a 2° or 3° pad under the toe of the foot and allowing the horse to stand on it[10,19] (Fig. 9). If the horse does not resent the tension this places on the DDFT, this test allows the farrier to safely trim the hoof wall at the heels in a tapered fashion starting in a palmar direction from the widest part of the foot using the thickness of the degree pad as a guide. The toe is shortened by trimming the outer surface of the dorsal hoof wall with a rasp. The trimmed foot is fitted with a shoe that has the breakover forged or ground into it starting just dorsal to the apex of the frog and tapering toward the toe to further decrease the stresses on the DDFT. There are also commercial shoes available that have a rockered toe that provide appropriate breakover.

With the more advanced cases of club feet, the heels should still be lowered to load the heels and unload the toe, but the addition of heel elevation following the trim is necessary to compensate for the shortening of the musculotendinous unit. The concept of lowering the heels with the trim then wedging the palmar aspect of the hoof back up is often not understood. When the heels are trimmed back to the widest point of the frog, the load bearing surface area of the foot increases, and this is necessary for normal function of the hoof. However, the musculotendinous unit must be accommodated and maintained without excessive tension and pain. This is accomplished by decreasing the breakover and by adding elevation to the palmar aspect of the hoof. The degree of wedge that is applied often mimics the amount of heel removed, but in many cases may be less due to mechanical contributions made by rockering or rolling the toe of the shoe. The amount of heel elevation needed if necessary can be demonstrated following the trim by placing the trimmed foot on the ground 6 to 8 inches palmar to the contralateral limb. A space will generally appear between the heels of the foot and the ground (Fig. 10). The author uses either a wedge shoe or places a degree pad or a bar wedge between the heels of the foot and the shoe to compensate for the shortening of the muscle-tendon unit (Fig. 11). This method allows the heels to be weight-bearing but at the same time decreases the stresses on the musculotendinous unit. Creating breakover in the shoe to further relieve stress in the DDFT, as described above, is essential. It is important to note that when the heels are elevated with a wedge shoe, the normal

Fig. 10. Post trimming, the foot is placed 6 to 8 inches palmar to the contralateral limb. A space under the heels indicates shortening of the musculotendinous unit and the necessity of heel elevation.

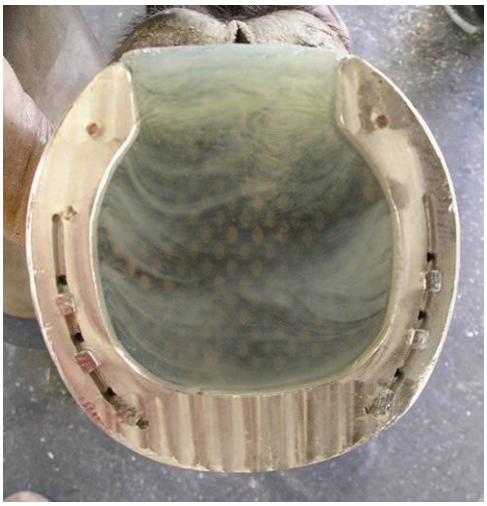

Fig. 12. Pour-in pad used to change the distribution of pressure between the branches of a shoe with wedge heels.

ground reaction forces and load bearing structures are altered. To redistribute the load, it is beneficial to apply a "pour-in" pad or impression material to the sole of the hoof between the branches of the shoe to reestablish load sharing of the weight bearing structures of the hoof (Fig. 12). Without increasing the surface area over which the ground reaction force is distributed, the heels may become overloaded over time and possibly result in quarter cracks, contracted heels, and subsolar bruising of the heel region. Following the farriery, it is necessary to see the horse have a flat strike pattern rather than a toe first landing. If the horse does not land flat, heel elevation should be considered.

In severe cases of club feet, the hoof wall consistency may be compromised such that alternative methods of application (other than nailing) may need to be integrated. The most common method of "gluing" a shoe on is termed "direct gluing," which attaches a shoe directly to the weight bearing surface of the hoof wall and sole via some type of adhesive. Aluminum rather than steel shoes are typically used for this purpose because aluminum is very porous and the adhesives adhere with more affinity. The same principles for shoe fit are used with glue-on shoes vs. shoes that are nailed. Recently, polyurethane shoes[c] have reached the marketplace and can be applied via the direct gluing technique (Fig. 13). These shoes are mentioned because they are flexible, allow the heels to expand, yet still carry the advantage of being attached with an

Fig. 11. Club foot illustrated in Fig. 7. Following the appropriate trim, the horse is shod with a wide web steel shoe with heel elevation and breakover created in the shoe.

Fig. 13. Polyurethane shoe attached directly to the foot with an acrylic adhesive (photo courtesy of Curtis Burns).

Fig. 14. Shoe with three removable 2° wedge pads attached to a plastic treatment plate.

adhesive. Although glue-on shoes will not take the place of traditional farriery, they provide an alternative when necessary to prevent the compromised club foot from continuing to lose sole depth and develop further hoof capsule distortion by not having a shoe applied. It is always the goal, however, to transfer the horse into the most normal and simple shoeing/trimming protocol when possible.

12. Farriery Combined With Surgery

In selected cases, horses with a severe flexural deformity or horses that have not responded to appropriate farriery and remain lame may benefit from a desmotomy of the AL-DDFT.[2,10,18,19,21,23,25] This release procedure, along with therapeutic farriery, allows realignment of the distal phalanx within the remainder of the digit, loads the entire surface of the hoof capsule, and readily allows the accompanying distortion of the hoof capsule to be improved. It is the author's opinion that if this surgery is being contemplated, it should be performed early in the horse's athletic career, before there is a significant hoof capsule distortion and before pathological changes involving the DIPJ and or the margin of the distal phalanx become evident on radiographs.

The surgery is generally performed under general anesthesia but in the mature horse, it can be performed standing using sedation and local or regional analgesia if necessary. If surgery is performed with the horse standing, the heel should be elevated by taping a 12° wedge to the foot to decrease tension in the AL-DDFT/DDFT complex allowing the ligament to be easily identified, separated, and transected away from the cutaneous incision. The client should be forewarned that the surgery involves an extended recovery period, and a blemish or fibrous thickening at the surgery site is inevitable due to the mature nature of the tissue. Caution is advised when performing the farriery that accompanies the surgery because the soft tissue structures within the hoof capsule and the digit have adapted/accommodated for the distortion of the hoof capsule. The author will trim the palmar section of the foot moderately using the information obtained from the radiograph for guidance and then apply two or three 2° wedge pads using either a shoe or a cuff (Fig. 14). After surgery, the horse is walked daily, and a degree pad is removed every 7 to 10 days depending on the comfort of the horse. After 3 weeks, the horse is allowed turnout in a small paddock such as a round pen for an additional 3 weeks and then turnout in a larger area for 3 to 6 months before exercise is resumed. The appropriate farriery must be maintained post surgery or the foot will have a tendency to revert to the original foot conformation. The cosmetic appearance of the limb is maximized by keeping the limb bandaged for the first 6 weeks. In a limited number of cases that the author has managed or consulted on, the benefits returning the horse to soundness have far outweighed the rehabilitation process being labor intensive. The author has not realized any benefit in applying a toe extension to the shoe in the adult horse following surgery. Many mature horses with a club foot frequently have damage to the dorsal lamellae and a stretched white line similar to that found in horses with laminitis, and therefore, toe extensions may markedly exacerbate detrimental mechanical forces on the lamellae and dorsal hoof wall.

13. Conclusion

The club foot is an important cause of equine lameness, decreased athletic performance, and a challenge to the veterinarian and farrier. The clinician must recognize and understand the altered mechanics that are placed on the osseous structures within the hoof, the DIPJ, and on the hoof capsule that accompany a flexural deformity involving the DIPJ. This understanding allows the clinician to apply the appropriate treatment and appreciate subsequent improvement. Additionally, it is essential to look beyond the deformed foot to identify and remove, if possible, any underlying cause(s). As with most disorders, early recognition and intervention signif-

icantly increase the chances for a successful outcome. This is especially true when dealing with the young horse. A thorough physical examination, high quality radiographic imaging, understanding biomechanical principles, familiarity with composite materials, and surgical competence are all essential to properly treat horses presented with a wide range of club foot abnormalities. Owners and trainers must be informed regarding the severity of any individual horse's flexural deformity, the treatment options, the expected outcome, and the aftercare. Knowledge, skill, and interaction between the veterinarian and farrier are necessary for a successful outcome when treating a horse with a club foot, regardless of whether treatment is limited to farriery or combined with surgery.

Acknowledgments

Conflict of Interest

The Author declares no conflicts of interest.

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[a]Equilox® International, 110 NE 2nd Street, Pine Island, MN 55963.
[b]Vettec®, 600 E. Hueneme Road, Oxnard, CA 93033.
[c]Polyflex® Shoe No Anvil, LLC., Wellington, FL 33414.

Characterization of Equine Hoof Lamellar Tissue Microanatomy With Fluorescent Markers

Hannah L. Galantino-Homer, VMD, PhD, DACT*; Robert K. Clark, PhD; and Renata L. Linardi, DVM, PhD

Equine histological studies are impaired by the lack of reagents validated for use on horse tissues. This paper describes the use of fluorescently-conjugated lectins and antibodies for the identification of tissue and cellular microanatomy in equine hoof lamellar tissue. Structures that can be specifically labelled include connective tissue extracellular matrix, epidermal basal cells, and subcellular components of epidermal cells such as cell membranes, cytoskeletal intermediate filaments, and cytolinkers that join the cytoskeleton to adhesion complexes. Authors' addresses: Department of Clinical Studies, New Bolton Center, 382 West Street Rd., Kennett Square, PA 19348 (Galantino-Homer, Linardi); and STEM and Health Division, Cumberland County College, Vineland, NJ 08360 (Clark); e-mail: hghomer@vet.upenn.edu. *Corresponding and presenting author. © 2014 AAEP.

1. Introduction

Our long-term goal is to improve the understanding, treatment, and prevention of equine laminitis. Laminitis is a common and debilitating disease characterized by tissue damage, inflammation, and dysplasia of the epidermal and dermal lamellae that can result in mechanical failure of the suspensory apparatus of the distal phalanx causing chronic pain and lameness, frequently necessitating euthanasia.[1,2] Equine laminitis results in the generation of abnormal hoof capsule growth and formation of the lamellar wedge, a cornified but weak wedge of epidermal tissue produced by hyperplastic, metaplastic/dysplastic keratinocytes lying between the third phalanx and the hoof wall.[3,4] Relatively few studies have investigated the histological localization of the altered protein/glycoprotein expression during laminitis pathogenesis. These studies are essential to an improved understanding of laminitis pathogenesis since laminitis is, ultimately, a disease of tissue structural failure and altered differentiation. Indirect immunofluorescence microscopy is a useful tool for these studies due to its high sensitivity and specificity, ability to precisely determine antigen colocalization, applicability to confocal microscopy, and amenability to quantification.

We have demonstrated that the metaplastic epidermal lamellae in horses with chronic laminitis have significantly decreased expression of the nuclear transcription factor, p63, a marker of epidermal stem cell proliferative capacity, consistent with the altered growth, differentiation, and regenerative capacity of this tissue.[5,6] It has also recently been demonstrated that epidermal basal cell proteoglycan depletion in concert with increased metalloproteinase (ADAMTS-4) activity occurs early in the development of the lesion.[7,8] Wang and co-workers have shown that suppression of the canonical Wnt signaling pathway in lamellar tissue from horses

NOTES

with laminitis is associated with changes in lamellar epidermal expression of cell-cell and cell-matrix adhesion proteins, which is also consistent with altered cell and tissue differentiation.[9] We have characterized the keratin isoform composition of normal hoof lamellae by quantitative proteomic analysis, including the identification of 2 novel isoforms that comprise more than half of the keratin content,[10] but the expression of these keratin isoforms in laminitic horses and in relation to basal cell keratin markers has not been investigated. For all of these studies, indirect immunofluorescence utilizing commercial antibodies validated for use on equine tissues have provided an effective means of identifying protein localization. However, without appropriate fluorescence counterstains that identify specific tissue and cellular structures, the relative microanatomical localization of proteins of interest cannot be precisely documented.[11,12] Even nuclear counterstains fail to provide any information on the structure of matrix-rich connective tissues.[11,12] Suitable counterstains include double-label indirect immunofluorescence with cell type-specific, connective tissue and subcellular-localizing antibodies. In addition, plant lectins, which specifically bind to saccharide moieties within tissues ([13] and references therein), may have utility as counterstains. The object of this study was to identify and characterize antibody- and lectin-based fluorescent markers of lamellar microanatomy.

2. Materials and Methods

Blocks of hoof tissue from the mid-dorsal stratum internum and including the hoof epidermal and dermal lamellae were dissected from humanely-euthanized nonlaminitic and chronic laminitic horses according to a protocol approved by the Institutional Animal Care and Use Committee, as described.[5] Tissues were fixed in 4% paraformaldehyde for 24 hours and then dehydrated in 10% and 30% sucrose, respectively, for 24 hours each. Tissue blocks were coated with embedding medium[a] and snap-frozen in liquid nitrogen-chilled 2-methylbutane. Tissue blocks were stored at −80°C prior to cryostat sectioning at 6 microns. Sections were air-dried and stored at −20°C until needed. Indirect immunofluorescence histology was performed on cryosections as described elsewhere.[5,10] Tissues and cells were incubated with primary antibodies overnight at 4°C followed by fluorescent dye[b]-conjugated secondary antibodies incubation at room temperature for 1 h.

The following primary antibodies were used at the following dilutions: mouse monoclonal anti-K14, clone LL002[c] (1:100) and rabbit anti-desmoplakin I and II antiserum[c] (1:400). The following secondary antibodies were used: fluorophore 488-conjugated goat anti-mouse IgG[b] [green] (1:500) and fluorophore 488-conjugated goat anti-rabbit IgG[b] [green] (1:500). Nucleic acids were stained with 4′,6-diamidino-2-phenylindole, dilactate[d] (DAPI, 0.5 μg/ml) for a 5 min incubation at room temperature. Sections were mounted using mounting medium[e] for light microscopy with epifluorescence illumination[f] and confocal microscopy[g] using software for confocal imaging[h]. Epifluorescence microscopy images were acquired with a digital camera[i] and imaging software[j].

We utilized tetramethylrhodamine isothiocyanate (RITC)-conjugated wheat germ agglutinin (WGA) and Ulex europeas (UEA) lectins[k] to perform staining as follows: tissue sections were thawed and air-dried to room temperature; washed 3 times in PBS on ice, ten min each; incubated for 30 min in the dark, at room temperature (RT) with WGA diluted 1:300 or UEA diluted 1:100 in PBS; again washed 3 times in PBS on ice for ten min each prior to mounting with mounting medium[e]. Sections were stored at 4°C in the dark prior to scanning by confocal microscopy[g].

3. Results

As shown in Figs. 1 and 2, keratin-14 (K14) is restricted to the basal epidermal cells of the secondary epidermal lamellae (SELs) in nonlaminitic horses, as detected by indirect immunofluorescence with the mouse monoclonal anti-K14 antibody. K14 localization is abnormal in laminitic tissue and extends into suprabasal layers.

As shown in Fig. 2, fluorescently-conjugated WGA stains the plasma membrane regions of the SEL basal and suprabasal cells and connective tissue in the primary and secondary dermal lamellae from nonlaminitic horses. Double labeling using K14 indirect immunofluorescence and fluorescently-conjugated WGA allows the precise demarcation of the membrane and cytoskeletal components of epidermal basal cells (Fig. 2B). Fluorescently-conjugated UEA stains epidermal, but not dermal, lamellar cells with the highest intensity apparent in cytoplasmic and plasma membrane regions of suprabasal cells adjacent to the keratinized axis (data not shown).

As shown in Fig. 3, the localization of WGA to the epidermal cell membrane allows the precise localization of the cytolinker protein, desmoplakin, to the epidermal cell membrane, but not along the basal side of the cells adjacent to the extracellular basement membrane zone in the primary and secondary epidermal lamellae from nonlaminitic horses. Co-localized WGA (red) and desmoplakin (green) along epidermal cell membranes appear yellow to yellow-green in merged images (Fig. 3B). Figure 4 illustrates the use of fluorescently-conjugated WGA combined with K14 immunofluorescence and DAPI staining to demarcate cell nuclei, extracellular matrix connective tissue, epidermal lamellar cell membranes, and epidermal basal cell cytoskeletal intermediate filaments.

4. Discussion

The high prevalence and frequently poor outcomes associated with laminitis make it a high priority for equine practitioners and veterinary medical researchers alike.[14] The varied etiologic pathways of

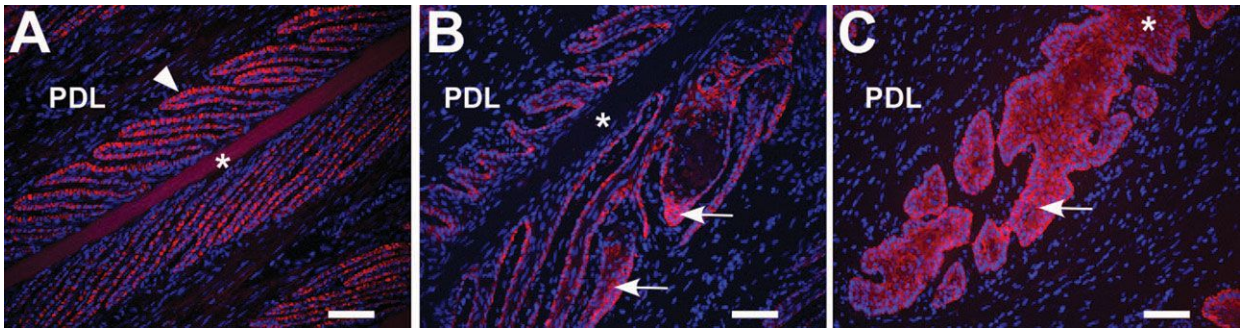

Fig. 1. Localization of K14, a basal epidermal cell marker, in nonlaminitic and laminitic mid-dorsal lamellae. (A) and (B) show comparable, mid-primary epidermal lamellar regions from a nonlaminitic (A) and laminitic (B) horse, with the primary epidermal lamellar keratinized axis and adjacent primary dermal lamellae indicated by an asterisk (*) and (PDL), respectively. (C) is from the axial region (primary epidermal lamellar tip) of a laminitic horse. (A) K14 (red) is localized exclusively to the basal epidermal cell layer (arrowhead) of the secondary epidermal lamellae (SELs) in nonlaminitic horses. In horses with chronic laminitis (B, C), K14 positive cells extend into the suprabasal layers (arrows) in areas of lamellar dysplasia and hyperplasia. Scale bar = 50 μm, images captured from epifluorescence microscopy with a 20× objective as described in the Materials and Methods section. This experiment was performed at least 5 times using lamellar tissue from 5 different nonlaminitic horses and 5 different laminitic horses. Representative images are shown.

this condition combine with the complex tissue architecture to make definitive answers regarding even the most basic questions elusive. Immunofluorescence techniques are frequently employed in efforts both to identify cells that may play important roles in the development of laminitis and to identify molecules that may be impacting those cells. Better localization of each of these target types is dependent on our ability to recognize the non-immunoreactive tissues immediately adjacent to the fluorescent signal. This precise identification is particularly difficult when good technique and reagents render the best results, specifically when there is a strong immunofluorescence signal with little to no background fluorescence. By identifying fluorescent markers for specific foci within the lamellae, we increase the power of our immunofluorescence technique to do exactly what we intend it to do—enable us to identify the precise location of our target antigen.

While this goal has been of interest to our laboratory for some time, we did not originally set out to identify such markers. We fortuitously saw the utility of fluorescent lectin markers as counterstains

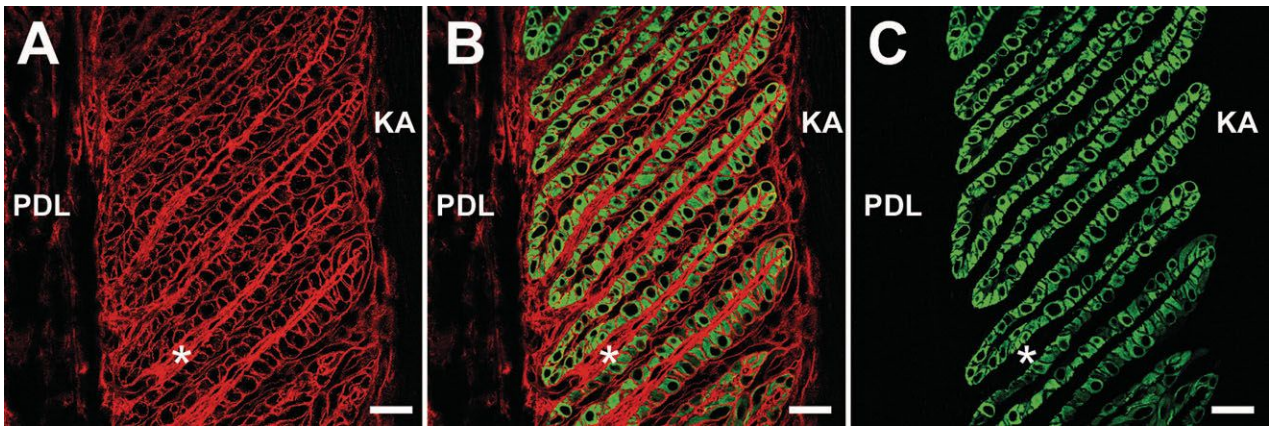

Fig. 2. Localization of K14 combined with WGA counterstaining on equine hoof lamellae. Red (A), green (C), and merged (B) images of a lamellar tissue region from a nonlaminitic horse that includes portions of a primary dermal lamella (PDL), secondary dermal lamellae, as indicated by the asterisk (*), keratinized axis of a primary epidermal lamella (KA), and secondary epidermal lamellae. Red fluorescence demonstrates localization of WGA binding to extracellular matrix fibers in the primary and secondary dermal lamellae and to cell membranes in secondary and primary epidermal lamellae (A, B). Green fluorescence demonstrates immunolocalization of K14 to the cytoplasm of basal cells of the secondary epidermal lamellae (B, C). Note that red WGA fluorescence augments the precise localization of the green K14-positive cells. Scale bar = 25 μm, images captured by confocal microscopy with a 63× objective as described in the Materials and Methods section. This experiment was performed at least 6 times using lamellar tissue from 6 different nonlaminitic horses. Representative images are shown.

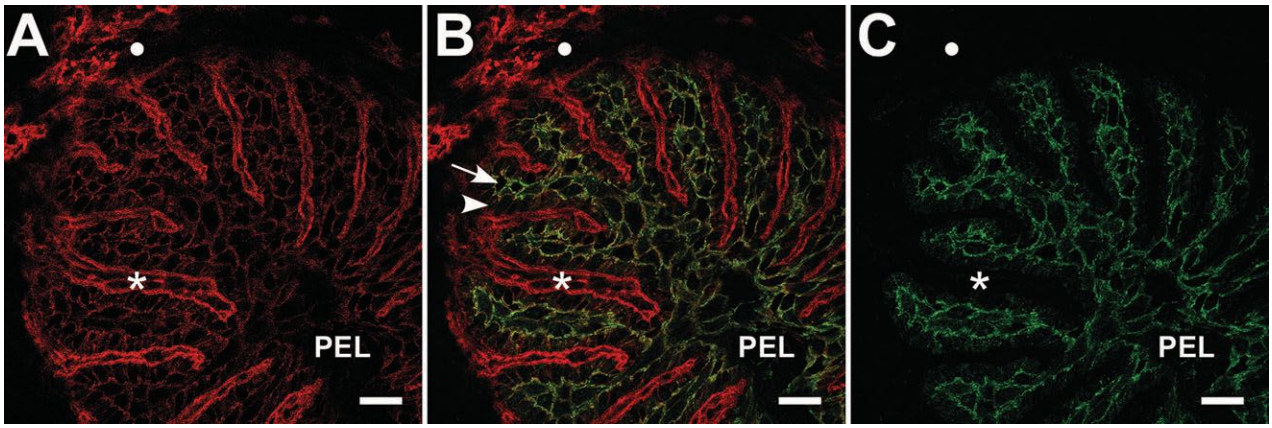

Fig. 3. Localization of desmoplakin combined with WGA counterstaining on equine hoof lamellae. Red fluorescence demonstrates localization of WGA binding to extracellular matrix fibers in the dermal corium (●) and secondary dermal lamellae (*) and to cell membranes in the primary epidermal lamella (PEL) and secondary epidermal lamellae (A, B) from a nonlaminitic horse. Green fluorescence demonstrates immunolocalization of desmoplakin to the cell membrane of basal (arrowhead) and suprabasal (arrow) cells of the secondary epidermal lamellae but not to the basal membrane of basal cells (B, C). Colocalization of red WGA fluorescence with green desmoplakin fluorescence appears yellow-green. Scale bar = 25 μm, images captured by confocal microscopy with a 63× objective as described in the Materials and Methods section. This experiment was performed at least 6 times using lamellar tissue from 6 different nonlaminitic horses. Representative images are shown.

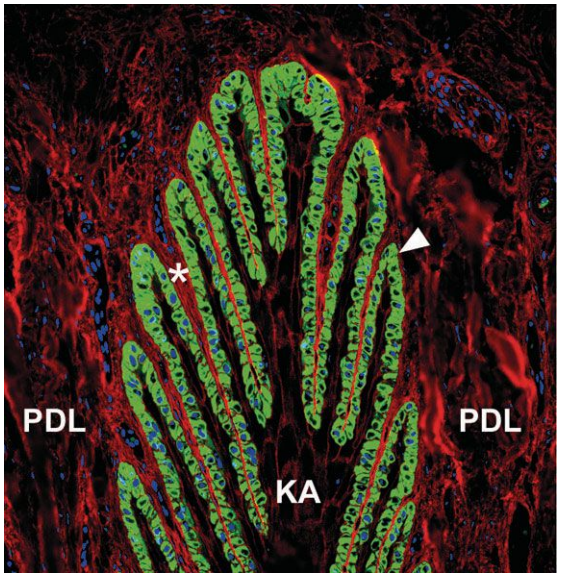

Fig. 4. Triple-fluorescence staining to identify multiple tissue components of equine hoof lamellae. Red fluorescence demonstrates localization of WGA binding to extracellular matrix fibers in the primary dermal lamellae (PDL) and secondary dermal lamellae (*) and to cell membranes in secondary and primary epidermal lamellae (keratinized axis (KA) indicated). Green fluorescence demonstrates immunolocalization of K14 to the cytoplasm of basal cells of the secondary epidermal lamellae (arrowhead). Blue fluorescence demonstrates localization of DAPI staining to cell nuclei. Image captured by epifluorescence microscopy with a 20× objective as described in the Materials and Methods section. This experiment was performed at least 5 times using lamellar tissue from 5 different nonlaminitic horses. A representative image is shown.

when employing this technique in our investigation of the breakdown of proteoglycans at the onset of laminitis. The cytoplasm of the basal epidermal cells of the SEL have been shown to contain aggrecan, a member of the chondroitin sulfate proteoglycan family.[8] This large molecule carries numerous covalently-linked chondroitin sulfate and oligosaccharide side chains.[15] Given its role in the extracellular matrix of cartilage, it may be expected that, through osmotic attraction of water, it contributes a cushioning effect within the hoof.[8] In fact, it may be degraded quite early in a number of disease processes including laminitis.[7,8,16]

It is against this backdrop that we sought to examine carbohydrate content of hoof lamellae epidermal basal cells through the use of lectins. Lectins have been used in the characterization of carbohydrate expression in epidermal cells and adnexae for decades and across many species including the dog,[13] horse,[17,18] and human.[19,20] The complex, stratified cytoarchitecture of these tissues is generated by pathways of differentiation that lead through distinct cell phenotypes.[21] The above referenced studies demonstrate that these phenotypes can be distinguished based on their lectin binding pattern.

Our studies quickly yielded interesting findings other than the answers we sought. The affinity of WGA for fibers in the extracellular matrix and membranes of epidermal cells render it a rapid, economical, and simple counterstain to immunofluorescence studies on equine hoof lamellae. We then questioned which other reagents might be similarly useful. The affinity of UEA for suprabasal cells, especially those at the intersection between SELs

and the KA, make it potentially useful as a marker of differentiation in the epidermal keratinocytes of the hoof lamellae. Moreover, altered UEA reactivity might be consistent with tissue metaplasia and the resultant widening of the keratinized axis of primary epidermal lamellae during lamellar wedge formation.[5] On the other hand, a marker that identifies the less differentiated epidermal cell population would be equally useful. Antibodies against the intermediate filament protein K14 specifically label this cell type. The abnormal localization of K14 to suprabasal cells in the lamellar wedge is consistent with the presence of dysplastic epidermal cells in this tissue.[5] Altered epidermal differentiation might contribute to laminitis disease pathogenesis.

Since the cytolinker desmoplakin interconnects intermediate filaments with desmosomal adhesion complexes, antibodies immunoreactive with this protein are useful in the localization of the plasma membranes of epidermal cells interlinking with other epidermal cells. The restriction of desmoplakin staining to the non-basal SEL cell membranes is consistent with its absence from hemidesmosomes linking these cells to the basement membrane.[22] We are currently using combinations of these lectins and immunoreagents to investigate K14 and desmoplakin localization during laminitis pathogenesis.

5. Conclusion

Fluorescently-conjugated lectins and antibodies are useful for the identification of tissue, cellular, and subcellular microanatomy in equine hoof lamellar tissue. Structures that can be specifically labelled include connective tissue extracellular matrix, epidermal basal cells, and subcellular components of epidermal cells such as cell membranes, cytoskeletal intermediate filaments, and cytolinkers that join the cytoskeleton to adhesion complexes. Ongoing studies in our laboratory are using these markers to investigate laminitis histopathology and molecular pathogenesis.

Acknowledgments

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Conflict of Interest

The Authors declare no conflicts of interest. The manuscript was not reviewed by any financial sponsors prior to submission.

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[a]Neg 50, Richard Allen/Thermo Scientific, Kalamazoo, MI 49008.
[b]Alexa Fluor®, Life Technologies, Grand Island, NY 14072.
[c]Anti-Cytokeratin 14 antibody [LL002] (ab7800) and Human Desmoplakin I + II antibody (ab71690), Abcam, Cambridge, MA 02139.
[d]DAPI Nucleic Acid Stain (D3571), Life Technologies, Grand Island, NY 14072.
[e]VectaShield™, Vector Laboratories, Burlingame, CA 94010.
[f]Model DM5000B, Leica, Bannockburn, IL 60015.
[g]Model Leica DMI6000 B with Leitz TCS SP5 confocal system; Leitz Microsystems CMS GmbH, Mannheim, DE.
[h]LAS (Leica Application Suite) Advance Fluorescence System software for confocal imaging, Leica, Bannockburn, IL 60015.
[i]EXi Blue, QImaging, Surrey, BC, V3S 6K3, Canada.
[j]Image-Pro Plus, v.7.0, MediaCybernetics, Rockville, MD 20850.
[k]Lectin Kit Rhodamine (RLK-2200), Vector Laboratories, Burlingame, CA 94010.

Safety of Tiludronate Disodium in the Management of Lameness Associated With Equine Navicular Syndrome

Valentine S. Williams, DVM, MS, DACVS

Intravenous infusion of tiludronate disodium used as directed is safe for the control of lameness associated with navicular syndrome. Author's address: Ceva Animal Health, LLC, 8735 Rosehill Rd., Suite 300, Lenexa, KS 66215; e-mail: valentine.williams@ceva.com. © 2014 AAEP.

1. Introduction

The safety studies characterized the drug's safety profile.

2. Materials and Methods

Safety was assessed in a target animal safety (TAS) study at 1× (1 mg/kg), 3×, and 5×, a renal safety study (1× and 3×), a hyperkalemic periodic paralysis (HYPP) carrier study (1×), an infusion rate study (1×), and two field studies (1×). Infusion times varied from 30 to 120 minutes.

3. Results

The majority of horses were treated with no adverse effects. Forty-four percent (44%) of horses infused over 90 minutes exhibited signs consistent with colic. The majority of horses resolved with hand-walking only or use of non-NSAID treatment. All cases resolved uneventfully.

In the TAS study, there were mild decreases in ionized calcium and mild elevations in blood urea nitrogen (BUN) and/or creatinine. In the renal study, horses had normal renal function and no evidence of toxicity on histopathology. Horses that developed azotemia in the field studies resolved with fluid therapy.

In the HYPP study, treatment did not induce HYPP episodes in carrier horses. All cases resolved without medical intervention.

4. Discussion

Tiludronate sodium (1 mg/kg IV infusion) offers a safe treatment for the management of navicular syndrome.

Acknowledgments

Conflict of Interest

This study was funded by Ceva Santé Animale. The author is employed by Ceva Animal Health, LLC, the U.S. subsidiary of the sponsor.

Footnote

[a]Tildren, Ceva Sante Animale, 33500 Libourne, France.

Research Abstract

NOTES

Tiludronate Disodium in the Management of Lameness Associated With Equine Navicular Syndrome

Valentine S. Williams, DVM, MS, DACVS*; and A. Kent Allen, DVM†

Intravenous tiludronate disodium significantly improved lameness associated with navicular syndrome. Authors' addresses: Ceva Animal Health, LLC, 8735 Rosehill Rd., Suite 300, Lenexa, KS 66215 (Williams); and Virginia Equine Imaging, 2716 Landmark School Road, The Plains, VA 20198 (Allen); e-mail: valentine.williams@ceva.com. *Corresponding author; †Presenting author. © 2014 AAEP.

1. Introduction

Navicular syndrome is a common cause of lameness and disability in performance horses. The objective of this study was to examine the effectiveness of the bisphosphonate tiludronate disodium[a] plus corrective shoeing on lameness associated with navicular syndrome in a blinded randomized placebo controlled clinical trial.

2. Materials and Methods

Cases were selected based on lameness examination (AAEP lameness score of 2 or 3), palmar digital nerve blocks, radiographic findings, and magnetic resonance imaging findings (navicular bone edema with minimal soft tissue lesions). Suitable cases were randomly assigned to a single treatment with tiludronate disodium (1.0 mg/kg) or control (250 mg mannitol) in 1 liter of 0.9% saline administered by intravenous infusion over 30 or 60 minutes. All horses were subjected to corrective shoeing. Effectiveness was defined as an improvement of $\geq$1 grade lameness score in the lamest limb with no worsening of lameness in the opposite limb at 2 months post treatment.

3. Results

Located at 12 sites, 181 cases (119 treated group and 62 control group) complied with all requirements for inclusion in data analysis. Tiludronate disodium was significantly more effective compared to the control group in reducing lameness score (64% vs 48%, respectively).

4. Discussion

Tiludronate disodium in conjunction with corrective shoeing effectively reduced lameness associated with navicular syndrome.

Acknowledgments

The authors thank all investigators who provided cases for this study: Drs. Erin Denney-Jones, Ron Genovese, Jake Hersman, Carter Judy, Douglas Langer, Paul McClellan, Rick Mitchell, Robert Schneider, Scott Taylor, Kelly Tisher, and Chad Zubrod.

Conflict of Interest

This study was funded by Ceva Santé Animale. Dr. Williams is employed by Ceva Animal Health, LLC, the U.S. subsidiary of the sponsor.

Footnote

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Research Abstract

NOTES

Comparison of Subjective and Objective Methods to Identify Mild Forelimb Lameness in Horses

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Subjective evaluation and inertial-sensors agree on mild forelimb lameness more frequently than either modality compared to force platforms. Subjective evaluation more reliably identified the limb with an osteochondral fragment 15 post induction. Authors' address: Orthopaedic Research Center, Colorado State University, Fort Collins, CO 80523; e-mail: david.frisbie@colostate.edu. *Corresponding author; †Presenting author. © 2014 AAEP.

1. Introduction

The goal of this study was to compare subjective and objective lameness detection methods to identify the presence of mild forelimb lameness using an established model of osteoarthritis.

2. Materials and Methods

A unilateral carpal osteochondral fragment (OCF) was created in 16 horses. Force platforms, inertial-sensors, and subjective evaluation were used to detect forelimb lameness at 4 time points. Agreement was measured for identification of the limb with an OCF using each individual method and for identification of the same limb identified as lame between methods. Pearson correlations were calculated between all output parameters.

3. Results

Fifteen days post OCF agreement was 87% for subjective evaluation, 63% for force platforms, and 50% for inertial-sensors identifying the OCF limb. Average agreement over all time points for identifying the same forelimb as lame was 53% between subjective evaluation and inertial-sensors, 45% between inertial-sensors and force platforms, and 33% between subjective evaluation and force platforms.

4. Discussion

The OCF limb was more reliably identified by subjective evaluation at day 15. It should be noted that the evaluator was aware of study day and design introducing potential bias. The agreement between the subjective evaluation and inertial-sensors may be due to the increased number of strides evaluated by each method as compared to the force platforms.

Acknowledgments

Conflict of Interest

The Authors declare no conflicts of interest.

Research Abstract

NOTES

Clinical Hindlimb Lameness is Frequently Associated With Significant Compensatory Forelimb Lameness

Sylvia Maliye, BSc, BVM&S*; and John M. Marshall, BVMS, PhD, DACVS, DECVS

Compensatory ipsilateral forelimb lameness commonly occurs with hindlimb lameness and must be ruled out as a cause of forelimb lameness before blocking. Observing the effect of hindlimb blocking on forelimb movement can assist in determining the response. Authors' address: Weipers Centre Equine Hospital, University of Glasgow, Bearsden Road, Glasgow G61 1QH, U.K. e-mail: sylvia.maliye@hotmail.com. *Corresponding and presenting author. © 2014 AAEP.

1. Introduction

Compensatory forelimb lameness associated with hindlimb lameness has not been fully characterized in a significant number of clinical cases. Therefore, we aimed to describe and quantify the compensatory effects of naturally occurring hindlimb lameness.

2. Materials and Methods

Data from lameness investigations using an inertial sensor-based system with positive response to hindlimb diagnostic anesthesia were reviewed. Horses were grouped into (1) clinical hindlimb lameness only (HL, $n = 16$, 60%), (2) HL and ipsilateral forelimb lameness (IFL, $n = 9$, 33%), or (3) HL and contralateral forelimb lameness (CFL, $n = 2$, 7%). The change in measures of head (HDMax, HDMin, HMA) and pelvic (PDMax, PDMin, PDA) movement following diagnostic anesthesia was determined. The data were analyzed to determine the effect of abolishing hindlimb lameness using a paired t-test or signed rank test as appropriate. Statistical significance was set at $P < 0.05$.

3. Results

In the HL and IFL groups, the abolition of HL significantly decreased ipsilateral forelimb (−38%, −41%) and increased contralateral forelimb movement asymmetry (+36%, +131%). A significant decrease in the difference in minimum head height (HDMin) occurred in both groups (−69%, −71%); a significant decrease in maximum head height (HDMax) occurred in the IFL group only (−69%).

4. Discussion

We identified a common and significant effect of hindlimb lameness on forelimb and head movement in horses with and without clinical forelimb lameness.

Acknowledgments

Conflict of Interest

The Authors declare no conflicts of interest.

Research Abstract

NOTES

Review of Mistakes That Can Be Made When Interpreting the Results of Diagnostic Analgesia During a Lameness Examination

Jim Schumacher, DVM, MS, DACVS; John Schumacher, DVM, MS*; Michael Schramme, DrMedVet, CertEO, PhD, DECVS, DACVS†; and William Moyer, DVM, DACVSMR

Results of diagnostic analgesia can be misinterpreted during lameness examinations. Many diagnostic tests have been known to produce both false positive and false negative results. Authors' addresses: Department of Large Animal Clinical Sciences, College of Veterinary Medicine, University of Tennessee, Knoxville, TN 37996 (Jim Schumacher); Department of Clinical Sciences, College of Veterinary Medicine, Auburn University, AL 36849 (John Schumacher); Equine Clinic, National Veterinary School of Lyon, Marcy L'Etoile, France (Schramme); and Department of Clinical Sciences, College of Veterinary Medicine, Texas A&M University, College Station, TX 77845 (Moyer); email: schumjo@auburn.edu. *Corresponding author; †Presenting author. © 2014 AAEP.

1. Introduction

Many diagnostic tests used during the clinical examination of a lame horse are known to produce both false positive and false negative results (e.g., application of the hoof tester, the distal interphalangeal extension test, flexion tests, and pressure applied to the proximal aspect of the suspensory ligament). Diagnostic intrasynovial and regional analgesia is, therefore, an important component of lameness examination if the site of pain causing lameness remains uncertain after the horse undergoes a thorough clinical examination. The results of diagnostic analgesia are usually subjectively evaluated, sometimes with difficulty, and errors in the subjective evaluation lead to errors in diagnosis and, therefore, in prognosis and treatment. To accurately evaluate the results of diagnostic analgesia, the clinician should have a thorough knowledge of the nuances of interpretation. Although interpreting the results of diagnostic analgesia is usually straightforward, the clinician should be aware of the many ways in which diagnostic analgesia can be misinterpreted.

The results of diagnostic analgesia can be misinterpreted if:

- The horse is inconsistently or insufficiently lame.
- Lameness improves or resolves as a result of exercise (i.e., warms out of lameness) rather than from diagnostic analgesia.
- The incorrect limb was chosen for evaluation.
- The clinician is biased by the expected or desired result of an analgesic technique.

NOTES

- The horse's gait has been altered by the use of sedatives.
- Testing of regional desensitization after diagnostic analgesia is inaccurate.
- Administration of anesthetic solution is inaccurate.
- Local anesthetic solution has migrated proximally following perineural injection.
- Anesthetic solution leaks or diffuses from a synovial structure to desensitize an adjacent nerve.
- Time of gait assessment after an analgesic technique is inappropriate.
- The clinician does not understand what structures are desensitized by the diagnostic block.
- Disease of the subchondral bone contributes to joint pain.
- The horse has aberrant nerves.
- Long-term pain causes the horse to develop an abnormal protective gait that is unchanged by diagnostic analgesia.
- An abnormal gait is caused by mechanical restrictions rather than pain.
- Lameness is caused by severe pain that cannot be significantly ameliorated by diagnostic analgesic techniques.

2. Effect of Subtle or Inconsistent Lameness on Interpretation

For subjective evaluation of a horse's gait, the horse should be consistently and sufficiently visibly lame before diagnostic analgesia is performed, so that any improvement in gait can be detected and attributed to the diagnostic analgesic injection. The clinician should be aware that lameness of some horses improves or resolves during exercise and, so, for these horses, a false positive response to diagnostic analgesia can occur if the horse has not been sufficiently exercised to attain a consistent state of lameness.

Often, the lameness may only be improved, rather than completely abolished, after administering diagnostic analgesia. Many clinicians consider a positive response to regional analgesia to be 70% or more improvement in gait[1] and a positive response to intrasynovial analgesia to be 50% or more (authors' opinion). If the lameness is subtle, a 50% or even a 70% improvement in gait may be difficult to appreciate. Keegan et al.[2] demonstrated, in a study comparing objective and subjective evaluations of mildly lame horses, that agreement among experienced equine clinicians on subjectively determined lameness scores is poor.

Methods by which a subtle lameness can be made more apparent include exercising the horse before the examination (e.g., by riding or lunging), changing the ground underfoot (e.g., from soft to hard ground or vice versa) and evaluating the horse's gait while the horse trots in a circle. Pain in the digit can usually be exacerbated by exercising the horse on a hard surface and pain more proximal in the limb can sometimes be exacerbated by exercising the horse on a soft surface.[3,4] In some cases, a lameness is subtle because the horse is bilaterally lame. When a nerve or joint block causes a temporary resolution of pain in one limb, lameness in the other limb may become obvious. When lameness is too subtle for consistent subjective evaluation, thereby prohibiting the use of diagnostic analgesia, the use of an automated lameness detection device, such as one that uses body-mounted, wireless, inertial sensors, may still enable measurement of mild but consistent gait asymmetry to allow the clinician to proceed with the use of diagnostic analgesia.[5–7]

3. Evaluating the Incorrect Limb

The results of diagnostic analgesia can be misinterpreted if the incorrect limb is chosen for evaluation. Lameness of a single limb can cause alterations in gait symmetry that make the horse appear lame on other limbs, causing the wrong limb to be chosen for evaluation.[8] Uneven loading in a fore limb can cause compensatory uneven loading in the hind limbs and vice versa. Keegan cited the "law of sides" to aid in deciding which limb is the truly lame limb.[8] According to this "law of sides," when a horse appears to be lame in the ipsilateral fore and hind limbs, the site of pain causing lameness is likely to be located in the hind limb. The ipsilateral fore limb appears lame because of a compensatory change in loading in the fore limbs. When a horse appears to be lame in a fore limb and the hind limb of the contralateral side, the site of pain causing lameness is most often in the fore limb. The hind limb appears lame because of a compensatory change in loading or push-off in the hind limbs. The second part of the "law of sides" is less frequently applicable because *only marked fore limb lameness* tends to cause a visibly noticeable compensatory decrease in impact loading in the ipsilateral hind limb and a compensatory increase in push-off in the contralateral (i.e., diagonal) hind limb. A mild lameness in a hind limb, however, may cause a change in gait that mimics a substantial lameness of a fore limb, whereas lameness of a fore limb is less likely to cause a visibly noticeable change in gait of a hind limb.[8]

4. Clinician Bias Towards the Expected or Desired Result of an Analgesic Technique

One clinical trial showed that clinicians are biased by knowing that a diagnostic block has been performed on a lame horse.[9] This bias may reveal itself in a positive or a negative way and a clinician may be inclined to see an improvement in a horse's lameness where there is none or fail to see the improvement. The former situation may occur when external circumstances, such as intractable behavior by the horse, time of day, and owner pressure, complicate continuation of the lameness examination with diagnostic analgesic techniques and either situation may arise when a clinician has a strong expectation of the site of pain causing the lameness

based on the findings of the clinical examination. The use of an automated lameness detection device, such as one that uses body-mounted wireless inertial sensors, removes subjective bias from assessment of the horse's gait after diagnostic blocks have been performed.[7]

5. Gait Alteration by Sedation or Tranquilization

Applying a lip twitch or lip chain to the horse usually provides sufficient restraint to allow diagnostic analgesia to be safely administered, but some horses must be sedated or tranquilized to administer diagnostic analgesia, either because the owner resents having a twitch or lip chain applied to his or her horse or because diagnostic analgesia cannot be safely administered even after a twitch has been applied. The degree to which sedation may interfere with assessment of gait may depend on the severity of lameness and the skill of the clinician performing the examination. The effect of sedation or tranquilization on gait should be observed before the effect of diagnostic analgesia on gait is evaluated. A change in gait of a sedated horse can be erroneously attributed to the effects of diagnostic analgesia rather than to the effects of sedation if the gait was not evaluated after the horse was sedated but before diagnostic analgesia was administered.

Xylazine (0.2 mg/kg, IV), detomidine (10 μg/kg, IV), or acepromazine (0.01–0.02 mg/kg) administered in the course of a lameness examination usually does not substantially interfere with assessment of gait[10–13] and lameness may even become more apparent after a horse is tranquilized.[14] A tranquilizer, such as acepromazine, has no analgesic effect, whereas a sedative, such as xylazine or detomidine, provides some analgesia.[15] Although a sedative may provide more restraint for administering diagnostic analgesia than does a tranquilizer, we believe that a tranquilizer is less likely to adversely affect a lameness examination.

If the clinician is concerned that the effects of sedation may confound the lameness evaluation, the horse can be examined after the effects of sedation dissipate, provided that analgesia imparted by the local anesthetic solution persists longer than the effects of the sedative. Some clinicians have advocated using bupivacaine hydrochloride when administering diagnostic analgesia to intractable horses after sedating the horse with xylazine so that regional analgesia remains in effect when sedation has completely dissipated.[16] Bupivacaine, however, appears to be chondrotoxic and its use in diseased joints may be harmful.[17] Waiting until the effects of the sedative dissipate may confuse the interpretation of the results of regional analgesia because the local anesthetic solution may diffuse with time from the site of injection, desensitizing unintended structures.[3,18–22]

After diagnostic analgesia is administered, the effects of an α_2-agonist, such as xylazine or detomidine, can be diminished by administering an adrenergic blocking agent such as yohimbine or tolazoline. Yohimbine, however, is not approved for use in the horse and administration of either drug to reverse the sedative effects of an α_2 agonist has been associated with adverse reactions in horses, including death.[23]

6. Inaccurate Testing of Regional Desensitization After Diagnostic Analgesia

The efficacy of a regional nerve block can be tested by observing the horse's response to stimulation of skin in the area meant to be desensitized by the block. Testing efficacy by testing for lack of skin sensation is not infallible, however, because a lack of reaction to stimulation verifies only that the skin has been desensitized. When lameness is still present, distinguishing between effective and ineffective desensitization of the section of the limb supplied by the innervation targeted by the block is not always easy.

Pain sensation, skin sensation, and deep sensation are supplied by different afferent fibers, each of which may require a different amount of time to become fully anesthetized by the local anesthetic solution, depending on the degree of myelination of individual fibers.[24,25] A local anesthetic injection can, therefore, remove pain without totally desensitizing the skin. For the same reason, there is frequently a time-lapse between removal of superficial sensation (e.g., skin sensation at the coronary band) and blockade of sensitivity to deep pressure (e.g., sensitivity to application of hoof testers or sensitivity to a flexion test) by the local anesthetic solution.[25] The clinician cannot be certain, therefore, that after administering regional analgesia, deeper structures have been fully desensitized, even though skin sensation has been abolished.

Local anesthetic solution deposited around a nerve diffuses from the periphery of the nerve towards the center of the nerve.[25] Sensation to the most distal portion of the limb is supplied by the core fibers of the main nerve trunk and, therefore, desensitization proceeds from the site of deposition of local anesthetic solution distally.[25] After administering a tibial and peroneal (fibular) nerve block, for example, the time required for analgesia of the distal hock joints should be less than the time required for loss of skin sensation at the bulbs of the heel.

After perineural injection of a local anesthetic solution, sensations disappear in the following order: pain, cold, warmth, light touch, joint proprioception, and deep pressure.[25] Re-examining a horse too soon after a nerve block, before all sensations have been abolished, may lead to erroneous interpretation of results of the nerve block. A clinician may, after accurately administering a nerve block that would effectively eliminate pain causing lameness, re-administer the block without first trotting the horse if skin sensation is still present. By not trotting the horse, the clinician fails to realize that the first administered block was effective in ameliorat-

ing lameness. As a consequence of increased volume of local anesthetic solution and increased time before the horse's gait is evaluated, the response to diagnostic analgesia may no longer be specific.

If skin sensation is no longer present after a diagnostic nerve block (e.g., 5 min after a palmar digital nerve block) and the horse, when trotted, still appears to be lame, the clinician may come to the inaccurate conclusion that the cause of lameness is not located in the area that should have been desensitized by the nerve block because sensitivity to deep pressure in the foot has been not yet been abolished. Consequently, when the horse's lameness is significantly improved by a more proximally administered nerve block in the diagnostic sequence, the clinician may conclude erroneously that the cause of lameness is located between the apparently ineffective block (e.g., a palmar digital nerve block) and the apparently effective block (e.g., an abaxial sesamoid nerve block) rather than in the area that should have been desensitized by the first nerve block (e.g., a palmar digital nerve block) because sensitivity to deep pain in that area has been abolished after a longer period of time.

Judging the effectiveness of a regional nerve block performed on the proximal aspect of the limb [i.e., anesthesia of the median, ulnar, deep peroneal (fibular), or tibial nerve] is often difficult. After a regional nerve block, skin can be tested for loss of sensation at a specific site on the limb for each nerve, but this method of testing may yield erroneous information for several reasons: (1) the horse may be stoic and show little reaction to noxious stimulation of skin, (2) the region of skin desensitized may vary somewhat among horses,[26] and (3) some horses react progressively more violently to the slightest provocation, making a positive reaction to skin testing difficult to interpret. In particular, the efficacy of a tibial nerve block cannot be reliably tested, especially if the distal portion of the limb has been desensitized by a low 4-point or 6-point nerve block; loss of skin sensation after a peroneal nerve block is extremely variable.[26]

A well-behaved stoic horse may not react to stimulation of the skin even though regional analgesia was ineffective. For such a horse, reaction to cutaneous stimulation should be assessed before regional analgesia is performed, or reaction to cutaneous stimulation of the same dermatome on the contralateral limb should be evaluated.

7. Inaccurate Administration of Diagnostic Analgesia

The effectiveness of regional analgesia can be assessed by evaluating the horse's gait. A positive response to regional analgesia indicates that the site of pain was located within the region desensitized by the block. A negative response indicates that the site of pain was not within the region desensitized or that diagnostic analgesia was inaccurately performed; the ability to make this distinction is an important part of the lameness examination.

A perineural injection of local anesthetic solution in the distal portion of the limb aims to result in deposition of injectate within the neurovascular bundle, thereby establishing direct contact between the local anesthetic solution and the nerve. Perineural injection to anesthetize a nerve may result in deposition of the local anesthetic solution superficial to the perineural fascia, thereby preventing the local anesthetic solution from rapidly contacting the nerve. As a consequence, therefore, the time between injection and diffusion of the local anesthetic solution through the perineural fascia to contact and anesthetize the nerve is likely to be prolonged.[18] The time required for diffusion of local anesthetic through the perineural fascia remains unknown.

Directly instilling local anesthetic solution into a joint, bursa, or tendon sheath leaves little or no doubt that intrasynovial structures are desensitized and is an advantage of intrasynovial analgesia over regional analgesia. The clinician cannot always be certain, however, that the local anesthetic solution was instilled within the intended synovial structure. The most accurate technique to assure accurate synoviocentesis is the use of diagnostic imaging. Radiography can be used to ascertain accurate positioning of the needle (e.g., for navicular bursocentesis) or accurate intrasynovial instillation of a mixture of local anesthetic solution and contrast medium (Fig. 1). Ultrasonography can be used to monitor needle placement and instillation of local anesthetic solution into the synovial space in real-time.[27,28]

Successful synoviocentesis can be assumed when synovial fluid is observed exiting the needle, but often synovial fluid cannot be aspirated even though the needle is correctly inserted within a synovial structure. The volume of synovial fluid in some synovial structures, such as the distal joints of the tarsus and the navicular bursa, may be so small that fluid cannot be aspirated. Or the clinician may fail to aspirate fluid if the opening of the needle lies against synovial villi or cartilage.

In some cases, the degree of resistance to pressure on the plunger of the syringe during injection may be the only means of determining the likelihood of having entered a synovial structure. If the needle was correctly placed, resistance to injection is slight, but for some synovial structures, such as the scapulohumeral joint or the synovial compartments of the stifle, lack of resistance to pressure on the plunger of the syringe is not a reliable indication that the joint has been entered.[29] The ability to aspirate a portion of the injectate is a good indication of accurate synoviocentesis for some synovial structures such as the coxofemoral, scapulohumeral, and cubital joints, and the bicipital bursa. When a periarticular injection of local anesthetic solution may affect motor nerves (e.g., a periarticular injection of the cubital, scapulohumeral, or coxofemoral joints), the authors inject isotonic saline solution, before instilling the

Fig. 1. Contrast radiography can also be used to verify accuracy of injection of a synovial structure. One-half mL positive radiocontrast solution was added to 2.5 mL mepivacaine HCL to verify accuracy of injection of the centrodistal joint. Positive radiocontrast solution can be seen in the dorsal pouch of the joint.

local anesthetic, to allow aspiration to verify placement of the needle within the synovial structure, rather than risk peri-articular injection of local anesthetic solution.

Another cause of misinterpretation of the results of regional analgesia due to inaccurate administration is inadvertent instillation of the local anesthetic solution into an adjacent blood vessel or synovial structure. This complication occurs most commonly when performing regional analgesia in the distal portion of the limb. For example, when anesthetizing the palmar nerves at the level of the distal aspect of the second and fourth metacarpal bones, as part of the low 4-point nerve block, the digital flexor tendon sheath is often inadvertently penetrated because, at this site, there is no connective tissue interposed between the neurovascular bundle and the wall of the tendon sheath.[19,30] The metacarpophalangeal joint is also occasionally inadvertently and unknowingly entered when blocking the palmar metacarpal nerves as part of the low 4-point nerve block.[30]

Techniques to anesthetize the proximal aspect of the palmar metacarpal nerves are associated with a risk of inadvertent injection of either the carpometacarpal joint or the carpal sheath,[20,31] potentially leading to a false negative or false positive result. Local anesthetic solution administered subtarsally to anesthetize the plantar metatarsal nerves can, on occasion, be deposited into the tarsometatarsal joint, the tarsal sheath, or a blood or lymphatic vessel to produce a false negative or a false positive result.[32] Besides possibly producing false negative or positive results, inadvertent administration of local anesthetic solution into a synovial structure while performing regional analgesia may result in synovial infection of that structure because skin preparation for perineural injection is generally less thorough than is skin preparation for synoviocentesis. Administering the local anesthetic solution as the needle is withdrawn decreases the likelihood of depositing the entire amount of solution in an unintended structure and results in deposition of the solution in more than one tissue plane, which increases the likelihood of the solution contacting the nerve.[33]

When using the lateral approach to ameliorate pain in the distal interphalangeal joint, the clinician should be aware that the navicular bursa or digital flexor tendon sheath can be entered inadvertently.[34] When inexperienced veterinary students attempted to inject the proximal interphalangeal joint of horses, 64% of injections were inadvertently placed into the digital flexor tendon sheath using the palmaroproximal approach (Fig. 2).[35]

8. Effect of Proximal Migration of Local Anesthetic Solution

When assessing the effects of anesthesia of nerves in the distal portion of the limb, the clinician should be aware that, after injection, anesthetic solution might migrate proximally along the neurovascular bundle to anesthetize more proximal structures, thus confusing the results of the examination.[3,18–20,36,37] For example, anesthetizing palmar digital nerves at the level of the cartilages of the foot may desensitize the pastern and fetlock joints of some horses, at least partially,[38–40] and anesthetizing the palmar digital nerves at the level of the proximal sesamoid bones (i.e., an abaxial sesamoid nerve block) may completely desensitize the fetlock joint.[41–43] Perineural analgesia of the deep branch of the lateral plantar nerve at the subtarsal level[44] may desensitize the distal tarsal joints and other structures of the tarsus.[36,37,45]

To help avoid unintended anesthesia of more proximal nerve branches when anesthetizing nerves in the distal portion of the limb, the needle should be directed distally during insertion. The degree of proximal extension of local anesthetic solution is likely related to the volume of local anesthetic solution administered.[25,30] A minimal amount of local anesthetic solution should be administered to anesthetize a nerve when performing diagnostic analgesia of the distal portion of the limb to minimize the flow of local anesthetic solution proximally along the

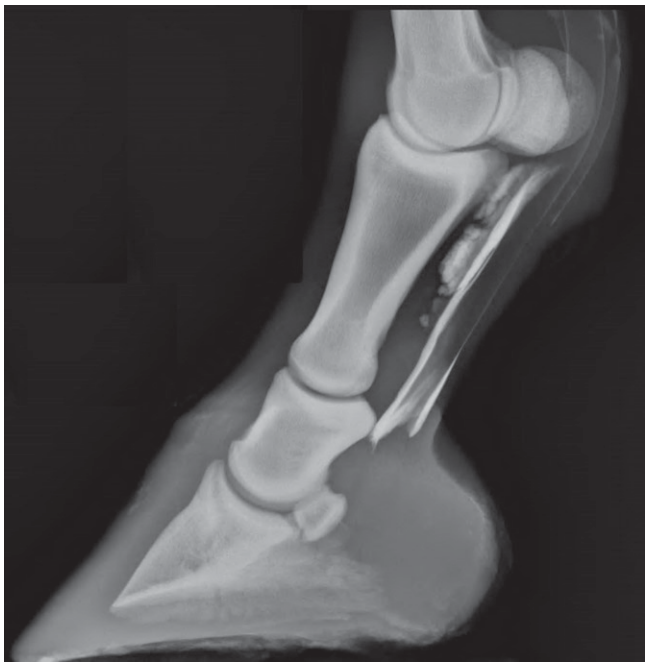

Fig. 2. Positive radiocontrast solution is seen in the digital flexor tendon sheath after an inexperienced clinician performed the palmaroproximal approach for arthrocentesis of the proximal interphalangeal joint as part of a study.[a] In one study, inadvertent injection of the digital flexor tendon sheath occurred in 64% of injections using this approach when attempted by inexperienced veterinary students.[35] If a lesion causing lameness exists within the proximal interphalangeal joint, a negative result would be falsely interpreted. If a lesion causing lameness exists within the digital flexor tendon sheath, a positive result would be falsely interpreted.

neurovascular bundle (Fig. 3). A very small volume of local anesthetic solution can be used when a nerve can be subcutaneously palpated because the solution can be placed more accurately. To the authors' knowledge, there are no studies to suggest the smallest volume of local anesthetic solution necessary to anesthetize a particular nerve. A volume of as little as 1 mL has been recommended for anesthesia of the palmar digital nerve administered at the level of the proximal sesamoid bone (i.e., an abaxial sesamoid nerve block).[1]

The likelihood of desensitizing more proximal structures increases as the time after injection increases. Nagy et al.[18] showed that substantial proximal migration of positive radiocontrast solution occurred within 10 min after perineural injection of the palmar nerves, yet they pointed out that a period of 10 min is often required for desensitization of the foot during clinical examination.

9. Leakage or Diffusion of Local Anesthetic Solution From a Synovial Cavity Can Desensitize Adjacent Nerves Leading to Misinterpretation

The results of intrasynovial analgesia can be misinterpreted if anesthetic solution leaks or diffuses from a synovial structure to desensitize a nerve. The most widely recognized example of this situation is the desensitization of nearly the entire foot after local anesthetic solution is injected into the distal interphalangeal joint.[46] Intrasynovial analgesia of the distal interphalangeal joint desensitizes the toe region of the sole[47] and the podotrochlear apparatus.[48,49] The coronary band may also be desensitized after intrasynovial analgesia of the distal interphalangeal joint. Diffusion of local anesthetic solution from the distal interphalangeal joint to the palmar digital nerves, which lie in close proximity to the palmar pouches of the distal interphalangeal joint, is likely responsible for desensitization of these structures. The palmar digital nerves also lie in close proximity to the navicular bursa and, so, intrasynovial analgesia of the navicular bursa also desensitizes the toe region of the sole.[50]

Another example is the potential desensitization of suspensory branch injuries and proximal lesions

Fig. 3. The volume of local anesthetic solution injected perineurally may affect the extent of the region desensitized. One mL of positive radiocontrast solution was injected perineurally at the base of the medial proximal sesamoid bone and 3 mL was injected over the lateral proximal sesamoid bone. The column of positive radiocontrast solution injected over the lateral palmar nerve extends more proximally than does the column of medium injected over the medial palmar nerve and, if the injectate was local anesthetic solution, more of the lateral aspect than the medial aspect of the metacarpophalangeal joint would be desensitized.

of the distal sesamoidean ligaments by intra-articular analgesia of the metacarpophalangeal or metatarsophalangeal joint.[51] Recent imaging studies using magnetic resonance imaging have identified these ligamentous injuries as the cause of lameness in horses with significant improvement or elimination of lameness after intra-articular analgesia.[51–54]

Leakage of local anesthetic solution at the injection site or its diffusion from the digital flexor tendon sheath may result in desensitization of the skin at the heel region of the foot by anesthetizing the palmar digital nerves of some horses receiving intrasynovial analgesia of the digital flexor tendon sheath and may, perhaps, result in desensitization of the distal portion of the limb.[55] The effect of leakage of local anesthetic solution from the digital flexor tendon sheath on the palmar digital nerves varies, however, according to the site of synoviocentesis. One study found that intrasynovial analgesia of the digital flexor tendon sheath using the palmar axial sesamoidean approach[56] did not ameliorate lameness caused by pain from the sole, navicular bursa, or distal interphalangeal joint, indicating that the palmar digital nerves were not anesthetized by direct analgesia of the digital flexor tendon sheath performed at this site.[57] Nevertheless, to avoid misinterpreting the results, skin sensitivity at the heel bulbs should always be tested after intrasynovial analgesia of the digital flexor tendon sheath to determine if leakage of local anesthetic solution at the injection site has caused inadvertent anesthesia of one or both palmar digital nerves.[55]

Intrasynovial injection of a large volume of anesthetic solution or repeated punctures of a synovial membrane should logically result in leakage of local anesthetic solution.[58] Even when a small volume of local anesthetic solution is injected into the scapulohumeral joint after numerous attempts at centesis, leakage of that solution through the multiple punctures of that joint can temporarily paralyze the surpraspinatus and infraspinatus muscles by anesthetizing the suprascapular nerve or part of the brachial plexus.[58]

When a large volume of local anesthetic solution (i.e., $\geq$10 mL) is injected into the tarsometatarsal joint, the joint capsule may rupture, causing leakage of local anesthetic solution, which may desensitize the insertions of the *tibialis cranialis* and *peroneus tertius* muscles and the tarsal sheath and anesthetize the medial and lateral dorsal metatarsal nerves or plantar metatarsal nerves.[32,59] Presumably, overdistention of other synovial structures with local anesthetic solution could also cause leakage of solution and inadvertent analgesia of peripheral nerves in close vicinity to the site of injection or synovial rupture.

10. Time of Assessment After an Analgesic Technique is Inappropriate

The results of diagnostic analgesia can also be misinterpreted if the horse's gait is assessed before the onset of relief of pain. Relief of pain and resolution of lameness after local anesthetic solution is administered near a nerve in the distal portion of the limb usually occurs within 5 min, but anesthesia of larger nerves in the proximal portion of the limb may take 20 to 40 min.[3,13,25] In a study concerning the extent of proximal migration after perineural injection of positive radiocontrast solution along the palmar nerves at the base of the proximal sesamoid bones, 11% of injections appeared to be outside the neurovascular bundle.[18] The amount of time required for local anesthetic solution to anesthetize a nerve when the anesthetic solution is deposited outside the perineural fascia is not known, but presumably, more time would be required for anesthesia of that nerve.

Some clinicians prefer to confine a horse after a nerve block because they fear that walking the horse may increase diffusion of local anesthetic solution causing desensitization of more proximal structures, thereby complicating interpretation of the nerve block. Walking, however, does not seem to influence the extent of proximal or distal migration of local anesthetic solution after perineural injection.[18]

The results of intrasynovial analgesia can be misinterpreted if the expectation for timing of onset of analgesia is incorrect. Lameness has been observed to resolve within 5 min after injecting mepivacaine into a painful intercarpal joint[60] and, therefore, administering local anesthetic solution into a synovial structure of the distal portion of the limb also probably results in synovial analgesia within 5 min. Onset of analgesia is delayed when local anesthetic solution is administered into a synovial structure larger than those of the distal portion of the limb. For instance, analgesia of the coxofemoral joint may not occur for 30 min after local anesthetic solution is administered into this joint (authors' observation).

11. The Clinician Does not Understand What Structures are Desensitized by the Diagnostic Block

The misinterpretation of regional analgesia can arise if clinicians do not realize that a diagnostic block may desensitize more than the target region or that the diagnostic block may not completely desensitize the target region. For example, the results of a low palmar nerve block, as part of a low 4-point nerve block, can be misinterpreted if one palmar nerve is anesthetized proximal to the *ramus communicans* and the other is anesthetized distal to the *ramus communicans*, because sensory fibers travel in both directions in the *ramus communicans* to connect the medial and lateral palmar nerves.[61] When administering a low palmar nerve block, both palmar nerves should be anesthetized distal or proximal to the *ramus communicans* to avoid leaving nondesensitized sensory nerve fibers traveling through this neural connection. Alternatively, local anesthetic solution could also be deposited adjacent to the *ramus communicans* when anesthetizing the palmar nerves. Because the *ramus communi-*

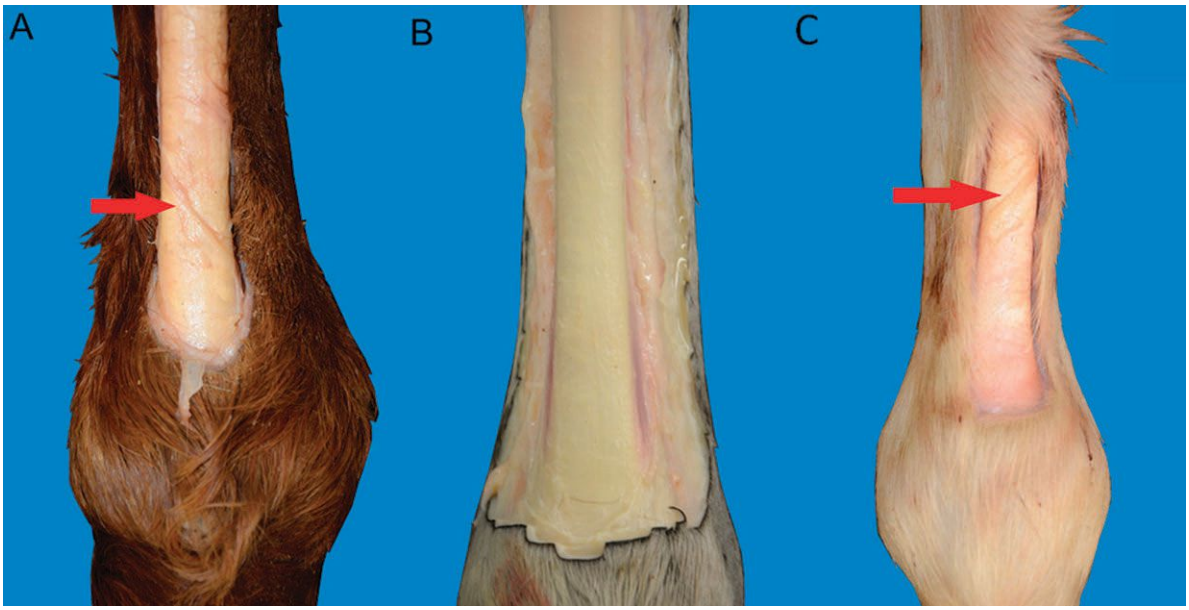

Fig. 4. The *ramus communicans* on the hind limb is often rudimentary (A) or missing (B) and, if present, its position on the hind limb is variable (compare A to C). Because the *ramus communicans* is difficult to palpate on the hind limb, knowing if the plantar nerves are anesthetized proximal or distal to this potential communication is difficult. The degree of communication between the medial and lateral plantar nerves via the *ramus communicans,* to the authors' knowledge, is not known.

cans of the hind limb is difficult to palpate (and does not exist for many horses), ascertaining if the plantar nerves are anesthetized proximal or distal to this communication may not be possible (Fig. 4; authors' observation).

An advantage of intrasynovial analgesia over regional analgesia is that intrasynovial analgesia can be administered at any level on the limb, if pain in a particular synovial structure is suspected by clinical findings, because intrasynovial analgesia does not preclude the subsequent use of regional analgesia. There are several situations, however, where a positive response to intrasynovial analgesia does not exclusively verify intrasynovial pain as the cause of lameness. For instance, analgesia of the distal interphalangeal joint anesthetizes the palmar digital nerves as they course close to the joint capsule and, therefore, a positive response to analgesia of the distal interphalangeal joint could implicate any structure within the foot as the site of pain causing lameness.[46] In some cases, local anesthetic solution injected into the tarsometatarsal joint results in anesthesia of the plantar metatarsal nerves, which lie in close proximity to plantar outpouchings of this joint (Fig. 5A).[32] Resolution of lameness after a tarsometatarsal joint block may, therefore, be erroneously interpreted as resolving pain within that joint, even though pain from the proximal portion of the suspensory ligament is the cause of lameness, because the plantar metatarsal nerves innervate the proximal portion of the suspensory ligament.

Similarly, the palmar outpouchings of the carpometacarpal joint extend into the fibers of the proximal portion of the suspensory ligament (Fig. 5B) and, therefore, local anesthetic solution injected into the intercarpal joint and entering the carpometacarpal joint may desensitize the origin of the suspensory ligament.[31,62] The proximal portion of the palmar metacarpal nerves is located in close proximity to the palmar outpouchings of the carpometacarpal joint capsule and diffusion of local anesthetic

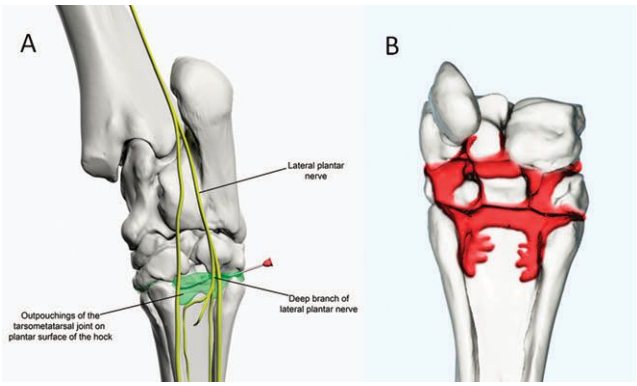

Fig. 5. A, In some cases, local anesthetic solution injected into the tarsometatarsal joint results in perineural anesthesia of the plantar metatarsal nerves, which are in close proximity to the plantar outpouchings of this joint. Resolution of lameness after a tarsometatarsal joint block may, therefore, be erroneously interpreted as resolving pain within that joint even though pain from the proximal portion of the suspensory ligament is the cause of lameness. B, Outpouchings of the carpometacarpal joint may extend into the fibers of the proximal portion of the suspensory ligament and, therefore, local anesthetic solution injected into the intercarpal joint enters the carpometacarpal joint desensitising the origin of the suspensory ligament.

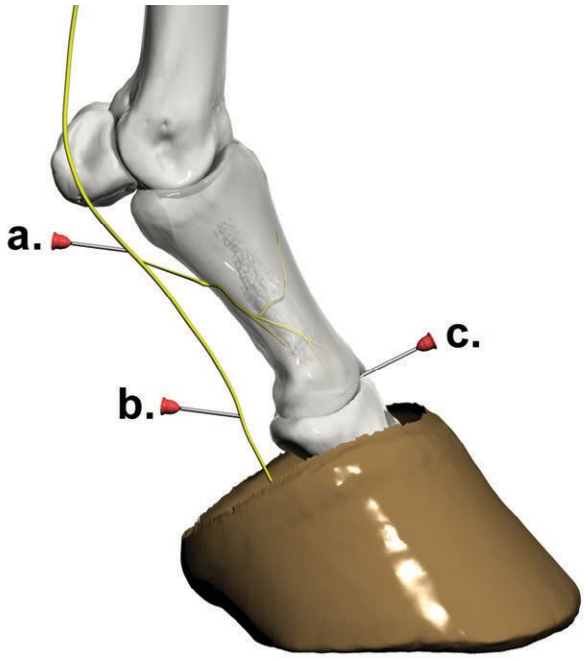

Fig. 6. A, When pain originates in the subchondral bone, local anesthesia of the nerve distal to the level at which the nerve enters the nutrient foramen to supply the subchondral bone (B) does not attenuate lameness, while local anesthesia of the nerve proximal to the nutrient foramen (A) does. C, Direct analgesia of the joint may not substantially attenuate lameness.

solution to these nerves from the carpometacarpal joint may have the same effect in desensitizing the origin of the suspensory ligament.[63]

Because analgesia of the intercarpal joint or the tarsometatarsal joint may desensitize the proximal portion of the suspensory ligament and improve lameness caused by pain in the proximal aspect of the suspensory ligament and because subcarpal or subtarsal nerve blocks can, on occasion, improve lameness caused by disease of the distal carpal or distal tarsal joints, performing "cross blocking" to determine more accurately which structure is painful may be prudent. For example, if lameness is ameliorated with a subtarsal nerve block, analgesia of the tarsometatarsal joint could be performed after the effects of the subtarsal block have dissipated. If both analgesic techniques ameliorate lameness, the block with the highest percentage of improvement most likely identifies the most important site of pain.[51]

12. Disease of Subchondral Bone Contributes to Joint Pain

A negative response to intra-articular analgesia does not always exclude joint pain as a cause of lameness. Intrasynovial analgesia may not resolve lameness if disease of the subchondral bone contributes to joint pain because the subchondral bone is innervated by branches of nerves that enter the bone through its nutrient foramen (Fig. 6).[64,65] Subchondral bone pain of the metatarsophalangeal or metacarpophalangeal joint is a common cause of lameness in young racehorses that is commonly inadequately ameliorated by intra-articular analgesia of these joints.[66] Intra-articular analgesia of the antebrachiocarpal joint of horses lame because of a chip fracture of the distal portion of the antebracheum may fail to abolish lameness when the cartilage remains intact, preventing local anesthetic solution from penetrating subchondral bone.[67]

13. Aberrant Innervation

Aberrant distribution of the appendicular nervous system is claimed to be uncommon[25] but Kainer[68] described four variations in innervation of the digit

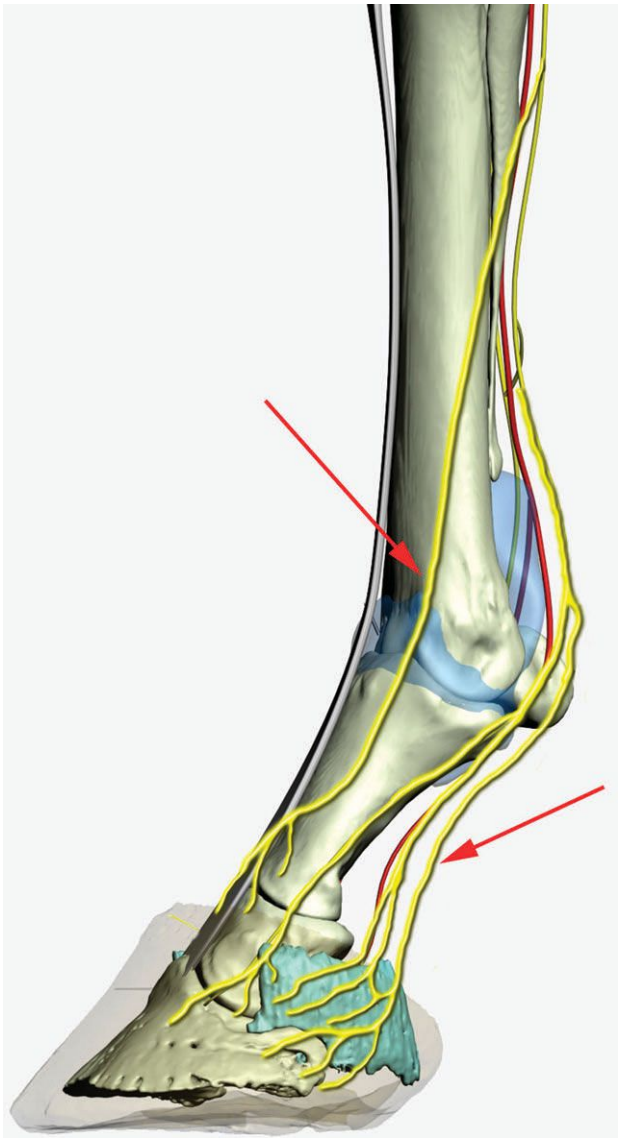

Fig. 7. Topography of branches of the principal nerves of the foot varies considerably.[69] Common variant branches of major nerves (arrows) as cited by Kainer[68] are shown in this illustration.

of the equine forelimb (Fig. 7). He found that 30% of horses possess an intermediate branch originating from the dorsal digital nerve, that 30 to 50% have a continuation of the medial palmar metacarpal nerve to the coronary corium and, that occasionally, an extra palmar branch of the medial palmar nerve, originating in the middle third of the metacarpal region, courses distally into the digital cushion. He also found that, occasionally, an extra branch from the lateral palmar nerve, originating in the proximal metacarpal region, turns dorsally and extends to the coronary band. In a study of the nerves of the equine digit, Sack[69] concluded that the topography of the branches of the principal nerves of the foot varies considerably. Aberrant nerve branches may be responsible for ineffective desensitization of the foot after a palmar digital nerve block, leading to misinterpretation of the block, especially when the skin at the coronary band is no longer sensitive. When aberrant nerve routes are suspected, ring blocks may be a useful adjunct to perineural analgesia.[25]

14. Development of a Protective Gait

We believe that chronic pain causing lameness (months to years) may cause the horse to acquire a protective stance and/or gait as an accommodation to lessen the pain creating the lameness. Some horses, therefore, may still retain an abnormal gait even after diagnostic analgesia has completely relieved the pain causing the lameness. This abnormal gait may persist for a long time after the horse has been adequately treated for the condition causing lameness.

15. Mechanical Lameness

When an abnormal gait is caused by restrictive tissue rather than by pain, diagnostic analgesia does not identify the site of the lesion. Examples of mechanical lameness include fibrotic myopathy usually involving adhesions between the semitendinosus, semimembranosus, or biceps femoris muscles, but other muscles as well.[70] Momentary upward fixation of the patella and fibrosis of various ligaments and tendons may also cause abnormal gait unassociated with pain.

16. Evaluating Horses With Severe Limb Pain

In the experience of the authors, extremely painful disease of the foot (e.g., fracture, laminitis, or sepsis) often cannot be substantially ameliorated by regional analgesic techniques, especially when they are performed close to the lesion causing pain. Analgesic techniques performed more proximally on the limb seem to ameliorate severe foot pain better than palmar/plantar digital or abaxial sesamoid nerve blocks. The site of pain causing lameness might be misconstrued if the influence of severe pain on the results of diagnostic analgesia are not considered (authors' experience).

Acknowledgments

Conflict of Interest

The Authors declare no conflicts of interest.

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[a]Schumacher, unpublished data, 2012.

Long-Term Outcome of Standing Medial Patellar Ligament Splitting to Manage Horses Exhibiting Delayed Patellar Release: Sixty-Four Horses

Sarah J. James, DVM, DABVP*; Timothy G. Eastman, DVM, MPVM, DACVS; and Justin D. McCormick, MS, DVM

Standing medial patellar ligament splitting is a successful, long-term, surgical option for treatment of delayed patellar release. Authors' addresses: Steinbeck Country Equine Clinic, 15881 Toro Hills Avenue, Salinas, CA 93908 (James, Eastman); and College of Veterinary Medicine, Department of Large Animal Medicine and Surgery, Texas A&M University, College Station, TX 77843 (McCormick); e-mail: sjames@steinbeckequine.com. *Corresponding and presenting author. © 2014 AAEP.

1. Introduction

This study describes a standing technique for medial patellar ligament splitting and reports long-term (average 4.5 years) efficacy in horses exhibiting delayed patellar release.

2. Materials and Methods

The medical records of 64 horses that had a standing medial patellar ligament splitting surgery performed to treat delayed patellar release were retrospectively analyzed. Horses were sedated in standing stocks. A number 15 scalpel blade was used to percutaneously split the medial patellar ligament from just proximal to its insertion on the tibial tuberosity to its attachment on the parapatellar fibrocartilage, with the goal of inducing a localized desmitis and subsequent thickening of the ligament. Aftercare consisted of oral antibiotics, 14 days stall rest with hand walking, light exercise for 14 days, and full work at 4 weeks. Follow-up information was obtained through telephone calls to owners and/or clinical evaluation by a veterinarian.

3. Results

Eighty-nine percent of horses benefitted from the procedure, with complete resolution in 58% of horses and improvement in 31% of horses. Seventy-three percent of horses were able to perform at the desired level of performance following the procedure. Sixty-three percent of horses showed signs of improvement or resolution within 30 to 60 days. Two horses had complications following the procedure: one had an incisional infection and one had a medial patellar ligament rupture.

4. Discussion

This study shows that standing medial patellar ligament splitting is a successful long-term sur-

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Research Abstract

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gical option for treatment of delayed patellar release. The procedure has few complications and allows a rapid return to desired performance.

Acknowledgments

Conflict of Interest

The Authors declare no conflicts of interest.

How to Perform Centesis of the Bicipital Bursa Using a Trans-Tendinous Approach

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1. Introduction

Lameness caused by disease associated with the bicipital bursa in horses is uncommon,[1,2] but centesis of this synovial structure is often performed when attempts at localizing pain to other structures in the limb using regional and intra-articular analgesia have failed to ameliorate lameness or when clinical signs of bicipital bursitis are obvious. Signs of bicipital bursitis include signs of pain when the affected limb is extended, reluctance to advance the limb, and signs of pain when pressure is applied to the tendon of the *biceps brachii* muscle.[1,3,4]

Centesis of the bicipital bursa can be performed by inserting a spinal needle near the deltoid tuberosity and advancing the needle proximomedially toward the bursa.[2,5–8] For this technique, a 9 cm spinal needle is usually used. The bursa can also be accessed by inserting a 3.8 cm hypodermic needle medial to, or slightly distal to, the palpable edge of the cranial prominence of the greater tubercle of the humerus, in a plane parallel to the bearing surface at about a 45° angle to the longitudinal axis of the horse and advancing the needle until it strikes the cartilage of the intertubercular groove.[6,9–11] The intent of these methods is to access the bursa while avoiding placing the needle through the tendon of the *biceps brachii* muscle. In a previous study,[11] we found that clinicians who had no previous experience performing centesis of the bicipital bursa are unlikely to be successful in centesis of the bursa using either approach.

Because other synovial structures are accessed by advancing a needle through a tendon or ligament without apparent deleterious effects to those structures, we reasoned that penetration of the tendon of the *biceps brachii* muscle approximately 2 cm medial to the cranial prominence of the lateral tuberosity of the humerus would provide easy, consistently accurate access to the bicipital bursa.

2. Materials and Methods

To attempt centesis of the bicipital bursa using a trans-tendinous approach on an unsedated horse, three operators are likely necessary: one to restrain the horse, another to elevate the limb, and one to perform the centesis. The horse is restrained with sedation or by applying a lip twitch. Desensitizing skin at the site of injection is optional. The limb is pulled forward to extend the scapulohumeral joint

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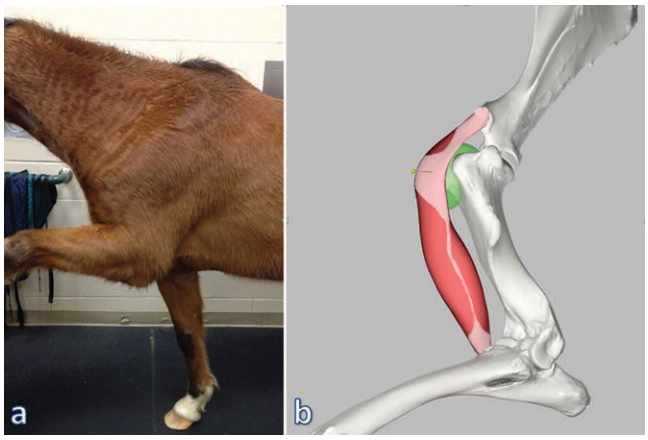

Fig. 1. To perform a trans-tendinous approach to the bicipital bursa, the limb is pulled forward to extend the scapulohumeral joint and flex the cubital joint. The radius is held horizontal (or slightly lower) to the bearing surface. This maneuver likely lifts the tendon from bone to create a space for the needle tip to enter.

and flex the cubital joint. The radius is held horizontally (or slightly lower) (Fig. 1).

An 18-gauge 9 cm spinal needle is used for centesis of the bicipital bursa. The needle is inserted at a site approximately 2 cm medial to the cranial part of the greater tubercle of the humerus (Fig. 2) until the needle tip strikes cartilage. The tip of the needle enters into the lateral portion of the intertubercular groove if the needle is inserted at an angle approximately parallel to the longitudinal axis of the horse (Fig. 3). Resistance is felt as the needle is advanced through tendon, and then, as the bursa is entered, a sudden lack of resistance can be appreciated. Unless the bursa is inflamed, synovial fluid does not fill the needle hub; synovial fluid, however, can usually be aspirated (Fig. 3C). Solution is easily injected; difficulty injecting solution indicates the needle was inserted inaccurately. The ability to aspirate injected solution is another indication that centesis was accurate. Local anesthetic solution injected inadvertently outside the bursa may temporarily paralyze the extensor muscles of the forearm (Fig. 4).

Accurate injection can be confirmed radiographically if positive iodinated (ionic or nonionic) contrast medium is added to the solution injected, or by using ultrasonographic visualization. Examination of a mediolateral radiographic projection obtained with the horse standing and its limb extended with the radius horizontal to ground provides good visualization of the bursa outlined by contrast medium. Centesis can be considered successful if a cylinder-shaped radiopacity created by the contrast medium is seen cranial to the cranial prominence of the greater tubercle of the humerus and pooled in two sac-like structures several centimeters ventral to the cranial prominence of the greater tubercle (Fig. 5A); the injection is likely outside the bursa if the medium outlines the contour of the cranial border of the tendon (Fig. 5B).

To determine the extent of damage to the tendon caused by a trans-tendinous approach to the bicipital bursa, we inserted an 18-gauge spinal needle through the lateral head of each tendon of the *biceps brachii* muscle of two horses immediately after they were euthanized. The tendons were removed immediately from the cadavers and examined grossly for damage caused by the needle; two of these tendons were also examined histologically.

3. Results

A radiocontrast study that examined the accuracy of a trans-tendinous approach to the bicipital bursa indicated that the approach is highly accurate.[12] In that study, no attempt was made to aspirate synovial fluid. Rather, before radiographic assessment of accuracy of synoviocentesis, accuracy of synoviocentesis was based on appreciation of the texture of the tissues as the needle was advanced, sudden loss of resistance as the needle entered the bursa, and ease of injection. Since that study, we have performed centesis of the bicipital bursa in each shoulder of 12 horses free of evidence of bicipital bursitis. We found, in every instance, that synovial fluid could be aspirated using the trans-tendinous approach.

Minimal damage was found in the tendon of the *biceps brachii* muscles of two horses that underwent a trans-tendinous approach to each bicipital bursa immediately after euthanasia. Evidence of needle-induced damage, other than plaques of hemorrhage on the cranial surface of each tendon, could not be found. Neither the site of needle entry on the cranial surface nor needle exit on the caudal surface could be found during gross examination of three tendons; the probable site at which the needle exited was found on one tendon. A needle tract could not be found in the two tendons examined histologically.

4. Discussion

A similar trans-tendinous approach to the bicipital bursa was suggested by Wyn-Jones[3] who advised

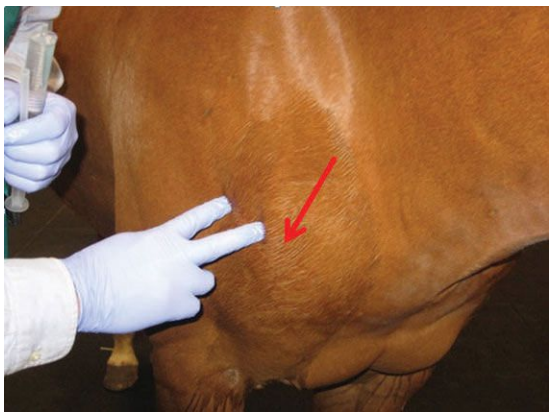

Fig. 2. The needle is inserted at a site approximately 2 cm medial to the cranial part of the greater tubercle of the humerus (arrow).

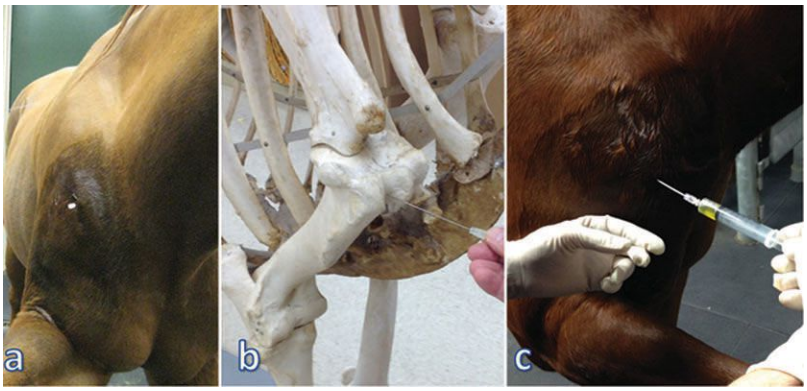

Fig. 3. A, If the needle is inserted at an angle approximately parallel with the longitudinal axis of the horse, B, its tip will enter into the lateral portion of the intertubercular groove. C, Synovial fluid can usually be aspirated to verify accuracy of synoviocentesis.

that the bursa be accessed by inserting a 6.35 to 9 cm (2½ to 3½ in.) needle at the point of the shoulder and directing it to the intertuberal groove. He advised, however, that the shoulder be flexed to tense the bursa to ease penetration. This method may be suitable for aspirating fluid from a fluid-distended bursa, but the authors have found that even with the scapulohumeral joint in a neutral position, injecting fluid into the bursa of horses without disease of the *biceps brachii* tendon or tendon sheath to be difficult.[12] The authors believe that manipulation of the limb to extend the scapulohumeral joint and flex the cuboidal joint lifts the bicipital tendon from the humerus to create a space for the needle to penetrate.

We selected a 9 cm (3½-inch) spinal needle to perform centesis of the bicipital bursa not only for its length but also because, with its short bevel, its orifice is more likely to reside entirely within the synovial space than is a standard hypodermic needle with its longer bevel. Additionally, spinal needles are particularly flexible and more likely to bend than break[6] and, thus, safer to use if there is a possibility the horse may move the limb. Using a flexible needle is especially important when the difference in the range of movement between skin and deeper tissues is large. We have directed 20-gauge spinal needles through the dense tendon of the *biceps brachii* muscle, but needles of this gauge tend to bend as they are advanced. It is the authors' experience that a 3.8 cm (1½-inch) hypodermic needle is too short to penetrate the bicipital tendon where the tendon is the thickest; the authors be-

Fig. 4. Injection of local anesthetic solution outside the bursa may result in temporary paralysis of extensor muscles of the forearm.

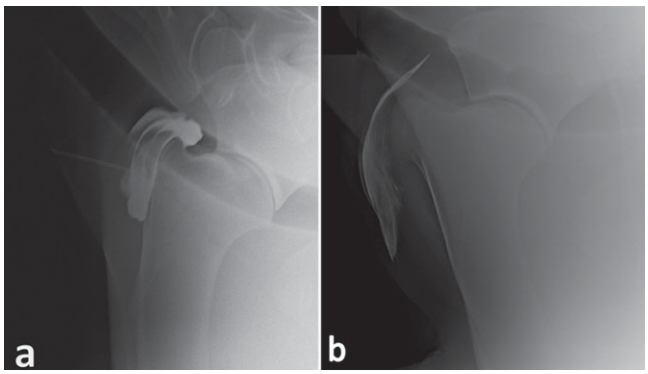

Fig. 5. A, Centesis can be considered successful if a cylinder-shaped image created by the radiopaque contrast medium is seen cranial to the cranial prominence of the greater tubercle of the humerus and radiopaque contrast medium is seen pooled in two sac-like structures several centimeters ventral to the cranial prominence of the greater tubercle (30 mL of radiocontrast medium was injected into this bursa); B, if the medium follows the contour of the tendon on its cranial border, the injection is likely outside the bursa.

lieve, however, the bicipital bursa can sometimes be entered with a needle of this length if the needle is directed through a thin portion of the tendon, toward the periphery of the groove.[12]

Substantial damage to the tendon of the *biceps brachii* muscle seems unlikely, provided that movement of the limb is minimal during centesis. The authors are not aware of reports of damage to a tendon associated with other procedures of synoviocentesis in which the needle is directed through a tendon, such as during centesis of the navicular bursa[13] or during centesis of the digital flexor tendon sheath (palmar/plantar axial sesmoidean approach).[14]

Clinicians should be aware that the bicipital bursae may communicate with the scapulohumeral joint. Direct communication between these two synovial structures has been noted by Sisson,[15] Dyson,[6] and Cole et al.[12] but to the authors' knowledge, the frequency of this communication, direct or indirect, has not been examined.

Acknowledgments

Conflict of Interest

The Authors declare no conflicts of interest.

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Gumshoe Sleuthing in the World of Infectious Disease and Neonatology: Discoveries That Changed Equine and Human Health

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1. Introduction

I wish to thank the AAEP for the honor of delivering the Milne lecture at the 60th annual AAEP convention. The purpose of the Milne program as described by the AAEP is: "The lecture is intended to honor the accomplishments of the individual and bring a meaningful learning experience to the AAEP membership. The lecture should be a perspective on the state-of-the-art in your particular area of expertise."

My career started as a private practitioner in Mendocino County in 1975 where I developed a rural veterinary practice for horses and built the first large animal hospital in the county. I have always identified with the equine practitioner in the field and private practice clinics. I returned to UC Davis in 1983 as an assistant professor. It was at UC Davis that I was given the freedom and opportunity to pursue discovery in the manner that I began in private practice. My investigation of disorders was a bit different, and more like detective work, than traditional epidemiology. Therefore, I am taking this once in a lifetime opportunity of the Milne lecture to share the investigative steps, the leads, the clues, the "thinking out of the box," which led to discoveries in infectious disease and neonatology, which I have been requested to focus on in this presentation. Each section of the science of a topic will be preceded with the "Gumshoe Sleuthing" component for the readers, my fellow equine practitioners, who I hope can use this information for the pleasure of being who we are and for making a difference in the life of a horse or a foal.

"The joy is in creating, not maintaining."
—Vince Lombardi, NFL Hall of Fame Coach

2. Equine Granulocytic Anaplasmosis

(*Anaplasma phagocytophilum*, *Ehrlichia equi*, The California Ehrlichia Agent)

Gumshoe Sleuthing

Gumshoe definition: *Slang* (1) An investigator, especially a detective. To move about stealthily. (2) To use the power of keen observation to obtain leads and develop ideas to solve mysteries. (3) A person whose job is to find information about someone or something.

When I graduated from veterinary school in 1975, there were 6 cases of the disease termed *Ehrlichia equi*, now known as *Anaplasma phagocytophilum* or Equine Granulocytic Anaplasmosis, worldwide.

NOTES

There were no known human cases, and all the diagnosed equine cases were in the sierra foothills of California. The first case seen at UC Davis was diagnosed by Racheal Smith, a hematology technician, who saw the unique and characteristic inclusion bodies in the white blood cells of a horse presented for persistent fever, ataxia, reluctance to move, limb edema, and anorexia. The blood from the second horse, seen a year later, was transfused into a research horse that subsequently came down with a fever within 72 hours. The blood from that horse was frozen and used as a source for an extensive PhD project by David Gribble on the pathogenesis and pathology of the disease in horses. In the fall of 1975, 3 months after my graduation from veterinary school, I examined a 3-year-old horse with a fever, lethargy, ataxia, icterus, and mild petechiation in the parking lot of the small veterinary clinic where I was employed. I performed a complete blood count (CBC) in our laboratory and noted several characteristic inclusion bodies within neutrophils that were identical to the agent Gribble had identified. The horse had many ticks visible throughout the hair coat. The horse was treated with tetracycline; the fever defervesced within 24 hours and with 7 days of treatment the horse recovered. Two weeks later, I saw another case and had the inclusion bodies confirmed at UC Davis by sending the hematology slides to the lab. I traveled to various veterinary clinics in Mendocino and Humboldt County with the slides in my pocket showing other veterinarians, who expressed modest but polite interest. In 1981, thanks to accumulating many other horses diagnosed with *A. phagocytophilum*, I presented 41 cases at the AAEP meeting. I was hired by UC Davis in 1983 as an assistant professor coming directly from private practice to the University. In 1988, I began a research project with Dr. J. Stephen Dumler after the first human cases of granulocytic anaplasmosis were diagnosed by the same method of examining a blood film in a sick person who had tick exposure after a hike. By 1990, hundreds of human cases of what was then called Human Granulocytic Ehrlichia (HGE) were being diagnosed, with a significant number of fatalities from lack of prompt administration of tetracycline. The Center for Disease Control named HGE the second most common tick transmitted disease in the United States.

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Etiology

Anaplasma phagocytophilum (formerly *Ehrlichia equi*) is the etiologic agent of equine granulocytic anaplasmosis (EGA; formerly equine granulocytic ehrlichiosis). *A. phagocytophilum* has recently been classified, based on genetic analysis, in the genus Anaplasma with *Anaplasma marginale*, which causes infectious anemia in cattle by infecting erythrocytes, and *Anaplasma platys*, which causes canine cyclic thrombocytopenia by infecting platelets.[1]

Because 16 S ribosomal ribonucleic acid (rRNA) gene sequences differ only up to 3 bases (99.1% homology) among former *E. equi, Ehrlichia phagocytophila* (cause of tick-borne fever in Europe), and the recently discovered HGE agent, these organisms are now all considered strains of *A. phagocytophilum*. *E. equi, E. phagocytophila*, and the HGE agent are also closely related on the basis of morphology, host cell tropism, and antigen analysis by indirect fluorescent antibody tests.[2] DNA sequences of the 16 S rRNA gene from the peripheral blood of naturally infected horses in Connecticut and California are identical with those of the HGE agent.[3] Moreover, infected human blood from HGE patients injected into horses causes typical EGA, which can be transmitted to other horses. It induces protection in horses to subsequent challenge with *A. phagocytophilum*.[4,5]

Anaplasma species are small (0.2–1.0 μm in diameter) obligate intracellular bacteria with a gram-negative cell wall[6], but lack lipopolysaccharide biosynthetic machinery.[7] The bacteria reside in an early endosome, where they obtain nutrients for binary fission and grow into a cluster called a morula. Genomic studies demonstrated a type IV secretion apparatus, which could facilitate transfer of molecules between the bacterium and the host.[8,9] *A. phagocytophilum* is found within the cytoplasm of infected eukaryotic host cells, primarily neutrophilic and eosinophilic granulocytes. These inclusion bodies consist of one or more coccoid or coccobacillary organisms approximately 0.2 mm in diameter, as well as large granular aggregates called morulae, which are approximately 5 mm in diameter. Organisms are visible under high, dry, or oil-immersion objectives with light microscopy. They stain deep blue to pale bluish-gray with Giemsa or Wright–Leishman stain. Electron microscopy reveals loosely packed, ovoid to round *A. phagocytophilum* organisms in several membrane-lined vacuoles of equine granulocytes. The size of vacuoles ranges from 1.5 to 5 mm in diameter.

Epidemiology

Equine granulocytic anaplasmosis occurs during late fall, winter, and spring. The horse represents an aberrant host, and it seems unlikely that infected horses could serve as effective reservoirs of *A. phagocytophilum* because the presence of the organism in an affected animal is generally limited to the acute phase of the disease. Horses of any age are susceptible, but the clinical manifestations are less severe in horses younger than 4 years old.[10] Horses from endemic areas have a higher seroprevalence of antibody to *A. phagocytophilum* than horses from nonendemic areas, suggesting the occurrence of subclinical infection in some animals.[11] Further-

more, horses introduced into an endemic area are more likely to develop EGA than native horses.

Persistence of *A. phagocytophilum* has not been demonstrated in naturally infected horses. However, infection with *A. phagocytophilum* can persist in experimentally infected horses for at least 129 days, but the continued presence of the organism is not associated with detectable clinical or pathological abnormalities.[12] The disease is not contagious, but infection can be transferred readily to susceptible horses with transfusion of as little as 20 ml of blood from horses with active infection. Most often, one infected horse is observed in a group of horses in the same pasture. The disease, first reported in the late 1960s in the foothills of northern California, has since been reported in horses in Washington, Oregon, New Jersey, New York, Colorado, Illinois, Minnesota, Indiana, Connecticut, Florida, and Wisconsin and outside the United States in Canada, Brazil, and Europe. Recent surveillance studies in the Southeast United States have been conducted to determine *A. phagocytophilum* prevalence in *Ixodes scapularis* ticks at horse-inhabited sites to evaluate the potential risk for equine exposure to *A. phagocytophilum*-infected ticks in these areas. The collective prevalence of *A. phagocytophilum* in *I. scapularis* ticks was 20%.[13]

In recent years, EGA has been experimentally transmitted by the western blacklegged tick (*Ixodes pacificus*)[14] and the deer tick (*Ixodes scapularis*).[15] Furthermore, an epidemiologic study in California showed that the spatial and temporal pattern of EGA cases closely paralleled the well-characterized life history and distribution of *I. pacificus* but not other ticks typically associated with horses.[16] In the eastern and midwestern United States, *I. scapularis* is the vector of granulocytic anaplasmosis, and small rodents such as white-footed mice, chipmunks, and voles, as well as white-tailed deer are potentially important reservoirs.[17] In California, white-footed mice, dusky-footed wood rats, cervids, lizards, and birds have been proposed as reservoirs.[18,19] In Europe, where granulocytic anaplasmosis is transmitted by the sheep tick (*Ixodes ricinus*), the reported reservoir hosts are wild rodents, deer, and sheep.[20]

Pathogenesis

The pathogenesis of EGA is poorly understood. Clearly, after entering the dermis by tick bite inoculation and spread, presumably through lymphatics or blood, ehrlichiae invade target cells of the hematopoietic and lymphoreticular systems. Ehrlichiae replicate within vacuoles of professional phagocytes. Whether or how these granulocytic ehrlichiae directly injure cells is not known, despite clear evidence of cytolytic activity in vitro.[21] Granulocytic ehrlichiae are suspected of initiating a cascade of localized pathologic inflammatory events after invading organs such as spleen, liver, and lungs. Subsequent tissue injury is thought to be mediated locally by accumulating inflammatory cells and systematically by induction of proinflammatory responses.[22] The mechanism by which sufficient cells are removed to cause pancytopenia is unknown. However, peripheral sequestration, consumption, and destruction of normal blood elements are thought to be the major mechanisms for ehrlichia-induced pancytopenia. This is supported by the presence of normal cellularity or diffuse hyperplasia of bone marrow, hemophagocytosis in spleen and lymph nodes, and the presence of infected granulocytes in spleen and lung.[22]

Granulocytic anaplasmosis caused by *A. phagocytophilum* is a disease that triggers dysfunction or suppression of host defenses. It is well established that horses infected with *A. phagocytophilum* are predisposed, as are humans and sheep, to develop opportunistic infections and secondary infections with bacteria, fungi, and viruses.[23] These animals develop defects in both humoral and T-cell-mediated immunity and abnormalities in normal neutrophil phagocytic and migratory functions.[24]

Immunologic studies of *A. phagocytophilum* indicate both a cell-mediated and a humoral immune response to clinical infection. Horses that recover from experimental infections develop these responses by 21 days after infection.[25] In naturally infected horses, antibody titers peak 19 to 81 days after the onset of clinical signs. Immunity persists for at least 2 years and does not appear to be related to latent infection or carrier status.[26,27]

Clinical Signs

The incubation period after experimental exposure of horses to infected ticks is 8 to 12 days and 3 to 10 days after needle inoculation of infectious blood. The incubation period for natural infection is believed to be less than 14 days. This estimate is based on the time of onset of clinical signs in horses that had presumptive exposure to ticks while on a trail ride before returning to a nonendemic area for EGA.

The severity of clinical signs of EGA varies with the age of the horse and the duration of the illness.[10] This can make clinical recognition of EGA difficult at the first examination. Adult horses over 4 years of age generally develop characteristic progressive signs of fever, depression, partial anorexia, limb edema, petechiation, icterus, ataxia, and reluctance to move. Clinically and experimentally, it appears that horses less than 4 years old tend to develop milder signs, including moderate fever, depression, moderate limb edema, and ataxia. In horses less than 1 year old, clinical signs may be difficult to recognize, with only a fever present. During the first 1 to 2 days of infection, fever is generally high, fluctuating from 39.4 to 41.3°C (102.9 to 106.3°F). Initial clinical signs are reluctance to move, ataxia, depression, icterus, and petechiation of the nasal septum and oral mucosa (Fig. 1).

Fig. 1. Horse infected with *A. phagocytophilum* showing petechiation of the oral mucosal membranes. From: Pusterla N, Madigan JE. Equine granulocytic anaplasmosis. *J Equine Vet Sci* 2013;33:493–496.

Weakness and ataxia can be severe, to the point that horses will sustain fractures after falling. Staggering is often seen, and the tendency to assume a base-wide stance suggests proprioceptive deficits. Partial anorexia develops in most affected horses. Limb edema (Fig. 2) and more severe signs of disease develop by days 3 to 5, with fever and illness lasting 10 to 14 days in untreated horses. Heart rate is often modestly high (50–60 beats/min). Rarely, there is cardiac involvement with development of cardiac arrhythmias. Ventricular tachycardia and premature ventricular contractions have been observed with the usual clinical signs. The clinical course of the disease ranges from 3 to 16 days. The disease is normally self-limiting in untreated horses; fatalities can result from secondary infection and from injury secondary to trauma caused by lack of coordination. Abortion has not been observed in pregnant mares, and laminitis has not been reported as part of the clinical syndrome.

The initial stage of the disease is characterized by the development of a fever and may be mistaken for a viral infection. The differential diagnoses for EGA include purpura hemorrhagica, liver disease, equine infectious anemia, equine viral arteritis, and encephalitis.

Fig. 2. Horse infected with *A. phagocytophilum* showing distal limb edema. From: Pusterla N, Madigan JE. Equine granulocytic anaplasmosis. *J Equine Vet Sci* 2013;33:493–496.

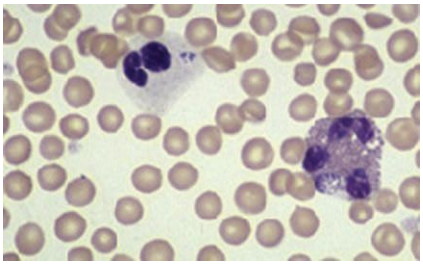

Fig. 3. *A. phagocytophilum* inclusions in neutrophilic and eosinophilic granulocytes of a horse with equine granulocytic ehrlichiosis (buffy coat smear, Giemsa stain, original magnification × 1000). From: Pusterla N, Madigan JE. Equine granulocytic anaplasmosis. *J Equine Vet Sci* 2013;33:493–496.

Laboratory abnormalities in horses affected with EGA may include leukopenia, thrombocytopenia, anemia, icterus, and characteristic inclusion bodies (morulae) in neutrophils and eosinophils. The morulae are pleomorphic, bluish-gray to dark blue in color, and often have a spoke-wheeled appearance.

Diagnosis

Diagnosis is based on awareness of geographic area for infection, typical clinical signs, abnormal laboratory findings, and visualization of characteristic morulae in the cytoplasm of neutrophils and eosinophils in a peripheral blood smear stained with Giemsa or Wright stain (Fig. 3). Because affected horses are leukopenic, a greater percentage of neutrophils can be examined by use of the buffy coat preparation and subsequent staining. The number of cells containing morulae varies from less than 1% of cells initially to between 20% and 50% of neutrophils by days 3 to 5 of infection. However, more than 3 ehrlichia inclusion bodies need to be seen on a blood smear to consider the diagnosis definitive.

Culture is rarely attempted for horses infected with *A. phagocytophilum*. Alternatively, an indirect fluorescent antibody test is available, and paired-titer testing with a significant (fourfold or greater) rise in antibody titer to *A. phagocytophilum* can be performed to confirm recent exposure retrospectively.[11] However, because inclusion bodies are always visible during the mid-stage of the febrile period, antibody testing is not usually required to make a definitive diagnosis.

Recently, several polymerase chain reaction (PCR) assays have been developed for members of the *A. phagocytophilum* genogroup and are considered highly sensitive and specific.[28–30] PCR analysis is useful for the diagnosis of EGA, particularly during early and late stages, when the number of organisms may be too small for diagnosis by microscopy.

Pathology

The characteristic gross lesions observed in experimentally infected horses are hemorrhages, usually petechiae and ecchymoses, and edema. Edema is observed in the legs, ventral abdominal wall, and prepuce. Hemorrhages are most common in the subcutaneous tissues, fascia, and epimysium of the distal limbs. Histologically, there is inflammation of the small arteries and veins, primarily those in the subcutis, fascia, and nerves of the legs, as well as in the ovaries, testes, and pampiniform plexus.[23] Vascular lesions may be proliferative and necrotizing, with swelling of the endothelial and smooth muscle cells, cellular thromboses, and perivascular accumulations, primarily of monocytes and lymphocytes but also, to a lesser extent, neutrophils and eosinophils. Mild inflammatory vascular or interstitial lesions have also been reported in the kidneys, heart, brain, and lungs of animal necropsies during the course of the disease.[22] The ventricular tachycardia and premature ventricular contractions occasionally observed in affected horses are thought to be associated with myocardial vasculitis. Furthermore, horses with a pre-existing chronic bacterial infection may develop an exacerbation of the primary lesion (bronchopneumonia, arthritis, pericarditis, lymphadenitis, cellulitis).[23]

Therapy and Prevention

The intravenous administration of oxytetracycline at 7 mg/kg, once daily for 5 to 7 days, has been an effective treatment for EGA.[10] Alternatively, doxycycline at 10 mg/kg orally, twice daily, can be given for 7 to 10 days following two initial intravenous oxytetracycline treatments. Prompt improvement in clinical appearance and appetite and decrease in fever are noticed within 12 hours of appropriate treatment. Indeed, a failure of defervescence within 24 hours would strongly indicate another cause of illness. On rare occasions, horses treated for less than 7 days relapse within the following 30 days. When untreated, the disease can be self-limiting in 2 to 3 weeks if no concurrent infection is present, but weight loss, edema, and ataxia are of increased severity and duration. In treated horses, ataxia will persist for 2 to 3 days, and limb edema may persist for several days. Inclusion bodies generally are difficult to find after the first day of treatment and are no longer present within 48 to 72 hours. Supportive measures are recommended in severe cases, including fluid and electrolyte therapy, supportive limb wraps, and stall confinement of severely ataxic horses to prevent secondary injury. The prognosis for EGA is considered excellent in uncomplicated cases, in sharp contrast to some of the differential diagnoses.

At present, no vaccine is available against EGA, and prevention is limited to the practice of tick control measures such as the use of permethrin repellent products.[31]

Public Health Aspects

Human granulocytic anaplasmosis (HGA) was first identified in 1990 in a Wisconsin patient who died with a severe febrile illness 2 weeks after a tick bite.[32] HGA is now increasingly recognized as an important and frequent cause of fever after tick bite in the Upper Midwest, New England, parts of the mid-Atlantic states, northern California, and many parts of Europe, all areas where *Ixodes* ticks bite humans.[6,33,34] This tick-borne infection has great capacity to infect and cause disease in humans while maintaining a persistent subclinical state in animal reservoirs.[2] The major mammalian reservoir for *A. phagocytophilum* in the eastern United States is the white-footed mouse, *Peromyscus leucopus*, although other small mammals and white-tailed deer (*Odocoileus virginianus*) can also be infected. White-footed mice have transient (1–4 weeks) bacteremia; deer are persistently and subclinically infected. Human infection occurs when humans impinge on tick-small mammal habitats.[6,33–35]

The most frequent manifestations are malaise (94%), fever (92%), myalgia (77%), and headache (75%); less frequently, patients have arthralgia or involvement of the gastrointestinal tract (nausea, vomiting, diarrhea), respiratory tract (cough, pulmonary infiltrates, acute respiratory distress syndrome), liver, or central nervous system.[6,33–35] A rash is observed in 6% of patients, although no specific rash has been associated with HGA. Frequent laboratory abnormalities identified in up to 329 patients include thrombocytopenia (71%), leukopenia (49%), anemia (37%), and elevated hepatic transaminase levels (71%).

Recent seroepidemiologic data suggest that many infections go unrecognized, and in endemic areas as many as 15% to 36% of the population has been infected.[36,37] Discrepancy between the seroprevalence and symptomatic rate may result from underdiagnosis of infection, asymptomatic serologic reactions, or even infections that produce cross-reactive serologic responses.[2] Symptomatic infection can occur often in tick-endemic regions and varies in severity from mild, self-limited fever to death. Severity sufficient for hospitalization is observed in half of symptomatic patients and is associated with older age, higher neutrophil counts, lower lymphocyte counts, anemia, the presence of morulae in leukocytes, or underlying immune suppression.[33] Approximately 5% to 7% of patients require intensive care, and at least 7 deaths have been identified,[6,22,33,35,38] in which delayed diagnosis and treatment were risk factors. Unlike results of animal observations,[39] no evidence has shown *A. phagocytophilum* persistence in humans.

The discrepancy between bacterial load and histopathologic changes with HGA suggests that disease relates to immune effectors that inadvertently damage tissues.[2] Infection by *A. phagocytophilum* results in significant disruption of normal neutro-

phil function, including endothelial cell adhesion and transmigration, motility, degranulation, respiratory burst, and phagocytosis, and an intact immune system appears important in recovery.[2] Immunological control of *Anaplasma phagocytophilum* is incompletely understood. Despite *A. phagocytophilum*'s mechanisms to subvert neutrophil antimicrobial responses, whether these mechanisms lead to disease is unclear. The inflammatory basis for disease is most likely a result of immunopathologic injury after interferon (IFN)-γ activation of effector cells not adequately tempered by the dampening effects of IL-10.[40,41] A subset of patients that do poorly owing to serious inflammatory injury could occur because of a higher degree of polarization in this IL-10:IFN-γ axis, resulting in a macrophage activation-like syndrome, that if accompanied by defects in cytotoxic effector molecule delivery, whether genetically predisposed or acquired via infection, could lead to the most severe infection complications.[40–42] The ability to affect disease progression by targeting anti-inflammatory treatment during infection in horses could provide additional guidance to help those severely affected.[42,43] Recent evidence suggests that Stat1 signaling is involved in IFN-γ-mediated immunopathologic lesions and disease in *A. phagocytophilum* infection.[44] Stat1 operates as a transcription factor central to the generation of effectors of inflammatory injury and could be an important target for intervention in this disease.

3. *Neorickettsia Risticii* Infection

(formerly *Ehrlichia risticii*, other names include Potomac Horse Fever (PHF), Shasta River Crud, Equine Neorickettsiosis (EN), *equine monocytic ehrlichiosis*, or *equine ehrlichial colitis.*)

Gumshoe Sleuthing

In the late 1970s a syndrome affecting horses in Maryland along the Potomac River was being reported in a newspaper article and the reporter termed the syndrome "Potomac Horse Fever." Horses adjacent to the Potomac River appeared to be at risk for spring and summer seasonal fever, diarrhea, and mild colic followed by a high percentage developing laminitis and 30% mortality. The investigation was negative for known bacterial, parasitic, and viral agents. Ronald Reagan was in the White House and, as a horse lover, his constituents pleaded for more investigation. The USDA was subsequently given funding to pursue the cause of the syndrome.

Private practitioners would call Dr. Allen Jenny, a research investigator, and report insights and cases. Several practitioners reported seeing "inclusion bodies" in neutrophils of the affected horses, and so Jenny contacted me at UC Davis to review the slides and to see if it appeared to be an ehrlichia-like agent. I examined several slides and all "inclusion bodies" were simply Dohle bodies in toxic neutrophils. Jenny was feeling pressure to pursue this lead, so I suggested he perform a blood inoculation experiment from a case of PHF into a research horse to rule out *Ehrlichia equi*. He did and, to everyone's surprise, the research horses came down with fever and diarrhea, but no clinical signs of *Ehrlichia equi*. The blood was sent to the United States leader on ehrlichia diseases at that time, Dr. Miodrag Ristic at the University of Illinois, who put the blood on every cell culture he had in his lab. Ehrlichia are intracellular parasites and require a cellular media for replication. Ristic's Masters Student Cindy Holland, who was studying *Ehrlichia sennetsu,* a rare tropical disease of humans, had a canine monocyte cell culture system for her agent. The blood inoculation procured growth in the cell culture of monocytes and another experimental horse came down with the clinical disorder following subsequent inoculation.

This started a 20 year search for the agent in nature pursuing the vectors knows to transmit ehrlichia—ticks. Study after study was negative and mode of transmission and location of the agent in nature was unknown until a new piece of evidence showed up on the crime scene. The Ohio State group, lead by Harvard trained world renowned rickettsiologist Y Rickihhisa, performed sequencing analysis of the several ehrlichial agents including *E. equi* and *E. sennetsu* and demonstrated how related some of these agents were. I was struck by the close relationship of the agent of salmon poisoning *Neorickettsia helminthoceca* and *E. sennetsu*, which had grown in the same monocyte cell culture as the unidentified PHF agent.

At the same time, Ristic's group published serological evidence that PHF was in California. We performed serological testing of Californian horses, including those with any history of colitis compatible with PHF. We found a uniform distribution of about 15% of horses from San Diego to the Oregon border with antibodies to *E. risticii*, as the agent was named. This made no biological sense for a vector borne disease, which should have clusters where certain tick vectors live in the environment. So we developed a nested PCR test and surveyed active cases of colitis using the presence of the agent in blood via PCR as the diagnostic criteria. We got a hit on a focal area in northern California by the Shasta River. Our team travelled there and found local horsemen and veterinarians recognized a syndrome identical to PHF, but they called it Shasta River Crud. It had similar epidemiology—spring and summer onset, fever, and diarrhea followed by laminitis. The local veterinarians had figured out early on that treatment with a tetracycline was therapeutic in many cases. We tested ticks in the area and all were negative.

We made a bold decision to pursue a new line of investigation using the syndrome of salmon poisoning as a model. With salmon poisoning, dogs eat the tissue of a river salmon or trout thereby ingest-

ing a metacercariae containing the rickettsia agent *Neorickettsia helminthotheca* and then develop fever and severe gastroenteritis. The fish get the infection from a fluke stage originating in a freshwater stream snail. We formed the hypothesis that the agent of PHF had something to do with snails. The immediate response from colleagues was not positive, and indeed I was ridiculed at meetings and in the hallways when other PHF researchers asked me how many snails horses had to eat to come down with PHF, knowing of course that horses don't eat snails. Undeterred, we tested 400 freshwater snails and got the first DNA evidence of *E. risticii* in nature in about 15% of snails in the Shasta River adjacent to the pastures and barns where horses had developed Shasta River Crud. We attempted transmission by stomach tubing research horses with infected snails but got no clinical disease. So our team of Gerhart Reubel, Nicola Pusterla, Christian Leutennegger, Jeff Barlough, Joon Seok Chae, and Elfriede DeRock went to the river, brought the snails back, and put them in an aquarium in the lab. There, the snails one day released "white stuff" which, when examined under a microscope, contained swimming cercariae. We DNA tested the cercariae and they were positive for *E. risticii*. We attempted transmission via stomach tube and, again, there was no infection in horses given the infected cercariae. We next thought that maybe the cercariae penetrate and burrow into the skin when horses cross the river. So we taught horses how to stand in buckets of water and we added the infected cercariae from the aquariums but again no transmission. We tried putting the cercariae in drinking water; no transmission. We knew we needed an isolate and not just DNA evidence so we did a bold experiment involving transmission by dissecting the infected snails and injecting the infected tissue into 3 research horses, who were given penicillin and gentamicin which have no killing spectrum against *E. risticii*. We had done it! We got disease transmission with fever, diarrhea, and mild colic. We isolated the agent from the blood of that horse on cell culture. We inoculated the cell culture and the next horse got the same disease. We had therefore achieved the first isolation of the agent from nature.

The next question was how the agent gets from the river to the horse. We used gumshoe sleuthing to answer this question interviewing several older wranglers at the ranch who remembered cases of Shasta River Crud. We asked if all the cases had direct access to the river and the answer was no. Several were stall confined and lived over 100 yards from the river. So we pursued this lead. The killer (*E. risticii*) had to walk or be transported by a carrier on ground or flight to the barn. We then began to collect, with the direction of a UC Davis freshwater entomologist, all insect larvae on the rocks next to the snails that released the cercariae. The cercariae would rapidly float downstream following release so they had to go to the next stage somewhere close to form metacercariae. We determined over 17 species of insects were infected with the agent. We brought caddisfly larvae back to the lab and put them in our aquarium culture. Our lab looked like the Petco section for fish. One afternoon in the cold room on the second floor of Tupper Hall, the caddisfly larvae hatched and adult caddisflies were flying around the room. Pusterla caught 8 of them, which we fed to a research horse. This horse came down with severe disease; fever, diarrhea, mild colic followed by laminitis. No other IV transmission studies with *E. risticii* had produced laminitis, as developed in naturally occurring cases. Our next step was determining how snails became infected. We knew something had to eat the flying insects and return the agent to the river to complete the fluke cycle. We saw lots of bats on the river in the evening and began a study, which determined that the bats had a fluke that was infected with *E. risticii*. The eggs of the fluke contained the agent, which were released by the bats while flying over the river allowing transmission to the snails.

The modus operandi of the killer was revealed and is this: The killer struck its victims by emerging from the creek within the body of a small insect, mayfly, stonefly, caddisfly, etc., and flew to pastures, or perhaps where lights were on in the barn, dropped into feed buckets or grass or hay and the horse ingested them and came down with the infection. The horse is a dead end host and not contagious.

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Etiology

Neorickettsia risticii (formerly *Ehrlichia risticii*) is the etiologic agent of EN, also called Potomac Horse Fever, *equine monocytic ehrlichiosis*, or *equine ehrlichial colitis*. *Neorickettsia risticii* has recently been classified based on genetic analysis in the genera *Neorickettsia* among three other species: *Neorickettsia sennetsu* (human agent of Sennetsu fever), *Neorickettsia helminthoeca* (agent of salmon poisoning in the dog), and an ehrlichia-like bacterium present in the metacercarial stage of the fluke *Stellantchasmus falcatus* (SF agent).[1] Based on sequence analysis of the 16S rRNA gene, *N. risticii* shares 98.9% homology with *N. sennetsu*, 94.8% with *N. helminthoeca*, and 99.1% with the SF agent. Strain variance has been determined among 11 *N. risticii* strains, with a maximum divergence of 0.7%.

Neorickettsia risticii is a Gram-negative coccus and stains dark blue to purple with Giemsa stain and Romanowsky stain, red with Macchiavello stain, and pale blue with hematoxylin and eosin (H&E). The organism tends to occupy one side of the cytoplasm rather than being symmetric and is

generally round. *Neorickettsia risticii* divides by binary fission and is found in membrane-lined vacuoles within the cytoplasm of primarily macrophages and glandular epithelial cells in the intestine of the horse. The organism is rarely observed in peripheral blood monocytes. In cell culture or host cells, *N. risticii* occurs in two different forms, either singly or in groups (morulae), the former being 0.8 to 1.5 μm electron-lucent and the latter 0.2 to 0.4 μm electron-dense bodies. *Neorickettsia risticii* has been successfully cultured in human histiocytic lymphoma cells and in canine, equine, and murine monocytes.

Epidemiology

Equine neorickettsiosis was recognized originally in 1979 along the Potomac River in the state of Maryland.[45] Equine neorickettsiosis is known to occur in 44 of the United States, three Canadian provinces (Nova Scotia, Ontario, Alberta), South America (Uruguay, Brazil), Europe (The Netherlands, France), and India. Isolation or detection of the causative agent from clinical cases of the disease using conventional cell culture or molecular detection by PCR has only been reported from 14 states (California, Illinois, Indiana, Florida, Kentucky, Maryland, Michigan, New York, New Jersey, Ohio, Oregon, Pennsylvania, Tennessee, Texas, and Virginia), Nova Scotia, Uruguay, and Brazil.

The epidemiology of *N. risticii* has been the subject of intensive research efforts for more than 20 years. The disease typically occurs near freshwater streams and rivers and on irrigated pastures, mainly during middle to late summer (May to November). The seasonal incidence of the disease, the geographic distribution of EN, and the experimental transmission by the intradermal route implied the involvement of a blood-sucking arthropod as a vector. The historic connection between other ehrlichial agents and tick vectors prompted many to regard ticks as prime candidates for the transmission of *N. risticii*. Therefore, many studies focused on identifying an arthropod vector for EN. Despite intensive investigation, however, no evidence was found for spread of the disease by arthropod vectors such as ticks.[46]

The causative organism is present in the feces of experimentally infected horses and can be experimentally transmitted by the oral route using feces from infected horses. These findings, together with the close serologic and molecular relationship between *N. risticii* and *N. helminthoeca* isolated from flukes, suggest that the vector of *N. risticii* may not be an arthropod but instead a helminth closely associated with aquatic habitats. Barlough et al provided strong evidence that trematodes, which use operculate freshwater snails as intermediate hosts, may be involved in the life cycle of *N. risticii*.[47] This theory was confirmed when DNA of *N. risticii* was detected by nested PCR in operculate snails (*Pleuroceridae: Juga* spp.) collected from stream waters in a northern California pasture where EN is endemic. The results of sequencing PCR-amplified DNA from a suite of genes (16S rRNA, groESL heat shock operon, and 51-kDa major antigen genes) indicated that the source organism was clearly related to the type strain of *N. risticii*. The PCR-amplified product is associated with the presence of virgulate cercariae in the snail secretions (Fig. 4).[14] The number of snails harboring the trematode stages varied from 3.3% to 93.3%, and the number of PCR-positive snails (3.3–20%) appears to depend on the size of the snails, the month of collection, and geographic origin.

Fig. 4. Photomicrograph of virgulate cercaria released by pleurocerid snails of genus Juga (bar = 0.01 mm). From: Pusterla N, Madigan JE. *Neorickettsia risticii* infection. In: Sellon D and Long M, eds. *Equine infectious diseases*. 2nd ed. Amsterdam: Elsevier, 2013.

In northern California, the species of snail incriminated in the life cycle of *N. risticii* is *Juga yrekaensis,* a common pleurocerid snail, which inhabits fresh or brackish stream water in the northwestern United States (Fig. 5). Investigation of the role of a district irrigation canal in Nevada County, CA as the point source of infection for *Neorickettsia risticii*, found 4 out of 568 freshwater snails tested PCR positive for *N. risticii*, including the snail species *Planorbella subcrenata*, which has not previously been reported.[48] Phylogenetic analysis showed 99.8% to 100% homology between the different snail and horse *N. risticii* isolates.

Additionally, DNA from *N. risticii* has been detected in virgulate cercariae in lymnaeid snails (*Stagnicola* spp.) from northern California, in virgulate xiphidiocercariae isolated from pleurocerid snails (*Elimia livescens*) in central Ohio, and from pleurocerid snails (*Elimia virginica*) in central Pennsylvania, suggesting that other types of snails may also harbor infected trematodes.[14,49,50] This type of trematode is known to become encysted in

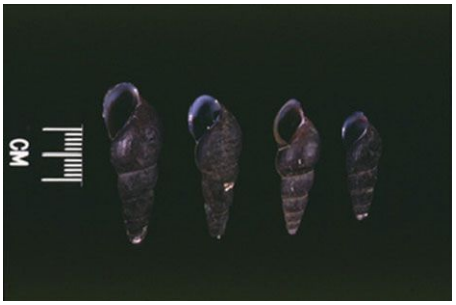

Fig. 5. *Juga yrekaensis* pleurocerid snails collected from equine neorickettsiosis endemic region in northern California (bar = 1 cm). From: Pusterla N, Madigan JE. *Neorickettsia risticii* infection. In: Sellon D and Long M, eds. *Equine infectious diseases*. 2nd ed. Amsterdam: Elsevier, 2013.

the second intermediate host. *Neorickettsia risticii* DNA has been detected by PCR in mesocercariae and metacercariae in various aquatic larval, nymphal, and adult insects such as caddisflies, mayflies, damselflies, and dragonflies in northern California and in central Pennsylvania (Fig. 6).[50,51] Polymerase chain reaction investigations suggest that the prevalence of aquatic insects harboring *N. risticii* varies from 10% to 80%.

Recently, two potential helminth vectors, *Acanthatrium* spp. and *Lecithodendrium* spp., both infected with *N. risticii,* were found in the intestine of bats and birds collected in northern California and Pennsylvania (Fig. 7).[52,53] Further, transstadial transmission of *N. risticii* in the vector *Acanthatrium oregonense* was recently documented by molecular characterization.[54]

These trematodes belong to the *Lecithodendriidae* family, common parasites of bats, birds, and amphibians in North America, which use pleurocerid freshwater snails as first intermediate hosts and aquatic insects as second intermediate hosts. Additional trematodes, members of the *Lecithodendriidae* or other families, may also act as vectors of *N. risticii* in other endemic regions of the United States.

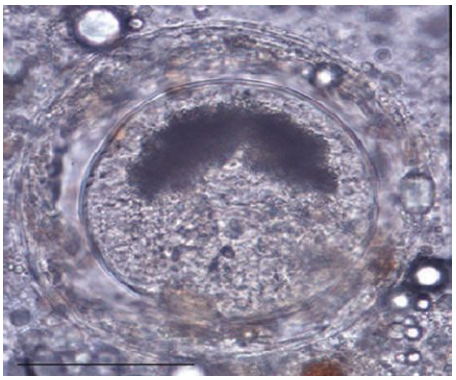

Fig. 6. Photomicrograph of metacercaria collected from caddisfly larva (bar = 0.2 mm). Reprinted with permission from Madigan JE, Pusterla N. Ehrlichial diseases. *Vet Clin North Am Equine Pract* 2000;16:487–499.

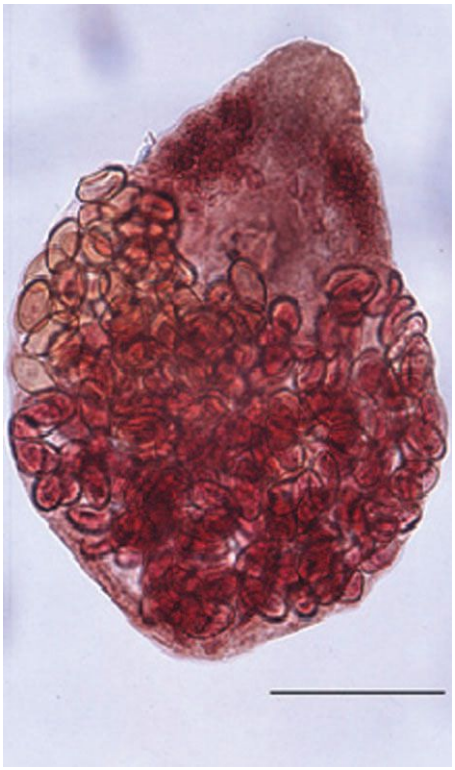

Fig. 7. Photomicrograph of adult Acanthatrium trematode collected from intestine of *Myotis yumanensis* bat (bar = 0.5 mm). Reprinted with permission from Madigan JE, Pusterla N. Ehrlichial diseases. *Vet Clin North Am Equine Pract* 2000;16:487–499.

Since *N. risticii* was first identified, no definitive reservoir host of the organism has been proposed. Seroepidemiologic studies have revealed the presence of antibody titers specific to *N. risticii* in domestic and wild animals, such as dogs, cats, coyotes, pigs, and goats, from regions in which EN is endemic.[55] A variety of nonequine mammalian species, such as mice, dogs, cats, and cattle, are susceptible to *N. risticii*.[56–58] Based on vertical transmission of *N. risticii* in the trematode *Acanthatrium oregonense* and detection of *N. risticii* DNA in the blood, liver, or spleen of bats and swallows, it is speculated that these insectivores act as both definitive host of the helminth vector and natural reservoir of *N. risticii.*

The biologic activity of *N. risticii* in infected vectors has been recently investigated by the inoculation of PCR-positive trematode stages into horses and mice. Horses injected subcutaneously with *N. risticii* PCR-positive trematode stages (virgulate cercariae and sporocysts) collected from *J. yrekaensis* snails developed clinical signs and hematologic changes consistent with EN.[59] Furthermore, *N. risticii* was transmitted to mice using PCR-positive metacercariae isolated from caddisfly larvae (*Dicos-*

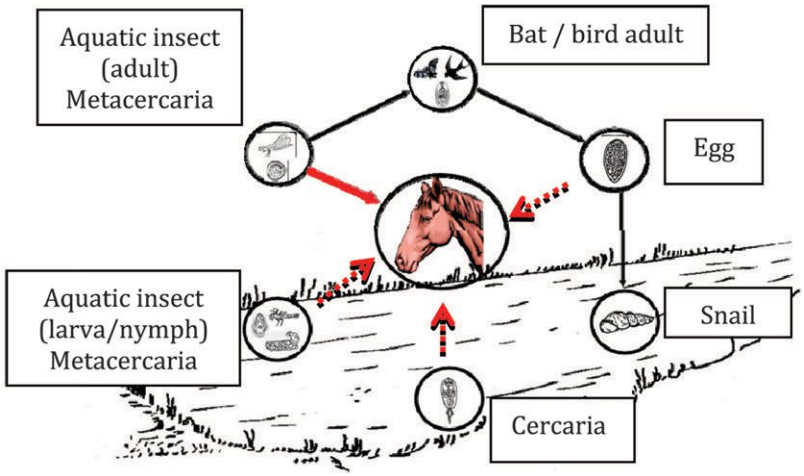

Fig. 8. Life cycle of helminthic vector of *Neorickettsia risticii* and natural route of transmission. Solid red arrow represents demonstrated route of transmission with adult aquatic insects. Dashed red arrows represent possible routes of infection with trematode eggs, free cercariae, or larval/nymphal aquatic insect stages. From: Pusterla N, Madigan JE. *Neorickettsia risticii* infection. In: Sellon D and Long M, eds. *Equine infectious diseases*. 2nd ed. Amsterdam: Elsevier, 2013.

moecus spp.).[51] These data confirm that *N. risticii* is associated with a helminth vector and illustrate the value of PCR technology as a screening method for epidemiologic studies.

Pathogenesis

The mode of transmission of *N. risticii* has remained one of the greatest mysteries of EN. *N. risticii* has been successfully transmitted by the intravenous, intramuscular, subcutaneous, intradermal, and oral routes using whole blood from naturally infected horses or with infected cell culture material.[60–64] In light of recent epidemiologic discoveries concerning the vector of *N. risticii* and its helminth hosts, horses could conceivably be exposed to *N. risticii* through skin penetration by infected cercariae or by consuming infected cercariae in water or metacercariae in a second intermediate host such as an aquatic insect. One horse fed adult caddisflies (*Dicosmoecus gilvipes*) in northern California[65] and two horses fed adult caddisflies (*Cheumatopsyche campyla*, *Hydropsyche hageni*) or a mixture of adult caddisflies and mayflies (*Leucrocuta minerva*) in central Pennsylvania developed EN.[50] These studies attempted to mimic the natural route of infection with *N. risticii* and showed that oral transmission using infected aquatic insects was not only possible but also that the clinical disease produced was similar to that seen in naturally infected horses. Aquatic insects, such as caddisflies and mayflies, represent a likely source of infection because of their abundance in the natural environment, their high infection rate with *N. risticii* as determined by PCR, and the mass hatches regularly observed during summer and fall. Under natural conditions, horses grazing near rivers or creeks will ingest adult insects along with grass (adult insects live near water and are likely to die there) or consume adult insects trapped on the water surface. Horses also may consume insects that are attracted by stable lights and subsequently accumulate in feed and water (Fig. 8). A serosurvey performed at two Ohio racetracks in 1986 reported that cases of PHF were associated with certain barns, as well as specific stalls in those barns.[66] Aquatic insects might have been present in larger numbers in those locations, perhaps attracted by specific lighting, and were accidentally ingested by the horses in their food. A recent outbreak of EN in Minnesota and Iowa was the first one to incriminate mayflies as significant vectors for horses.[66] The use of night lighting was determined to be a consistent risk factor during that outbreak.

After natural or experimental transmission in horses, *N. risticii* infects blood monocytes. Although the pathogen is readily phagocytized by monocytes, it appears to elude the host's defense mechanisms by inhibiting lysosomal fusion with phagosomes.[67] *Neorickettsia risticii* can be isolated by cell culture from the peripheral blood monocytes of infected horses as early as 6 days after ingestion of adult aquatic insects harboring the organism, and bacteremia can persist up to 2 weeks after spontaneous resolution of clinical signs.[51] *Neorickettsia risticii* also has a predilection for the intestinal wall, especially that of the large colon. Colonic epithelial cells, mast cells, and tissue macrophages are the targets of infection. Lesions are confined to the gastrointestinal tract. The resultant diarrhea is thought to be caused by loss of epithelial cell microvilli, reduction in electrolyte transport, and increase in intracellular cyclic adenosine monophosphate (cAMP) in infected intestinal cells. All these mechanisms contribute to the reduced luminal ab-

sorption of electrolytes (sodium and chloride) and increased water losses in the large and small colon.[68]

Neorickettsia risticii causes significant immune depression in mice and detectable alterations of the immune system in horses. Whether a clinically significant immune depression occurs in horses is unclear. Recovered horses are resistant to development of clinical disease by rechallenge for a least 20 months. Humoral and cell-mediated immune responses appear to have significant roles in conferring protection against *N. risticii*. Antibodies can be protective when they block the pathogen's attachment to or penetration of host cells. This occurs by several mechanisms such as blocking ehrlichial binding to its specific receptor, by inhibiting ehrlichial metabolism, or by conferring antibody-dependent cell-mediated cytotoxicity. However, the presence of antibodies does not always correlate with clearance of ehrlichial organisms and presence of protective immunity. This has been shown with horses that have been vaccinated with a killed *N. risticii* vaccine and subsequently developed clinical disease after natural exposure.[69] Antibodies induced by a killed vaccine may not be effective because protective antigens may only be expressed during cell invasion or replication. Cell-mediated immunity likely plays a dominant role in protecting the host from *N. risticii* infection, as shown for other rickettsial infections.

Clinical Findings

The incubation period for *N. risticii* infection in horses is approximately 1 to 3 weeks. In two recent studies, horses fed aquatic insects harboring *N. risticii*-infected metacercariae developed clinical signs 9 to 15 days after oral challenge.[50,65] The clinical features of EN have been extensively reported over the years. Naturally occurring cases of EN are typified initially by an acute onset of mild depression and anorexia, followed by a biphasic increase in body temperature ranging from 38.9 to 41.7°C (102–107°F). At this stage, decreased intestinal sounds can be auscultated.

Within 24 to 48 hours, moderate to severe diarrhea ranging from "cow pie" to watery consistency develops in approximately 60% of affected horses. The onset of diarrhea is often accompanied by mild abdominal discomfort. Some horses develop severe toxemia and dehydration, which result in cardiovascular compromise characterized by increased heart rate and respiratory rate and congested mucous membranes. Subcutaneous edema along the ventral abdomen has also been observed in horses with EN. Laminitis can supervene as a severe complication of EN in as many as 40% of affected horses. Laminitis may progress, despite resolution of other clinical signs. Interestingly, laminitis has only been reported in naturally infected horses and probably reflects as-yet undetermined pathophysiologic mechanisms related to the natural route of transmission. It should be emphasized that a horse with EN may present with all or any combination of these clinical signs.

Retrospective analysis of clinical and clinicopathological features of 44 horses with EN by Bertin et al demonstrated the most common clinical signs included diarrhea (66%), fever (50%), anorexia (45%), depression (39%), colic (39%), and lameness (18%).[70] The median duration of hospitalization was 6 days, and 73% of horses survived to discharge. Laminitis was present in 36% of horses, 88% of which were affected in all 4 feet. Electrolyte loss, hemoconcentration, and prerenal azotemia, indicative of severity of colitis, were significantly higher in non-survivors. Serum chloride and sodium concentrations as well as duration of hospitalization were significantly lower in non-survivors. The results of forward stepwise logistic regression indicated that blood hemoglobin concentration on admission and antimicrobial treatment with oxytetracycline were independent factors associated with survival.

Case-fatality rates vary from 5% to 30% and depend mostly on the strain involved. Fatalities are associated with toxemia and severe laminitis. Long-term problems appear to be related to sequelae such as laminitis. To date, no evidence exists that *N. risticii* infection results in chronic disease. Attempts to isolate *N. risticii* by culture or PCR after clinical signs have abated have been unsuccessful.

Transplacental transmission of *N. risticii* has been reported in natural and experimental infections, and the organism may induce abortion or resorption of the fetus or produce weak foals, which require extensive neonatal care. Pregnant mares, which exhibit clinical signs of EN, can subsequently abort around 7 months of gestation, regardless of the severity of infection.[71,72] In mares experimentally infected at 90 to 120 days of gestation, abortion occurred at 65 to 111 days after inoculation.[73] Abortions are spontaneous with a fetus in fresh condition. Gross findings of the fetuses include meconium staining and petechiation of external surfaces.

Hematologic findings vary in the early stage of EN from a transient leukopenia (white blood cell count $<5000/\mu L$), characterized by a neutropenia and a lymphopenia, to a normal hemogram, despite evidence of systemic toxicity.[74] A common finding in cases of EN is a marked leukocytosis ($>14{,}000/\mu L$), usually observed within a few days of disease onset. Increases in both packed cell volume and plasma protein concentration secondary to dehydration and hemoconcentration can occur. A transient nonregenerative anemia and thrombocytopenia may develop and can be profound in some horses. Horses often present with evidence of a hypercoagulable state, characterized by significant changes in plasma fibrinogen, fibronectin, factor VIII, and plasminogen. In contrast to the tick-borne *Anaplasma phagocytophilum* infection, visual observation of *N. risticii* in peripheral blood monocytes is rarely successful.

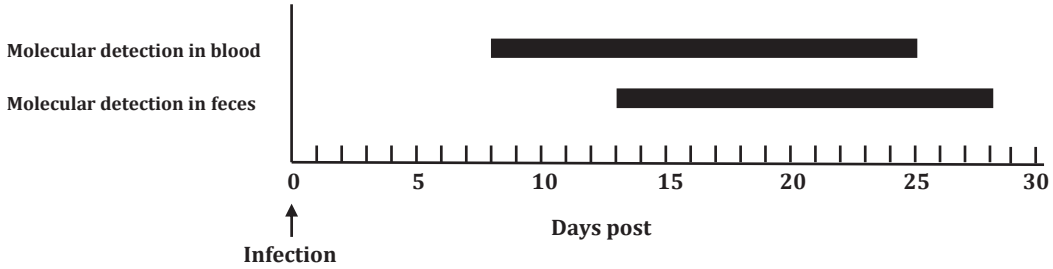

Fig. 9. Molecular detection time of *Neorickettsia risticii* by real-time polymerase chain reaction in blood and feces of horses with Potomac horse fever. From: Pusterla N, Madigan JE. *Neorickettsia risticii* infection. In: Sellon D and Long M, eds. *Equine infectious diseases*. 2nd ed. Amsterdam: Elsevier, 2013.

Diagnosis

A provisional diagnosis of EN is often based on the presence of typical clinical signs and the seasonal and geographic occurrence of the disease. A definitive diagnosis of EN, however, should be based on the isolation or detection of *N. risticii* from the blood or the feces of infected horses. Serologic testing using indirect fluorescent antibody or enzyme-linked immunosorbent assay (ELISA) test formats is of limited value as a diagnostic tool because antibody levels to *N. risticii* may not be detectable for some time after infection. Paired serum titers must be evaluated; single titers are useless for confirmatory testing of EN. The reliability of the indirect immunofluorescence technique for antibody detection has been questioned because the test yields a high percentage of false-positive results.[75]

Isolation of the agent in cell culture from the peripheral blood of affected patients, although possible, can take from several days to weeks of culture before detection is successful and is not routinely available in many diagnostic laboratories. The recent development of *N. risticii*-specific PCR assays has greatly facilitated and hastened the diagnosis of EN.[76,77] In experimentally and naturally infected animals, PCR performed on feces and peripheral blood was more sensitive than culture.[78] Conventional PCR assays, however, are time-consuming and prone to contamination. Real-time PCR platforms associated with automated nucleic acid extraction allow the detection of *N. risticii* DNA within the same day of sample receipt, making this technology a much more practical assay for routine diagnostic testing.[59] To enhance the chances of detection of *N. risticii*, the assay should be performed on blood, as well as fecal samples, because the presence of the organism in blood and feces may not necessarily coincide (Fig. 9). Another routine application of PCR is the detection of *N. risticii* DNA in fresh or formalin-fixed and paraffin-embedded colon tissue, allowing postmortem diagnosis.

Differential diagnoses should include peritonitis and any clinical syndrome of enterocolitis such as salmonellosis, clostridial diarrhea, or intestinal ileus secondary to displacement or obstruction. Diagnostic tests specific to ruling out these diseases should be concurrently pursued.

Pathologic Findings

Gross necropsy findings in the acute stage of EN disease include distended large colon and cecum filled with watery contents. Mucosal hyperemia and ulceration and areas of necrosis and hyperplasia of lymphoid follicles and lymph nodes may also be observed. Microscopic changes include areas of moderate to severe lymphohistiocytic infiltration of the submucosa and lamina propria of the cecum and large colon.[68] Lack of severe lesions and absence of neutrophil infiltration are important in the differential diagnosis of EN. Both silver stain and immunoperoxidase procedure using a specific antibody to *N. risticii* can demonstrate rickettsial organisms in intestinal epithelial cells and macrophages in paraffin-embedded tissue specimens. Although not routinely done, electron microscopy can be used to detect *N. risticii* infection during disease.

Changes in fetuses aborted because of *N. risticii* infection are consistent, unique, and diagnostic of this abortion syndrome. Fetuses have increased volume of feces within the small and large intestine and liver discoloration. Microscopic findings include lymphohistiocytic enterocolitis, periportal hepatitis, lymphohistiocytic myocarditis, and severe splenic inflammation characterized by both intense lymphohistiocytic infiltration and lymphoid necrosis.[71–73] *N. risticii* can be recovered by cell culture from bone marrow, spleen, lymph node, colon, and liver of aborted fetuses.

Therapy

Horses with EN can be treated successfully by the intravenous (IV) administration of oxytetracycline at 6.6 mg/kg twice a day, when given early in the clinical course of the disease. A response to treatment is usually seen within 12 to 24 hours, associated with a decrease in rectal temperature followed by an improvement in demeanor, appetite, and borborygmal sounds.[79] The disease does not progress after initiation of treatment. If therapy is begun early in the course of EN, clinical signs frequently

resolve by the third day of treatment. No more than 5 days of antimicrobial therapy are usually needed. Whether treatment of clinically affected broodmares during the diarrheal stage of disease prevents subsequent abortion remains unknown. In horses exhibiting signs of enterocolitis, IV administration of polyionic fluids is extremely important to prevent hypovolemia and shock. Addition of calcium, magnesium, and potassium to fluids may be necessary in horses with prolonged anorexia and fluid losses. Concurrent use of nonsteroidal antiinflammatory drugs (NSAIDs), such as flunixin meglumine (0.25 mg/kg IV or orally [PO] every 8 hours [q8h]) or phenylbutazone (2.2–4.4 mg/kg IV or PO q12h) is indicated. Horses developing severe protein-losing enteropathy associated with decreased albumin concentrations may benefit from plasma transfusion. Preventive measures for laminitis, the most common potentially lethal sequela of EN, should be implemented as well. Although no specific therapy is universally recognized to prevent laminitis, the authors recommend stall confinement of affected horses, use of foot support (deep bedding, padded support), ice for the feet, and administration of NSAIDs as previously described.

Prevention

Several inactivated, whole-cell vaccines based on the same strain of *N. risticii* are commercially available and have been used in endemic areas for several years to protect horses from EN. Vaccination has been reported to prevent all clinical signs except fever in 78% of experimentally infected ponies.[57] Protection conferred by this vaccine appears to be much shorter in duration than protection after natural infection, which can last up to 2 years. For unexposed horses, considering the time required to develop immunity after vaccination, the short-lasting humoral immunity, and the existence of antigenic variations in the field, it is questionable how much benefit the vaccine will provide under field conditions. Vaccine failure has been reported and attributed to antigenic and genomic heterogeneity among *N. risticii* isolates.[69] Vaccine failure may also be caused by lack of protection at the site of exposure because the natural route of transmission seems to be the oral route. An improved vaccine for EN is strongly desired in the future.

If vaccination of horses is performed using inactivated vaccines, the primary series should include two vaccines given 4 weeks apart. A third dose should be given if the patient is a foal that received the first dose at less than 5 months of age. Thereafter, boosters should be administered at 4- to 6-month intervals. *Neorickettsia risticii*-induced abortion is not prevented by vaccination.

The ingestion of aquatic insects carrying infected trematodes is probably the only means of transmission of *N. risticii* under natural circumstances. In endemic regions, control measures should limit access of susceptible horses to freshwater streams, ponds, and irrigated pastures during peak incidence, as well as reducing night lights on horse facilities to minimize the attraction of water insects during mass hatches. Methods to control snail populations where possible could lower infection rates in aquatic insects.

4. Neonatal Septicemia

Copyright use authorized by AAEP: Madigan JE. Method for preventing neonatal septicemia, the leading cause of death in the neonatal foal, in *Proceedings*. Am Assoc Equine Pract 1997;43: 17–19.

Gumshoe Sleuthing

Several years ago, I was involved with an outbreak of neonatal salmonellosis on a large Thoroughbred farm.[80] The foals were born healthy and had >800 mg/dl IgG (often >2000 mg/dl). However, they still became infected by 12 hours and developed clinical signs of fever, colic, diarrhea, swollen joints by 24 to 48 hours when foaling in a clean barn, with clean feed and water and biosecurity (gloves, gowns, foot baths, etc.) procedures used by personnel. We performed 2860 Salmonella cultures during the course of that outbreak investigation. Mares were found to be asymptomatically shedding low numbers of *Salmonella ohio* obtained from contaminated feed of a broodmare mix.[81] When the mares defecated during stage two labor, the placenta and perineum became contaminated. During udder seeking the foals ingested Salmonella prior to obtaining any colostrum and *S. ohio* bacteremia resulted first detectable at 12 hours of age via blood culture of an apparently healthy foal. This was clue number 1 regarding the open gut as an immediate access for bacteria despite adequate IgG levels.

Clue number 2 came from colostrum deprivation models in foals where, despite rigorous hygiene, over 50% of foals became bacteremic and of these, most died despite therapy.[82] These foals were allowed 30 to 60 min of bonding, which consisted of udder seeking, perineum licking, and so on. They were then removed from the mares and promptly became bacteremic with the usual organisms such as *Escherichia coli.* Many other colostrum deprivation studies have had similar results, and lack of IgG has been inappropriately implicated as the sole cause. Exposure of the foals to pathogenic bacteria during udder seeking appeared to be the principal route of infection and demonstrated the magnitude of bacterial exposure during this udder seeking activity.

Clue number 3 came on a trip to the International Conference on Equine Infectious Disease in Tokyo. During a tour of a facility used for *Rhodococcus equi* studies, I was brought to a stable where colostrum deprived foals were born. For the past 2 years, 12 foals per year had been raised with no illness. The mares foaled in dirt floor, straw bedded barns. Foals were administered a single injection of procaine penicillin and the navel disinfected with beta-

dine. In addition, milk replacer was used for 24 hours (cow's milk purchased at a market). None of these procedures would have an influence on Gram-negative bacteremia. We had just concluded a study indicating betadine was worse than nothing at disinfecting the navel.[83] Upon further questioning, I found that immediately following delivery the foal was moved to the stall adjacent to the mare and no mare contact occurred. Prior to rising, the foal was fed as much cow's milk as it wanted from a bottle and continued to be fed upon demand for 24 hours, whereupon the foal was returned to the mare that had been milked out of colostrum. In my opinion, the procedure that was effective in this system was the rapid closure of the open gut and lack of udder seeking with the associated bacterial absorption across the open gut. In the other two scenarios of the *Salmonella* outbreak and colostrum deprivation, during udder seeking foals ingested pathogenic organisms, which crossed the open gut directly into the bloodstream producing septicemia. The effectiveness of rapid gut closure even without IgG is demonstrated in that model.

Clue number 4 came with a lecture on management of foal septicemia in England.[84] At a farm with good management and routine use of antibiotics for 72 hours after birth, the incidence of septicemia was 0.3%. Routine use of antibiotics had been in place for 20 years on this farm. This demonstrated the relative safety of routine antibiotics as well as an extremely effective program at preventing septicemia.

Early on in foal medicine, the umbilicus was considered the route of infection for most foals with septicemia and septic arthritis. Numerous studies have shown the umbilicus is not involved in the majority of foal septicemia cases. We developed the hypothesis that delayed gut closure and exposure to bacteria during udder seeking or due to delayed feeding or nursing and subsequent environmental licking or ingestion of bacteria by the newborn foal is the risk factor and source of bacteria for most septicemias in foals. Early administration or ingestion of colostrum may be associated with reduced illness in foals because of early (rapid) gut closure preventing absorption of bacteria across the gut wall. Thus, a foal with high IgG could be a marker for wellbeing based on rapid and early feeding prior to bacterial access across the open gut. Additionally, this would explain healthy foals that stood and nursed vigorously but did not become ill despite low serum IgG. Delayed nursing and early exposure to pathogens (prior to any colostrum) are the key factors in risk of infection in this hypothesis. Therefore, conditions that may be associated with delayed gut closure, such as neonatal maladjustment syndrome, prematurity, dystocia, musculoskeletal problems, weak at birth, twins etc. would have significant incidences of septicemia, which they certainly do. Good management for preventing infections are clean stalls, clean mares, factors that aid early ingestion of colostrum and short-term post birth antibiotics in the newborn.

Jeffcott demonstrated the indiscriminate active absorption of large molecules by the "open" gut shortly after birth.[85] An iodinated marker with a mean molecular weight 160,000 was well absorbed from the intestine of the newborn foal. Absorption was maximal (22%) 3 hours after birth but fell rapidly in a linear decline to less than 1% by 20 hours of age.[85] There was reduced absorption (10%) when the foals were deprived of colostrum although the time of cessation of uptake did not alter.[85] This potential major route of pathogen exposure to the foal has been largely overlooked. Specialized cells line the newborn gut and will nonspecifically ingest various large molecular weight compounds via pinocytosis not just immunoglobulin. Additionally, the lack of tight junctions between gut barrier cells allows molecules of >70,000 MW to freely pass into the lymphatics and circulate between cells.[85] When these specialized cells are used up, the gut assumes its normal structure and no further absorption of large molecules can take place. Ingestion of adequate amounts of colostrum during the first few hours of life plays an important non-immunological role in preventing acquisition of infection by "closing the neonatal gut" to translocation of macromolecules, including bacteria.[86]

Etiology

The period of greatest risk of disease and also of death within the first year of a foal's life is the first 7 days of life with septicemia the major cause of death <7 days old.[87] Both American and British studies indicate that the majority of septicemic foals have a Gram-negative component of infection.[88–90] *E. coli*, *Actinobacillus* spp., *Klebsiella* spp., *Enterobacter* spp., *Pseudomonas* spp. are the most common isolates. Streptococcal infection does occur but is usually in conjunction with Gram-negative bacteria. Although Gram-negative bacteria, particularly Enterobacteriaceae, remain the most common isolates from neonatal foals with sepsis, the prevalence of Klebsiella infection is decreasing whilst that of Gram-positive bacteria is increasing.[88–90] The increased prevalence of *Enterococcus* spp. is of concern because antimicrobial susceptibility patterns for enterococci are unpredictable and enterococci can also act as donors of antimicrobial resistance genes to other bacteria.[90]

Onset is usually within 3 to 4 days of age, although some infections develop *in utero* and will be present at birth. Foals frequently show first clinical signs after infection has already been established for a considerable period of time.

Route of Infection

Routes of microorganism entry include the umbilicus, respiratory tract, and wounds, although the gastrointestinal tract is now believed to be the predominant portal of entry for infection.[86] Fre-

quently, failure of passive transfer is listed as the leading cause of septicemia[91–94] and the practice of assessing passive immunity has been associated with decreased morbidity due to septicemia.[87] However, many (>25%) confirmed septicemic foals have greater than 800 mg/dl IgG. Additionally, many foals with low IgG are sick at birth and have poor vigor and vitality. With good management, healthy foals with serum IgG of 200–400 mg/dl have only slight risk of acquiring illness.[95] Efforts to raise IgG by various means seem not to have eliminated the problem of septicemia over the last 10–15 years. Well-conducted studies have indicated that low IgG per se is not a risk factor for disease and that foals with only 200 mg/dl IgG at 24 hours of age do not get sick on some farms.[80,95] It is my opinion that this high rate of infection in this age group is best explained by delayed gut closure and bacterial invasion across the "open" gut rather than low IgG.

Predisposing Conditions

Predisposing conditions include: prematurity; delayed access to colostrum; failure to ingest adequate quantity of colostrum and specific antibody; maternal risk factors—concurrent illness or fever, vaginal discharge, poor nutritional status, colic, endotoxemia, premature lactation, recent transport stress, agalactia, poor mothering; neonatal maladjustment syndrome (NMS); twins; and adverse environmental conditions.

What all these conditions have in common is exposure to pathogens prior to colostrum ingestion.

Clinical Signs

Clinical signs often cannot be differentiated from NMS. Early clinical signs are vague and include depression, lethargy, decreased mammary sucking, and a behavior change. Fever (>102°F, 39°C) occurs in less than 50% of cases and hypothermia <100°F (37.8°C) is not uncommon. In advanced cases petechiation of pinnae and mucous membranes of the oral cavity and vulva is seen. It should be noted that episcleral hemorrhages are common after normal foalings from birth canal pressure. Other signs include anterior uveitis, diarrhea, obtundation, coma, convulsions, respiratory distress, dehydration, poor pulse quality, and swollen joints.

Clinical Pathology

Clinical pathology should be obtained as soon as possible. Serum IgG concentration of <400 mg/dl is common although some are within the 400 to 800 mg IgG range. Both neutropenia (<4000/ul) and neutrophilia (>12,000/ul) can occur (it should be remembered that premature, noninfected foals have neutropenia). Additional hematological findings include >50/ul band neutrophils and Dohle bodies, toxic granulation, or vacuolization in neutrophils. Fibrinogen concentration is frequently elevated >400 mg/dl indicative of inflammation. Hypoglycemia occurs in approximately 50% of cases (<80 mg/dl) and arterial oxygen is <70 mmHg in 40% of cases. Acid-base status indicating a mild to severe acidosis is common.

Blood culture is indicated in any suspected case of sepsis and should be performed on all foals entering the intensive care unit. If blood culture medium is not readily available, the sample can be transferred in a yellow top tube containing anticoagulant citrate (ACD). Sampling should be performed before antibiotics or at trough periods before next administration. One blood sample should be collected initially upon admission and then repeated in 1 to 2 hours. Do not delay antimicrobial treatment of suspected septicemia to complete a series of cultures. Intravenous antimicrobial therapy should be initiated if laboratory work does not rule out sepsis. Negative blood cultures do not rule out septicemia; over 50% of foals with *E. coli* septicemia have negative blood cultures.[96] Organisms found most commonly are *E. coli,* Actinobacillus spp.*, Klebsiella pneumoniae, Pseudomonas* spp., *Citrobacter* spp., *Enterobacter* spp., *Salmonella*, and gram-positive organisms such as Streptococcus, Staphylococcus, Enterococcus.

Sepsis scoring is a method of attempting to predict infection based on history, physical exam, and clinical pathology designed by Brewer and Koterba, 1988. The system uses 14 historical, clinical and laboratory weighted variables to derive 14 scores, which are then added together to give the sepsis score. The sepsis score is reported to have a sensitivity of 93%, a specificity of 86%, positive accuracy rate of 89% and negative accuracy rate of 92%.[97] Subsequent studies have suggested the sepsis score is less reliable than initially reported.[98,99]

Therapy

Antimicrobials

Almost all systemic neonatal bacterial infections involve Gram-negative (often enteric) organisms, with or without accompanying gram positive organisms. The opposite is usually the case in adults. Septicemic foals deteriorate rapidly and, therefore, antibiotic treatment should be started as soon as cultures have been collected and later modified, if necessary, after culture and susceptibility results are available. Front line antibiotics should have excellent activity against Gram-negative bacteria and specific combination therapy is rational to broaden the spectrum. The most useful antibiotics for initiating treatment of suspected or confirmed sepsis are the aminoglycosides, e.g., amikacin or gentamicin, in combination with penicillin G, ampicillin, ticarcillin, or a cephalosporin antibiotic. Depending on the susceptibility of bacterial isolates, other antibiotics that may prove useful include trimethoprim/sulfonamide, 3rd-generation cephalosporins, or ticarcillin/clavulanic acid. Bactericidal drugs are preferred because neonates have suboptimal defense mechanisms and most infected foals have total or

partial failure of passive transfer of colostral antibodies. The disease process can interfere with drug absorption; therefore, parenteral routes should be used in systemically ill foals; the IV route is preferred initially. Avoid using antimicrobials that require extensive hepatic metabolism prior to excretion (e.g., chloramphenicol, erythromycin), especially in systemically ill foals with impaired liver function and in premature foals.

Several trends in antimicrobial susceptibility have been identified over the last 40 years in the United States including a decrease in the percentage of isolates sensitive to gentamicin, an increase in minimum inhibitory concentration (MIC) values of several bacteria, including Enterobacteriacae, to amikacin, a decrease in the percentage of isolates sensitive to ceftiofur and an increase in MIC values of *Enterococcus* spp. and *Pseudomonas* spp. to ceftiofur.[90] Based on a review of UC Davis equine neonatal septicemia isolates from field and in-house cases, the probability for antimicrobial susceptibility: 100% imipenem, 90% to 99%; ciprofloxacin, ceftazidime; 80% to 89% ceftriaxone, amikacin, netilmicin, cefaperazone, ceftizoxime; 70% to 79% aztreonam, gentamicin; 60% to 69% ceftiofur, chloramphenicol, ticarcillin/clavulanate, trimethoprim/sulfamethoxazole, ipericillin, azlocillin; 50% to 59% amoxicillin/clavulanate, ampicillin/sublactam, tetracycline, cephalothin; 40% to 49% ticarcillin; 20% to 39% ampicillin, penicillin G, sulfamethazine; <20% rifampin, oxacillin, erythromycin, tylosin. The choice of starting antimicrobial therapy is a clinician's choice. One popular combination is ceftiofur 10 mg/kg IV slowly BID and amikacin 21 mg/kg IV or IM once daily; this is based on our studies, with isolates we have found, and may vary geographically. If you have nothing to go on in your area try using procaine penicillin 20,000 units/kg IM and gentamicin 6.6 mg/kg IM, both given once daily, or ceftiofur (5 mg/kg IM).

Plasma Therapy

Use of commercial USDA approved plasma is desirable as donors may be hyperimmunized, tested for disease conditions, and plasma is free of red blood cells. United States sources of plasma include: Plasmavac Inc. (formerly Veterinary Dynamics)[a], Lake Immunogenics, Inc.[b], MgBiologics[c]. Canadian sources include: Centaur Pharmaceuticals[d].

If commercial plasma is not available, plasma can be collected from a suitable donor; however, caution is advised as anaphylaxis is much higher with untested plasma. Preselection of suitable blood/plasma donors on a large breeding farm or in a large practice is possible using blood typing procedures. The preferred donor is negative for A, Q, and C erythrocyte antigens and contains high level of antibody to indigenous pathogens. Alternately, blood from the dam, a Shetland pony, or unrelated gelding with no history of transfusion, may be used if the situation warrants. Plasma collected by plasmapheresis is preferred as centrifugation obtained plasma may not be red blood cell (RBC) free. Plasma immunoglobulin content should be quantitated if possible and assessed for anti-RBC alloantibodies, which could produce neonatal isoerythrolysis. Plasma can be administered immediately after collection or frozen and subsequently thawed at the time of administration although frozen storage should not exceed 3 years. Storage of non-frozen plasma increases the risk of contamination; use immediately after collection or thawing and do not refrigerate non-commercial harvested plasma longer than 12 to 24 hours.

Fluid Therapy

Briefly, any hypoglycemia, acidosis, and dehydration should be corrected and renal perfusion maintained. Once initial deficits have been replaced, administration of an isotonic electrolyte fluid containing a physiologic concentration of sodium to neonatal foals that require *maintenance* fluid therapy leads to sodium retention. Therefore, reserve isotonic fluids for correction of initial deficits and then replace with hypotonic fluids such as 5% dextrose. Additionally, relative to body weight, total body water, plasma volume, and extracellular fluid volume (ECF) are much larger in foals. Compared to adults, water content of the body is 10% higher (75–80% versus 65–70%). The extracellular and intracellular ratio is 50%:50% in neonates compared to 40%:60% in adults with 75% of the extracellular fluids within the interstitium. The colloid oncotic pressure of the plasma is lower than in adults (20 mmHg versus 25 mmHg); therefore, increased hydrostatic pressure can lead to interstitial edema more rapidly than in adults (overhydration).[100,101] As such, it is absolutely essential that the initial body weight be measured and subsequently recorded at regular time intervals (at least daily). Foals on maintenance fluids should have small daily weight gain. Significant weight loss indicates inadequate, inappropriate therapy, or excessive fluid losses, and the cause should be determined. Large, rapid and continued weight gain suggests fluid accumulation and the cause should be determined. Urine output should be high (6 ml/kg/h). Oliguria or anuria in the face of fluid therapy indicates a serious problem and the cause must be promptly and fully determined. Further specifics and guidance on fluid therapy can be found in Madigan.[102]

Nutritional Support

Partial or total parenteral nutrition is routinely used and is an integral part of neonatal intensive care. Advances in delivery systems and development of long-term, double or triple lumen intravenous catheters have allowed greater utilization of parenteral nutrition. In contrast to human infants, foals should gain a significant amount of weight daily (1–3 lb, ≈1 kg) following birth. Further spe-

cifics and guidelines for administration can be found in Madigan.[103,104]

Other Therapies

Polymixin (6000 U/kg IV TID) has proven useful in improving attitude scores and blood glucose concentrations and decreasing lactate and cytokine values in an endotoxemic model of foal sepsis.[105] It should be noted that use in clinically compromised foals is still lacking.

Prognosis and Outcome

Prognosis for survival is guarded with blood culture positive foals; mortality may be 50% even with intensive care. If foals present collapsed and comatose, the prognosis is very poor. Complicating potential sequelae to septicemia include osteomyelitis, corneal lesions, pneumonia, patent urachus, arthritis, joint infections, and gastric ulcers.

Short-term survival is negatively associated with age at admission, septic arthritis, band neutrophil count, and serum creatinine concentration whereas factors positively associated with survival include diarrhea, rectal temperature, neutrophil count, and arterial blood pH.[89] Reports of the effect of neonatal sepsis on future athletic performance vary. Sanchez et al state that surviving Thoroughbred foals (n = 102) did not differ from siblings with regard to percentage of starters, percentage of winners, or number of starts; although surviving foals had significantly fewer wins and total earnings.[89] In contrast, however, a longitudinal study of 35 foals showing clinical symptoms indicating septicemia within their first 18 hours postpartum and 88 control foals determined that a significantly higher proportion of septic foals (29%) compared to control foals (7%) were killed or died before 2 years of age ($p = 0.001$).[106] The majority of the remaining septic foals were poor performers and some were used only for pleasure riding.[106]

Take Home Point: Preventing Septicemia

1. Keep the mare in the facility in which foaling will take place to allow production of antibodies to pathogens within this environment. Clean foaling stalls twice daily and disinfect stalls between use. Wash the mare before foaling from the withers caudally.
2. Immediately following delivery, prevent the foal from contacting the mare until steps 3 and 4 are completed.
3. Wash the mare after foaling with large volumes of soap and water to remove bacteria around the perineum, udder, and rear quarters, which the foal may lick during udder seeking. Dry the mare.
4. Milk the mare's cleaned mammary gland of 2 to 4 oz of colostrum (preferably greater than 1060 specific gravity) and bottle feed the foal, prior to the foal rising, upon obtaining a suck reflex. Use colostrum from colostrum bank if necessary.
5. If the foal is weak, tube feed the foal within 1 hour of birth with 6 to 8 oz of colostrum or, if none available, use mare milk replacer or, if none available, use cow's milk. In orphan foals, continue feeding from a bottle or pan until 10% of body weight is fed. Feed when the foal is hungry.
6. For any foal from an unobserved birth and for foalings in an unclean area without the above precautions, I recommend veterinarians prescribe and commence antibiotic therapy within 8 hours of birth and treat for 48 to 72 hours only. Longer treatment may produce antibiotic resistance and should be reserved for ill foals. The choice of antibiotic therapy will vary with the geographical area. If you have nothing to go on in your area, try using procaine penicillin 20,000 units/kg IM and gentamicin 6.6 mg/kg IM—both given once daily or ceftiofur (5 mg/kg IM). Post birth antibiotics have been a routine part of management on stud farms in the United Kingdom for the past 40 years, and the incidence of sepsis is much lower than the United States. For those of you concerned about aminoglycoside antibiotics in foals, monitor serum creatinine or urinalysis if you so desire. I find aminoglycosides safe in foals that are kept hydrated; that is most important. In a bright, alert foal receiving short-term antibiotics, this should be no problem.

5. Management of the Umbilicus

Gumshoe Sleuthing

Data on umbilical care and disinfection in domestic animals is scarce. I was invited by the AAEP to give a presentation on Management of the Newborn Foal in 1990. I wanted to use the literature to obtain evidence for various post birth procedures we routinely perform. Studies on the care of the umbilicus were very limited with a paucity of evidence based medicine. Treatment of the umbilical stump is performed in an attempt to limit the number of microorganisms colonizing the area and prevent local and systemic infections which, in foals, can have severe consequences. In the 1990s, we recognized the need for a study describing the types of microorganisms that colonize the equine neonatal umbilical cord in the immediate post-birth period and to evaluate the effect of common umbilical disinfectants on the microflora of the external umbilical stump 6 hours after initial treatment.

We recruited 139 foals from two sites in northern California during the 1990 to 1991 foaling season.[83] Both study sites used clean, dirt floored stalls bedded with straw for foaling. Inclusion criteria were normal events of labor and delivery, and no physical signs of infection or congenital abnormality at birth.

Foals were randomly assigned to the following treatment groups:

- 2% iodine solution
- 0.5% chlorhexidine diacetate solution
- 1% povidone iodine solution
- 7% iodine tincture
- no dip used (dry cord)

Umbilical stumps were dipped at birth and then again when foals were 6 to 8 hours of age by submerging the stump in 12 cc of dip solution for 3 seconds. Only 20 foals were available to the 7% iodine tincture and dry cord groups because it was anticipated that these treatments would be detrimental.[107,108] Samples for aerobic and anaerobic bacterial culture were aseptically collected prior to the first dip, generally within the first 10 minutes postpartum, and just prior to the second umbilical treatment at 6 hours postpartum. By 6 hours, most umbilical stumps were dry and partially closed and, therefore, the sample was taken from the most distal part of the stump, underneath any attached epithelial flaps. The first swab was used to evaluate the types of bacteria that initially colonized the umbilical stump at birth. The second swab reflected changes in the bacterial flora after the single application of an umbilical dip.

The most common colonizers of umbilical stumps were skin and soil bacteria that are generally nonpathogenic in foals. Coagulase-negative staphylococci were the most prevalent organisms found (59% of all foals) with diphtheroids the next most common organism (40%). All other bacteria and fungi identified had a prevalence of less than 20%, with most less than 10%. A single *Clostridium* sp. was the only anaerobic bacterium found on umbilical stumps at birth and was reported from only one foal. One fungus was isolated (*Scopulariopsis*) from a single foal. Enteric bacteria were not frequently recovered from umbilical stumps despite the most common bacterial organisms recovered from umbilical abscesses reported in the literature to beGram-negative rods, especially *Escherichia coli*, and gram positive cocci, especially B-hemolytic *Streptococcus* species.[94,109] Four foals developed umbilical abscesses limited to the external stump following treatments; two in the dry cord group, one foal treated with 2% iodine, and one foal treated with povidone iodine.

Foals that did not receive any umbilical dip showed continued growth of all bacterial species seen at birth. Frequency of umbilical stumps with no organism growth at 6 hours after birth were 3% foals receiving 2% iodine, 24% foals receiving chlorhexidine, no foals receiving 1% povidone iodine, 40% foals receiving 7% iodine, and 13% of dry cord care foals. There was no significant change in bacterial flora within any single treatment group when bacterial colonizers at birth and 6 to 8 hours later were compared. Both 2% iodine and 1% povidone iodine groups had approximately the same frequencies of microflora seen at birth, and both showed an increase in the relative number of colonies that could be recovered at 6 hours. When compared to no treatment, these two dips reduced the number of colony-forming units on the umbilical stumps but had greater numbers of colonies compared to chlorhexidine treated stumps. The inactivation of iodine solutions by contact with organic debris and secretions is well documented[110] and may have occurred on application to a freshly severed umbilical stalk. A single application of chlorhexidine resulted in a marked reduction in the quantitative recovery of organisms from the umbilical stump and appears to be more effective than 2% iodine or 1% povidone iodine in suppressing the number of organisms colonizing the umbilicus. Chlorhexidine is reported to have sustained residual activity,[111] not to be inactivated by organic matter, and to bind to the stratum corneum leaving a persistent residue.[110]

The use of 7% iodine resulted in the lowest recovery of viable organisms and the highest percentage of umbilical stumps showing no recoverable growth of microorganisms; however, this dip rapidly desiccated the stump, leaving a long tail, and occasionally caused sloughing of adjacent skin, both of which negate its value for routine use. Almost half (4 of 10) of the foals in this group developed patent urachus when the epidermal tail broke off after 3 to 5 days. The caustic nature of iodine at this strength and its potential damaging effects on the skin do not make it an appropriate routine umbilical dip. Martens suggested that 7% iodine, a strong irritant, may cause bacteria to become trapped inside the umbilical stump after use, with the potential for focal abscessation.[108]

Take home point: 0.5% chlorhexidine solution is a superior foal umbilical disinfectant.

Comparative Human Data

Umbilical infections in human neonates are now rare in developed countries. Since 1998 the World Health Organization (WHO) has advocated for the use of dry umbilical cord care (leaving untreated, open to the air, or loose covering), and cleansing with water only if soiled, and the use of topical antiseptics (e.g., chlorhexidine) in poor hygiene births such as in developing countries.[112] A 2004 Cochrane review[113] (n = 8959 newborns, 21 studies) found no benefit of antiseptic or antibiotic umbilical stump application on neonatal mortality or rates of disseminated or localized infection compared with dry cord care; however, this may not be applicable in the developing countries as most included studies were from high-income countries, and all but 1 were conducted in hospital settings. Data on the incidence of omphalitis in developing countries is generally scarce; the available data estimate the risk to range between 2 and 77 per 1000 live births in hospital settings and 217 per 1000 live births in community-based births.[114] Subsequent meta-

analysis of data from developing countries has shown that the use of 4% chlorhexidine reduces neonatal mortality and morbidity among infants born at home.[115] Similarly, cord cleansing with 4% chlorhexidine immediately after birth in rural Bangladesh reduced overall and organism-specific colonization of the stump with greater reductions and longer effect with daily cleansing through the first week of life.[116] A subsequent comprehensive Cochrane review[117] of 34 trials involving 69,338 babies published in 2013 reviewed three large, cluster-randomized trials conducted in community settings in developing countries (78% of the total number of children) and 31 studies conducted in hospital settings, mostly in developed countries. There were 22 different interventions studied across the included trials. The most commonly studied antiseptics were 70% alcohol, triple dye (brilliant green, crystal violet, and proflavine hemisulfate) and chlorhexidine, but only chlorhexidine was studied in community settings. Combined results of the three community trials showed a reduction of 23% (average risk ratio (RR) 0.77, 95% confidence interval (CI) 0.63–0.94) in the chlorhexidine group compared with control. The reduction in omphalitis ranged from 27% to 56% depending on the severity of infection. Cord separation time was increased by 1.7 days in the chlorhexidine group compared with dry cord care (mean difference 1.75 days, 95% CI 0.44–3.050). Washing of umbilical cord with soap and water was not advantageous compared with dry cord care in community settings. Among studies conducted in hospital settings, no antiseptic was advantageous to reduce the incidence of omphalitis compared with dry cord care; however, topical triple dye application reduced bacterial colonization with *Staphylococcus aureus* compared with dry cord care (average RR 0.15, 95% CI 0.10 to 0.22, four studies, n = 1319) or alcohol application (average RR 0.45, 95% CI 0.25 to 0.80, two studies, n = 487). There was no advantage of application of alcohol and triple dye for reduction of colonization with *Streptococcus*. Topical alcohol application was advantageous in reduction of colonization with *Enterococcus coli* compared with dry cord care (average RR 0.73, 95% CI 0.58 to 0.92, two studies, n = 432) and in a separate analysis, triple dye increased the risk of colonization compared with alcohol (RR 3.44, 95% CI 2.10 to 5.64, one study, n = 373). Cord separation time was significantly increased with topical application of alcohol (MD 1.76 days, 95% CI 0.03 to 3.48, nine studies, n = 2921, random-effects, $T^2 = 6.54$, $I^2 = 97\%$) and triple dye (MD 4.10 days, 95% CI 3.07 to 5.13, one study, n = 372) compared with dry cord care in hospital settings. The review concluded that topical application of chlorhexidine to the umbilical cord reduces neonatal mortality and omphalitis in community and primary care settings in developing countries; however, there is insufficient evidence to support such application in hospital settings in developed countries. Whilst chlorhexidine application may increase cord separation time, there was no evidence that it increases risk of subsequent morbidity or infection.

6. Meconium Impaction

Gumshoe Sleuthing

During our weekly foal rounds reviewing neonatal cases in our critical care unit, we were discussing a poor outcome on a meconium retention case that went to surgery. Sitting in the audience was our MD advisor Dr. Boyd Goetzman, a neonatologist at the UC Davis Sacramento Medical Center. He asked why we did not use an acetylcysteine enema. We knew nothing of this and began a study of volume and safety of acetylcysteine solution to soften meconium. We first collected meconium from a foal and placed one meconium pellet in each of 4 plastic jars containing water, dioctyl sodium sulfosuccinate (a common stool softener), 4% acetylcysteine, and mineral oil. We waited 30 minutes and put a glove on and had a blindfolded person squeeze each meconium pellet and rate the softness. We found acetylcysteine did a great job of softening. So we began using it in foals after several safety studies. In this situation, as in others, the evidence came later in a review by Pusterla.[118] Our clinic has not done a single meconium retention surgery since implementing this procedure.

Etiology

Meconium consists of digested amniotic fluid, glandular secretions, mucus, bile, and epithelial cells, is greenish black to light brown, has little odor, and has a tarry consistency. It is usually first seen to be evacuated from the foal within 3 hours after birth. Meconium is considered retained if the foal makes frequent attempts but fails to produce meconium by 12 hours of age. Meconium impaction is the most common cause of rectal obstruction in foals[119] with colts appearing to be more commonly affected[108,118] suggesting a narrowed pelvis plays a role. Foals display restlessness, tail swishing, frequent posturing to defecate or urinate, tail elevation, and disinterest in sucking or colic and abdominal distention if advanced. Most impactions are located at the pelvic inlet in the small colon (low retention), but they may also be located in the dorsal or transverse colon (high retention). Diagnosis is based on clinical signs and detection of a firm mass upon digital rectal examination (low impaction), lack of passage of milk stool, and abdominal palpation or radiography of fecal masses in the colon.[120] Our work with treating severe colic due to meconium retention with acetylcysteine solution enemas[121] suggests to me that "high" meconium retention is not a common syndrome and most of the problem is at the pelvic inlet.

Treatment With Acetylcysteine

Traditional treatment consists of administration of multiple enemas, such as commercial phosphate en-

emas, soapy-water enemas, mineral oil, and liquid paraffin. Enemas can be administered per rectum through a soft, flexible tube by gravity flow. Caution should be used to avoid mucosal trauma with a tube in the rectum. Forceps or firm metal instruments to grasp the meconium are not recommended due to risk of trauma and mucosal penetration. If 3 or more enemas are unrewarding, consider using 4% acetylcysteine. These have been used successfully in human infants with meconium plug syndrome.[122] Acetylcysteine cleaves disulphide bonds in the mucoprotein molecules and decreases the tenacity of the abnormal meconium, working best at pH 7 to 8.[123] The 4% solution is hypertonic and will cause some loss of fluid into the bowel, an action which may help detach the meconium from the bowel wall. Acetylcysteine has been shown to be safe when used as a 4% solution and did not induce mucosal damage in an animal model.[124] Acetylcysteine requires 30 to 45 minutes before maximum effect occurs and, therefore, the enema must be retained within the colon using a Foley catheter.

A commercial acetylcysteine retention enema[e] is now available although enemas can also be prepared in situ. To prepare acetylcysteine solution from powdered N-Acetyl-L-Cysteine: add 1.5 level tablespoons (20 gm) of baking soda ($NaCO_3$) powder to 200 ml of water and then add 8 g of acetylcysteine (1 packed tablespoon); this makes a pH 7.6 solution. In very sick foals, hypernatremia from the baking soda may result. To prepare solution from Mucomyst[f] add 40 ml of the 20% solution (10 ml vials) to 160 ml of water to make the 4% solution; this costs approximately $30.00. The solution is pH balanced and, therefore, is preferable.

Administration:

1. Restrain foal, with sedation if required.
2. Insert a size 30 French Foley catheter with 30 cc balloon[g] into the rectum approximately 1 to 2 in.
3. Inflate balloon on end of catheter slowly.
4. Administer 4 to 8 oz (120–240 ml) of 4% acetylcysteine slowly.
5. The Foley catheter allows retention of the enema; keep in for up to 45 minutes, then deflate balloon, remove catheter.
6. May repeat enema in 1 hour.
7. May require 1 to 3 hours to soften meconium and pass stool.

This treatment of refractory meconium retention has been very successful in our hands.[118,121] Of 41 foals treated with acetylcysteine retention enemas at UC Davis, 24 foals received one acetylcysteine enema, 12 received 2 enemas, and 5 received 3 enemas (average of 1.5 enemas/foal).[118] No complications associated with the administration of acetylcysteine enemas were encountered. Time for meconium retention to resolve ranged from 1 to 96 hours (mean ± SD 11 ± 16 hours). The meconium retention resolved within 6 hours in 24 foals, up to 12 hours in 8 foals, up to 24 hours in 7 foals, and after 48 and 96 hours in one foal each. Intravenous fluids and analgesic drugs were administered to 32 and 25 foals, respectively. The fluid of choice was lactated Ringer's solution, and butorphanol (0.01 mg/kg bwt) or flunixin meglumine (1 mg/kg bwt) were used to relieve pain. Hospitalization time for 37 foals treated medically was 1 to 5 days (average 1.9 days).

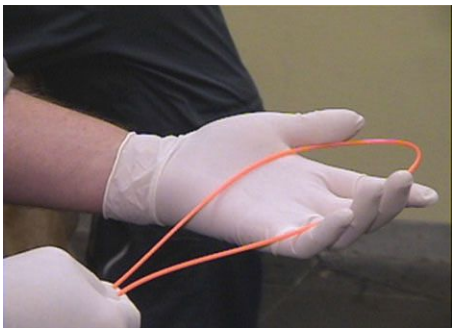

Fig. 10. Weed wacker (strimmer) wire bent for use in meconium retention enema evacuation.

Meconiumectomy

If the administration of two acetylcysteine enemas has not worked, consider the use of plastic "weed wacker" (strimmer) wire (smooth, not serrated) such as used to cut weeds on a machine (Fig. 10). Cut the "plastic wire" about 14 inches in length and bend it to form a loop at one end. Insert the bent part of the loop into the rectum and continue passage so that it goes through the pelvic inlet. The loop then expands around the meconium; pull it back and a big wad of meconium will be on the end within the loop (Fig. 11). Repeat as needed and use plenty of lube. If the foal's rectum becomes very swollen, the foal may need one dose of (2 mg) dexamethasone and antibiotics because bacteria may translocate across the inflamed gut. Flunixin can be administered for pain.

Fig. 11. Evacuation of meconium using weed wacker (strimmer) wire.

7. *Rhodococcus Equi* Infection

Gumshoe Sleuthing

A local Thoroughbred breeding farm, which had a resident veterinarian, Dr. Noel Muller, was having significant *R. equi* problems. One year they had 8 deaths and 26 confirmed cases, and Dr. Muller was asking us for some help. Sharon Heitala had just completed her PhD on *R. equi* and found there was some evidence for humoral immunity. Dr. Muller said they were willing to try anything, so we made a bacterin (a killed or weakened bacteria for use as a vaccine) out of an isolate from the farm, vaccinated donors and harvested the plasma, froze the plasma, and administered it within 24 hours of birth one time. The results were stunning. The first year we used the plasma there were 68 foals and no deaths or foals requiring treatment for *R. equi*. The next year we gave plasma (1 liter post birth) to 101 foals, and 14 foals did not receive plasma. Only 3% of plasma-treated foals developed *R. equi* pneumonia, with no deaths, compared to 6 of the 14 (43%) foals that did not receive plasma, 2 of which died. Why this worked so well compared to today's plasma treatments to prevent *R. equi* is part of an ongoing study to be discussed in the Milne lecture.

Etiology

Rhodococcus equi, a gram positive coccobacillus, causes chronic purulent bronchopneumonia in foals less than 6 months of age and is a significant cause of wastage to the equine breeding industry, especially on farms where the disease is endemic. An 80 to 90 kb plasmid encoding nine virulence-associated proteins (Vaps), termed VapA, VapC-VapI, and pseudo-VapE, is important for pathogenicity. VapA appears to be the most significant of these proteins. The organism is ubiquitous in the soil, particularly dry and dusty soil, and bedding on equine farms such that foals are exposed to *R. equi* within the first few days of life. The majority of exposed foals develop protective immune responses; however, some foals appear susceptible to infection due to an immaturity of their immune system.[125] Epidemiological study of *R. equi* pneumonia has determined a seasonal incidence that peaks late spring and summer when the high number of foals coincides with an increased risk of aerosol challenge from the environment and/or herd mates.[126] An excellent review of the immunological response to *R. equi* is provided by Dawson et al.[127]

Overview of the Use of Hyperimmune Plasma

The observation that *R. equi* pneumonia typically coincides with the decline in maternal antibodies suggests that antibodies play a protective role and is the basis for administering hyperimmune plasma.[128,129] The goal of specific hyperimmune plasma is to provide the foal with a broad spectrum of specific anti-*R. equi* antibodies, and perhaps other immunomodulators, to enhance the humoral response to infection. Use of hyperimmune plasma for prevention of *R. equi* pneumonia has shown inconsistent results. A reduction in foal morbidity and mortality has been reported by some authors[128,130–134]; however, other studies have described hyperimmune plasma as unsuccessful in preventing *R. equi* disease.[135–137]

The mechanism by which hyperimmune plasma may have a protective effect is unknown. Purified immunoglobulin specific for VapA and VapC gave similar protection against *R. equi* disease as commercially available hyperimmune plasma suggesting a primary protective role for antibodies against *R. equi* VapA and VapC.[133] However, there appears to be no correlation between total serum IgG concentrations and the concentration of specific anti-*R. equi* antibody, and colostrum-derived *R. equi* antibody is not as protective as *R. equi* antibodies provided by hyperimmune plasma.[128,138] Hyperimmune plasma may provide other, unknown, nonspecific immune factors that are absent from colostrum, such as fibronectin, complement, and cytokines.[128]

The effectiveness of hyperimmune plasma is likely to be affected by the dose, timing of administration, innate immune system competence, management conditions, and number of virulent bacteria in the environment.[127] Plasma containing low quantity and/or quality of specific anti-*R. equi* antibodies, such as against VapA and VapC, is unlikely to be efficacious. Use of a vaccine strain genetically different to field strains or inappropriate donor vaccine dose and adjuvant are just two possible causes of such plasma.

Environmental management of contamination is also important in prevention of disease and should be used in conjunction with immunoprophylaxis. Infected foals are a major source of contamination, shedding up to 10^6 CFU of virulent *R. equi*/gram of feces.[139] Additionally healthy foals and mares may excrete up to 10^5 *R. equi*/gram of feces.[140,141] The removal of infected manure alone, without other preventative measures, has failed to reduce *R. equi* disease on an Australian farm.[142] Aerosol transmission between foals from the respiratory tract has also recently been suggested as another means of disease transmission in the field.[143] It is unknown if administration of hyperimmune plasma reduces bacterial shedding from infected foals via the respiratory or gastrointestinal tract.

8. Studies in Transitions of Neonatal Consciousness: Why Foals Do Not Gallop *In Utero*

Gumshoe Sleuthing

Since starting our neonatal critical care unit at UC Davis in the late 1980s, we have treated many of what is termed the "dummy foal." Many different names for this condition have been proposed, all revolving around the assumption that the cause is low oxygen or poor perfusion; Hypoxic Ischemic En-

cephalopathy (HIE), Perinatal Asphyxia Syndrome, and other names have been used. The question for me was why do 80% of foals with this severe hypoxia recover within 2 to 7 days, with no residual neurological deficits? In any other mammal, hypoxia that is sufficient to cause clinical signs of hypothermia, reduced gut motility, compromise of the renal system, hypoventilation, seizures, weakness, disorientation, failure to recognize the maternal source of milk, etc., causes long-term adverse outcomes. This is not the case with the maladjusted foal. We have found in the dummy foal and other sick foals, a persistence of the sedative neurosteroids, which play a role in keeping foals from galloping *in utero*. This can, and does, cause the clinical signs seen in dummy foals and opens a huge avenue of potential for prompt reversal of symptoms. Learning about these neurosteroids is important. We believe that the pressure of the birth canal during stage 2 labor, which is supposed to last 20 minutes, is an important signal that tells the foal to quit producing the sedative neurosteroids and wake up. We are in the early stages of treating the dummy foal by recreating the birth process via a squeeze system to mimic 20 minutes of birth canal pressure. The preliminary results have been dramatic.

Neurosteroids: Background

Certain steroidal compounds, predominantly 5α-reduced pregnanes, can cross the blood brain barrier and have neuromodulatory effects as neuroactive steroids (neurosteroids).[144] These pregnane metabolites are primarily synthesized within the central nervous system (CNS) from cholesterol, via progesterone, by 5α-reductase action but can also be synthesized in other tissues and readily cross the blood brain barrier.[145] These neuroactive steroids modulate gamma-aminobutyric acid (GABA), glutamate and opioid neurotransmission affecting brain development and functioning.[146] Steroids exert organizational and activational actions during brain development and modulate neurotransmission either by directly interacting with neurotransmitter receptors or by genetic mechanisms. Pregnenolone sulphate and dehydroepiandrosterone (DHEA) sulphate are excitatory, being allosteric antagonists of $GABA_A$ receptors and agonists of N-methyl-D-aspartate (NMDA) receptors, whereas pregnenolone, allopregnanolone, and androsterone are allosteric agonists of $GABA_A$ receptors and are neuroinhibitory.[146] The final neurophysiological outcome may, therefore, depend on the relative ratios of excitatory and inhibitory steroids.

The Transition From Fetus to Neonate

Unequivocal changes in mammalian fetal steroid hormones are a prerequisite for the transition from the quiescent intrauterine fetal state to active extrauterine life where suckling and following the dam within a few hours of birth is required. The uterus plays a key role in providing the chemical and physical factors that together help to keep the fetus continuously asleep. This is thought to be achieved through the combined neuroinhibitory actions of a powerful EEG suppressor and sleep inducing agent (adenosine), two neurosteroidal anesthetics (allopregnanolone, pregnanolone), and a potent sleep-inducing hormone (prostaglandin D2), acting together with a putative peptide inhibitor and other factors produced by the placenta, further supported by the warmth and cushioned tactile stimulation of the uterine environment.[147] Injections of progesterone or its metabolites into the ovine fetal circulation in late gestation reduce fetal electroencephalograph, electrocorticograph, and electrooculograph activity, breathing movements and behavioral arousal, while inhibition of placental progesterone enhance these parameters.[148–151]

The loss of placentally-derived precursors at birth and the switch to adrenal or other derived precursors causes a dramatic decline in pregnane concentrations shortly after birth in healthy neonates.[152] Studies of healthy neonatal foals have shown high concentrations of pregnanes at birth that decrease rapidly, to essentially zero, over the first 48 hours of life.[153,154] Reduction of these CNS depressant pregnanes is accompanied by increased alertness and arousal.

Infusion of Pregnanes to Healthy Neonates: An Inducible Model of Neonatal Maladjustment Syndrome?

Infusion of two healthy neonatal foals with the pregnane allopregnanolone induced obtundation, lack of affinity for the mare and decreased response to external stimuli,[155] similar to clinical signs observed in neonatal maladjustment syndrome (NMS) foals. Similarly, infusion of 5α-reduced pregnanes into rodents leads to anesthesia or marked behavioral effects[156] suggesting that these pregnanes cross the blood-brain barrier and exert neuromodulatory effects. The effects observed following allopregnanolone infusion were short-lasting and associated with measurable concentrations of pregnanes, which peaked in conjunction with maximum neurobehavioral effects, and EEG abnormalities such as slow wave sleep while standing (Estell et al, unpublished data, 2013). Allopregnanolone also caused bradycardia and subjectively decreased gastrointestinal borborygmi due to either general sedative effects of the steroid or potentially increased vagal tone (Estell et al, unpublished data, 2013).

A range of neurobehavioral abnormalities are observed in NMS including obtundation, seizures, and hyperesthesia. Whilst the infused steroid allopregnanolone has a dampening effect in the CNS, others within the large spectrum of neurosteroids, including metabolites of allopregnanolone, have excitatory effects that may be associated with seizures and hyperesthesia.[157]

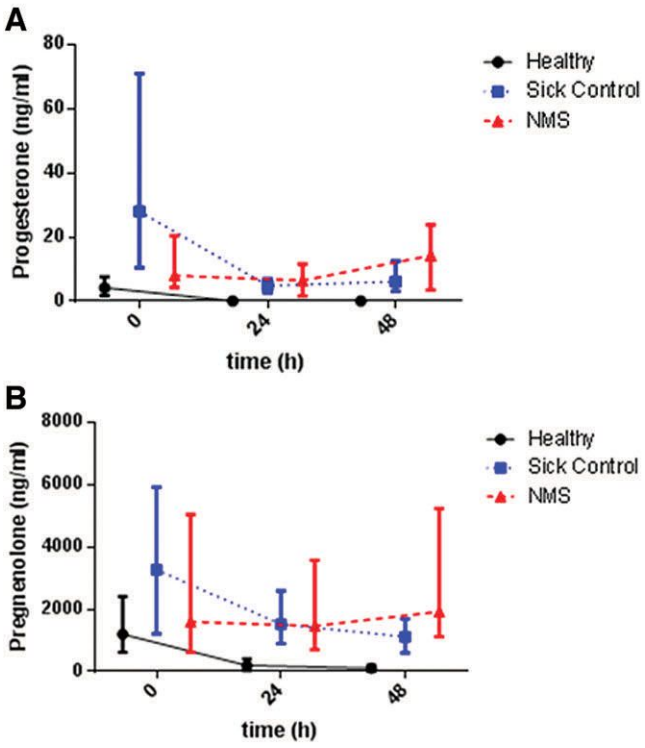

Fig. 12. Progesterone concentrations in healthy, NMS, and sick control foals. Pregnenolone concentrations in healthy, NMS, and sick control foals. Reprinted with permission from Aleman M, Pickles KJ, Conley AJ, et al. Abnormal plasma neuroactive progestagen derivatives in ill, neonatal foals presented to the neonatal intensive care unit. *Equine Vet J* 2013;45(6):661–665.

Neurosteroids and the Sick Neonate

Sick foals presented to the neonatal intensive care unit have elevated concentrations of pregnanes compared to healthy neonates.[154] In this study, sick foals comprised foals with NMS (n = 32) and foals with other neonatal disorders, including sepsis (sick control, n = 12). Healthy foals showed a significant decrease in pregnane concentrations over the first 48 hours of life ($p < 0.01$). Foals with NMS and sick control foals had significantly increased progesterone, pregnenolone, androstenedione, dehydroepiandrosterone, and epitestosterone concentrations compared to healthy foals ($P < 0.05$). Progesterone and pregnenolone concentrations of sick control foals decreased significantly over 48 hours ($P < 0.05$), whereas concentrations in NMS foals remained elevated and showed a trend of increasing concentration over time (Fig. 12A and 12B). Serial blood sampling and pregnane measurement may, therefore, prove useful in aiding differentiation between NMS and sepsis.

These observations support the hypothesis of a delayed, or interrupted, conversion from intra- to extrauterine life in ill, neonatal foals, particularly those with NMS. We propose that NMS may comprise of more than one phenotype: foals with hypoxia and ischemia and foals with persistence of, or reversion to, fetal hypothalamic-pituitary-adrenocortical (HPA) axis and increased pregnanes. Multiple phenotypes would explain the lack of histological evidence of hypoxia in many maladjusted foals as well as their rapid and full recovery. Specific enzymes may be inhibited in these foals and the roles of 5α-reductase, 3β-hydroxysteroid dehydrogenase and 3α-hydroxysteroid dehydrogenase are being further evaluated. The underlying cause of any possible abnormal adrenal function is also not known; it may reflect a state of dysmaturity in which the foal fails to transition to extrauterine life or may reflect hypoxic injury to the HPA axis.[158] Another potential reason for persistence of fetal hormones is a failure of normal events of parturition that are essential for the transition from the *in utero* fetal cortical status to extrauterine behavioral status. Regulation of the neuroactive steroid content in the fetal ovine brain is independent of adrenal steroidogenesis and hypothalamic-pituitary factors[159]; however, in the neonate, concentrations of some neurosteroids and their precursors in the peripheral circulation dramatically affect concentrations in the brain.[160] Lastly, another possible mechanism would be the reversion to fetal cortical status when adverse post-birth circumstances occur. The syndrome of reversion to fetal circulation is a well-known and accepted consequence of adverse birth and post birth events, which is seen in both maladjusted and foals with other neonatal diseases and causes the neonate to revert to mechanisms that regulated the cardiovascular system *in utero*.

It is also possible that the increased pregnanes are acting in a neuroprotective role as has been reported in other species. Stress (hypoxia, endotoxin) in the neonatal period increases neurosteroid concentrations in the brain of newborn lambs,[152,161] suggested to represent an endogenous protective mechanism. Similarly, acute, but not chronic, hypoxic stress during pregnancy increases fetal neurosteroid concentrations[152] and inhibition of neurosteroid synthesis increases asphyxia-induced brain injury in late gestation fetal sheep.[162]

Manipulation of Elevated Neurosteroid Concentrations

The observed elevated concentrations of pregnanes in sick neonatal foals and the known sedative and anesthetic properties of such compounds invite speculation that decreasing plasma pregnane profiles would be correlated with positive clinical outcome.

The enzyme 5α-reductase is believed to be the rate limiting step in the production of 5α-reduced pregnanes.[160] This enzyme can be very efficiently blocked by the 5α-reductase inhibitor drugs finasteride and dutasteride, which have been extensively studied in laboratory animals and humans due to their use in treatment of prostate cancer. Dutasteride, the more efficient of these drugs, is well tolerated in humans, with a profile comparable with that of placebo. A safety study of dutasteride ad-

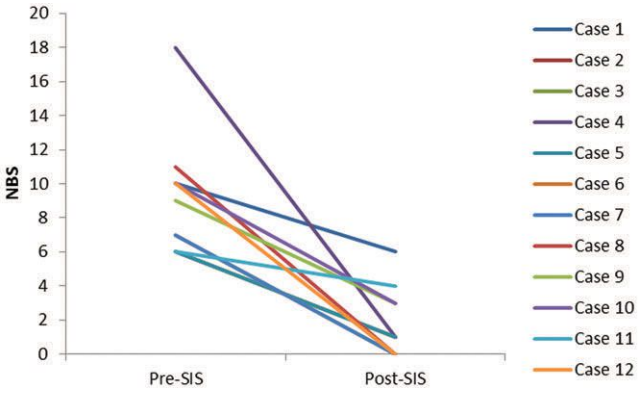

Fig. 13. Neurobehavioral scores (NBS) at time points pre- and post-SIS in 12 neonatal foals with clinical signs compatible with neonatal maladjustment syndrome. Foals had significantly lower NBS post-SIS ($p = 0.003$).

ministration in five foals at ten times the recommended dose did not result in any adverse effects (Madigan et al, unpublished data, 2010). Investigation into the treatment of maladjusted foals with dutasteride is ongoing with preliminary observations appearing promising (Madigan et al, unpublished data, 2012).

Another potential method of reducing circulating neurosteroids is the use of strong tactile stimulation using a modified soft rope "squeeze" technique[163] to simulate compression of the young by the cervix and vagina during birth. The use of this squeeze procedure in foals with NMS, as an aid to transition to the extrauterine environment, has recently been investigated in 12 foals (Madigan et al., unpublished data, 2013). Foals underwent a 20 minute period of physical restraint using a rope applying constant pressure around the thorax (squeeze induced somnolence, SIS). All foals tolerated the squeeze procedure well, and no adverse effects were observed. All foals showed marked clinical improvement and survived to discharge. Clinical improvement such as an improved ability to stand and nurse unaided were observed very rapidly in some foals (within minutes of restraint release) but, in all foals, marked improvement was evident at 2 hours post-SIS. Neurobehavioral scores were significantly decreased following SIS (Fig. 13).

The mechanism by which SIS restraint appears to exert a positive effect is unknown. It may provide strong tactile stimulation, similar to that experienced during labor and passage through the birth canal, which elicits locus coeruleus (LC) noradrenaline-mediated neuroactivation.[147] The LC is a pontine nucleus located near the pontomesencephalic junction and is the largest group of noradrenergic neurons in the central nervous system having extensive projections to widespread areas of the brain and spinal cord.[164] The LC is known to be a major wakefulness promoting nucleus, with activation of the LC resulting in an increase in EEG signs of alertness and also plays an important role in controlling autonomic function, where LC activation produces an increase in sympathetic activity and a concomitant decrease in parasympathetic activity.[165] The LC is also important in sense of smell having dense inputs into the olfactory lobe, and, in the neonate, maternal odor recognition and imprinting is dependent on an intact LC.[166] The fetal experience of labor compressions (real or simulated) has been shown to exert a positive effect on neonatal rat pup suckling, respiration, and behavior.[167,168] Likewise, human babies exposed to labor, even if subject to later caesarean, display better olfactory exposure learning in the first hour postpartum than babies not exposed to labor.[169] Caesarean section and rapid birth are recognized risk factors for NMS.[91,170] The neonate may experience inadequate tactile stimulation in these parturition events for optimal LC activation.

An alternative explanation for the beneficial effect of SIS could be that the increase in DHEA-S elicited in foals during the procedure[163] is the cause of neuroactivation. DHEA-S is an NMDA agonist at nanomolar concentrations,[171] and such concentrations have been reported in the serum of foals during SIS restraint.[163] Additionally, DHEA-S is a $GABA_A$ receptor antagonist at low micromolar concentrations.[172] Whilst serum concentrations of DHEA-S undergoing SIS restraint were in the nanomolar range, local concentrations in the brain are likely to be significantly higher. DHEA and DHEA-S are also precursors of estrogens, which is interesting given that administration of 17β-estradiol has been reported to induce behavioral arousal, breathing, and subsequent survival in lambs that were initially "flat" or inactive after delivery by hysterectomy close to the time of normal birth.[173]

It is occasionally reported in the press that very sick human neonates, pronounced medically unlikely to survive, have made spontaneous and miraculous recoveries following continued, skin-to-skin holding by the grieving parents.[174] Perhaps these babies also benefit from squeeze induced LC stimulation or neuroactivation via other means.

Comparative Neonatal Neurosteroid Data

Pregnenolone concentrations in healthy human neonates also display a rapid and significant fall in both early preterm infants [95.78 nmol/liter (0 hours) to 36.69 nmol/liter (d 14)] and in full-term infants [66.62 nmol/liter (0 hours) to 14.81 nmol/liter (d 6)] (Table 1).[175] After 12 hours, significantly higher levels for pregnenolone were found in early preterm infants (98.01 nmol/liter and 69.13 nmol/liter) compared with full-term neonates (36.29 nmol/liter and 28.55 nmol/liter, $P < 0.05$) (Table 1), which was proposed to reflect increased fetocortical activity as a response to the stress of delivery in the premature infant.

Table 1. Serum Pregnenolone Concentrations in Human and Equine Neonates,[175] Mean and Range Data,[154] and Median and Range Data

	Pregnenolone (ng/ml)	
	Birth	**24 h**
Healthy, term infants[175]	21.1 (4.5–74.9)	8.8 (4.4–18.0)
34–37 week premature infants[175]	32.2 (10.8–78.9)	11.9 (11.3–18.6)
Term, healthy foals[154]	1199 (290–6005)	193 (28–545)
Sick foals[154]	3270 (996–12,891)	1514 (211–3473)
NMS foals[154]	1598 (82–16,828)	1463 (35–16,277)

Neurosteroids in Autism Spectrum Disorder

Autism and autism spectrum disorder (ASD) encompass a group of behaviorally defined neurodevelopmental disorders typified by communication impairment, social withdrawal, emotional deficits, anxiety, stereotypic behavior and movement, and sensory disturbance. It is typically diagnosed by behavioral manifestations, and biological markers are not well defined. ASD is 4 to 5 times more prevalent in male individuals,[176,177] which may suggest a role for steroid hormones in its pathobiology. Recent comparison of saliva concentrations of 22 steroids in prepubertal autistic male and female children from 2 age groups (3–4 years old and 7–9 years old) with those from healthy controls demonstrated that ASD children had significantly greater concentrations of many steroids, including androstenediol, dihydroepiandrosterone, androsterone, the steroid precursor pregnenolone, and allopregnanolone (Fig. 14).[178] Furthermore, moderate strength correlations existed between some neurosteroid concentrations and measures of autism severity (Fig. 15). Steroids found in greatest concentrations in the saliva of autistic children were polar conjugates of DHEA, pregnenolone, androsterone, epiandrosterone, and 20α-dihydroprogesterone. Cortisol concentrations were not different. Salivary steroid concentrations are thought to represent 1% to 10% of serum concentrations, depending on their conjugation status.

The behavioral and clinical appearance of the maladjusted foal shares some similarities with those of autistic children. It is difficult to compare serum

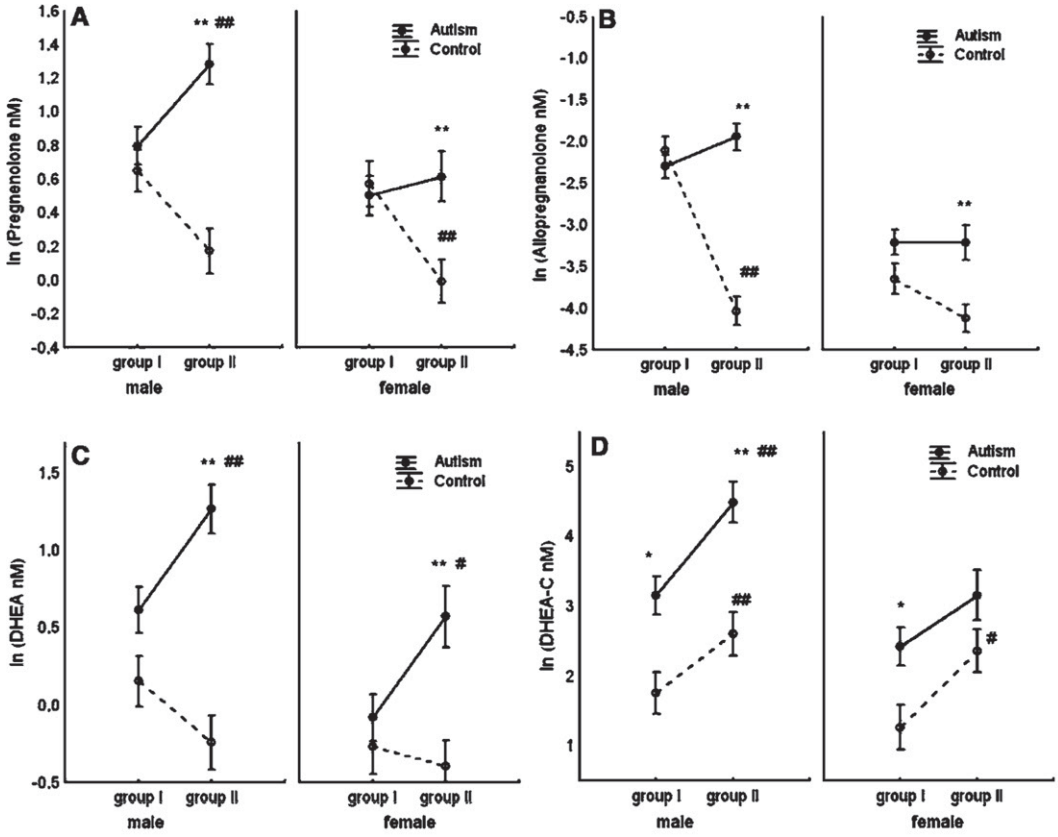

Fig. 14. Distinct patterns of developmental changes in salivary levels of neuroactive steroids (pregnenolone, allopregnanolone, DHEA, and DHEA-C) in autistic children than in healthy controls. Groups I and II refer to experimental age groups. Data represent natural logarithms of mean (nM) concentrations. Significant differences between autistic and control groups: *$p < 0.05$ and **$p < 0.01$. Significant differences between older and younger groups of either autistic or control children: #$p < 0.05$, ##$p < 0.01$. Reprinted with permission from Majewska M, Hill M, Urbanowicz E, et al. Marked elevation of adrenal steroids, especially androgens, saliva of prepubertal autistic children. *Eur Child Adolesc Psychiatry* 2014;23(6):485–498.

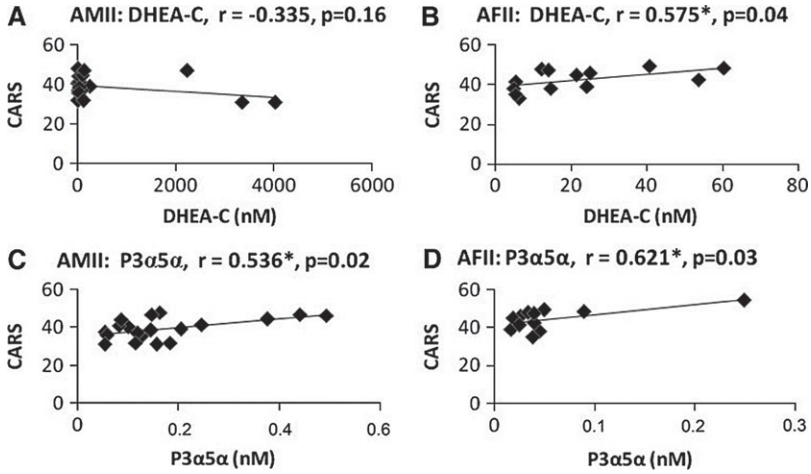

Fig. 15. Scatter plots of correlations between salivary levels of two major neurosteroids (DHEA-C and allopregnanolone/P3a5a) and CARS scores in older autistic boys and girls. A, C, Autistic boys (AMII); B, D, autistic girls (AFII). Stars denote statistically significant correlations; $p < 0.05$. Reprinted with permission from Majewska M, Hill M, Urbanowicz E, et al. Marked elevation of adrenal steroids, especially androgens, saliva of prepubertal autistic children. *Eur Child Adolesc Psychiatry* 2014;23(6):485–498.

neurosteroid concentrations from maladjusted foals with salivary concentrations in the above study due to the different ages of the populations and differing biological fluids. In general, however, serum concentrations in maladjusted foals were 100 to 1000 times greater than salivary concentrations from autistic individuals. We are currently exploring the role of neurosteroids in ASD in collaboration with medical colleagues at UC Davis.

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Material has been reproduced with permission from Pusterla and Madigan. *J Equine Vet Sci* 2013;33:493–496; Pusterla N, Madigan JE. *Neorickettsia risticii* infection. In: Sellon D and Long M, eds. *Equine infectious diseases*. 2nd ed. Amsterdam: Elsevier, 2013; Madigan JE. Method for preventing neonatal septicemia, the leading cause of death in the neonatal foal, in Proceedings. Am Assoc Equine Pract 1997;43:17–19.

Conflict of Interest

The Author declares no conflicts of interest.

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[b]Lake Immunogenics, Inc., Ontario, NY, 14519.
[c]MgBiologics, Ames, IA 50014.
[d]Centaur Pharmaceuticals, Guelph, Ontario N1H 6T9.
[e]EZ-Pass™, Animal Reproduction Systems, Chino, CA 91710.
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Lameness Imaging in the Sports Medicine Era: It's Not Fractures Anymore

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1. Introduction

The use of imaging modalities such as radiographs, ultrasound, nuclear medicine, computed tomography, and magnetic resonance imaging (MRI) have all aided in creating methods to identify abnormalities in the equine patient. Ten years ago, equine patients would have a lameness that was easily identified and imaging yielded a diagnosis. However, as owners and trainers have become more in tune with the equine athlete and the imaging modalities have become more advanced, it is not uncommon to have 6 to 10 imaging abnormalities identified without a clear or single diagnosis as the cause for the lameness. This shift from surgical lameness to sports medicine with the prevalent diagnosis being soft tissue injuries has led to the creation of the American College of Veterinary Sport Medicine and Rehabilitation.

With all of the existing imaging modalities and the increased availability of standing MRI and nuclear medicine to the practitioner, it is rarely the availability of the modality that is the limitation. In certain cases, an accurate or specific diagnosis is still lacking. In the equine realm, most imaging interpretation is done by practitioners or specialists with experience; however, board-certified radiologists have an integrated knowledge of all of the modalities and how they work together to help determine the specific modalities that have the best chance of seeing lesions and can help make a diagnosis. This knowledge allows for understanding the limitations of the modality and furthermore, understanding that a negative study provides both a list of differential diagnoses and excludes many differential diagnoses, which is something that is sometimes overlooked.

The purpose of this review is to discuss the use of radiographs, nuclear medicine, and standing MRI in the equine patient and describe the limitations and methods to overcome these limitations.

2. Radiographs of the Neck: How Good are We at Injections?

Radiographs of the cervical spine are now available to practitioners, thanks to digital radiographic systems. With digital radiography and a small X-ray generator, which used to only be available with larger X-ray tubes, images of the caudal cervical spine to T1 have become part of standard practice. This has led to increased diagnosis and treatment of neck pain in horses. However, have you ever wondered how skilled we are placing a needle in the joint and keeping the material in the joint recess?

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At Michigan State University, a resident injected the cervical facet joints of 10 horse from C2–3 to C6–7 using contrast medium and 2 different techniques. The results are what you would expect. The C2–3, C3–4, C4–5, and even C5–6 were repeatably injected with a majority of contrast material present inside the joint. Injection of C6–7 was much more variable. When the facet joints were missed at C6–7, the contrast material was injected in C7-T1 or just less than 50% of the contrast medium within the C6-C7 joint.

The technique to inject cervical facets with ultrasound guidance will be reviewed,[1,2] but briefly, a curved 8 MHz microconvex or linear >7 MHz ultrasound probe should be used. A 20-gauge needle can be used to inject the joint; however, if hyaluronic acid is being administered, an 18-gauge needle is recommended. The probe should be oriented in a dorsoventral direction and the joint identified as a V-shaped hypoechoic region and the joint capsule can be seen. The needle should be directed into the V-shaped joint space. When injecting, minimal fluid should be seen around the bone and fluid placed in the joint will cause expansion of the joint capsule.

3. Nuclear Medicine: The Underused and Over-Interpreted Modality

Nuclear medicine (also called scintigraphy) is performed using a radiopharmaceutical that binds specifically to hydroxyapapite crystals.[3] Basically, rather than a radiograph that sends radiation through a bone to a detector to see how much can pass through, nuclear medicine provides a radioactive source to the horse and then, with the help of a binding agent, finds areas where osteoblasts are turning over bone. The difficulty with this modality is that bone turnover occurs for numerous reasons. The physis of a foal will normally have a high degree of osteoblast activity and even fractures can continue to heal for up to 3 years after the initial insult. Therefore, the main limitation of nuclear medicine is that it shows bone remodeling, not a source of inflammation or pain. Increased activity can help determine a region of interest for follow-up with radiographs. However, even osteophytes on radiographs do not necessarily correlate to a lameness. The main goal of the nuclear medicine study is to take a complicated multilimb lameness and see if it can be narrowed down to specific regions that cannot be characterized during the physical examination.

Soft tissue can be evaluated by nuclear medicine. This is done based on the premise that increased blood flow will be present when an inflammatory process is present and, therefore, performing the imaging scan at 5 min after injection will show the "soft tissue phase" of imaging. This can also be done using technetium pertechenate without a binding agent to just see the circulation of the radiopharmaceutical. Imaging immediately after injection can be done to assess blood flow (called the "vascular phase"); if a vascular anomaly is suspected, such as blood clot or vasculitis, unbound technetium can be used to look at the perfusion of the distal limbs. Case examples of this will be provided in the oral presentation.

4. Standing Magnetic Resonance Imaging and Elastography: A Fad or Here to Stay?

There is probably no more widespread expansion of imaging in the last 10 to 15 years that is greater than the use of MRI on the equine athlete. From soft tissue injuries to cartilage damage, MRI has taken the equine market by storm. Also, with the availability of standing MRI, general practitioners and specialists have developed equine imaging facilities to focus on the equine athlete and subtle lameness that is the most common presentation being seen by the equine practitioners.[4]

In addition, a new modality called elastography using ultrasound has recently been reported and shown to be repeatable and easy to use on normal horses to assess tendons and ligaments in horses.[5] A paper from North Carolina State University showed a difference in the elastography or "stiffness" of tendons in weight bearing as well non-weight bearing positions. This program is now available with portable ultrasound units and may become more widespread. More research needs to be done; however, there is a chance this may serve as a method to identify microtears prior to core lesions in horses.

Standing MRI is by far more widespread; however, the limitation of this system is the field strength and patient motion. Since standing units have a small magnetic field, allowing them to be put in any facility and requiring minimal maintenance and power, the resolution is considerably less when compared to high-field systems, especially when smaller structures are being imaged, such as cartilage that is less than 5 mm thick. The level of detail that is discussed by clinicians versus what can be seen in a research environment is quite different. Standing MRI can be used to evaluate tendons and ligaments, bone edema, and even fractures.[6] The size and severity of the lesion must be enough to allow detection using a low-field system. Limitations with magic angle artifacts and simple image noise makes over-interpretation easy, especially when a subtle lameness is present. Also, it is not uncommon to find 8 to 10 subtle lesions in a foot on standing MRI and the question is "which one is causing the problem?"

Ultimately, this one-hour lecture will show several case examples of state-of-the-art imaging available to practitioners and specialists alike. A major limitation in imaging of the equine patient is that currently there seems to be a lack of understanding of some of these modalities and, therefore, they are not used to their full potential. That said, researchers and clinicians are constantly studying

these modalities and continue to improve our understanding of how best to use and interpret the images. In the realm of noninvasive imaging, the future looks bright and the availability to start critically evaluating treatments is just around the corner.

Acknowledgments

Conflict of Interest

The Author declares no conflicts of interest.

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Comparison of Radiography, Scintigraphy, and Magnetic Resonance Imaging Techniques: How Does One Image Compare to Another?

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Each imaging modality has its own strengths and weaknesses. Knowing the caveats of each will help determine what modalities to use and how to interpret the findings. Author's address: Alamo Pintado Equine Medical Center, 2501 Santa Barbara Ave., Los Olivos, CA 93441; e-mail: carter@alamopintado.com. © 2014 AAEP.

1. Introduction

Advanced imaging techniques such as magnetic resonance imaging (MRI) and scintigraphy have helped to improve the diagnostic capabilities in the horse. They have allowed a unique perspective into many problems that previously may have been difficult to determine otherwise. With the proliferation of these different imaging modalities, they are being performed much more commonly and, often, in conjunction with each other. The purpose of this presentation is to discuss how MRI, scintigraphy, and radiographic imaging techniques relate to each other and to discuss some of the benefits and pitfalls of each and how over- and under-interpretation of various imaging modalities occur.

2. Scintigraphy and MRI

Nuclear scintigraphy has been used in equine orthopedic imaging to identify physiologic changes in bone and soft tissue metabolism and has been used to identify regions of lameness for decades. While it requires a regulatory license and involves the use of radioactive labeled isotopes, it is still a simple relatively non-invasive diagnostic technique that is performed standing under light sedation. The entire horse can be screened using this technique.

Magnetic resonance imaging (MRI) is the gold standard in imaging human orthopedic conditions and is quickly becoming more commonly available to equine veterinarians. It can be performed in the standing patient using a low field system or in the anesthetized patient using either a low or high field imaging system. The high field systems provide very detailed information about both physiology and anatomy.

Both of these techniques have traditionally been considered in isolation when considering imaging a patient. Scintigraphy is often used when the region of lameness is unknown or indeterminate using routine techniques such as nerve blocks to localize the area of pain. Magnetic resonance imaging is used when the area of pain is known but the cause has not been well established using other techniques. The question that often comes up when using scintigraphy is "what does that scintigraphy

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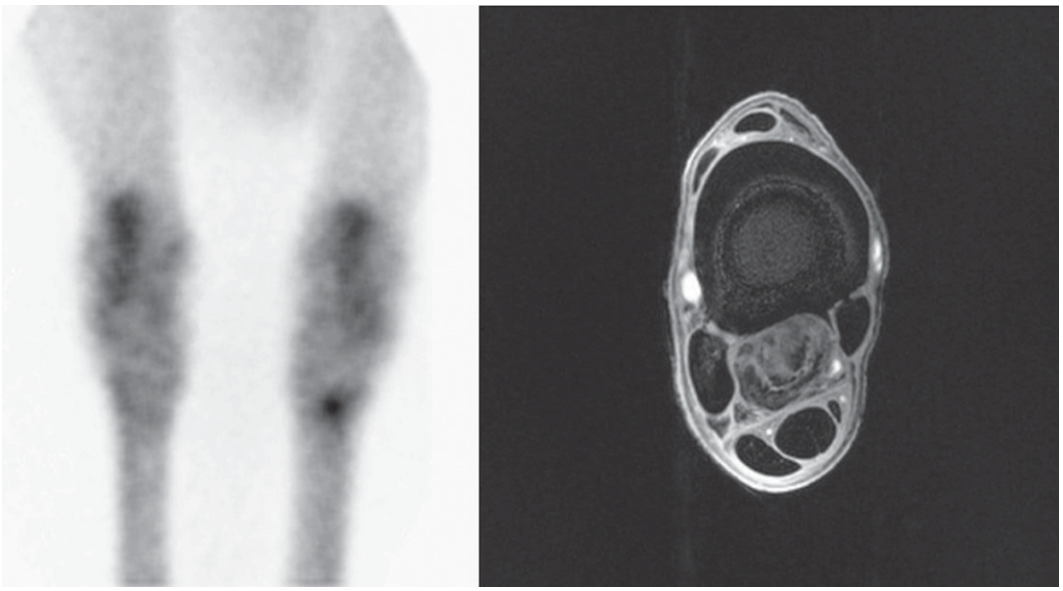

Fig. 1. Plantar scintigraphic projection of the hock region with focal increased uptake of the right hind proximal metatarsus. Transverse T1 image of the proximal metatarsus of the right hind limb in the same region as the scintigraphic uptake showing only suspensory ligament pathology.

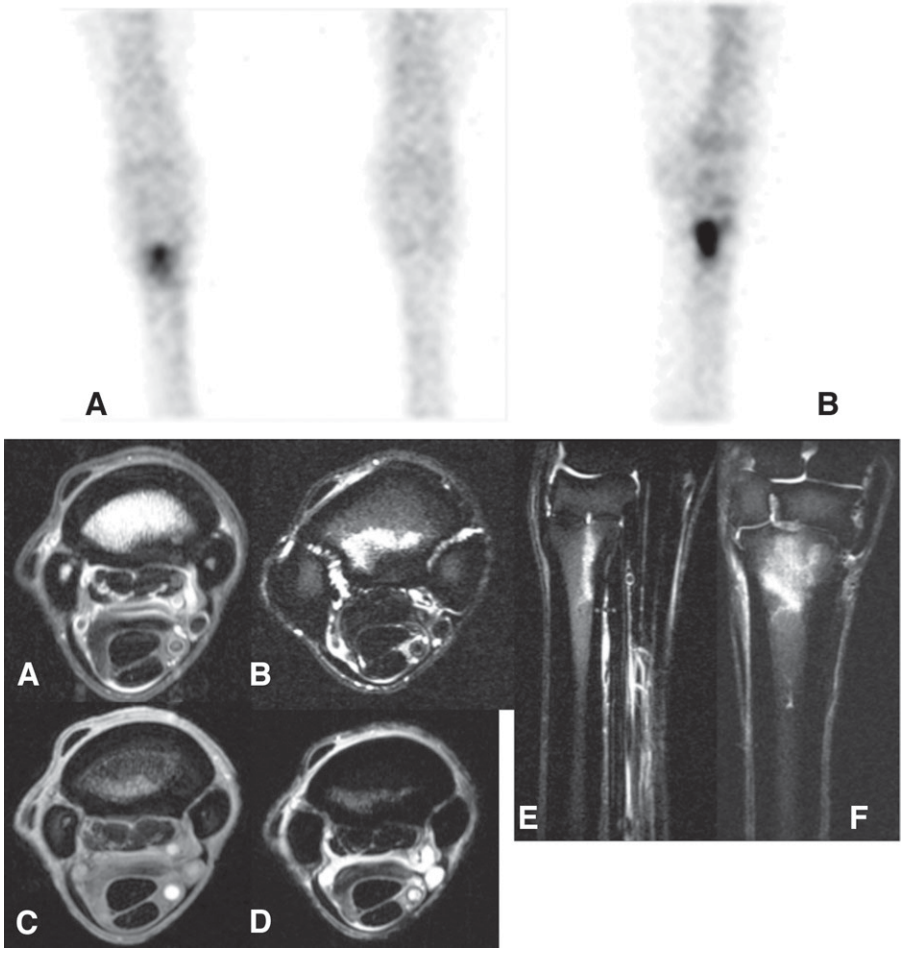

Fig. 2. MRI images of the region of increased uptake on the scintigraphy examination. A, Proton density axial, B, T2 short tau inversion recovery (STIR) axial, C, T1 VIBE FS axial, D, proton density FS axial, E, T2 STIR sagittal, and F, T2 STIR dorsal. Note the increased signal (white area in the palmar aspect of MC3) on images B–F and decreased signal on A. This is consistent with bone "edema." Note the normal appearance to the suspensory ligament, which contains fat and muscle, creating a region of light gray and white within the black ligament fibers.

uptake pattern mean," and "what might that look like on an MRI?" What the client often asks is "is it necessary to do an MRI?"

The comparison of MRI to scintigraphy has been previously reported and initially showed that the correlation of soft tissue injuries is poor between MRI and scintigraphy while the correlation of bone injuries was high between the two modalities in the foot.[1] This was expanded upon by the evaluation of scintigraphic uptake in the proximal metacarpus (tarsus) and the findings on MRI of the same region in the same horse.[2] This study showed a trend in the ability to use the relative degree of uptake on the scintigraphic examination to predict the likelihood of a soft tissue-only injury, bone-only injury, or both in the proximal metacarpus (tarsus). While a direct correlation was not possible, it does allow for a relative ability to predict the likelihood of relative involvement of bone, the associated soft tissues such as the suspensory ligament, or both. Interestingly, the more intense the uptake on the scintigraphy exam, the more likely that the lesion is going to involve a majority of bony tissue. Alternatively, if the uptake is relatively faint, it can represent a soft tissue injury. Figures 1 and 2 show examples of these correlations.

There are pathologies that are only evident on MRI and have negative scintigraphic findings. An example of this is the medial collateral ligament injury that does not involve either the origin or insertion. A true soft tissue injury is much less likely to have scintigraphic abnormalities when compared to MRI (Fig. 3). This is likely due to the fact of the relative lack of blood flow to the injury and decreased perfusion of the radioisotope, the specificity of the radionuclide for osteoblasts and bone, and the lack of fidelity of small structures on scintigraphy.

There are situations where the scintigraphic examination proves much more sensitive for a particular problem than the MRI. Certain pathologies involving the coffin bone, navicular bone, and long pastern bone have been identified on scintigraphy with "normal" MRI examinations of these regions. Figure 4 shows an example of severe inflammation of the coffin bone on the scintigraphic exam with a "normal" MRI examination. The reason for studies with discrepancies such as these are unclear. The affinity of the technetium 99m methylene diphosphonate for remodeling bone is extremely high, thus an abnormal bone uptake pattern would be anticipated with a bone type of injury. The MRI signal characteristics usually will show abnormalities in the fat-suppressed (short tau inversion recovery [STIR] of other fat suppression techniques) or T1 weighted images consistent with edema or inflamed tissue. It is possible that the intra-osseous pressure within the bone is too high for the extravasation of fluid that can be detected on MRI, but the cellular activity is great enough to attract the technetium 99m methylene diphosphonate and result in a positive scintigraphic examination and a negative MRI. This type of situation appears to be relatively rare, but does occur, and should be considered when

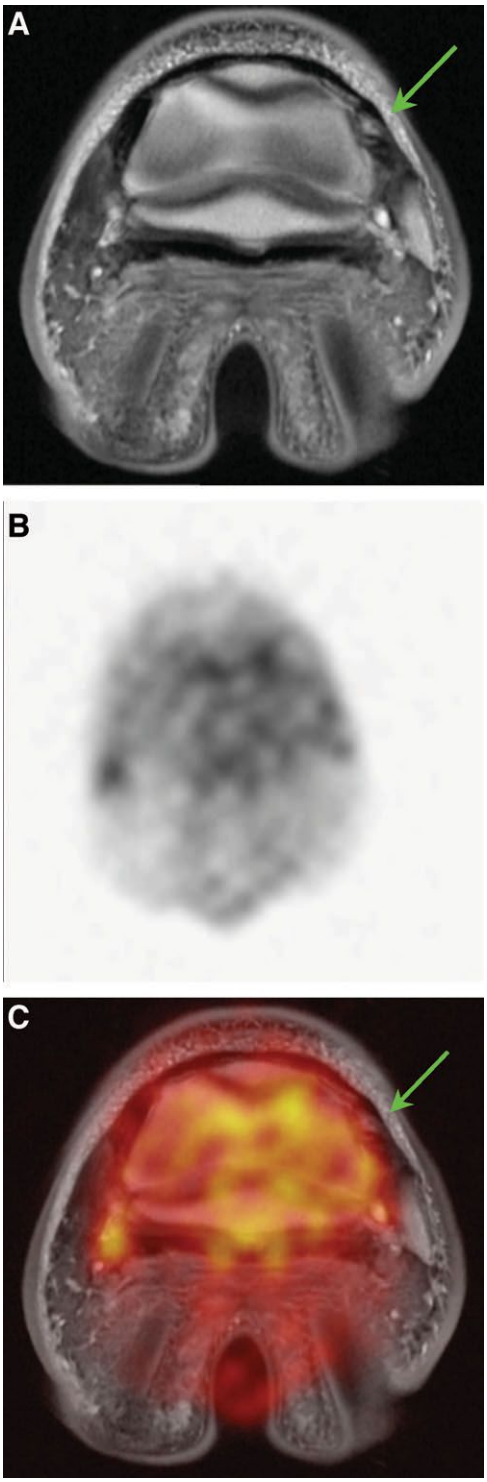

Fig. 3. Failure of scintigraphy to identify a problem in the foot: Proton density transverse images of the foot (A), solar margin scintigraphy projection (B), and image fusion (C) of scintigraphy (B) over the MRI image (A) showing no uptake in the region of the medial collateral ligament injury (green arrow) on the scintigraphy examination.

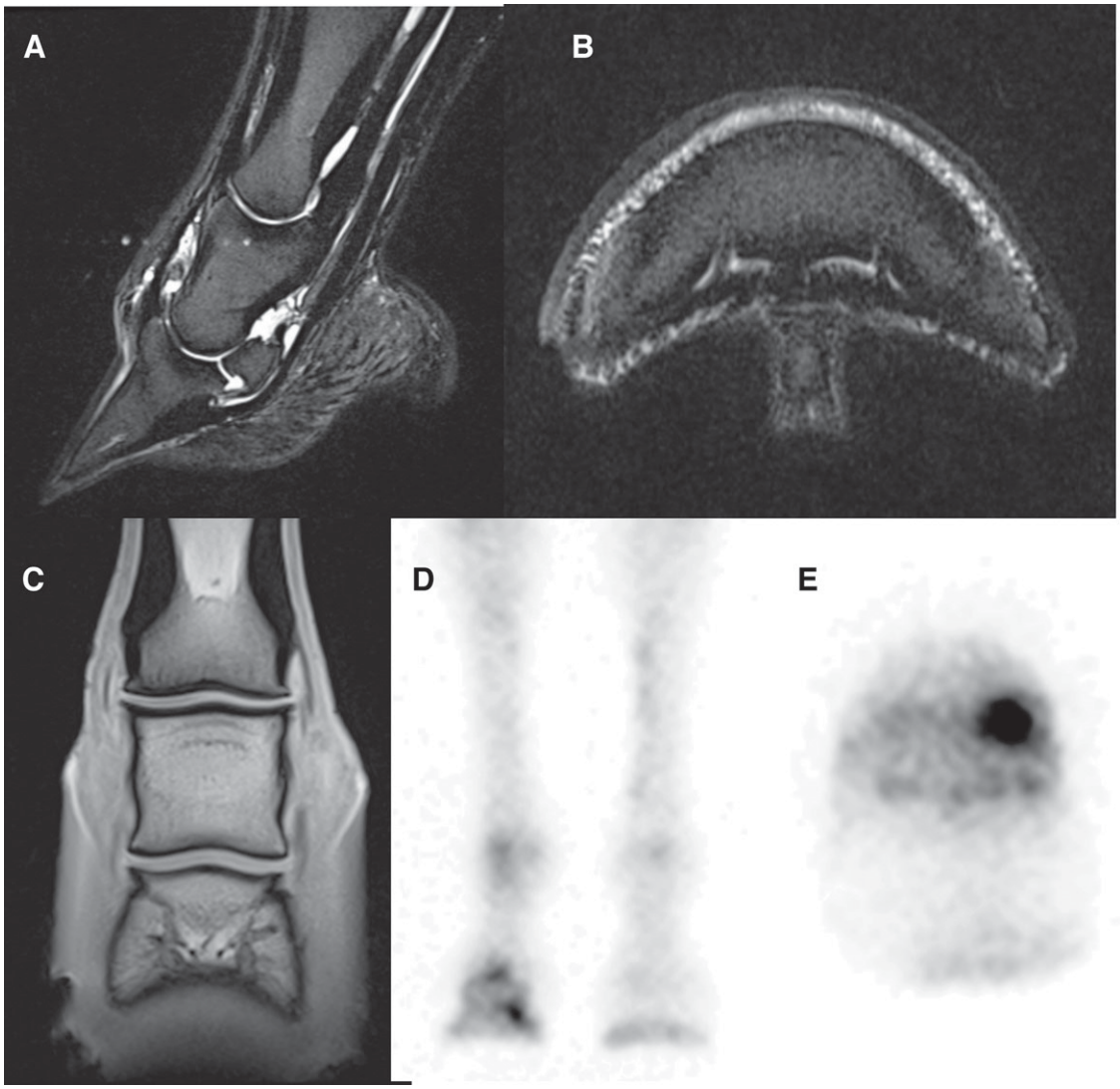

Fig. 4. Failure of MRI to identify bone inflammation: The marked uptake in the dorsal medial aspect of the coffin bone is shown in the scintigraphy images (D,E). The MRI images do not identify any bone pathology (A, T2 STIR sagittal, B, T2 STIR axial, and C, T1 VIBE dorsal). The MRI images are through the region of pathology on the scintigraphy examination.

a lameness has been precisely localized to a particular region and the MRI is negative.

In summary, MRI and scintigraphy can be thought of as complementary modalities and the use of one should not exclude the use of the other. Scintigraphy is a useful topographical technique to help localize difficult lamenesses and some patterns can infer that certain pathologies are occurring, especially when it relates to a bony issue. However, with soft tissue lesions, the underlying pathology from a positive scintigraphy examination is less clear.

3. Radiographs and MRI

The use of radiographs for the equine patient is paramount for accurate diagnoses and is probably the most commonly used diagnostic modality in the horse. From routine osteochondral fragments in the joints of horses to the most complex fractures, traditional radiography has proven to be essential in equine practice. With the advent of digital imaging modalities and the instant feedback provided by digital radiographic units, the use of radiography has become even more important.

One of the main problems associated with radiography is that it is typically limited in scope to bony problems. While some soft tissue changes can be ascertained, the focus of radiographs is to provide an evaluation of the bony structures of an object. Digital and computerized systems have improved upon the soft tissue evaluation over traditional film based systems. A large change in bone mass or density is required for pathologic changes to be visible to the observer. This change in density is reported to be as great as a 30 to 50% change in density before a pathologic change is identified. This means that a

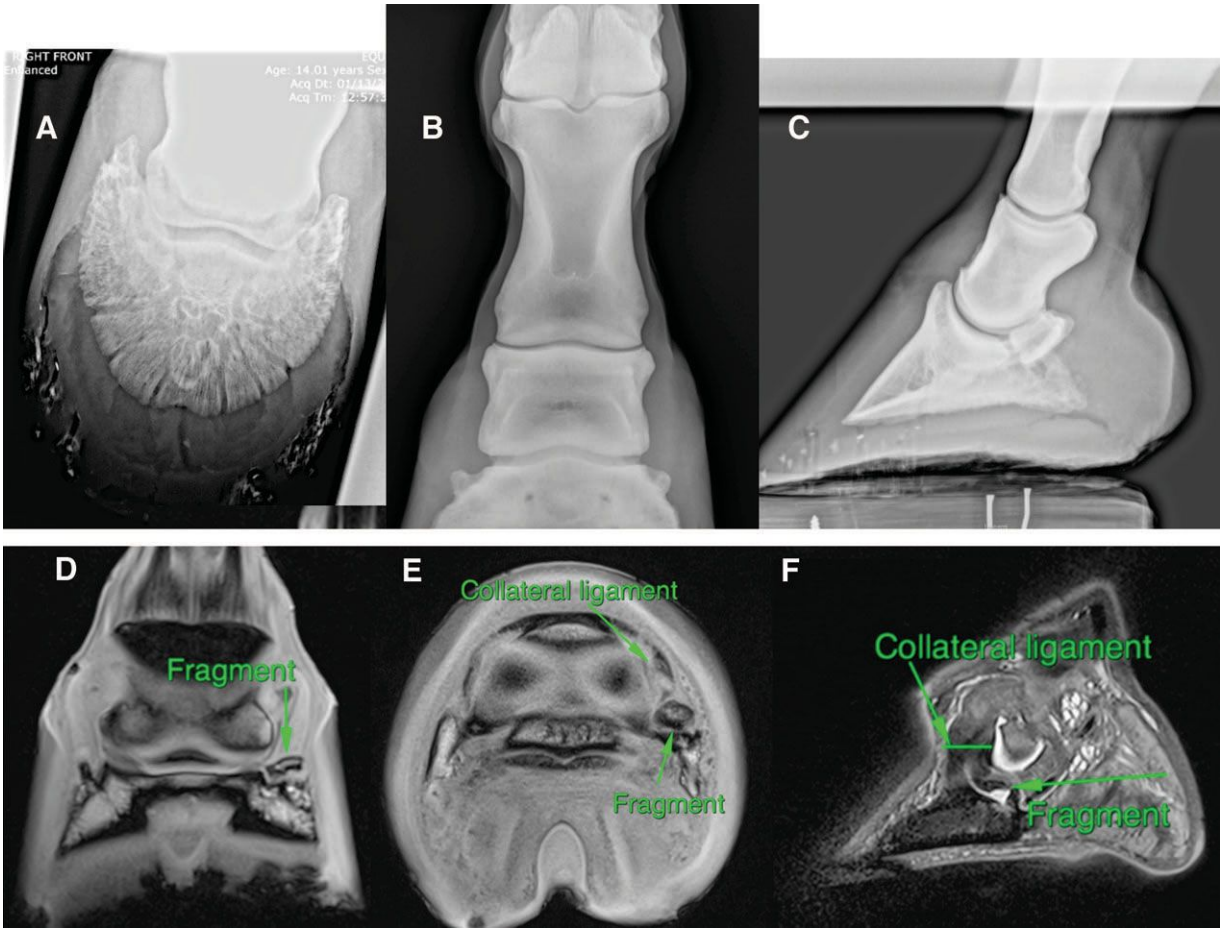

Fig. 5. Limitations of radiographs: The radiographs failed to identify the fracture fragment on the coffin bone (A–C). Some remodeling of the insertion of the medial collateral ligament insertion on the coffin bone is present on the radiographs. Oblique radiographs were not available prior to the MRI. The MRI (D–F) shows the osseous fragment along with its involvement with the medial collateral ligament of the coffin joint.

large change in bone density is required before the observer can adequately determine it.

Additionally, the traditional radiograph is a two-dimensional representation of a three- dimensional structure. This results in summation of structures, which can obscure lesions and be a significant disadvantage when trying to interpret radiographs. However, in some cases summation can actually serve as an advantage and make the observation of the abnormality more noticeable. For example, osseous proliferation at the margins of joints are most frequently easier to observe on a radiograph than on an MRI. This is due to the fact that the osseous proliferation is summated and combined on the radiograph, whereas on the single-slice MRI it is isolated on each area. This can make radiography easier to interpret peripheral bony changes and osseous proliferation.

In most situations however, the MRI is more sensitive than radiographs for deciphering the pathology occurring in the area of concern. While the radiographs look at the bony density of a structure and relies on changes in that density to detect pathology, the MRI uses the chemical composition of the tissue to derive its images. This makes physiological changes in the bone more likely to appear on the MRI than on a radiograph. A prime example of that is the identification of bone edema in the absence of radiographic abnormalities (Fig. 2).

Magnetic resonace imaging is also more sensitive at detecting small obscure issues that might be missed using routine radiographic projections. A small osseous "avulsion" fragment of the coffin bone at the palmar insertion of the medial collateral ligament of the coffin joint is very hard to detect on radiographs, but is a relatively simple diagnosis on MRI (Fig. 5).

In summary, radiographs remain a very pertinent diagnostic modality and may be more sensitive for certain bony pathologies such as osteophytes. However, a lame horse with a negative radiograph may have more bony pathology occurring than is present on even the best set of radiographs.

4. Conclusion

Imaging the equine patient can be a complicated process. Negative results on one modality do not preclude the absence of a problem in the patient and an alternative imaging technique may provide more insight into the pathology of a particular patient.

Acknowledgments

Conflict of Interest

The Author declares no conflicts of interest.

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Ultrasound in Equine Practice—Where We've Been, Where We Are Now, and Where We Need to Go

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1. Introduction

Ultrasound has been in use in equine veterinary medicine since the early 1980s, steadily gained traction in the late 1980s and early 1990s, and has gradually become widely available for veterinarians to diagnose all types of equine medical and surgical disorders. Ultrasound has been propelled forward by a particularly diverse group of equine veterinarians, ranging from anatomists to internists to surgeons to radiologists and nonboarded specialists. As we look back over the past 35 to 40 years, it is most impressive that ultrasound remains an imaging modality not fully embraced in veterinary education or residency training, despite its widespread use in equine practice. While we have made significant strides, it is curious that exams and indications remain unstandardized, education at the veterinary school level is minimal at best and, with few exceptions, ultrasound is often omitted or minimized within comparative imaging studies. It is important to understand the history of ultrasound in equine veterinary medicine to appreciate the path we have taken to ultrasound in practice today, especially as we plan for the future in this important imaging modality. The purpose of this report is to look back at where we've been, how it led us to where we are today, and where we still need to go. It represents the author's views as a nonboarded specialist in academia combined with historical commentary and viewpoints from several of today's thought-leaders in equine ultrasound.

2. Where We've Been—The Early Years

The roots of ultrasound in equine veterinary medicine can be traced back to the late 1970s. Dr. Virginia Reef, then a veterinary student at the Ohio State University, worked with cardiologist, Dr. Frank Pipers, to perform blind M-mode exams of the equine heart. Meanwhile, Dr. Norm Rantanen, then a radiologist at Washington State University (WSU), became interested in pursuing "alternative" imaging, which, at the time, included ultrasound, nuclear scintigraphy, and computed tomography (CT). Ultrasound ultimately won out due to its relatively low costs, and efforts were put in place at WSU to purchase the first B-mode ultrasound machine for use at a veterinary school. This machine was purchased for $62,500 from Unirad Ultrasound in Denver, Colorado. It was a static (not real-time) B-mode scanner. Image acquisition required that the animals remain completely still. The machine was huge and weighed several hundred pounds (Fig. 1). Images were acquired through the use of a cumbersome reticulated arm, which limited studies to tho-

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Fig. 1. Abdominal ultrasound on a horse using a static B-mode scanner (Unirad Ultrasound) at Washington State University with technician, Bob Ewing. Notice the extremely large size of the machine and the reticulated arm that prevented imaging of distal limbs in the standing horse. Image provided courtesy of Dr. Norm Rantanen, circa 1980.

racic and abdominal exams since the arm could not reach the equine distal limb unless the horse was under general anesthesia. The first real time B-mode scanner was obtained by WSU in 1982 (Fig. 2). This machine expanded the use of ultrasound to many regions, including musculoskeletal exams in the standing horse. This piqued the interest of Michael Hauser, who was then a veterinary student at WSU. Hauser subsequently worked with Dr. Rantanen to publish some of the first works on equine musculoskeletal ultrasound.[1–3] Shortly thereafter, Dr. Rantanen relocated to Kentucky, where he then collaborated with internist Dr. Doug Byars to develop techniques to evaluate and characterize many disorders, but especially pneumonia in foals and twin reductions in broodmares.

Fig. 2. Dr. Norm Rantanen, then faculty radiologist at Washington State University, performs a renal ultrasound exam on a horse using a real-time B-mode scanner (MK100 Ultrasound System, Advanced Technology Laboratories, Inc). Image provided courtesy of Dr. Rantanen, circa 1982.

Meanwhile, on the East Coast, Dr. Virginia Reef had taken over "Heart Station" at the University of Pennsylvania's New Bolton Center while an internal medicine intern in the early 1980s. Ultrasound equipment at Heart Station was similar to that used by Dr. Rantanen at WSU until the mid-1980s when a more portable real-time system became available. This machine facilitated expansion beyond echocardiographic studies. Although all types of ultrasound exams were, and continue to be, performed by this service, the term "Heart Station" has endured over time. Dr. Reef managed to lead Heart Station through her internal medicine residency and remains in charge to this day. Heart Station is the first known service dedicated to equine/large animal ultrasound at any U.S. veterinary school. The roots of many ultrasonologists, both in the U.S. and abroad, can be traced to Dr. Reef and her team at New Bolton Center. This includes the author and many of today's ultrasound leaders, including Dr. Johanna Reimer, who became both a boarded internist and cardiologist, published one of the first books on equine ultrasound in 1998,[4] and led ultrasound at one of the largest private practices in the country for several years.

The history of ultrasound in equine practice would not be complete without mention of Dr. Ron Genovese, owner of Randall Veterinary Hospital near Cleveland, OH (currently at Cleveland Equine Clinic). Dr. Genovese is considered one of the early pioneers and visionaries of equine ultrasound. With a busy racetrack practice, Dr. Genovese had become frustrated with the limitations of radiography and the number of racehorse breakdown injuries. He was looking for alternative means to detect injuries that could potentially predispose horses to breakdown. Dr. Genovese found it in a hotel room at the 1982 AAEP meeting. Without a trade show, vendors hosted sessions in hotel suites to show their wares. Steve Dumond, later the owner of Classic Ultrasound, hosted a session with Dr. Norm Rantanen to show ultrasound images he had been acquiring at Washington State University. Dr. Genovese was immediately intrigued, purchased a machine (Fig. 3), and invited Dr. Rantanen to Cleveland to learn techniques for distal limb ultrasound (Fig. 4). Within a few years, these efforts were presented at the first in depth session on ultrasound at the 1985 AAEP Convention in Toronto, Canada[5–7] and also within the first major work on equine ultrasound, the April 1986 edition of Veterinary Clinics in North America.[8] Soon after, Dr. Genovese became host to veterinarians from all over the world who wanted to learn more about this new imaging modality. Drs. Genovese and Rantanen also began teaching short courses to disseminate their knowledge. Such wet labs eventually became part of AAEP meetings.

Fig. 3. One of the first portable ultrasound machines (ATL) in equine practice purchased in 1983 by Dr. Ron Genovese (Randall Veterinary Hospital, Warrensville Heights, OH). Images were "captured" with the Polaroid camera mounted on the left side of the machine. Once an image was frozen and labeled, the camera was swung over the screen and a photo taken of the image for documentation of the exam. Image provided courtesy of Dr. Genovese, Cleveland Equine Clinic, Ravenna, OH.

Back on the west coast, University of California (UC) Davis was incubating its own large animal ultrasound service. In the late 1980s, Dr. Carol Gillis began performing musculoskeletal ultrasound exams during her equine surgery residency to resolve a client service problem. At the time, horses were being kept overnight until a radiologist became available to perform an ultrasound exam. Similar to many institutions, it was difficult for the radiologists to prioritize equine ultrasound exams over their busy small animal imaging caseload. Because Dr. Gillis had already developed a reputation for ultrasound as a private practitioner in the Northern California Bay Area, she was the ideal solution to this problem. Dr. Gillis had been inspired by Dr. Genovese's presentations on distal limb ultrasound at the 1985 AAEP Convention in Toronto, Canada. Dr. Gillis eventually staffed the Large Animal Ultrasound Service full time after her residency until she left for private practice in 1999. Although the Large Animal Ultrasound Service at UC Davis was modeled after New Bolton Center's Heart Station, the two services have retained somewhat distinct personalities, traceable to their surgical and internal medicine origins, respectively.

Publications

A PubMed search for "ultrasound and horse" reveals one of the earliest publications to be authored by Dr. Frank Pipers in 1977 on the topic of equine echocardiography.[9] Dr. Pipers authored several ultrasound publications in the early 1980s, primarily on echocardiography but also on the use of ultrasound for pregnancy diagnosis and monitoring with transrectal and transabdominal approaches.[10–14] The use of ultrasound to diagnose early single and twin pregnancies had also been described by a group from Newmarket in 1982.[15] Other early publications included the use of ultrasound to diagnose one of 4 horses with cholelithiasis in a 1982 Journal of the American Veterinary Medical Association (JAVMA) article[16] and to detect fistulous tracts and foreign

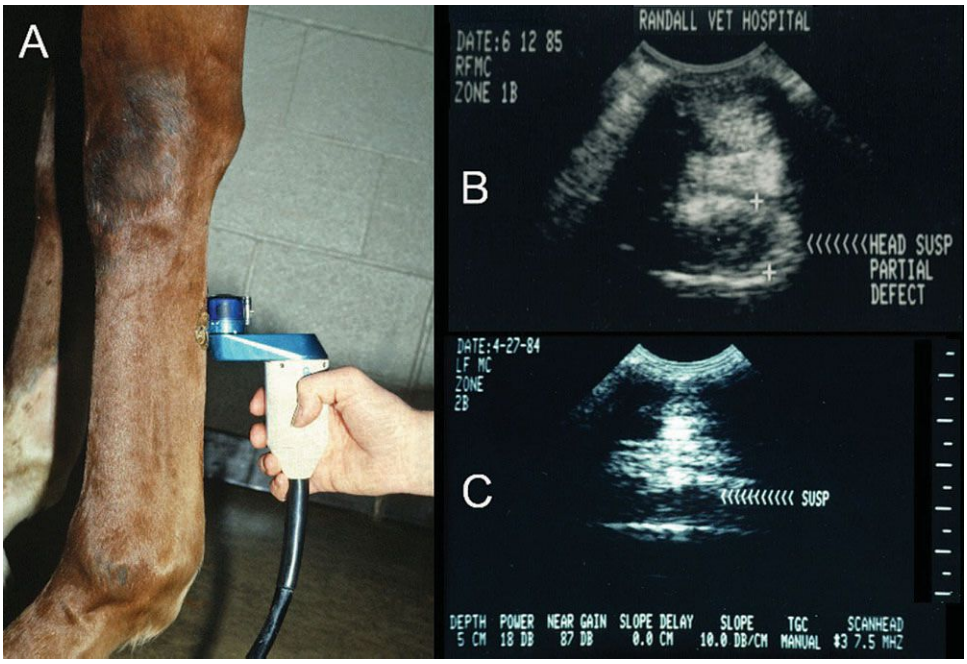

Fig. 4. Metacarpal ultrasound (1984–85). A, Placement of a mechanical sector transducer to obtain transverse metacarpal images. B, Transverse ultrasound image of a large tear of the suspensory ligament body. C, Longitudinal image showing fiber disruption in a similar injury in another horse. Images provided courtesy of Dr. Ron Genovese, Cleveland Equine Clinic, Ravenna, OH.

bodies in horses in 1984.[17] Ultrasound for musculoskeletal imaging was first embraced by the Journal of Equine Veterinary Science.[1–3,18,19] The first substantial works on ultrasound were published in two editions of Veterinary Clinics of North America dedicated to cardiology (1985) and ultrasound (1986).[8,20]

Equine ultrasound steadily gained traction throughout the 1980s, and the early- to mid- 1990s represented the culmination of many groups' efforts. By this time, ultrasound visionaries had sufficient time to accumulate cases to publish both retrospective and prospective studies. Many studies focused on the use of abdominal ultrasound, but several reported on musculoskeletal ultrasound. Much of the latter described the normal and abnormal appearance of the superficial digital flexor tendon, especially in racehorses.[21–27] While such intense focus on the superficial digital flexor tendon (SDFT) provided tremendous information on tendon injuries and healing; it may be argued that this information has hindered our knowledge of musculoskeletal soft tissue injuries. Unfortunately, and sometimes erroneously, this information continues to be extrapolated to other tendon or ligament injuries, especially as it relates to injury staging and ultrasonographic monitoring of healing. While the SDFT garnered most of the attention, publications on other distal limb injuries also began to emerge, including studies on injury distribution[28–33] and individual structures such as the suspensory ligament, inferior check ligament, and deep digital flexor tendon.[33–40] Veterinary Clinics of North America published a pair of editions in 1994 and 1995 on the topic of tendon and ligament injuries with Dr. Sue Dyson as the guest editor.[41,42] This era also featured early descriptions of ultrasound to evaluate bone, specifically to diagnose pelvic fractures in racehorses.[43–44]

In terms of abdominal ultrasound, multiple descriptions of renal, hepatic, and splenic abnormalities were published in the early 1990s, including the use of ultrasound to diagnose cholelithiasis, urolithiasis, and lymphosarcoma in horses.[45–48] Ultrasound to evaluate the gastrointestinal tract also began to take hold as authors published techniques to diagnose left dorsal displacements, right dorsal colitis, and intussusceptions in foals, among others.[49–54] Of these, a 1996 prospective study out of Chino Valley Equine Hospital in Southern California was one of the most influential publications on the use of ultrasound in the acute abdomen. The authors reported findings from 226 horses with colic and found ultrasound to be highly reliable to detect small intestinal strangulating obstructions.[52]

Surprisingly, it wasn't until the late 1990s that textbooks dedicated to equine ultrasound were finally published. These included *Equine Diagnostic Ultrasonography*, edited by Drs. Norm Rantanen and Angus McKinnon,[55] *Equine Diagnostic Ultrasound* by Dr. Virginia Reef,[56] and the *Atlas of Equine Ultrasonography* by Dr. Johanna Reimer.[4] All were published in 1998 and have remained the primary resources available to equine veterinarians for more than 15 years.

Equipment

The evolution of ultrasound equipment has been impressive. The articulated arms of the 1970s and 1980s were gradually replaced by sector and then linear array transducers in the mid-1980s and 1990s. Whereas the size and expense of the original ultrasound machines limited their use to academic institutions, ultrasound gradually migrated into equine private practice in the mid to late 1980s when somewhat portable machines capable of real-time B-mode imaging became available. Early transrectal exams for reproductive imaging were often performed with large sector transducers with an offset beam. Reproductive ultrasound with linear transducers was initially problematic because of their poor lateral resolution and lack of focal zones. This significantly affected the examiners' ability to distinguish between pregnancies and uterine cysts or ovarian follicles or between twin and singleton pregnancies. Sector transducers were the norm until the mid- to late 1990s when linear transducers became more widely available for musculoskeletal and rectal imaging. Linear transducers of this decade provided improved resolution compared to their sector counterparts but, more importantly, allowed evaluation of fiber pattern across the entire image when performing longitudinal views of tendons and ligaments (Fig. 5). Low frequency (2–5 MHz) curvilinear array transducers became available in the mid-1990s, but their value was initially limited by poor penetration relative to sector transducers. Over time, curvilinear transducers improved in terms of penetration, resolution, and detail and have now replaced sector transducers for equine abdominal work (Fig. 6).

The Ausonics Opus and its next generation, the Ausonics Impact, became the machine to own in equine hospitals in the mid- to late 1990s. While this machine could be taken "mobile," ambulatory practitioners bought more portable machines from Aloka, Pie Data, and Medison. These machines were primarily geared for reproductive use but could also be used for musculoskeletal imaging. Driven by advances in medical ultrasound, image quality steadily improved, especially in larger clinic based equipment. Linear rectal transducers were available for most machines marketed to equine veterinarians. With frequencies of 5 to 7 MHz, these transducers could also be used for basic tendon and ligament ultrasound and were certainly adequate to diagnose acute SDFT or suspensory ligament injuries. In the late 1990s and early millennium, linear transducers designed for musculoskeletal use became increasingly available for portable machines. These "tendon probes" were more ergonomic for musculoskeletal imaging than rectal transducers and offered higher frequencies (10–13 MHz) to im-

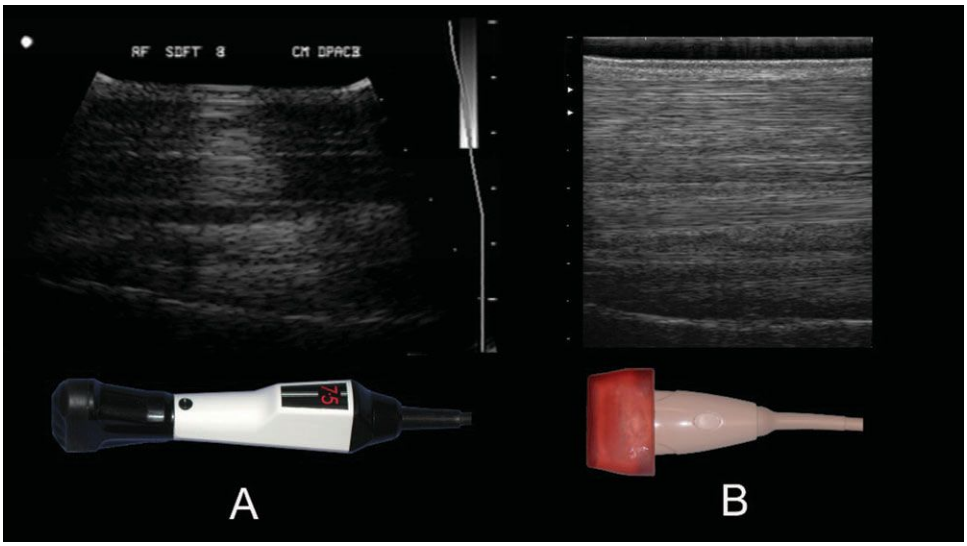

Fig. 5. Comparison between sector (A) and linear (B) transducers to evaluate fiber pattern. Both longitudinal images were obtained at the proximal metacarpus. Image A was obtained with the 7.5 MHz fluid-offset sector transducer shown below the ultrasound image. Note that the fiber pattern can only be evaluated in the central part of the image with this type of transducer. Image B was obtained using the 7 to 10 MHz linear array transducer with a standoff shown below the image. Note the superior evaluation of fiber pattern across the width of the ultrasound image with linear transducers compared to sector transducers. Linear transducers became available in the mid-1990s and have replaced sector transducers for musculoskeletal imaging.

prove resolution of tendons and ligaments (Fig. 7). This began the rapid expansion of equine musculoskeletal ultrasound, both in terms of diversity and quantity.

Specialists and Education

The 1990s and new millennium began to see veterinarians emerge from the fellowship program at New Bolton Center. Fellows received extensive training for 1 to 2 years, during which time they learned systematic techniques to evaluate a busy and diverse caseload from the surrounding Chester County horse country. UC Davis followed with its own fellowship program in 2001. Several graduates of these fellowships have gone on to academic positions and some have developed their own programs. Others have set up their own ultrasound consulting services in private practice.

The AAEP began holding annual ultrasound wet labs in association with its annual convention and then in Lexington at the Kentucky Horse Park.

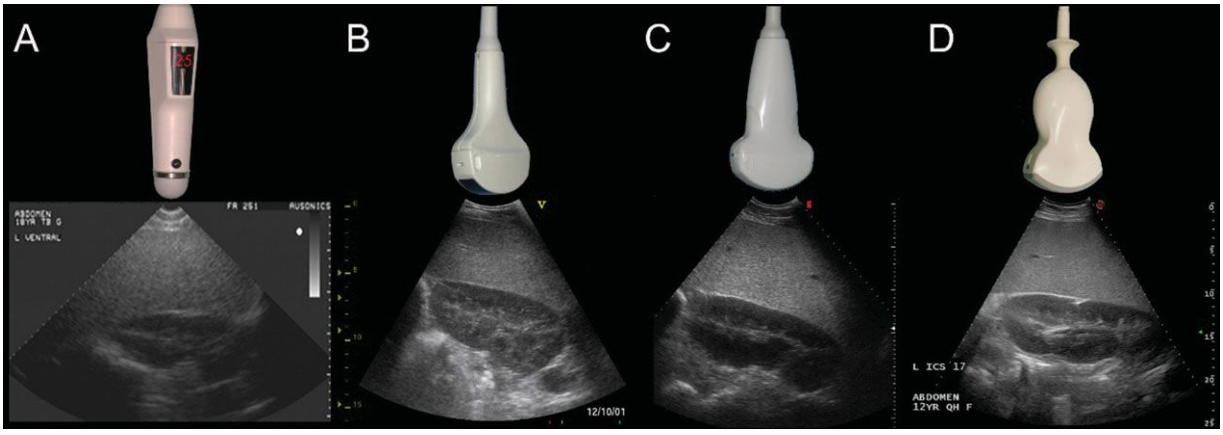

Fig. 6. Abdominal ultrasound images of the spleen and left kidney from 1999 through 2014 with the transducers shown above each image. A, A 2.5 MHz sector transducer from 1999 produces an image of the spleen and left kidney but does not provide substantial detail of the kidney compared with that seen in images B to D. B, A 2 to 5 MHz curvilinear transducer[a] from 2001 shows improved detail of the left kidney. C, A 2 to 5 MHz curvilinear transducer[b] from 2007 produces a softer image with improved resolution compared to the previous images. D, A 2 to 5 MHz curvilinear transducer[c] from 2012 shows an even softer image. Note that image quality has improved (more detail and less coarseness), despite the fact that the frequency range of these transducers has remained essentially unchanged.

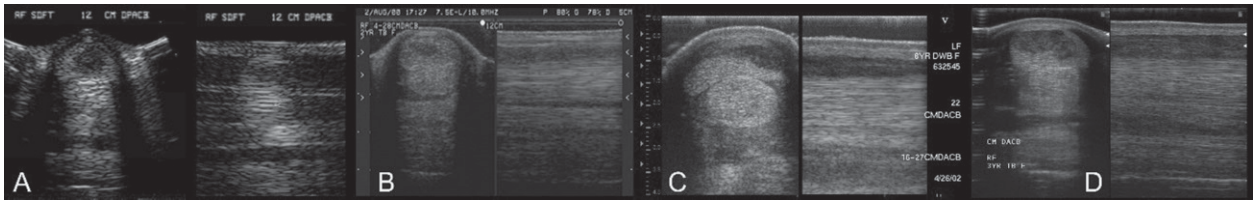

Fig. 7. Ultrasound images of superficial digital flexor tendon (SDFT) injuries obtained from 1996 through 2012 to illustrate improvement in musculoskeletal image quality over the past 20 years. A, A 7.5 MHz fluid offset sector transducer[d] was used to document an acute core lesion of the SDFT from 1996. Note the relatively poor detail/resolution compared to subsequent images. B, A 5 to 10 MHz linear transducer[e] shows significantly improved resolution of this SDFT injury from 1999. Note also the improved visibility of fiber pattern produced by the linear transducer compared to that produced by the sector transducer in image A. C, A 5 to 10 MHz linear transducer[a] produced an even higher resolution image of this SDFT injury from 2002. D, A 10 to 13 MHz linear transducer[b] shows continual improvement in image quality with exceptional resolution of this SDFT injury from 2012.

Ultrasound short courses and wet labs began to proliferate throughout the country, usually headlined by ultrasound specialists and supported partially or wholly by ultrasound vendors.

This was also a time where Dr. Jean-Marie Denoix emerged onto the American ultrasound scene. As an anatomist and imager, Dr. Denoix can be credited for the widespread acknowledgement that ultrasound *really is* about anatomy. Although others had been emphasizing this relationship for years, Dr. Denoix's in-depth lecture on joint ultrasound at the 2001 AAEP convention caused practitioners to stand up, take notice, and revisit their understanding of anatomy.[57] The subsequent formation of ISELP (International Society for Equine Locomotor Pathology) has continued to emphasize this crucial relationship between anatomical knowledge and imaging. Dr. Denoix's book on the equine distal limb was published in 2000 and continues to be the "go-to" resource on anatomy and imaging of this important region in the horse.[58]

The Imaging Wars

The early millennium marked the emergence of magnetic resonance imaging (MRI) onto the imaging scene.[59–65] With it, came the beginning of the imaging "wars" between MRI and ultrasound. Some early adopters could even be heard to proclaim that MRI would spell the end for ultrasound. Obviously, this has not been the case. Ultrasound remains in heavy use today for the same reasons that CT has not spelled the end for radiography. There is no doubt that MRI offers significant advantages over ultrasound from a diagnostic standpoint, but it remains out of reach for many horse owners (and veterinary clinics), primarily because of affordability issues. Early authors often seemed heavily biased to prove that MRI was superior to ultrasound to visualize soft tissue injuries. In many of these studies, ultrasound had either not been performed, was performed in an inconsistent manner prior to referral, or techniques were not defined within the body of the report. Unfortunately, these studies seemed to create an unprecedented rift between imaging modalities which did little to drive us forward from an ultrasound standpoint. To date, only a few comparative imaging studies have been published in a fair, unbiased, and comparative manner. Ultrasound and MRI are complementary and there is much to be learned from both imaging modalities.[66]

3. Where We are Today

What We are Scanning

The last 10 years have brought about tremendous change in the world of equine ultrasound. Ultrasound has become accepted to diagnose all types of musculoskeletal injuries, from cartilage lesions (Fig. 8),[67,68] to pelvic disorders (Fig. 9),[69–72] to meniscal tears (Fig. 10),[73,74] to cranial nuchal bursitis.[75] Ultrasound is now in widespread use in academic institutions and by private practitioners, both in the hospital and ambulatory setting. Continuing education (CE) courses have proliferated across the country. Motivated practitioners have sought out CE to learn ultrasound of "basic" regions such as the metacarpus, metatarsus, and pastern as well as more advanced regions such as the shoulder, tarsus,

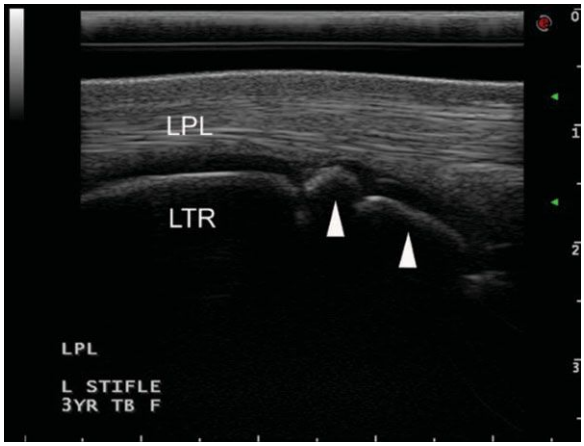

Fig. 8. Longitudinal image of an OCD lesion (arrowheads) of the lateral trochlear ridge (LTR) obtained with an 18 MHz linear transducer. The linear fibers of the lateral patellar ligament (LPL) are visualized overlying the LTR.

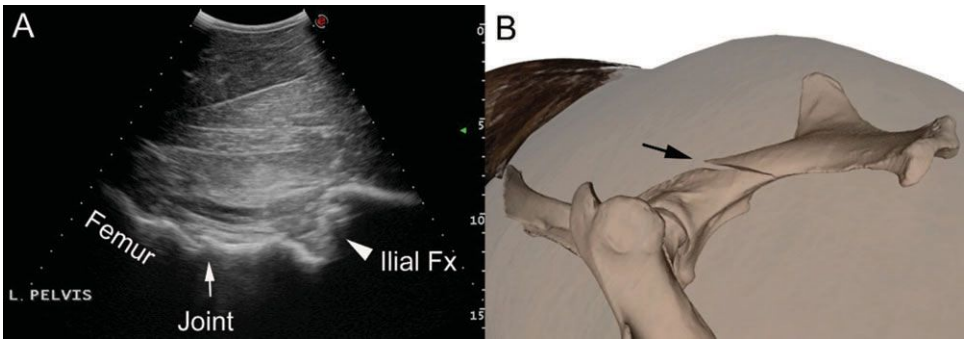

Fig. 9. Ultrasound image (A) and image capture from a 3D simulation movie (B) of a displaced ilial body fracture in a barrel horse. The coxofemoral joint is unremarkable, despite the close proximity of the fracture to the joint.

and stifle.[76–81] We have also capitalized upon ultrasound's advantages to diagnose osseous abnormalities of the cervical, lumbosacral, and sacroiliac regions; regions where radiography is often limited in terms of viability and availability.[82–87] Such widespread use of ultrasound underscores the importance of machine buttonology to prevent misdiagnoses. Even with today's sophisticated machines, there is no substitute for a working knowledge of frequency, scanning depth, focal zones, time-gain-compensation (TGC), and overall gain; the same controls emphasized nearly 30 years ago at the 1985 Toronto AAEP Convention.[7,88]

Whereas ultrasound-guided procedures had previously been performed by specialists to biopsy or aspirate abdominal organs, the value of ultrasound-guidance to access many synovial structures has become well-documented. Ultrasound has significant advantages over blind techniques to inject such structures as the navicular bursa, bicipital bursa, shoulder joint, cervical and lumbar articular facet joints, femorotibial joints, coxofemoral joint, and sacroiliac joint.[83,89–99] Additionally, the burgeoning use of regenerative therapies for tendon and ligament injuries has pushed many practitioners to learn ultrasound-guided techniques for intralesional injection (Fig. 11).[100–112] The entry of these products into the market, including platelet-rich plasma, stem cells, bone marrow concentrate, etc., coincided somewhat with the 2008 economic downtown and created somewhat of a perfect storm. Despite conclusive evidence of long-term efficacy, these therapies have been so rapidly embraced by horse owners that nearly any hypoechoic area could become a potential recipient of

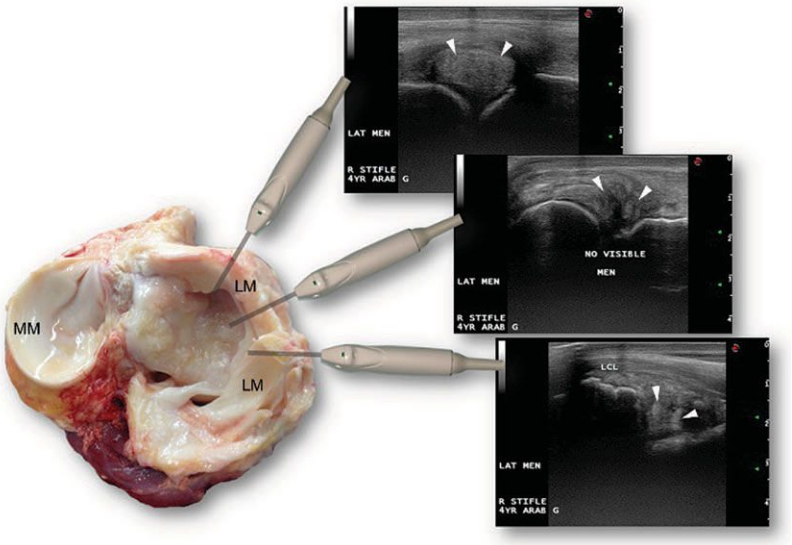

Fig. 10. Gross necropsy specimen (cranial to the top) and corresponding ultrasound images of severe tearing with a midbody rupture of a lateral meniscus (LM) in a 4-year-old Arabian gelding with lameness at the walk. The medial meniscus is normal. The lateral tibial plateau is severely irregular secondary to loss of stability by the torn meniscus. The ultrasound images were acquired at each transducer position prior to euthanasia. The LM (arrowheads) is markedly abnormal in each image. In the top image, the LM shape is recognizable but the meniscus appears extruded. In the middle image, recognizable meniscal tissue is not visible due to rupture at this location. In the bottom image, meniscal tissue is recognizable but the meniscus is severely misshapen.

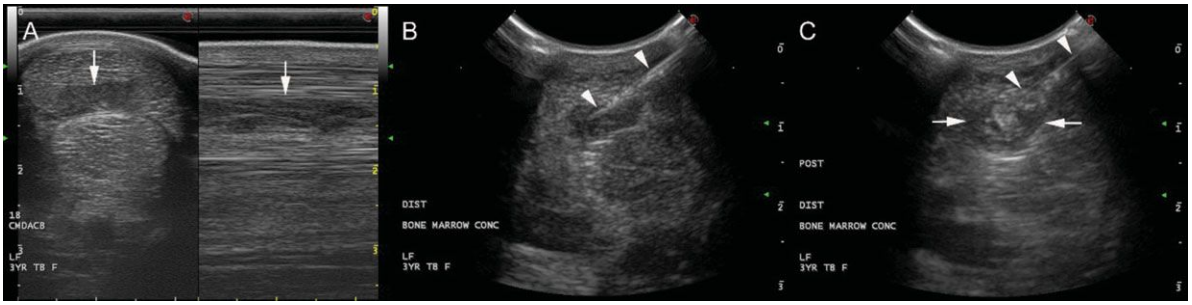

Fig. 11. Ultrasonographic diagnosis (A) and ultrasound guided injection (B,C) of a dorsal lesion (arrows) of the SDFT in a 3-year-old Thoroughbred racehorse. B, The injury was diagnosed using a linear transducer, but a small curvilinear (microconvex) transducer was chosen for injection purposes due to its small footprint and maneuverability. The needle (arrowheads) is placed using a lateral approach into the lesion (lateral to the right). C, Simultaneous imaging throughout needle placement and injection ensured intralesional product deposition, visible as hyperechoic material (arrows).

some type of regenerative medicine product. This subject was frequently voiced as a major concern among ultrasonographers interviewed for this presentation. It was generally felt that the efficacy of these products must be evaluated for longer time periods (i.e., throughout rehabilitation) and compared with controlled exercise and rehabilitation programs. Unfortunately, few studies have been performed in this manner.[113,114]

Another current and emerging trend is the practice of imaging *prior* to lameness localization. This issue is an area of concern for many interviewed and must be addressed. In the absence of swelling, ultrasound should be performed after sequential distal-to-proximal blocking of the limb, even when veterinarians (or the trainer or owner) are "sure" it's the suspensory ligament, the stifle, shoulder, or sacroiliac region. While this is understandable in a fractious patient, it should be otherwise discouraged. The excuse that owners won't approve of blocking is not acceptable. It is up to us as a profession to educate owners of the value of lameness localization. It is a short-term investment that could prevent them from spending thousands of dollars for treatment and rehabilitation for an injury that does not exist or is clinically insignificant. Additionally, it often prevents the discovery of the actual source of lameness.

Abdominal ultrasound has become routine upon referral of a colic patient to a surgical facility and is gradually making headway into the ambulatory setting. Whereas ultrasound was primarily used to detect evidence of strangulating small intestinal lesions, left dorsal displacement, and right dorsal colitis, we now look for evidence of right dorsal displacements (Fig. 12),[115–116] colon torsions,[117] and cecal lymphadenopathy (Fig. 13).[118] The recently described fast localized abdominal sonography of horses (FLASH) technique has prompted veterinarians to scan beyond the left kidney and inguinal regions.[119] Abdominal ultrasound has also become more accepted to evaluate horses for fever of unknown origin, weight loss, and suspect neoplasia (Fig. 14)[120–124] and even to perform transrectal evaluation of the equine ureters (Fig. 15).[125] Thoracic ultrasound is used routinely in academic settings and private clinics with foals as a large percentage of their patient population. In other settings, thoracic ultra-

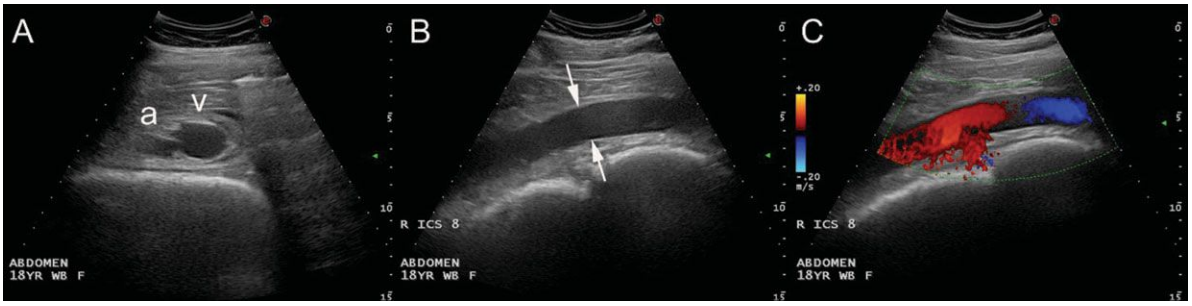

Fig. 12. Ultrasonographic images from an 18-year-old Warmblood mare showing severe venous distention within the colonic mesentery consistent with right dorsal displacement. All images were obtained from the right 8th intercostal space. A, With the transducer positioned perpendicular to the ribs, a transverse image of the distended vein (v) and artery (a) adjacent to the body wall is produced. B, With the transducer positioned parallel to the ribs, a longitudinal image is produced of the markedly distended vein (arrows) adjacent to the body wall. C, Color Doppler confirms blood flow within the vein. Exploratory laparotomy confirmed a right dorsal displacement.

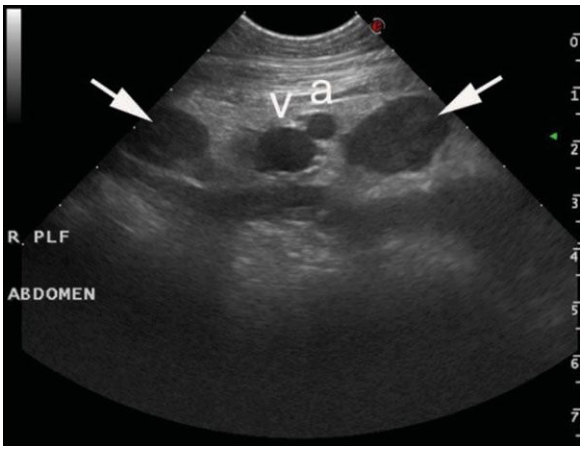

Fig. 13. Enlarged cecal mesenteric lymph nodes (arrows) in a 20-year-old Arabian gelding with recent weight loss and presumptive neoplasia based on other clinicopathologic findings. This image of the cecal mesentery was obtained with a 4 to 8 MHz microconvex (small curvilinear) transducer from the right paralumbar fossa/flank region. The lateral cecal artery (a) and vein (v) are unremarkable and are typically seen in this location adjacent to the body wall. Visibility of lymph nodes is not typical in the normal horse.

sound is often underutilized to evaluate horses in the field with suspect pulmonary or pleural disease, despite being relatively straightforward to perform and interpret. Echocardiography remains in the hands of a few specialists, usually in academic settings. Its use is most limited by the lack of available specialists. The demand for echocardiography is anticipated to rise as our horse population ages and more owners become aware of its value.

Equipment

It is fair to say that the majority of today's veterinarians own at least one ultrasound machine, although actual numbers are not known. Many practitioners have retained their first portable machine for reproductive use and subsequently purchased a higher quality machine for musculoskeletal examinations. From an availability standpoint, the most significant changes have come in the form of small, lightweight, and highly portable ultrasound equipment. If the budget allows, several laptop-type machines are available in the price range of $25,000 to 40,000. Most of today's ultrasound machines allow for digital storage. In less expensive machines, images may be saved on USB-enabled devices. More expensive machines usually have digital archival systems, where images are saved in the patient file system on the machine; most of which also allow for exportation of digital imaging communications in medicine (DICOM) images to picture archiving communication system (PACS) servers. Few veterinarians currently own a thermal printer to print hard-copy ultrasound images. Veterinarians are much more likely to burn images on a compact disc, upload images to a cloud-based system, or PACS server than to print images on thermal paper. Hard copy images, however, remain useful, especially when comparing between exams.

From a transducer standpoint, more of today's equine veterinarians own a high-frequency linear transducer designed for musculoskeletal use. Since their introduction in the mid-1990s, image quality has dramatically improved (Fig. 7). Fewer equine practitioners use a rectal transducer for both reproductive and musculoskeletal exams. This can be explained by the shift away from reproductive work in many practices but also to the increased demand for musculoskeletal ultrasound by the horse-owning public. From an availability standpoint, transducers are now available up to 14 to 18 MHz, which enable us to obtain incredible detail of superficial structures. This is especially true of small structures such as nerves, collateral

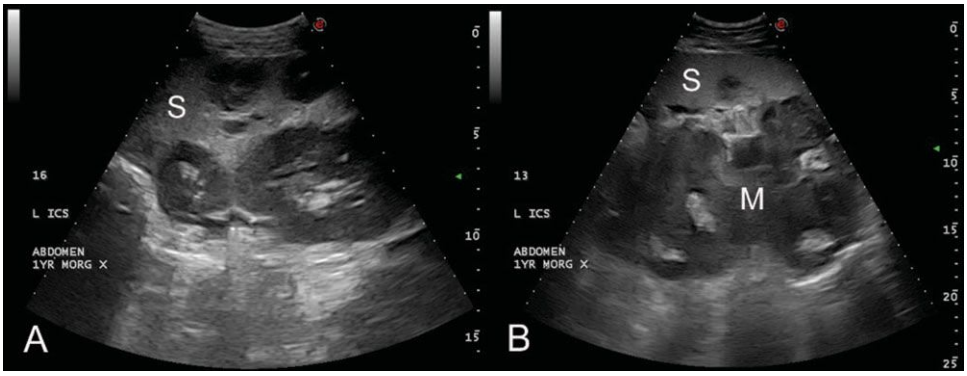

Fig. 14. Ultrasound images from the left 16th (A) and left 13th (B) intercostal spaces of a 19-month-old Morgan cross colt with extensive splenic lymphoma. Only a small amount of normal appearing splenic tissue (S) is visible in both images. Multiple hypoechoic masses, some with hyperechoic centers, are seen in image A. Similar coalescing masses are seen in image B and throughout the left abdomen. The horse was euthanized due to its poor condition and the extent of involvement. Splenic lymphoma was confirmed at necropsy.

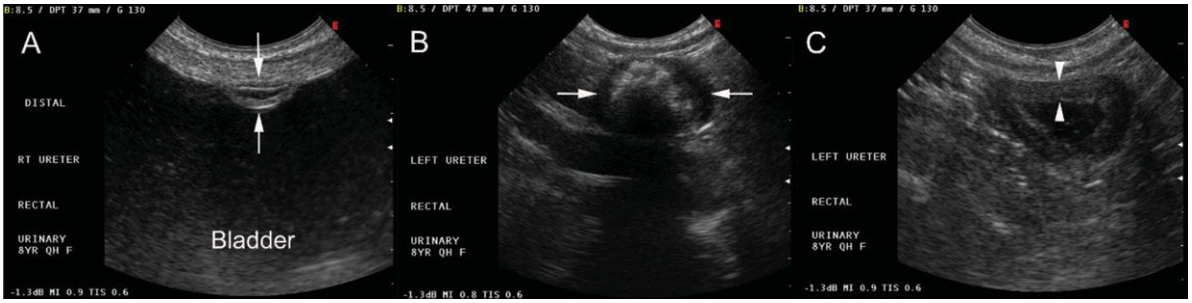

Fig. 15. Transrectal ultrasound images of the right and left ureter in a mare with urolithiasis. A, The right ureter (arrows) appears normal at the trigone region. B, An irregular ureterolith (arrows) was seen within the midportion of the left ureter. C, The left ureter shows marked thickening (arrowheads) and moderate distention proximal to the obstructive ureterolith. Transabdominal evaluation of both kidneys revealed bilateral nephroliths (not shown). No uroliths were found in the bladder.

ligaments, and ocular structures, among others. The drawbacks of these transducers is that they may not penetrate adequately to visualize the suspensory ligament body. Practitioners with a large musculoskeletal imaging caseload may opt to purchase a second linear transducer in the 5 to 10 MHz range for suspensory imaging. Additionally, some of these very high frequency transducers produce unacceptably slow frame rates.

For deep cavity imaging, low frequency transducers have remained in the 2 to 5 MHz range, yet image quality has steadily improved, mainly driven by advancements in image quality from human medicine. Increasing numbers of equine veterinarians now own this transducer, driven, in part, by the desire to perform abdominal and upper limb ultrasound such as the pelvis and sacroiliac region. The midrange frequency (4–8 MHz) transducer, commonly known as a microconvex, has begun to appear in the hands of veterinarians with a large sport horse population to evaluate the suprasesamoidean region, meniscus, and cervical articular facet joints, among other regions.

One of the most significant advances in equine musculoskeletal ultrasound is the availability of durable standoffs that last for more than a few weeks or months. Many practitioners remember the days of purchasing stand-offs for $200 to 300, only to see them tear within a few weeks or months of use. Today's standoffs are also more ergonomic and less challenging to manage than their bulky predecessors (Fig. 16).

Education

Despite the widespread use of ultrasound and owner expectations that veterinarians can perform ultrasound exams, we continue to lag behind in education at the veterinary student and resident level. Few veterinary schools offer meaningful coursework (didactic or hands-on) in large animal ultrasound, with the exception of those few schools with large animal ultrasound faculty and services. These numbers are growing but at a slow pace. In addition to New Bolton Center and UC Davis, Tufts University, Texas A&M, and Colorado State University have either established or are developing programs.

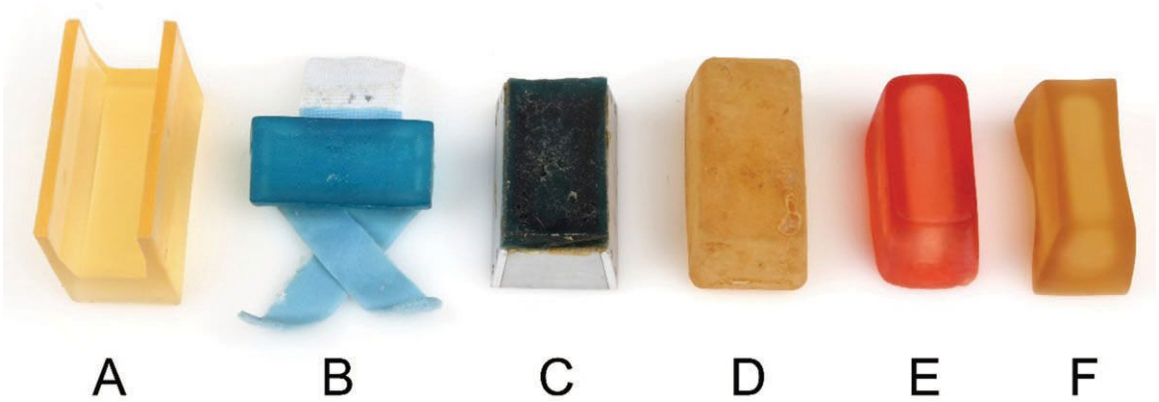

Fig. 16. Various standoffs available from the mid-1990s through 2014 for musculoskeletal ultrasound. Standoffs A to C were available in the 1990s and early millennium. These were notoriously difficult to handle and maintain on the transducer. They were also remarkably fragile, often tearing within 1 to 3 months. Standoffs D to F became available in the mid-2000s. They fit easily over linear transducers and are much more durable than their predecessors. Standoff's E and F have been in heavy use at UC Davis for over 2 to 3 years without tearing.

Many schools primarily rely upon residents to perform ultrasound exams with and without supervision. Some schools still wait for a radiologist to pull away from their lucrative small animal work to perform an equine ultrasound exam.

At the resident level, ultrasound training remains informal and unstructured. Neither the American College of Veterinary Surgeons (ACVS), American College of Veterinary Internal Medicine (ACVIM), nor the American College of Veterinary Radiology (AVCR) have any formal requirements for ultrasound training in large animal species, yet resident graduates are often called upon as "specialists" in this area. It is not difficult to understand why this has occurred in the radiology college where most residents are small animal oriented. This is further confounded by the high demand and financial incentives for small animal work as a board-certified radiologist.

Fig. 17. Veterinary student instruction in equine ultrasound begins in year 3 of the UC Davis curriculum. Multiple regions are scanned by students with a focus on teaching the principles of machine controls and transducer manipulations.

4. Where We Need To Go

Despite the use of MRI in equine veterinary medicine, ultrasound remains a dynamic and exciting area with tremendous potential for growth, but we must also take a critical look at the current state of ultrasound as we plan for the future. The following discussion represents the author's opinions and a compilation of those expressed by Drs. Ron Genovese (Cleveland Equine Clinic), Carol Gillis (Equine Ultrasound and Rehabilitation, Aiken, SC), Norm Rantanen, Virginia Reef (University of Pennsylvania), and Johanna Reimer (Lexington, KY); all well-respected ultrasound leaders with ties to the past and present of equine ultrasound.

Ultrasound has come a long way since its origins, but it is particularly curious that several colleagues felt vehemently that ultrasound had taken steps backwards in recent years. Concerns include a lack of accountability, especially when exams are not properly documented or cannot be traced to a patient or limb due to a lack of labeling. Additional concerns previously mentioned include the proliferation of costly yet unproven intralesional therapies (often client-driven), a lack of understanding of the significance of hypoechoic areas (created or real) within many anatomic structures, and the tendency to leap to imaging before appropriately localizing the lameness. Many also commented on the lack of education in veterinary school that leaves new practitioners untrained and ill-prepared to perform a diagnostic exam and the tendency to make a diagnosis without consulting on the significance of suspect lesions. With these concerns in mind, future directions for ultrasound will be consolidated into to the following three categories: Education, Best Practices, and Research.

Education

It is the strong opinion of the author and fellow ultrasound colleagues that education is the key to advancing ultrasound in equine practice and that it should begin with foundational skills at the veterinary school level (Fig. 17). Ultrasound is a highly operator-dependent imaging modality, with interpretation occurring during both real-time scanning and captured still images. The ability to perform and interpret ultrasound studies is a major challenge for many practitioners. Instruction at the veterinary school level provides students with the freedom to make mistakes since there are no preconceived notions of ability levels. Such an environment allows students to develop those foundational skills that can be used throughout their scanning career. These skills become much more challenging to teach after graduation, where CE attendees are often more focused on scanning specific regions rather than learning those foundational skills necessary to obtain diagnostic images.

Continuing education events provide the mainstay of today's ultrasound education, and CE dedicated to ultrasound has proliferated across the country. Because of the lack of training in veterinary school, these events are important to advance the state of ultrasound in equine practice. However, attendees must continually remind themselves that learning ultrasound requires practice, dedication, a critical eye and humility to reach out to experts on a regular basis. Although many of us teach at these events, we have become increasingly concerned with the predominance of events produced solely by the private profit driven sector of ultrasound companies. While their efforts are to be commended, this creates potential conflicts of interest. Practitioners can be led to believe they will obtain a level of competence after an afternoon, one day, or weekend ultrasound course. It cannot be overstated that ultrasound is an art with many nuances. Attendees must understand that ultrasound takes time to learn and that proficiency is not

rapidly learned. It takes years to develop the expertise to scan some of the regions presented in short courses, yet some instructors have felt discouraged from telling this truth to attendees.

From a residency training standpoint, large animal ultrasound should become a formal requirement for board certification in many residency programs. Some level of ultrasound expertise is often assumed after completion of an equine surgery, internal medicine, or imaging residency. While residents are exposed to varying levels of ultrasound experience, most residents receive little, if any, formal didactic or hands-on ultrasound instruction. It is surprising that ultrasound education is not a formal requirement for the ACVS, ACVIM, or ACVR. This does little to legitimize an imaging modality that is in widespread use and fraught with potential interpretative errors. Progress towards this end has been made within the European College of Veterinary Diagnostic Imaging (ECVDI), who recently began a large animal imaging residency program. To expand the number of qualified mentors for their programs, the ECVDI inducted several non-ECVDI boarded experts in large animal imaging as associate members. The ACVR has also made efforts to develop an equine imaging residency program, but the outcome is not yet clear. In any case, the formal incorporation of ultrasound into ACVR training programs should help to legitimize equine ultrasound, develop standardized exams and techniques, increase student exposure in veterinary school, and encourage a more balanced approach to comparative imaging studies.

Best Practices

The concept of best practices and education go hand-in-hand. Well-known in the business world, best practices are usually formulated by experts in the field who have a strong sense of what is necessary to develop guidelines that optimize the value of a technique or process. Specific to ultrasound, best practices should include the development of standardized exams, indications, exam documentation, proficiency standards, and ethics, among others. With no such standards or practices set in place, clinicians do not have a bar to which they can rise.

Proficiency Standards

Ultrasound is highly operator-dependent, much more so than any other imaging modality. Unfortunately, few veterinarians possess the natural spatial orientation skills to translate three-dimensional anatomical structures into two-dimensional ultrasound images. For those lucky few, ultrasound will seem more straightforward. For the majority of veterinarians, ultrasound is a struggle. This is further compounded by the fact that some regions are much more challenging to evaluate than others. It would, therefore, seem prudent to assign levels of difficulty to individual regions and structures (e.g., Levels 1–5, with 1 being easiest and 5 being most challenging). Doing so would help budding ultrasonographers better match their ability levels with structures of interest and leave the more challenging structures to advanced or expert imagers.

Proficiency standards would be more challenging to define, but attempts should be made to do so. Because of variations in natural ability level, it is impossible to state how many exams are required before a level of proficiency is achieved with a specific region. Proposed proficiency levels up for discussion could be: beginner (1–30 scans), intermediate (31–100 scans), advanced (101–300 scans), and expert level (>300 scans).

Study Indications

Indications will vary depending on the type of exam being performed, i.e., abdomen, cardiac, metacarpal, pastern, stifle, sacroiliac, etc. Although a detailed discussion of study indications for all regions is beyond the scope of this presentation, some regions deserve special mention. From a musculoskeletal standpoint, we must become more diligent and critical in lameness localization prior to performing imaging studies. Ultrasound is clearly indicated in the presence of swelling, wounds, effusion, or regional atrophy. Otherwise, lameness should be localized via nerve blocks and/or intrasynovial anesthesia prior to ultrasound. It is continually surprising how many owners and veterinarians believe they can see a shoulder, stifle, or sacroiliac lameness without the use of diagnostic nerve blocks. Referral clinics are often faced with such cases that subsequently block to the foot. It is recognized that clients sometimes resist the expense of blocking; however, it is good insurance against the expense of a long-term rehabilitation program for a clinically insignificant injury. We must also be diligent and consider the possibility that perineural blocks may have entered synovial structures, such as that which can occur in the proximal metacarpus and metatarsus.

Exam Standardization

Similar to that for radiographic studies, standardized exams for ultrasound studies should be defined. While it may be challenging to obtain a consensus on standardized studies, it should be relatively straightforward to define what would be considered basic "views" and what would be considered a "special" or perhaps "advanced" view. For example, a basic pastern ultrasound exam traditionally entails imaging of the deep digital flexor tendon and straight distal sesamoidean ligament along the palmar (plantar) midline from the ergot to the proximal interphalangeal (PIP) joint and then abaxial views to image the branches of the superficial digital flexor tendon and origins of the oblique distal sesamoidean ligament. Evaluation of the axial and abaxial palmar (plantar) ligaments or collateral ligaments of the pastern joint would likely be considered special

Fig. 18. An appropriately labeled image of an injury to the superficial digital flexor tendon. At a minimum, patient identification (1), documentation of the limb or region being scanned (2), and the site of image acquisition (3) should be included on every image. For publication purposes, the patient's name was removed from this image to preserve anonymity but is included on every image; however, the patient's ID number is shown. The clinic name should also be visible (4). We also find it helpful to write signalment on the image (5). This is especially helpful when sending images for review, where signalment is often omitted from the history. Practitioners should also check that the date of acquisition printed on each image (6) is correct.

views due to their inherent difficulty level. Evaluation of the suprasesamoidean region at the level of P2 between the heel bulbs is a regular part of the pastern exam at UC Davis, but it would likely be considered a special view since relatively few practitioners own the transducer (microconvex) necessary for this exam.[126] Such standards would better allow us to compare 'apples to apples.' Had standardization been present during the early years of MRI, it is possible that some injuries described as visible only with MRI could have been detected with ultrasound, provided that standardized exams had been performed on all cases.

Exam Documentation

Although ultrasound has been in routine use for 20 to 30 years, it remains the only imaging modality without standards for image acquisition and study documentation. At a minimum, every image should identify the patient, name of the performing clinic or veterinarian, and the limb, region, or structure being evaluated as well as some indicator of the location of the image (Fig. 18). It is quite surprising to the author and many ultrasound colleagues how frequently images are sent for review without *any* identifying information. This is widely considered unacceptable for radiographic, scintigraphic, CT, or MRI studies and should be considered equally unacceptable for ultrasonographic studies. Practitioners are not solely at fault. Ultrasound manufacturers and distributors are also partially responsible for this problem. On some machines, labels and text are sometimes set to disappear each time an image is unfrozen. The veterinarian is then left to relabel each image, which is both time-consuming and discourages proper labeling. This issue can sometimes be corrected within the menu settings, but sales representatives can be reluctant to do so and practitioners often are completely unaware of this possibility.

Documentation of the specific site of image acquisition is particularly important for metacarpal and metatarsal studies and especially for recheck purposes. The zone system described by Dr. Genovese and co-authors in the mid 1980s remains in use by some practitioners.[8] Another common technique is to measure in centimeters distal (or proximal) to the point of the accessory carpal bone in the forelimb or to the point of the hock (calcaneal tuberosity) in the hind limb. A ruler or measuring tape can be used for this technique. It is important to use the measurement that bisects the transducer in both transverse and longitudinal views (Fig. 19). Accurate localization of image acquisition is especially important when comparing images between exams. Tendon and lesion cross-sectional area measurements can vary substantially within a few centimeters of an injured area. This could lead to a misinterpretation of healing progress (or lack thereof) if an image is accidentally acquired slightly proximal or distal to the original site of image acquisition.

Finally, standards are also necessary to define the minimum number of images that adequately document an exam or injury (Fig. 20). Many ultrasound exams are performed without any documentation whatsoever or with inadequate documentation. While image capture or printing may not be practical for acute abdominal imaging or routine broodmare work, it is not acceptable for musculoskeletal ultrasound, just as it is not considered acceptable for radiographs, scintigraphy, CT, or MRI.

Ethics

Ultrasound ethics covers a wide range of issues; many of which have already been presented. These include recognition of one's limitations, divulging proficiency levels to clients, seeking expertise whenever possible, and remaining objective in interpretation, especially when the lameness has blocked to that region. One of the most challenging aspects of ultrasound is to tell a client that an ultrasound exam is unremarkable. Ultrasound is often the last resort to diagnose a lameness and one must resist the temptation to overdiagnose in such situations because "the lameness blocks there." This is especially a problem in horses with metacarpal or metatarsal lameness where hypoechoic areas (due to fat and muscle) within the suspensory ligament can be easily overinterpreted as injury. When in doubt, a consultation should be requested or, at a minimum, recommended in challenging situations.

Fig. 19. Use of a ruler to document the anatomic location of images obtained from the metacarpus. Cross-sectional area measurements of the tendon/ligament and injured region are also obtained on each captured image.

Perhaps more importantly, a certain responsibility comes with the use of an ultrasound machine. A user should feel obligated to become familiar with basic artifacts, machine instrumentation, and, of course, knowledge of regional anatomy. Its use requires a level of dedication to learning the modality, which includes CE and practice, practice, practice.

Research

As we look forward, there are many areas of research that would improve the value of ultrasound in equine practice. Although not meant to be exclusive, the following areas represent exciting areas of research as well as areas of perceived need, as identified by the author and ultrasound thought-leaders.

Healing/Rehabilitation

An improved knowledge of ultrasonographic features of tendon and ligament healing is necessary to better advise our clients throughout the rehabilitation process. This information is also relevant in horses with injuries diagnosed on MRI since ultrasound may be used to monitor after the initial MRI diagnosis. This information is important whether we are evaluating healing with or without intralesional therapies.

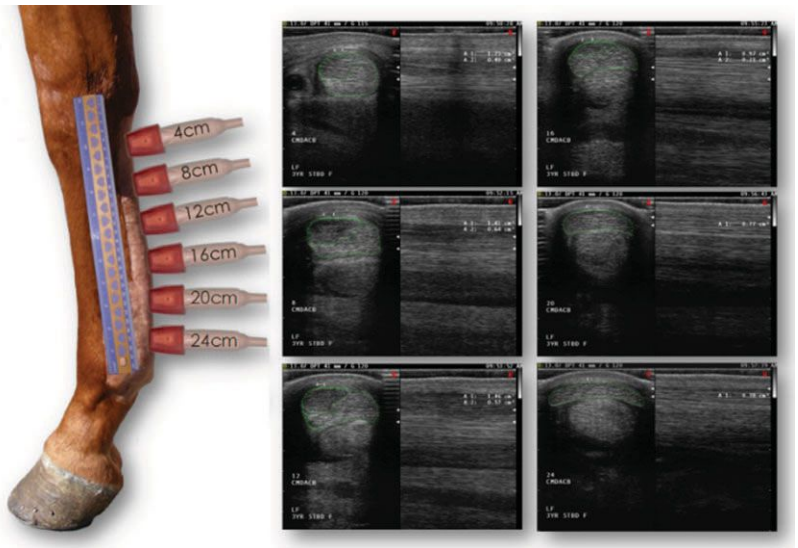

Fig. 20. A well-documented exam of a forelimb SDFT injury extending from 4 to 24 cm distal to the accessory carpal bone. Transverse and longitudinal images were obtained every 4 cm (in each zone). Cross-sectional area measurements were obtained of the tendon and lesion to calculate percent tendon damage. These images were then used for comparison during recheck ultrasound examinations.

We must first improve our knowledge of expected healing properties as they relate to the ultrasonographic appearance of injuries throughout rehabilitation. Much of our current knowledge of tendon and ligament healing is based on studies of SDFT injuries. Little information is available regarding short- or long-term healing patterns of other structures, yet the known ultrasonographic features of SDFT healing are routinely extrapolated to other tendons and ligaments. Experienced ultrasonographers with a discerning eye know that some structures show minimal, if any, ultrasonographic improvement on repeat ultrasound exams. Unfortunately, this information is not widely known, especially among practitioners with intermittent exposure to these injuries. In such a scenario, the lack of ultrasonographic improvement can be erroneously interpreted as inadequate or poor healing and recommendations made to repeat or begin intralesional therapies to "stimulate" healing. Such an approach entails substantial economic costs to the client and can unnecessarily prolong the rehabilitative process. This information is also important to define in horses treated with the various intralesional products so that we can better assess their efficacy.

Second, and perhaps more importantly, equine practitioners must become more critical regarding ultrasonographic assessment of healing during recheck exams. The temptation to describe an image as "improved," despite a nearly identical appearance, is not uncommon. This can be further influenced if the client has spent significant resources on intralesional therapies. We must also become more comfortable informing owners that the ultrasonographic appearance of their horse's injury has not changed in the recheck interim if, in fact, that is the case. Just as clients are made aware of the lack of radiographic healing of proximal and distal sesamoid bone fractures, clients should be aware of realistic expectations on recheck ultrasound exams.

Comparative Imaging Studies

As previously mentioned, comparative imaging reports tend to show heavy bias towards MRI and often dismiss the utility of ultrasound to diagnose soft tissue injuries. Admittedly, MRI provides more detailed information than ultrasound, but MRI remains impractical for many equine patients. It is possible that some injuries described as visible only with MRI could be detected with ultrasound, provided that standardized exams or appropriate techniques had been performed. More recently, some studies have been published with an eye to maximize the information obtainable from all imaging modalities rather than dismissing the value of one to enhance the value of another.[66,127]

New Techniques

Preliminary studies to evaluate innovative ultrasonographic techniques to detect early tendon/ligament injuries include elastography, acoustoelastography, contrast ultrasound, and off-normal incident angle scanning. *Elastography* measures tissue strain properties that may be altered in the presence of tendon and ligament injuries. The use of elastography was recently validated in normal equine flexor tendons in the metacarpus.[128] *Acoustoelastography* has also been recently described in normal equine superficial digital flexor tendons to evaluate tissue stiffness gradients.[129] A preliminary study on the use of *ultrasound contrast agents* in the distal extremity was recently presented at the ACVR.[130] Although the technique was performed in only three horses, no adverse effects were noted in the short term. None of these techniques are widely available. Their utility and acceptance by horse owners will remain to be seen, but all are interesting areas for research that may improve our ability to detect pathology in horses. *Off-normal incident angle ultrasound* has been used for some time to improve visualization of tendon borders or injuries that appear isoechoic with on-beam scanning (Fig. 21) and has recently gained traction to evaluate the suspensory ligament body using non-weight-bearing techniques. Recent studies have shed new light on this valuable technique to help differentiate between ligament, fat, muscle, and injuries within the suspensory ligament body.[127,131] Continued investigation into these techniques, along with MRI and histologic validation, should prove even more enlightening to help diagnose suspensory and other injuries.

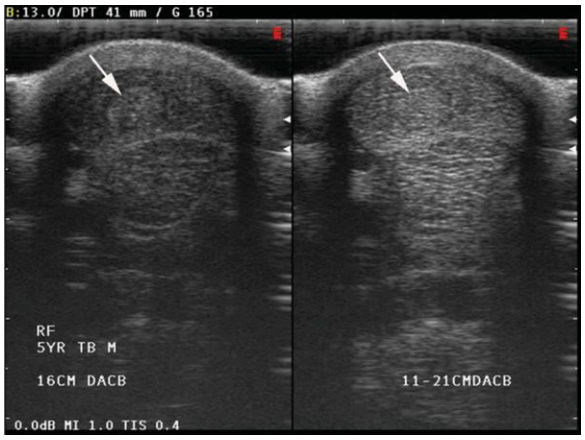

Fig. 21. Use of "off-beam" or off-normal incident angle ultrasound in a horse with a healing core lesion of the superficial digital flexor tendon. The left image was obtained with the transducer intentionally held "off beam." This technique improves visibility of the tendon margins and the hyperechoic core lesion compared with the image on the right. The right image was obtained with the transducer oriented 90° to the SDFT to produce an "on beam" or bright image of the tendon. Due to clot formation within the lesion (arrow), it appears nearly isoechoic to the surrounding normal SDFT and is therefore difficult to visualize.

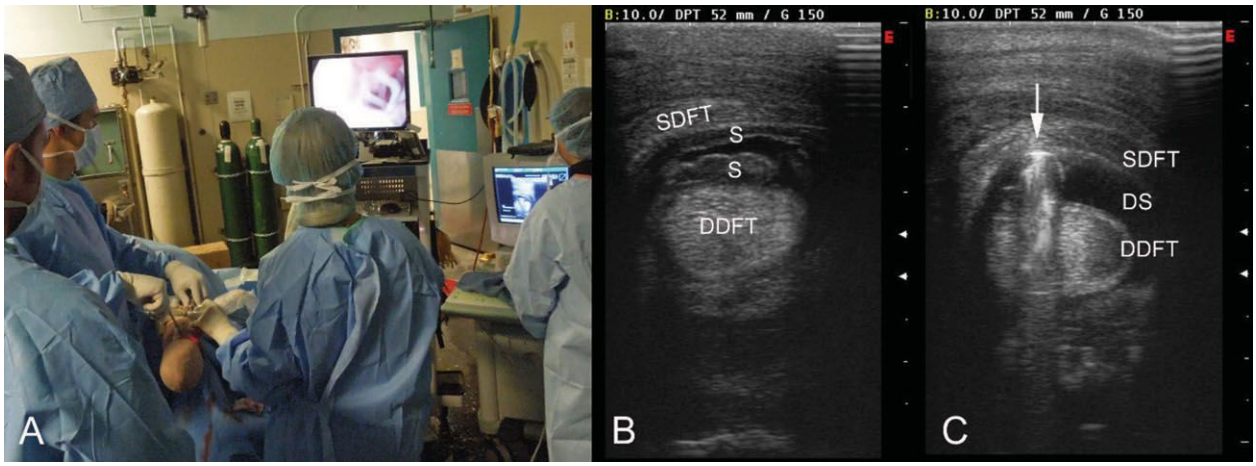

Fig. 22. A, Use of intra-operative ultrasound-guidance during tenoscopy in a bull with septic tenosynovitis of the lateral digital sheath of the left hind limb. Ultrasound was first used to place needles for synovial distention. B, Severe synovial thickening (S) was present along the length of the lateral digital sheath. The medial digital sheath (not shown) was unaffected. C, After exploration of the sheath, ultrasound was used for both orientation purposes and to monitor progress as the synovium was resected using a synovial resector (arrow). Note the improved appearance of the digital sheath (DS) in this image compared to B. Superficial digital flexor tendon (SDFT). Deep digital flexor tendon (DDFT).

Interventional Procedures

The widespread use of ultrasound-guidance to assist with intralesional and intra-synovial injections has improved our overall comfort level with ultrasound-guidance. Continued validation of ultrasound-guided versus blind techniques is important, similar to that recently performed for shoulder synovial structures.[132] The importance of dynamic real-time imaging throughout the entire ultrasound-guided procedure is another area of investigation. It is our experience that many practitioners use ultrasound to guide the needle to the site, but do not always monitor throughout the injection procedure (see Fig. 11).

Multiple techniques for ultrasound-guided intrathecal puncture for cerebrospinal fluid collection have been published in the past decade, including two recent studies that describe standing techniques at the level of C1-C2 and at the atlanto-occipital junction, respectively.[133–135] Although the authors of both studies reported good results, relatively few horses were used, the majority of which were normal. Further studies on a larger group of horses with a focus on safety would seem prudent, given the potential for serious consequences.

We would also benefit from the development of new minimally invasive procedures, such as ultrasound-guided removal of foreign bodies in standing horses. Ultrasound can also be integrated into the surgical suite in nontraditional ways, such as ultrasound-assisted tenoscopy or hardware removal. We recently performed both procedures at UC Davis and found that ultrasound provided assistance to direct instrumentation during digital sheath tenoscopy in a bull with synovial sepsis (Fig. 22). We also found it surprisingly valuable to locate screw heads for plate removal in a horse that had previously undergone pastern arthrodesis.

5. Conclusion

It is the author's hope that these proceedings and its accompanying presentation will serve as a 'call to arms' for the profession to raise the bar for ultrasound in equine practice. Ultrasound is a powerful tool and has much to offer our equine patients and their owners, even in situations where MRI is available and affordable. As we continue to expand the use of ultrasound, we must also continue to scrutinize and maintain a critical eye towards image quality, acquisition, and interpretation.

Acknowledgments

Conflict of Interest

The Author declares no conflicts of interest.

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[a]Vingmed System V, GE Healthcare, Wauwatosa, WI 53226.
[b]Biosound Technos, Biosound Esaote, Indianapolis, IN 46250.
[c]Biosound Twice, Biosound Esaote, Indianapolis, IN 46250.
[d]Ausonics Opus, Universal Ultrasound, Bedford Hills, NY 10507.
[e]Ausonics Impact, Universal Ultrasound, Bedford Hills, NY 10507.

Auditory Loss in Horses, Part I: Adult Horses

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Evaluation of hearing should be part of the physical and neurological examination since hearing loss occurs in horses and could result in altered behavior and performance. If hearing deficits are suspected, a brainstem auditory evoked response test should be performed. Authors' address: The William R. Pritchard Veterinary Medical Teaching Hospital, University of California, Davis, CA 95616; e-mail: mraleman@ucdavis.edu. *Corresponding and presenting author. © 2014 AAEP.

1. Introduction

Hearing loss in horses has been seldom investigated and can be a cause of difficulty in training, decreased performance, and behavior alterations. Brainstem auditory evoked response (BAER) testing is a noninvasive, objective, and easy to perform diagnostic modality that evaluates the integrity of the auditory pathway. The objective of the study was to describe BAER findings, common signs, and causes of hearing loss in adult horses.

2. Materials and Methods

BAER records from the Clinical Neurophysiology Laboratory were reviewed from the years of 1982 to 2013. Medical records were reviewed, and horses were grouped under disease categories. Descriptive statistics included mean, standard deviation, and range.

3. Results

Fifty-seven of 76 horses that had a BAER had auditory deficits. There was no breed, gender, or age predisposition with the exception of American Paint horses diagnosed with congenital sensorineural deafness. Auditory loss was bilateral in 74% and unilateral in 26% of the horses. The most common causes of auditory loss included temporohyoid osteoarthropathy (35%), congenital sensorineural deafness (30%) associated to certain coat and eye color patterns, multifocal brain disease (23%), and otitis media/interna (7%).

4. Discussion

Temporohyoid osteoarthropathy, multifocal brain disease, and otitis are common causes of hearing loss. Hearing should be investigated in horses with extensive white color markings.

Acknowledgments

Conflict of Interest

The Authors declare no conflicts of interest.

Research Abstract

NOTES

Auditory Loss in Horses, Part II: Foals

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Common neonatal diseases such as sepsis, ischemic hypoxia, neonatal isoerythrolysis, prematurity, and congenital anomalies can result or be associated with auditory loss in foals. Therefore, evaluation of hearing should be part of the physical and neurological examination in ill-neonatal foals. Authors' address: The William R. Pritchard Veterinary Medical Teaching Hospital, University of California, Davis, CA 95616; e-mail: mraleman@ucdavis.edu. *Corresponding and presenting author. © 2014 AAEP.

1. Introduction

Auditory deficits occur in foals but are rarely recognized. In human medicine, several neonatal diseases are common causes of permanent hearing loss in infants. Descriptions of auditory loss in neonatal foals are limited to 3 foals with lethal white foal syndrome. The association of auditory loss with common neonatal diseases has not been studied. Therefore, the objective of the study was to investigate auditory deficits in ill-neonatal and non-neonatal foals.

2. Materials and Methods

Brainstem auditory evoked response (BAER; a study to investigate auditory function) records from the Clinical Neurophysiology Laboratory were reviewed from the years of 1982 to 2013. Clinical data were extracted from the medical records. Descriptive statistics were performed.

3. Results

Ten of 15 neonatal foals had absent BAER and were associated with sepsis, neonatal encephalopathy, neonatal isoerythrolysis, and prematurity. Auditory deficits were also observed in foals with certain coat and eye color patterns such as completely or mostly white with blue irises and lavender with pale yellowish irises. One non-neonatal foal (n = ⅓) with an intracranial abscess had auditory loss.

4. Discussion

Hearing deficits occur in foals and are associated with common neonatal diseases and certain coat and eye color patterns. Neurologic and intracranial disease can also result in auditory loss in foals of any age.

Acknowledgments

Conflict of Interest

The Authors declare no conflicts of interest.

Research Abstract

NOTES

How to Manage Foal Rejection

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Foal rejection is an uncommon but important problem that can adversely affect the well-being of equine neonates. Various methods are available for managing and overcoming foal rejection. These include physical and/or chemical restraint, behavior modification, and hormone therapy. Surrogation is utilized when a foal's dam experiences severe illness, death, or outright rejection of the foal, all of which can prevent her from raising her foal. Methods commonly used to treat foal rejection are also used to graft foals onto surrogate mares. Authors' address: Claiborne Farm, PO Box 150, Paris, KY 40362; e-mail: cscoggin@claibornefarm.com. *Corresponding and presenting author. © 2014 AAEP.

1. Introduction

While relatively uncommon, foal rejection is a serious condition that can adversely affect the health and welfare of an equine neonate. Consequences can be costly and labor intensive for the human caretakers and they can cause untoward and sometimes fatal injuries to the foal. Foal rejection can be seen in all breeds of horses, with the highest rate reported in Arabians (5.1%), followed by Paint Horses (1.9%) and Thoroughbreds (<1%).[1] Another important consideration is that a mare that has previously rejected her foal is at an increased risk of doing so again.[2]

Normal Maternal Behavior

Most signs of normal maternal behavior are fairly easy to recognize. Nickering at and licking and nuzzling of the foal are commonly seen traits. A dam will shield her foal from humans and other horses and she will often stand over her foal while it is recumbent. More subtle signs include the mare stepping a hind leg back to offer greater exposure to the udder and gently nudging the foal as it is searching for a teat. These motherly instincts are thought to develop from various sensory, tactile, and mental stimuli. Stimulation can occur with olfactory recognition of fetal fluids and membranes, physical contact, nursing, and visual exchanges with the foal. These actions—along with other factors that might not be readily apparent—help foster normal attentive and protective maternal behavior.

Endogenous hormones are important for developing a female's instincts and ability to care for and nurture her offspring. The neuropeptide oxytocin seems to be important in developing proper maternal behavior. Oxytocin is synthesized in the hypothalamus and released from the posterior pituitary gland. While commonly thought of as an ecbolic agent whose primary target tissue is the myometrium of the uterus, oxytocin has been demonstrated to have effects within the central nervous system. Indeed, its importance in stimulating maternal behavior has been described in humans,[3] lab animals,[4] and farm animals.[5,6] In addition, progesterone and estrogen may also play a role in the development of maternal behavior. For example, a preliminary study showed lower serum concentrations of these hormones in rejecting mares compared to nonreject-

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ing mares.[1] Lactation—an important milestone in the immediate postpartum period—is mediated through a multitude of hormones, including the aforementioned sex hormones and oxytocin, as well as growth hormone, prolactin, dopamine, and insulin-like growth factor 1. Consequently, knowledge of the hormonal events involved in maternal behavior, lactogenesis, and milk letdown can become important when identifying causes for and managing cases of foal rejection.

Abnormal Maternal Behavior and Foal Rejection

Abnormal maternal behavior and foal rejection can be perplexing and, at times, disturbing to witness. Mares may kick, squeal, or chase their foal, while others may simply seem indifferent to or become elusive from their foals. Different forms of foal rejection have been previously described by Crowell-Davis and Houpt.[7] In our practice, we typically see two types of foal rejection. The first is aggressive rejection, which is typified by overt violence and hostility towards the foal. Signs include biting, kicking, and savage attacks. The second is avoidance rejection and is characterized by disinterest of the mare and/or evasion from the foal. For instance, the mare may walk away from the foal when it attempts to nurse or fail to display any interest in the foal, especially when the two are removed from eyesight of each other. A salient point regarding these classifications is that they are not mutually exclusive; indeed, variations of the two can occur depending upon the circumstances and individuals involved in each case.

In some cases, the cause for foal rejection is readily apparent. For example: the mare could be young and/or inexperienced; she could have previously rejected a foal; she could feel anxious or nervous around the foal due to a change in environment or management; she could have poor milk production secondary to a disease process; she could have produced an abnormal or sick foal; or the scent of the foal could have been altered through drugs, such as dimethyl sulfoxide (DMSO), which could interfere with olfactory recognition.[2] All of these instances can disrupt bonding time or hamper the natural progression of motherly behavior, thereby leading to foal rejection.

Other times, the reasons for rejection are not so clear. The clinician must obtain a thorough history and perform a careful physical examination of the mare and foal to decide if there are any issues that may be contributing to the abnormal behavior. Watching the pair interact can be useful for determining certain behavioral cues that might be contributing to rejection. It also behooves the clinician to carefully evaluate the management practices of the farm to ascertain whether changes in the environment have led to maternal aggression or avoidance.

One factor that is sometimes overlooked, yet highly important, is that of maternal pain. Pain can be secondary to either normal physiologic processes or certain disease conditions. Examples of the former include pain arising from postpartum uterine contractions or discomfort from a sore udder due to an aggressively nursing foal.[2] Examples of the latter include musculoskeletal injury, such as laminitis or osteoarthritis, and systemic illness, such as metritis associated with retained fetal membranes or gastroenterocolitis. Identifying the source of pain underscores the importance of performing a thorough physical examination, while proper management of the pain can result in a successful outcome for all parties involved.

The safety and health of the foal should be of primary concern when dealing with cases of foal rejection. As such, the foal should be immediately removed from a mare that displays overt aggression towards the foal. If necessary, the foal should be administered at least one pint of good quality colostrum through a nasogastric tube for passive transfer of immunoglobulins and nourishment. Alternatively, or in addition, intravenous plasma can be used to provide adequate passive transfer. Subsequent feedings can be performed every two to three hours with the mare's own milk (provided she is tractable for harvesting milk), goat's milk, or a commercial foal milk replacer formula[a] from a pan, bottle, or indwelling nasogastric feeding tube. Supportive care, in the form of intravenous fluids, and prophylactic administration of broad-spectrum antimicrobials may also be administered at the discretion of the clinician to prevent or treat dehydration and sepsis.

2. Materials and Methods

Strategies that can be employed to treat and manage foal rejection include physical restraint, behavior modification, pharmaceutical intervention, and surrogation. Depending on the circumstances and individuals involved, these strategies can be used alone or in conjunction with one another. Also, farm management practices and available resources may preclude the use of some of these strategies. The clinician must thus work in cooperation with the owner or caregivers to determine which strategies could be useful, as well as which ones are feasible with respect to their experience and wherewithal.

Success in establishing normal maternal behavior or adequate surrogation can be determined by using at least three subjective markers. The first marker is the mare's response following removal of the foal from eyesight. If the mare whinnies, becomes anxious, or attempts to follow the foal, then these are good indicators of the mare's acceptance of and bonding with the foal. The second marker is leaving the mare and foal free from restraint in a stall or small enclosed area and observing their interactions. Positive indicators include: witnessing the foal physically contacting the mare without the mare showing any signs of aggression or indiffer-

Fig. 1. Physical restraint of the mare with a halter and lead rope allows for the foal to nurse by minimizing the amount of movement by the mare.

Fig. 3. While a set of stocks or barricade is not available in all situations, it is both a safe and functional tool for managing foal rejection.

ence; seeing the mare willingly allow the foal to nurse; and observing the mare standing over the foal as it is lying down. The third positive marker is turning the pair out in a paddock and seeing them remain in close proximity to one another, with the mare permitting the foal to nurse her at its own volition.

Physical Restraint

Physical restraint is one of the most commonly used means of dealing with foal rejection. A mild method of restraint is that of having a handler hold the mare steady by her halter and a lead rope (Figs. 1 and 2). Doing so can prevent the mare from walking off, swinging her hind end away from, or turning towards the foal as it attempts to nurse, all of which can significantly impede the foal's ability to seek the udder. This particular method seems to be highly effective for inexperienced or maiden mares in that it can condition them to the presence of the foal when in the region of the udder.

There are several other options for restraint. These include the use of stocks or a barricade (Fig. 3), nose twitch (Fig. 4), lip chain (Figs. 5 and 6), neck pinch or "shoulder roll" (Fig. 7), and breeding hobbles. These methods are often employed when the mare demonstrates signs of aggression towards the foal, especially kicking or biting. Selection is dependent upon the available resources, experience of the handlers, and disposition of the mare. In the authors' practice, a barricade is routinely used that provides good protection to the foal but, at the same time, allows easy access to the udder. Nursing behavior can be controlled by the use of a sliding door to limit the number of suckling attempts (Figs. 8 and

Fig. 2. Physical restraint allows for controlled interaction between the mare and foal.

Fig. 4. Physical restraint with a nose twitch.

Fig. 5. Physical restraint with lip chain.

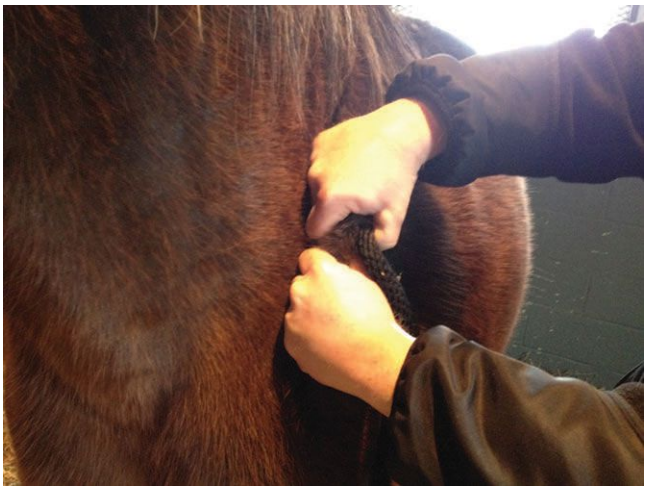

Fig. 7. A neck pinch, also called a "shoulder roll," is a method of physical restraint. The method of restraint used should be dependent on the horse, the handler, and the situation.

9). Doing so prevents the mare from developing a sore udder and protects the foal if the pair needs to be left unsupervised for a period of time. One obvious downside to this setup is that it does not allow the mare constant visual access to the foal, especially when it is attempting to nurse. It is also important to allow the mare time out of the barricade or stocks so that she does not become stiff or develop distal limb edema.

The reader is advised that these methods of restraint are only temporary measures. If used too frequently, mares may become resentful and develop a negative association with the foal and nursing behavior. What is more, caution must be exercised when using a twitch or hobbles because they can—if used too severely or for too long of a time period—put the mare, foal, and human handlers at risk for injury.

Behavior Modification

Behavior modification primarily involves the use of positive reinforcement. This method entails the handler offering encouragement in a soft and soothing voice when the mare demonstrates signs of good maternal behavior. Gentle rubbing along the neck or between the eyes can also be used to comfort the mare. Another option is to offer grain or treats as the foal nurses. Doing so presents a reward for good behavior and can, indirectly, create a mild distraction for the mare as the foal attempts to engage and nurse her. Negative reinforcement is less frequently used and is performed to dissuade a mare from acting aggressively towards her foal.

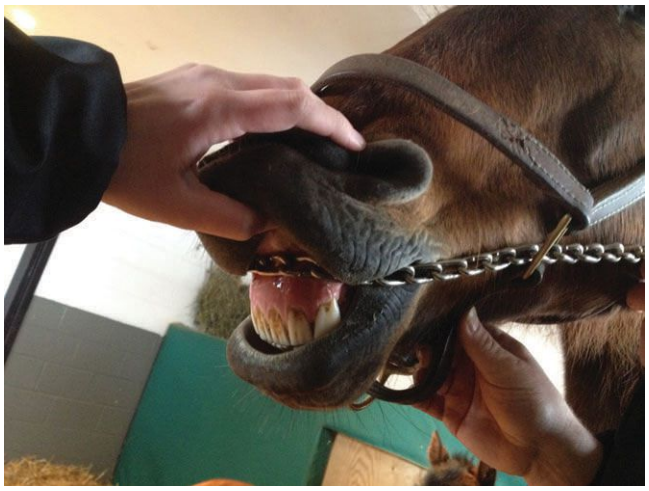

Fig. 6. Proper positioning of a lip chain.

Fig. 8. A padded barricade is used to contain the mare. It allows for some interaction between the mare and foal while greatly reducing the risk of injury to the foal. Note the sliding door in the barricade, which can be opened and closed to control the amount of nursing.

Fig. 9. A foal nursing through an opening in the barricade.

Oftentimes, behavior modification is used in conjunction with physical restraint. A good example is holding a mare via a lip chain as the foal attempts to connect with and nurse the mare. Pressure can be released on the chain when the mare demonstrates interest in the foal and a soothing voice and gentle rub along the neck can reward the mare for good behavior.

Pharmaceutical Intervention

Chemical restraint, through the use of tranquilizers and sedatives, is another option when dealing with mares that are actively rejecting their foals. The phenothiazine tranquilizer, acepromazine maleate[b] (0.05 mg/kg, IV or IM, q 8–12), is advocated by some authors[1] because it can provide both tranquilization and stimulate lactation via its antidopaminergic activity. Other agents, such as the α_2-agonists, xylazine[c] (0.5–1.1 mg/kg, IV, q 8–12 h) and detomidine[d] (0.001–0.002 mg/kg, IV or IM, q 8–12 h), and the opioid agonist/antagonist, butorphanol[e] (0.02–0.05 mg/kg, IV, q 8–12 h), may also be used either alone or in conjunction with acepromazine to provide tranquilization, sedation, and/or analgesia. Prudent use of tranquilizers and sedatives is important so as to prevent ataxia and ileus. Anecdotally, paradoxical reactions have been reported in horses administered xylazine whereby they can become aggressive and overly sensitive to external stimuli (i.e., the "xyla-mean" effect). Therefore, clinicians should be careful in their selection of and criteria for the use of tranquilizers and sedatives so as not to further compound problems associated with foal rejection.

Another means of pharmaceutical intervention is administration of a relatively high dose of prostaglandin-$F_{2\alpha}$ ($PGF2_{\alpha}$). This technique was first described by Daels and is hypothesized to trigger oxytocin release in the brain to promote concaveation and normal maternal behavior.[7] A hybridized protocol utilized by the current authors begins with removing the foal from eyesight of the mare and giving her a large dose of dinoprost tromethamine[f] (0.033–0.056 mg/kg, IM). Approximately 15 to 20 minutes after receiving $PGF_{2\alpha}$, mares will show signs of intense sweating and cramping, as well as stream milk. With one person holding the mare and another leading the foal, the foal is taken back in the stall and presented at the mare's head. Oftentimes, the mare will nuzzle and nicker at the foal. The foal is then directed towards the mare's udder and encouraged to nurse. Within a relatively short time period (~15–30 minutes), the mare usually accepts the foal and the two can be turned loose and left alone to interact and strengthen their bond. This protocol seems to effectively "reprogram" the mare's behavior to where she becomes highly attentive to and accepting of the foal. If the mare still rejects the foal after the first attempt, this method can be repeated 24 h later.

The peceding protocol is not without possible complications, most of which are often associated with the administration of exogenous prostaglandins in horses. These include signs of colic, diarrhea, and anxiety. Because a larger dose is used than what is normally given to induce luteolysis, these signs can be more intense but are usually transient and self-limiting. It should be noted that the likelihood and severity of adverse reactions seem to increase when multiple doses of $PGF_{2\alpha}$ are used over the course of several days, so it may be necessary to either scale back the dose of $PGF2_{\alpha}$ or simply utilize another means to get the mare to accept the foal. The synthetic $PGF2_{\alpha\text{-analog, cloprostenol}}$[g] (0.001–0.002 mg/kg, IM)[8] can be used instead of dinoprost because it is thought to produce less severe side effects compared to dinoprost.

Administration of progestogens is another option to promote proper maternal behavior. Use of progesterone in oil[h] (150 mg/horse IM q 24 h) and the synthetic progestogen, altrenogest[i] (0.044 mg/kg PO q 24 h), have been recommended by some clinicians.[1,2] These treatments are hypothesized to exert a calming effect, raise the threshold for aggression, promote motherly behavior, or a combination thereof.

Some mares may not produce sufficient amounts of milk to support and satiate their foals. They can subsequently become "teat sore" due to the foal constantly and aggressively attempting to nurse, which can cause mares to become aggravated and hostile towards their foals. In these instances—and as long as common causes of agalactia have been ruled out (e.g., metritis and fescue toxicosis)—treatment with nonsteroidal anti-inflammatory agents, warm compresses, and the dopamine antagonist, domperidone[j] (1.1 mg/kg, PO, q 12–24 h), can be utilized to reduce inflammation and promote lactation. Oxytocin[k] (5–20 IU, IM or IV, q 2–6 h) can be administered to promote milk letdown. Finally, improving the plane of nutrition can also be of benefit when attempting to improve milk quantity.

Surrogation

Surrogate mares are often utilized when a foal's dam experiences severe illness or death. In these instances, one of two options exists. The first is to utilize a wet nurse mare from either a commercial supplier or another mare that has recently lost her foal. Commercial nurse mare operations are fairly prevalent in Central Kentucky but are scarce in other parts of the country. As such, the other option is to stimulate lactation in a barren mare. Preferably, the mare should have raised foals in the past, possess an even temperament, be tractable to handling and restraint, have good udder conformation, and be free of disease and musculoskeletal issues.

Methods to induce lactation in nonpregnant mares have been described previously.[8,9] Lactation is stimulated with a combination treatment of progesterone and estrogen, as well as antidopaminergic agents, such as domperidone or sulpiride. Progesterone is responsible for promoting lobuloalveolar growth within the mammary gland, while estrogen stimulates cellular division and development of the mammary ducts. When combined with treatment with a dopamine antagonist, this protocol can be used successfully to promote lactogenesis in barren mares. The reader is directed to publications by Daels[8] and Steiner[9] for specific information regarding these protocols.

The actual grafting process utilizes many of the same previously described methods to counteract foal rejection. Moreover, since olfactory recognition plays an important role in stimulating normal maternal behavior, the authors find it useful to mask or alter the scent or smell of both the mare and foal. If a lactating mare is going to be used and fresh fetal fluids and membranes are available, they can be applied to the body of the foal and around the nostrils of the mare. If these are not available, or if the mare is barren, then a grafting spray[1] can be used to disguise the smell of the foal. Muzzling the foal for one to two hours is commonly done prior to introducing it to the surrogate. A hungry foal will usually seek the udder immediately upon presentation to the mare.

3. Results

Several of the previously described techniques have been utilized in the authors' practice for the past six years. During this time, we have treated eight mares (average age 5.6 years; range 4–6 years) that initially rejected their foals and required some form of intervention to make them accept their foals. Physical restraint alone was used in one case (12.5%), a combination of physical restraint with tranquilization was used in five of the eight cases (62.5%), and the high-dose $PGF_{2\alpha}$ method was used in two of the eight cases (25%). In all instances, we achieved a 100% success rate in inducing proper maternal behavior and foal acceptance within 72 hours (average = 52.5 h; range = 12–72 h) of foaling.

We have also employed these techniques on a total of 24 lactating nurse mares (ages unknown) with 22 foals (range of 6 h– to 32 days of age). All but two mares accepted the foals within five days (average = 59.0 h; range = 2–120 h) of first introducing a foal to them, for a grafting success rate of 91.7%. Physical restraint in combination with tranquilization was all that was required in 10 of these mares (10/24 or 42.0%). The high-dose $PGF_{2\alpha}$ method was used in the remaining 14 mares, and 12 of these mares (12/14 or 85.7%) adequately bonded with the foal within five days of being introduced to the foals. All of these mares were actively lactating, so exogenous progesterone and estrogen were not utilized in any of the mares. However, in three of the cases in which high-dose $PGF2_{\alpha}$ was administered (3/14 or 21.4%), domperidone was administered for two to three days following grafting because these mares' milk production was judged to be less than adequate.

4. Discussion

Foal rejection is a relatively uncommon occurrence in clinical equine practice. Nevertheless, when it does happen, it can be frustrating to deal with and unsettling to witness. Fortunately, several different options exist for treating and managing foal rejection, which range from physical restraint, to behavior modification, to pharmaceutical intervention. These methods can be used alone or in combination to fit each individual case or remain within the limits of available resources. We recommend starting with the simple methods (e.g., physical restraint with or without tranquilization) and then progressing to more involved techniques, such as behavior modification and high-dose $PGF_{2\alpha}$ administration, if the circumstances dictate the use of more aggressive tactics. Our experience indicates that avoidance rejection is easier and quicker to reconcile compared to aggressive rejection. In many cases, light manual restraint with light to moderate sedation works well in mares that are avoiding but not aggressive towards their foals. Aggression oftentimes requires more forceful and involved measures, such as moderate restraint (e.g., nose twitch or lip chain), behavior modification, and high-dose $PGF2_{\alpha}$, but not necessarily in that order. Luckily, we have been able to successfully treat all of our foal rejection cases within 72 hours from the birth of the foal, which is a shorter period of time than the two to three weeks previously reported in the literature.[1]

With respect to surrogation, there are a couple of concluding thoughts. First, the age of the foal and time frame in which the surrogate had her foal seems to impact the time required for adequate bonding. If the foal is relatively young (e.g., less than 7 days) and the mare has recently foaled (e.g., 24–48 h), the grafting process is often very swift and seamless. Older foals or mares that have been raising a foal for several days appear to require more time and effort. However, with persistence and pa-

tience, success can often be achieved within five days of introduction. Second, the authors are of the opinion that surrogation is a much better option than attempting to hand-raise an orphan foal. Anecdotally, orphan foals seem to display unsafe and unusual behavioral traits towards humans and other horses as they mature. This phenomenon has been coined the "orphan foal" syndrome. It is likely a manifestation of overly intensive handling and rearing by humans, as well as inadequate socialization with other horses during an early yet highly formative time period. Foals raised by nurse mares seem—on average—more respectful towards humans and better adjusted to a herd environment compared to orphan foals. Finally, surrogation does not appear to hinder foals' development or place them at a higher risk for disease relative to other foals. In the authors' practice, the rate of growth and adverse health events appear to be equivocal between foals raised on a nurse mare and those raised by their own dams.

Acknowledgments

Conflict of Interest

The Authors declare no conflicts of interest.

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[a]Foal-Lac Powder, Pet Ag, Hampshire, IL 60140.
[b]Aceproject, Henry Schein Animal Health, Dublin, OH 43017.
[c]AnaSed®, Lloyd Laboratories, Shenandoah, IA 51601.
[d]Dormosedan®, Zoetis, Florham Park, NJ 07932.
[e]Torbogesic®, Zoetis, Florham Park, NJ 07932.
[f]Lutalyse®, Zoetis, Florham Park, NJ 07932.
[g]Estrumate®, Merck Animal Health, Millsboro, DE 19966.
[h]Progesterone in oil, Rood and Riddle Veterinary Pharmacy, Lexington, KY 40511.
[i]Regumate®, Merck Animal Health, Millsboro, DE 19966.
[j]Equidone®, Dechra Veterinary Products, Overland Park, KS 66211.
[k]Oxoject, Henry Schein Animal Health, Columbus, OH 43204.
[l]MotherUp®, Pierre, SD 57501.

How to Build an Automatic Milk Feeding Device for Orphan Foals

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1. Introduction

Veterinarians often face the dilemma of having a newborn foal in their care that may require round the clock feedings of a supplemental milk source. Foals whose dams are deceased, have rejected them, or are simply not producing enough milk normally require either a nurse mare or an alternative supply of milk or milk replacer. Once a foal's antibody requirements have been met with the ingestion of colostrum or the delivery of an IV hyperimmune plasma, a decision will need to be made regarding the source and delivery method of its nutrition. A foal will require some form of milk for the first 2 to 5 months of life.[1] A commercially available nurse mare or an open mare who has been hormonally induced to lactate are two options that offer both a long-term source of naturally balanced nutrition and social upbringing. Unfortunately, both of these alternatives take valuable time to arrange, can be very costly, and still run the risk of foal rejection. In the meantime, it is necessary to provide feedings at 1–2 hour intervals for at least the first few days of the foal's life.[2] Owners may quickly become exhausted by feeding foals this frequently and paying staff can rapidly become cost prohibitive. Some caretakers are tempted to leave out large volumes of milk in the stall overnight for the foal to consume at will. This practice can lead to spoilage of the milk or overconsumption by the foal, which can cause colic, diarrhea, gastric ulceration, and lack of weight gain.[3] Constructing a relatively inexpensive automated device to store, refrigerate, and distribute a programmed volume of milk into a small receptacle for consumption at timed intervals can save the practitioner or owner time, money, and provide a more natural digestive process for the foal.

2. Materials and Methods

An area with a 110 V electrical outlet and protection from rain is required to run and maintain the feeding device. Always use caution to avoid flammable materials around any electrical device, and be sure the device does not sit in wet conditions. The materials required are readily available online, and overnight shipping can be used to expedite delivery. A busy breeding farm or veterinary hospital can construct the device before the beginning of foaling season to ensure it will be ready when needed. The unit as described here costs less than $400 in supplies to build, and our hospital was quickly able to reimburse costs by charging a deposit and monthly rental fee for at home use. Equipment to be purchased includes the following:

1. A compact (dormitory) refrigerator. We use a Kenmore Chill 3.3 cu. ft. model no. 93382[a],

NOTES

which averages $143.99 at www.sears.com, $129.99 at www.amazon.com, or is available at Sears appliance stores. This fridge allows a larger milk receptacle to be used inside, as some compact refrigerators do not provide a large enough ventral footprint on which to place a container large enough to store milk for 12 hours of use.

2. A rectangular milk storage container that fits inside the chosen compact refrigerator. We use a 2.5 gallon Sterilite[b] plastic trash can found at Dollar General or Walmart for around $2.00.
3. A peristaltic dosing pump with a timer. These can be purchased separately as a pump and programmable electric timer or as one unit. We have tried several methods with different pumps and timer combinations, and the listed all in one programmable peristaltic pump seems to be the most affordable for what it provides. We use a 115 VAC Beta DR-2000 Clock Based Chemical Feed System[c]. This unit can be purchased at www.cannonwater.com for $224.50 at the time of publication although they can occasionally be found new or used on www.ebay.com for less. This pump uses a standard 110 V outlet and pumps an average of 150 mL/min of fluid. It can be programmed to cycle once an hour for up to 20 minutes, which would provide a 3 L maximum output per cycle. It may be programmed to cycle more frequently than once an hour but only stores 24 programmed cycles to memory, so the internal clock would need to be reset every 12 hours for more frequent feeds. The pump is very easy to program once you read the enclosed instructions. Using a peristaltic type pump keeps the milk out of the moving pump parts to avoid contamination. Peristaltic pumps usually require very little maintenance or part replacement. The pump tube inside the roller assembly may need replacement every few years depending on how well it is cleaned and the conditions of storage. All the major components can be purchased from Cannon Water. The internal battery of this particular pump will only store programming for 6 months when unplugged, so if it is stored unplugged for more than 6 months in the offseason, then it will have to be reprogrammed at the beginning of each season and a new replacement battery may be purchased from the distributer. When purchased from the distributer, the pump is supplied with the remainder of the fittings including ¼ inch tubing, a mounting bracket, screws, drywall anchors, cable ties, squeeze tube fittings, and standpipe to stiffen the suction tubing and help keep it near the bottom of the milk receptacle.

Fig. 1. 115 VAC Beta DR-2000 Clock Based Chemical Feed System.

4. An optional clear storage container to protect the pump from water and dust in a barn type environment. We used a 4 L plastic food storage container found at the Dollar Store or Walmart for $2.00 to $6.00.

An electric drill, a box blade, a 3/16 inch drill bit, a ¼ inch drill bit, a marker, a hammer, and a Phillips head screwdriver are tools used to assemble the device.

Unpack the compact refrigerator and ensure the chosen milk receptacle fits within the closed refrigerator. Choose whether to mount the peristaltic pump (Fig. 1) to the top or the side of the refrigerator. The pump may be mounted with a sliding bracket or with 3 screws (Fig. 2). In this example, we have mounted the pump to the top of the refrigerator with 3 screws. If the pump is mounted within a protective container, ensure the pump fits within the closed container and use the box blade to cut exit holes for the power cord and intake and output tubing (Fig. 3). Place the container and pump in the selected mounting position on the top or side of the refrigerator, and use the marker to mark the mounting screw placement (Figs. 4 and 5). Use the 3/16 inch drill bit to drill the mounting holes, and use the hammer to place the drywall anchors provided with the pump. Mount the peristaltic pump within the protective container to the top or side of the refrigerator using the mounting screws or bracket provided with the pump and a Phillips head screwdriver (Fig. 6). Use the marker to mark the position of the suction hose entrance through the side of the refrigerator at a level just

Fig. 2. Mounting options on back of pump.

Fig. 4. Mark screw locations in protective box with a permanent marker then drill holes in clear plastic container.

below the freezer unit (Fig. 7). Once marked, use the ¼ inch drill bit to drill a hole through the thin metal housing and foam insulation on the side of the refrigerator. Cut the desired length of suction tubing to run between the pump and the bottom of the milk receptacle. Feed a section of suction tubing through this hole and to the bottom of the receptacle. Attach the stiff standpipe tubing provided with the pump to the distal end of the suction tube and place within the milk receptacle so it touches the bottom (Fig. 8). Attach the proximal end of the tube to the intake (suction) side of the peristaltic pump using the provided squeeze tube fittings. Silicone may be used to seal the exit hole through the wall of the refrigerator if desired. Attach the remaining section of tubing to the output port on the pump (Fig. 9). Run the section of output tubing to distribute milk into a chosen receptacle in the stall from which the foal is to drink. Use cable ties as needed to fasten the tubing in place.

Most foals can quickly be taught to drink from a bucket by placing a milk coated finger in their mouth and while they are suckling, slowly inserting

Fig. 3. Use the box blade to cut out an exit window for the electrical cords in the clear plastic container.

Fig. 5. Place the protective box in desired location on top or side of refrigerator then mark screw placement holes with a permanent marker. Use the 3/16 drill bit to drill through the refrigerator wall just deep enough to place the dry wall anchors. Hammer the dry wall anchors into the holes in the refrigerator.

Fig. 6. Place the pump inside the container, and use the Phillips screwdriver to screw in the mounting screws provided with the pump.

Fig. 8. Measure and cut the required length of tubing to run between the input port on the pump, through a hole drilled into the side of the refrigerator, and down to the bottom of the chosen milk receptacle. Attach standpipe to bottom of tubing and place at bottom of receptacle.

the finger into a shallow pan or bucket leading their mouth to the source of milk. For foals that drink well from a bottle but have difficulty learning to drink from a bucket, a Calf-Mate calf nursing bucket[d] can be purchased at www.eNasco.com and modified by cutting the end of a smaller human or goat nipple to fit the output.

Several commercially available foal specific milk replacers can be ordered online or found at local feed stores. The author's hospital most commonly uses Mares Match[e] by Land O'Lakes or Foals First[f] by Progressive Nutrition, as we have had more success with palatability and less incidence of diarrhea with these brands. Follow the packaged instructions carefully for preparation of the product. Some choose to dilute the foal replacer to half strength for

Fig. 7. Mark the area for milk tube placement through the side of the refrigerator just below the freezer coil shelf. Use the ¼ inch drill bit to drill a hole through the side of the refrigerator.

Fig. 9. Attach the remaining tubing to the output port of the pump. This will be used to run into the bucket where the foal is housed and secured with cable ties.

the first few feedings to acclimate the foal to taste if it was originally nursing from a mare and to avoid constipation. Avoid any sudden change in brands of milk replacer. The average foal requires 10 to 15% of its body weight in milk the first few feedings and then 25 to 35% of its body weight after that until it gradually begins to be able to digest disaccharides and fiber between 2 and 4 months when the Maltase and Sucrase enzymes increase in levels and cecal microflora are established.[4] The weight of milk is approximately 1 kg/1 L so a 50 kg foal will need at least 5 L a day in the first few days and gradually be increased to 12.5 to 15 L as it grows. The average foal will gain 2 to 3 pounds a day.[3] The timer on the machine is set to dispense the desired amount of milk per hour. We begin by setting the timer at 2 minutes per hour, which will dispense an approximated 300 mL an hour or 7.2 L a day. The chosen receptacle needs to be small enough, placed at the proper height, and possibly placed at a slight angle so that the foal's muzzle can fit to the bottom and drink the entire amount. As the foal grows, the timer is changed to pump for longer time periods distributing larger volumes of milk and at decreased intervals. The pump can be used until it is possible to get down to a more owner manageable 5 times a day feeding at around 6 weeks old or used for the first several months until it is eating a normal diet. Once the pump is done being used for a particular foal, 10% bleach water is pumped through the pump tubing followed by distilled water and then air to disinfect and store.

3. Results

The described device has worked well in our hospital setting, and as previously described, we often send them home with orphan foals for a monthly rental fee or more distant clients chose to purchase the device. We have used this automated method for over 20 orphan foals in our practice. The foals quickly learn the sound of the pump when it is activated and run to the bucket to drink. The foals gained weight at an average pace and no significant diarrhea, colic, or constipation was encountered. The owners gave positive reviews regarding the functionality and convenience of the device when it was returned for a deposit refund. We did have an instance in which the tubing required replacement due to damage by mice trying to reach the milk inside. The programmable pump was programmed as needed to gradually increase the volume and decrease the frequency of feedings as the foal grew and the intestines adapted to the introduction of roughage and grains.

4. Discussion

Having a programmable automated device available to offer allocated amounts of milk replacer to an orphaned foal can be a very convenient time and money saving option. Additionally, it can mimic a more natural feeding schedule. This can help avoid overfilling of the stomach, which can lead to delayed gastric emptying and proliferation of enteric pathogens.[3] More frequent meals can decrease the incidence of gastric ulceration as well as maintain glucose levels and gut motility in the foal.[3] The foals will also avoid being exposed to frequent human bonding as in a bottle feeding situation, which can lead to later behavioral problems. Presenting the option of a relatively inexpensive automatic feeding device is a good way to offer clients some peace of mind in a stressful situation. Although their mare may have passed away or rejected her foal, they do not need to worry about the foal's growth potential. Owners that typically visit the barn twice daily for morning and evening feeds have the ability to program and fill the device at these times without missing work or hiring extra staff to feed an orphaned foal. Veterinary hospitals without 24 hour staff can also benefit from the availability of this device for overnight feedings.

Acknowledgments

Conflict of Interest

The Author declares no conflicts of interest.

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[a]Kenmore 3.3 cu.ft. Compact Refrigerator, Kenmore, Hoffman Estates, IL 60169.
[b]Sterilite Corporation, Townsend, MA 01469.
[c]Cannon Water Technology Inc., Rocklin, CA 95765.
[d]Calf-Mate Calf Feeder, Fortiflex, Miami, OK 74354.
[e]Mares Match foal milk replacer, Land O Lakes Animal Milk Products Co., Shoreview, MN 55126.
[f]Foals First Milk Replacer Powder, Progressive Nutrition LLC, Brookville, OH 45309.

Frequency of Ultrasonographic Visualization of Liver in Published Sites for Blind Biopsy in Healthy Horses

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The practice of blind liver biopsy in the published recommended locations in horses carries significant potential risks. Ultrasound-assisted liver biopsy is recommended to reduce the risk of serious complications. Authors' address: Department of Large Animal Clinical Sciences, College of Veterinary Medicine and Biomedical Sciences, Texas A&M University, College Station, TX 77843; e-mail: SSammons@cvm.tamu.edu. *Corresponding and presenting author. © 2014 AAEP.

1. Introduction

Percutaneous liver biopsy is regarded as the best antemortem test for equine hepatopathy. Blind liver biopsies are still performed by practitioners, despite potential associated risks such as pneumothorax or hemorrhage. Direct or indirect ultrasound guidance allows for identification of the most appropriate site for liver biopsy. Our goal was to identify the frequency of sonographic identification of adequate liver tissue for biopsy in the published blind liver biopsy locations.

2. Materials and Methods

Serum liver parameters were evaluated in 36 middle-aged Quarter Horses to ensure that only horses with normal liver function were included in the study. The published blind liver biopsy region, just below a line drawn between the dorsal aspect of the tuber coxae and the point of the elbow in the right 11th to 14th intercostal spaces, was examined sonographically.

3. Results and Discussion

Only 39% of horses had liver visualized in the published blind liver biopsy region. None of the 36 horses had liver of adequate thickness for biopsy in the published region. When liver was not visible, lung was visualized instead in 55% of horses, bowel in 36% of horses, and both bowel and lung in 9% of horses. In many horses, liver tissue may be inadequate for biopsy in the published blind biopsy locations.

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Conflict of Interest

The Authors declare no conflicts of interest.

Research Abstract

NOTES

Owner-Reported Response to Treatment of 130 Headshaking Horses

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Nose nets, face masks, and magnesium supplementation were the most efficacious nonmedical therapies for treatment of headshaking. Of the pharmacological treatments, cyproheptadine, corticosteroids, and melatonin resulted in greatest improvement. Each of these treatments resulted in improvement in approximately 50% of headshaking horses. Authors' addresses: Department of Veterinary Medicine and Epidemiology, University of California Davis, CA 95616 (Pickles, Aleman, Madigan); Science Supplements, Unit 2A, Chase Road, Bury St. Edmunds, Suffolk, IP32 6NT, UK (Marlin); and PO Box 80, Bury St. Edmunds, Suffolk UK IP28 9BF, UK (Adams); e-mail: jemadigan@ucdavis.edu. *Corresponding author; †Presenting author. © 2014 AAEP.

1. Introduction

Idiopathic headshaking has been recognized for over a hundred years[1,2] and is a spontaneously occurring disorder of mature horses causing violent head flicking, snorting, and muzzle rubbing. Due to this localization of clinical signs to the head, and in particular muzzle area, it has long been suspected that the disease pathogenesis involves the trigeminal nerve.[1,3,4] Recently a decreased threshold for trigeminal nerve activation has been confirmed in headshaking horses compared to controls.[5] The limited pathological studies performed to date have failed to determine any structural abnormalities of the trigeminal nerve, and therefore a functional disorder is proposed.[5,6] Other unexplained features of headshaking include the predisposition of geldings to the disease and the seasonality of clinical signs.[7,8] This seasonality and the fact that some horses go into spontaneous remission suggests that the documented aberrant activity of the trigeminal nerve may be reversible. However, due to our limited understanding of the etiopathogenesis of headshaking, current treatments are primarily directed at minimizing clinical signs and discomfort rather than correcting anomalous trigeminal neurophysiology and, consequently, have limited efficacy in many horses.[4,9] There are few large scale studies reporting success rates of the various treatments for headshaking, which makes it difficult for veterinarians to provide such advice to owners. The aim of this study was to undertake a survey of owners of headshaking horses to determine the reported success rate of commonly utilized therapies.

NOTES

2. Materials and Methods

Questionnaires

An online questionnaire was designed using Survey Monkey[a]. The questionnaire contained 3 sections. The first section comprised 27 questions regarding the horse's signalment and general management. Section 2 contained 15 questions regarding the horse's headshaking including if the horse was currently headshaking, which months of the year headshaking occurred, which month headshaking was worst, evidence of seasonality and weather related headshaking behavior, and if headshaking had been diagnosed by a veterinarian. Additionally, owners were asked to complete a checklist of 20 particular headshaking behaviors for their horse and a 15 item checklist about activities associated with their horse's headshaking. Section 3 comprised 11 subsections regarding 11 possible headshaking treatments: nose net, face mask, fly control, cyproheptadine, carbamazepine, corticosteroids, non-steroidal anti-inflammatories (NSAIDs), antihistamines, melatonin, magnesium, and combined melatonin and magnesium therapy. If a particular treatment had not been used, the respondent was directed to the next treatment subsection. Within each treatment subsection, owners were asked to specify what time of year the treatment had been trialed, describe the particular treatment (e.g., type of nose net) and dosage and administration details if appropriate (including if product was generic or compounded), if any response was observed (completely stopped headshaking, marked improvement, some improvement, no change, or worsened headshaking), and if any side effects of treatment were noted. The questionnaire was advertised via headshaking website forums and via Facebook[b]. Links to the questionnaire remained open for 2 months.

Data Analysis

Results are reported descriptively. A positive treatment outcome was deemed as improvement in headshaking behavior and was calculated as the sum of completely stopped headshaking, marked improvement, and some improvement responses. A negative treatment outcome was described as no improvement or worsening of headshaking and was calculated as the sum of no change and worsened headshaking responses. For geographical comparisons, data was assigned a location of North America, Europe, or Australasia. Mann Whitney tests were used to examine 2 non-paired variables.

3. Results

Questionnaire Response

Questionnaires were completed by 137 respondents. The treatment section was not completed by 7 respondents giving 130 useable questionnaires. Some respondents gave incomplete details for a particular treatment giving a useable response range of 122 to 130 (median 127) for individual treatments. Responses were received from the UK (n = 49), U.S. (n = 45), Canada (n = 8), mainland Europe (n = 11, 3 each from France and Germany and 2 from The Netherlands), Australia (n = 3), and New Zealand (n = 5).

Table 1. Prevalence of Owner-Reported Clinical Signs and Effect of Weather in 132 Headshaking Horses

Clinical sign	Prevalence (%)
Shaking or flipping the head in a vertical plane	121 (92)
Acting as if an insect had flown up the nose	112 (85)
Rubbing the nose/muzzle on objects	108 (82)
Snorting	92 (62)
Rubbing nose/muzzle on ground while moving	81 (61)
Striking at nose/muzzle with front foot	70 (53)
Anxious expression	59 (45)
Headshaking reduced at night	59 (45)
Stumbling	42 (32)
Staring into space	38 (29)
Horizontal headshaking	37 (28)
Reluctance to move	36 (27)
Panic following staring episodes	32 (24)
Avoids light	32 (24)
Seeks shade	32 (24)
Licking or smacking of lips or excessive opening and closing of mouth	28 (21)
Worse when sunny	33 (25)
Worse when windy	29 (22)
Worse when rainy	13 (10)
Worse when humid	7 (5)
Better when cloudy	14 (11)
Better when rainy	13 (10)

Description of Headshaking Horses

The median (range) age of the population of headshaking horses was 11 (2–27) years with a median (range) length of ownership of 6 (0–20) years. Most breeds were represented with Thoroughbred (including crosses) and Quarter Horse being most frequent comprising 22% and 14% of the population, respectively. Almost half of the horses were used for light activities only with 24% used for hacking/trail riding, 16% for pleasure riding, and 8% being retired/companion animals. Dressage was the most frequent competitive discipline performed by headshakers, reported by 13% of owners.

Of the 132 horses for which data were available, 84 (64%) had been diagnosed with idiopathic headshaking by a veterinarian. Headshaking behavior was seasonal in 79/132 (60%) horses whilst 53/132 (40%) displayed clinical signs all year or randomly intermittently, without any apparent predictability. The most common headshaking signs displayed were "vertical shaking of the head" (121/132; 92%), "acting as if an insect flew up its nose" (112/132;

Table 2. Owner Reported Response of 130 Headshaking Horses to Various Treatments

Treatment	Used	Positive outcome	Completely stopped	Marked improvement	Some improvement	Negative outcome	No change	Worse	Side effects
Nose net (n = 128)	110 (86%)	58 (53%)	6 (5%)	21 (19%)	31 (28%)	52 (47%)	40 (36%)	12 (11%)	32 (29%)
Face mask (n = 130)	83 (64%)	44 (53%)	3 (4%)	13 (16%)	28 (34%)	39 (47%)	32 (39%)	7 (8%)	18 (22%)
Fly control (n = 126)	84 (67%)	18 (21%)	0	3 (4%)	15 (18%)	66 (79%)	66 (79%)	0	2 (2%)
Cyproheptadine[a] (n = 128)	29 (23%)	14 (48%)	4 (14%)	3 (10%)	7 (24%)	15 (52%)	13 (45%)	2 (7%)	14 (48%)
Carbamazepine (n = 125)	8 (6%)	2 (25%)	0	0	2 (25%)	6 (75%)	6 (75%)	0	3 (38%)
Corticosteroids (n = 127)	31 (24%)	17 (55%)	1 (3%)	7 (23%)	9 (29%)	14 (45%)	13 (42%)	1 (3%)	3 (10%)
NSAIDs (n = 126)	22 (17%)	4 (18%)	0	1 (5%)	3 (14%)	18 (82%)	18 (82%)	0	1 (5%)
Antihistamines (n = 122)	36 (30%)	12 (33%)	1 (3%)	4 (11%)	7 (19%)	24 (67%)	23 (64%)	1 (3%)	7 (19%)
Melatonin (n = 128)	17 (13%)	8 (47%)	0	1 (6%)	7 (41%)	9 (53%)	9 (53%)	0	7 (41%)
Magnesium (n = 128)	58 (45%)	25 (43%)	1 (2%)	11 (19%)	13 (22%)	33 (57%)	33 (57%)	0	4 (7%)
Melatonin and magnesium (n = 122)	11 (9%)	6 (55%)	0	1 (9%)	5 (45%)	5 (45%)	5 (45%)	0	4 (36%)

NSAIDs = non-steroidal anti-inflammatories; n = number of responses.
[a]Fifteen horses received compounded cyproheptadine, 12 horses received generic cyproheptadine, and 1 owner was unsure if cyproheptadine was compounded or not.

85%), and "rubbing of the muzzle on objects" (108/132; 82%). Headshaking clinical signs varied with the weather in 91/132 (69%) of horses and was worse when exposed to bright light in 52 horses (39%). The prevalence of headshaking clinical signs is shown in Table 1. Horses with a veterinary diagnosis of headshaking were more likely to show excessive snorting of the nose ($P = 0.05$), reluctance to move ($P = 0.007$), striking at nose/muzzle with front foot ($P = 0.02$), have an anxious facial expression ($P = 0.0009$), and stare out into space as if seeing imaginary objects ($P = 0.009$) compared to owner-diagnosed headshaking horses. These horses were also more likely to display clinical signs when at rest in the stable ($P = 0.05$), when lunged without tack ($P = 0.01$), when ridden at canter ($P = 0.003$), when walked in hand in a bridle ($P = 0.04$) or halter ($P = 0.02$), and when eating grass ($P = 0.05$).

For the 131 owners that gave data, 9 (7%) described their horse's headshaking as mild (occasional headshaking, rarely interferes with riding activity), 35 (27%) as moderate (some headshaking, interferes somewhat with riding activity), 41 (31%) as severe (frequent headshaking, interferes greatly with riding activity), and 46 (35%) as very severe (frequent headshaking, impossible to perform riding activity). Owners of horses with a veterinary diagnosis rated their horse's headshaking as more severe than owner-diagnosed horses ($P = 0.007$).

Treatment Outcome

Nose Net

Nose nets had been used by 110/128 (86%) respondents. Of those that had tried nose nets, 91 (83%) had used half nets extending from the noseband to cover the upper jaw and lip, 43 (39%) had used full nose nets covering both the upper and lower jaws and lips below the noseband, and 24 (22%) had tried both types of nose nets. A further 4 owners had used homemade nose nets made from nylon stockings, 1 used ropes dangling from the noseband and 1 used a dangling fringe from the browband.

A positive outcome was reported by 58/110 (53%) owners; 6 horses (5%) completely stopped headshaking, 21 (19%) showed marked improvement, and 31 (28%) showed some improvement (Table 2). Any improvement was seen immediately upon placement of the nose net although some horses required several sessions of wearing the net to accommodate its use. Six respondents said that the positive effect of the nose net had waned over time. A negative outcome was reported by 52 (47%) of owners with 40 horses (36%) showing no response and 12 horses (11%) headshaking worse with use of a nose net (Table 1). Negative side effects were shown by 32 horses (29%) of horses using nose nets with irritation by the net (24 horses) and panic (5 horses) described most frequently. There was no significant difference between half or full nets in outcome or the presence of adverse effects.

Face Mask

Face masks had been used as a treatment on 83/130 (64%) headshaking horses with 42 (51%) of these masks containing UV eye shades. Forty-four (53%) horses had a positive outcome from face mask use with 3 (4%) horses completely stopping headshaking, 13 (16%) showing marked improvement, and 28 (34%) showing some improvement (Table 2). A negative outcome was observed in 39 (47%) horses with 32 (39%) showing no response and 7 (8%) horses becoming worse following face mask use (Table 2). Negative side effects were noted in 18 (22%) horses with "spookiness," tripping due to diminished vision and worsening of headshaking most frequently cited. The presence of UV eye shades did not affect outcome or occurrence of adverse effects.

Insect/Fly Control

Insect control had been used by 84/126 (67%) of owners in an attempt to decrease headshaking. Of these, 18 (21%) reported a positive outcome, with 3 (4%) horses showing marked improvement and 15 (18%) some improvement immediately upon use (Table 2). No horses displayed increased headshaking following insect control measures. Two horses (2%) had negative side effects (skin irritation) following treatment.

Cyproheptadine

Cyproheptadine treatment had been used by 29/128 (23%) of owners to control their horses' headshaking. Fifteen horses received compounded cyproheptadine, 12 horses received generic cyproheptadine, and 1 owner did not know if cyproheptadine was compounded. Six owners reported administering 0.3 mg/kg, 2 owners stated 0.1 mg/kg, 15 owners did not know the administered dose, and dosage was unable to be determined from information given by 6 owners using compounded product. Nineteen owners reported administering cyproheptadine twice daily, 2 owners once daily, and 8 owners did not report frequency of dosing.

Fourteen horses (48%) had a positive outcome with cyproheptadine treatment: 4 (14%) stopped headshaking completely, 3 (10%) showed marked improvement, and 7 (24%) showed some improvement (Table 2). Fifteen horses (52%) had a negative outcome with 13 (45%) having no change and 2 (7%) having worsening of headshaking. The median (range) time to observe a positive response occurred 3 (2–14) days following initiation of therapy. Additionally, 1 owner reported that clinical signs resumed within 2 days of cyproheptadine withdrawal. Three owners described a transient effect such that headshaking recurred during administration; 2 of these horses received generic cyproheptadine and 1 received compounded product. One additional owner commented that cyproheptadine had stopped working when changed to compounded product. Adverse effects were reported in 14 (48%) of horses receiving cyproheptadine including lethargy, drowsiness, incoordination, colic, and irritability.

Carbamazepine

Carbamazepine therapy had been tried by 8/125 owners (6%), 2 of which reported some improvement and the remaining 6 reported no change in headshaking (Table 2). Improvement occurred within several weeks in 1 horse and was not reported for the other improved headshaker. One owner reported administration of 5 mg/kg 3 times daily, 6 owners did not know the dosage administered, and the dosage could not be extrapolated from the information given by 2 respondents. Five of these horses with an unknown dosage received carbamazepine twice daily and 1 three times daily. Adverse effects of lethargy and drowsiness were reported in 3 horses.

Corticosteroids

Corticosteroid therapy had been given to 31/127 (31%) of headshakers. Of these, 9 received oral steroids, 12 were administered injectable steroids, 6 were given dexamethasone pulse therapy (DPT), and 2 horses received inhalational medication. A positive outcome was reported in 17/31 (55%) of horses of which 1 completely stopped headshaking, 7 (23%) showed marked improvement, and 9 (29%) had some improvement (Table 2). Improvement was most frequently described by owners (7/12) as occurring a few days after treatment commenced. Three owners reported a transient improvement only. A negative outcome was noted in 14 (45%) of horses with 13 (42%) having no change and one becoming worse following therapy. Adverse effects were observed in 3 horses (10%) and comprised 1 each of increased appetite, lethargy, and mania (as described by owner).

NSAIDs

NSAID therapy had been used by 22/126 (17%) of owners for treatment of their horses' headshaking. Phenylbutazone was listed as the most frequently administered NSAID (13/18 responses). Four horses (18%) improved with NSAID therapy with 1 horse showing marked improvement and 3 horses (14%) showing some improvement whilst the remaining 18 horses (82%) showed no change in headshaking (Table 2). Hives were listed as an adverse effect of treatment in one horse.

Antihistamines

Antihistamine treatment had been used in 36/122 (30%) of headshaking horses. Most frequently used drugs were chlorpheniramine (9 horses), hydroxyzine hydrochloride (8 horses), loratadine or desloratidine (8 horses), diphenhydramine (7 horses), and pseudoephedrine hydrochloride pyrilamine maleate (3 horses). A positive outcome was described within a few days to a few weeks in 12 (33%) of horses with 1 horse completely ceasing headshaking, 4 horses (11%) showing marked improvement, and 7 horses (19%) mildly improving (Table 2). A negative outcome was seen in 24 (67%) horses with no change in 23 (64%) and increased headshaking in 1 horse. Adverse effects were observed in 7 horses (19%) with drowsiness cited most frequently.

Melatonin

Melatonin had been administered to 17/128 (13%) of horses, all of which were located in the U.S. except one in Canada and one in the Southern hemisphere. Dose range was 12 to 20 mg with 15 mg once daily cited most frequently (6 responses). Ten respondents reported giving melatonin at 5 PM/dusk, 1 administered melatonin in the morning, and 1 gave melatonin twice daily. Six horses started melatonin therapy in winter, 6 in spring, and 5 in the

summer. Eight horses (47%) responded positively to melatonin within 1 to 4 weeks of therapy; marked improvement was seen in 1 horse (the horse given melatonin in the morning) and some improvement in the remaining 7 (including the horse given melatonin twice daily) (Table 2). No change in headshaking was reported in 9 (53%) of horses. Seven horses (41%) had a longer hair coat or did not shed their winter coat whilst on melatonin.

Magnesium

Dietary supplementation with magnesium had been used by 58/128 (45%) of owners as a headshaking treatment. Magnesium oxide was the most common formulation reported (26 responses) followed by malate (7 responses). Doses of 5 to 40 g were reported. A positive outcome was reported in 25 horses (43%) within 2 days to 4 weeks of initiating therapy; additionally 1 owner reported headshaking worsened within a couple of days of stopping magnesium supplementation. One horse completely stopped headshaking, 11 (19%) showed marked improvement, and 13 (22%) showed mild improvement (Table 2). A negative outcome was noted in 33 horses (57%) in which no change in headshaking was observed. Two owners reported that their horse seemed calmer whilst on magnesium, whereas 1 owner each reported nervousness and diarrhea as adverse effects.

Melatonin and Magnesium

Only 11/122 (9%) owners had used melatonin and magnesium combination therapy to treat their horses' headshaking. Six horses (55%) responded positively with 5 horses (45%) showing some improvement and 1 horse (10%) marked improvement in headshaking whereas 5 horses (45%) had no change (Table 2). All respondents who gave data (n = 7) used a dosage of 15 to 20 mg melatonin (median 15 mg) at 5 PM and 5 to 40 g magnesium. Two owners initiated combination therapy in spring, 5 in summer, and 4 in winter. Adverse effects were observed in 4 horses (36%), 3 of which did not shed their winter coat and 1 which gained weight and became lethargic approximately 1 month after therapy was initiated.

4. Discussion

Treatment of headshaking horses is challenging as therapies are largely directed towards managing clinical signs rather than treating the cause itself, which remains elusive. There are a plethora of treatments recommended and utilized for management of headshaking; however, reported rates of success of such treatments are rare and often contain limited numbers of horses. This study describes the owner-reported outcome of 11 treatments used in the management of idiopathic headshaking in 130 horses.

The demographics of the survey population and prevalence of clinical signs were similar to that described in other headshaking studies.[7,8] Some variation in observed clinical signs existed between horses diagnosed by owners and veterinarians, with the latter group representing a more severely affected cohort, as previously reported.[10] Owners have proven reliable at using the clinical signs of vertical headshaking, rubbing the nose on the thoracic limb, and snorting for the diagnosis of idiopathic headshaking.[10] As vertical headshaking was observed in over 90% of horses in this study, it is likely that owner-diagnosed horses were true idiopathic headshakers; however, other causes of headshaking cannot be excluded. Likewise, details of the veterinary diagnostic procedure of the veterinarian-diagnosed horses are also unknown. In field situations, veterinarian diagnosis of headshaking, like owner assessment, is usually based on observation and history. A single veterinary exam has limitations, and there is no diagnostic test for headshaking. Idiopathic headshaking is a constellation of signs, which persist in the absence of a rider, independent of tack, and apparent lack of underlying pathology (i.e., sinus mass, iris cyst, etc.).[4,11,12] The authors, therefore, feel that veterinary diagnosis and owner assessment of clinical signs, which are the basis of any headshaking diagnosis, are both equally valid.

Nose nets were the most frequently used treatment for headshaking having been used by almost 90% of respondents. Whereas some degree of improvement was seen in over 50% (58/110) of horses, complete resolution of headshaking was rare and observed in only 5%. These improvements are lower than that previously reported where use of nose nets resulted in some decrease in clinical signs in 75% of 36 headshakers in the UK[13] but greater than the 33% improvement reported in the U.S.[7] Geographical location was not analyzed in this study due to the heterogeneous nature of the data. In this study, nose nets resulted in one of the highest rates of positive outcome and, therefore, represent a simple first-line therapeutic option. Such physical treatments avoid competition medication regulations; indeed, the use of nose nets is now permitted in some equestrian disciplines. Adverse effects of nose nets have not been previously reported, to the authors' knowledge, but were observed in approximately ⅓ of horses and were reported for both types of nose net. Owners should, therefore, be advised of the possibility of irritation or panic upon their application. The method by which nose nets reduce clinical signs of headshaking is unknown; however, it is postulated that they reduce aversive stimulation of hyperesthetic areas or, alternatively, the constant presence of the net might work by adjacent receptor field inhibition or receptor adaptation of the contact area.[14] Interestingly, some owners reported a waning of clinical effect with time raising the possibility of habituation.

A positive treatment outcome of 53% (44/83) of headshaking horses with a face mask was similar to

that previously reported[7,9] and equivalent to use of a nose net in this study, although 20% fewer respondents had tried them. Again, adverse effects not previously reported such as "spookiness" and stumbling due to presumed reduced vision were frequent and observed in over ⅕ of horses using face masks. Whereas observed less frequently than with use of a nose net, these reported adverse effects were of a more disruptive nature.

A surprisingly large percentage of horses (21%; 18/84) were reported to derive some benefit from the use of fly control, in contrast to only 2% previously described.[7] The current study population may have comprised more mildly affected horses than the aforementioned study,[7] despite ⅔ of owners describing their horse's headshaking as "severe" or "very severe." It is likely that a positive outcome rate of approximately 20% is heavily influenced by response bias, as no treatment achieved lower than 18% positive outcome. Such bias may have arisen from multiple sources including the difficulty in objective assessment of headshaking and any treatment response, a proxy-placebo effect, the phenomena of spontaneous and seasonal remission, fluctuation of clinical signs, regression to the mean, any contemporaneous treatments, and recall bias. Whereas such bias limits interpretation of all observed responses to treatments, the variability in reported outcomes gives confidence that a genuine treatment effect, above and beyond any bias, was noted for some treatments.

The positive response of over 50% (17/31) of headshakers to corticosteroid therapy was also unexpected given the documented aberrant trigeminal nerve activity[5] and the failure of a recent blinded, clinical trial of pulsed high dose dexamethasone therapy to have any effect on idiopathic headshaking.[15] Whereas not a first-line treatment for neuropathic pain, corticosteroids have been shown to reduce spontaneous discharge in injured nerves and might be useful as adjunct therapy.[16] Although nerve injury is not suspected in idiopathic headshaking, a reduced threshold for activation of the trigeminal nerve has been documented[5] and corticosteroids might act to decrease such aberrant activity. It is also possible that, in the responding subset of headshaking horses, there was a degree of low grade inflammation or allergy contributing to clinical signs observed by owners. As prolonged, ongoing management of headshaking is usually required, corticosteroids are unlikely to be useful as a therapeutic option due to the risk of serious adverse effects such as laminitis.

One-third of owners reported general antihistamine treatment improved their horse's headshaking (12/36). Such improvement may suggest an allergic component to headshaking in these horses; however, the reported effect may be largely due to bias as previously discussed. A positive response to cyproheptadine treatment was observed in approximately 50% of horses (14/29). Cyproheptadine is a first generation antihistamine with additional anticholinergic, antiserotonergic, calcium channel blocking, and local anesthetic activity,[17] which is used to treat human vascular headaches. Previous reports describing the response of headshaking to cyproheptadine treatment has varied widely, from no improvement[4,18] to 70%.[7] Such variation in efficacy is difficult to explain. The percentage of horses with a photic component to their headshaking is variable across these studies (none[4]; 39%, current study; 60%[7]), which may be relevant in responsiveness to cyproheptadine. Inappropriate dosage may have affected treatment outcome with cyproheptadine in the current study as, whereas only 2 owners reported using an inadequate dosage, many owners reported they did not know or omitted administration details. Adverse effects such as lethargy and drowsiness were noted in almost 50% of horses treated with cyproheptadine, which is likely to limit its use in ridden horses.

Carbamazepine, an anticonvulsant, which stabilizes voltage-gated sodium channels, had one of the lowest positive outcome rates of all treatments (25%; 2/8) and, therefore, does not appear to be an efficacious treatment for headshaking. Additionally, negative side effects were reported in ⅓ of treated horses. Although carbamazepine is the treatment of choice for human trigeminal neuralgia,[19] the pharmacokinetics of this drug in horses is unknown, which may explain its lack of effect. Additionally, recent trigeminal electrophysiological data suggests the etiopathogenesis of equine headshaking and human trigeminal neuralgia may differ.[5] NSAIDs were also poorly efficacious (22%; 4/22) as would be expected in the treatment of a neuropathic condition. The noted response in 4 horses may be due to the aforementioned biases.

Melatonin reduced headshaking in almost half (47%; 8/17) of horses in this study. The hypothesis for melatonin treatment of headshaking is that a late afternoon (5 PM) dose of melatonin artificially manipulates photoperiod such that, biologically, the horse remains in winter. Approximately 40% of treated horses did not shed their coat due to this phenomenon. Therapy is proposed to be most successful when melatonin is started before the onset of spring for seasonal headshakers[7]and, therefore, initiation of therapy when the horse was already headshaking may have reduced efficacy. A positive outcome in the two horses given melatonin inappropriately and the fact that suppression of the vernal increase in gonadotropins did not reduce headshaking[20] may question the proposed rationale. However, melatonin is also able to modulate pain, including neuropathic pain, via various antinociceptive effects including activation of opioid receptors, inhibition of pro-inflammatory cytokine production, modulation of $GABA_A$ receptor function and acting as a free radical scavenger.[21] Melatonin receptors have been identified in the trigeminal ganglion and trigeminal nucleus of mammals[22] and interestingly,

melatonin has been shown to attenuate an inducible model of trigeminovascular nociception in rats.[23] Melatonin might, therefore, act to reduce headshaking by mechanisms other than photoperiod manipulation. Only 13% of owners had used melatonin treatment, and all except one of these were based in North America, where the drug is cheaply available as an "over the counter" product. Whereas melatonin can be purchased with a veterinary prescription in other countries such as the UK, this does not appear to be occurring, most likely due to veterinarians being currently unaware of the protocol. Melatonin, as an endogenous hormone, also has the advantage of having no restrictions for use in competition horses. Given that almost half of headshakers improved with melatonin therapy, with only winter coat retention as an adverse effect, this treatment warrants further attention.

Magnesium supplementation had been used by almost 50% of owners, with just under half of these (25/58) reporting an improvement in their horse's headshaking. Magnesium increases the activation threshold of nerves such that a greater stimulus is required for depolarization.[24] Magnesium, therefore, appears a rational therapy given the reduced activation threshold of the trigeminal nerve in headshaking horses.[5] Formulations of magnesium vary in their oral bioavailability with that of magnesium oxide (the most frequently administered in this study) reported to be questionable in the horse.[25] Generally availability of magnesium in horses is high and feeding high percentages of roughage enhances its absorption from the gastrointestinal tract.[26] From this study, toxicity from daily magnesium supplementation of up to 40 g appears to be rare, although it may be advisable to periodically measure ionized magnesium concentrations. Magnesium may be another useful treatment for competing headshaking horses. Combining magnesium and melatonin therapy appeared to be beneficial, resulting in an improved outcome than use of either treatment alone (55%; 6/11). Indeed, combined therapy had the highest overall positive outcome; however, this should be interpreted with caution, as only 11 owners had used this treatment.

This study can be used to guide owners in the management of idiopathic headshaking. Owner-reported responses to treatment in 130 headshaking horses suggest that nose nets, face masks, and magnesium supplementation are the most efficacious nonmedical therapeutic options. Adverse effects such as facial irritation were reported in ⅓ of horses with nose nets. Cyproheptadine, corticosteroids, and melatonin were the most successful pharmaceutical treatments in this study; however, the number of horses using these therapies was low and, therefore, these results should be interpreted with caution. Of these pharmaceutical drugs, only melatonin is without significant adverse effects or allowed in competition horses. Veterinary diagnosis of idiopathic headshaking is advised before commencing any treatments. Further investigation of the pathophysiological mechanism underlying the aberrant trigeminal nerve activity in headshaking horses is warranted in order to develop more successful therapeutic options.

Acknowledgments

Conflict of Interest

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Review of the Discovery of the Basis for a Seasonal Pasture Myopathy/Atypical Myopathy

Stephanie Valberg, DVM, PhD, DACVIM, DACVSMR

The amino acid hypoglycin A is present in the seeds of box elder and sycamore maple trees. When pastured horses ingest seeds in the fall and early spring they develop a highly fatal form of rhabdomyolysis. A definitive diagnosis is made by measurement of serum acylcarnitines, which accumulate because the toxin blocks muscle lipid metabolism. Author's address: Department of VPM, University of Minnesota, 1365 Gortner Avenue, St. Paul, MN 55108; e-mail: valbe001@ umn.edu. © 2014 AAEP.

1. Introduction

Seasonal pasture myopathy,[1,2] atypical myopathy,[3,4] and atypical myoglobinuria[5] are terms used to describe an often-fatal form of rhabdomyolysis that develops in unexercised horses kept on pastures. Although initial clinical signs resemble colic, the apparent discomfort, trembling, tachycardia, short stiff stride, periods of recumbency, tachypnea, and myoglobinuria actually arise from severe degeneration of postural and respiratory muscles.[5–7] The first reports date back to 1939 in Wales[8] and 1959 in Southern Ontario.[9] Although described for 75 years, the cause of this disorder has remained a mystery until very recently. The purpose of this review is to highlight the sentinel findings that led to the discovery of the toxin associated with cases of seasonal pasture myopathy in European and North American horses.

2. Epidemiology

Vital epidemiological information on a large number of cases of pasture myopathy arose from the Atypical Myopathy Alert Group (http://www.myopathieatypique.fr/en/amag-2/). Many hundreds of cases were documented in Denmark, France, Germany, Ireland, United Kingdom, Latvia, Luxembourg, Spain, Switzerland, The Netherlands, Austria, Czech Republic, Italy, Norway, and Sweden.[7,10–12] Fewer carefully researched cases were documented in North America.[1,2,13] It became apparent that the number of outbreaks of pasture myopathy varied from year to year, were preceded by stormy weather, and occurred in pastures with trees in or around them that also contained dead leaves, dead wood and wet areas. The clustering of cases to specific pastures where horses were housed for at least 12 h a day, the seasonal occurrence in fall or the subsequent spring, the cessation of cases from December to January, and the lack of gender or breed predilection suggested a toxin was present in pastures on a seasonal basis. The wide variety of plants that could be implicated in causing pasture myopathy made the search for potential toxins confounding. Potential etiologic agents initially under investigation included white snakeroot,[1] clostridium toxins in soil,[14,15] toxins in dead leaves,[10,12] and the tar spot fungus growing on the leaves of *Acer pseudoplatanus*,[16] the European sycamore maple. Studies from 1988 in the United Kingdom[5] and

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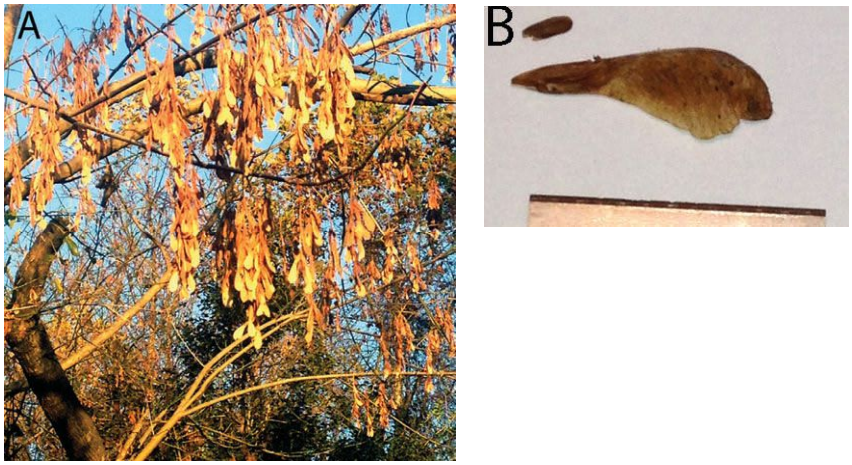

Fig. 1. A, Branches of a box elder tree (*Acer negundo*) laden with seeds in the late fall. B, One samara (husk) from the box elder tree with the seed removed (above and to the left) relative to the size of a razor blade (below).

2010 in the Netherlands[16] had identified the sycamore maple as a consistent presence on affected pastures. Information on the mechanism by which the toxin produced rhabdomyolysis was an important missing piece of the puzzle.

3. Pathophysiology

Serum biochemistry profiles of horses with pasture myopathy provided little direction for toxin investigation. Profiles revealed marked elevations in serum creatine kinase (CK) and aspartate transaminase and a moderate elevation in gamma-glutamyl transferase activities. Electrolyte derangements (high potassium and phosphorus, low sodium, chloride, and calcium), hyperglycemia, and lactic acidosis were often present.[1,7,13,17,18] Similarly, hematoxylin and eosin stains of formalin fixed muscle merely confirmed severe myodegeneration of postural and respiratory muscles and inconsistent degeneration of cardiac muscle.[4,5] Advances were made, however, when special stains were applied to muscle tissues and a lipid storage disorder was identified in postural and respiratory muscles of horses with pasture myopathy in both North America and Europe.[1,3]

A critical piece of the puzzle was contributed by Westerman et al. in 2008 that both narrowed the list of potential toxins causing pasture myopathy and led to a specific means to diagnose the condition.[19] An acquired defect in the enzyme multiple acyl-coA dehydrogenase (MAD) that represents the first step of β-oxidation of short and medium chain fatty acids as well as branched chain amino acids was found in muscle cells from Dutch horses with pasture myopathy. A diagnosis of MAD deficiency could readily be established by measuring the backlog of byproducts of lipid (acylcarnitines) and amino acid metabolism (glycine conjugates) in the bloodstream or urine. The specific diagnostic indicators of MAD were high short (C2-C5) and medium chain (C6-C10) acylcarnitines, variably high long chain (C14-C18) acylcarnitines, and marked elevations in urine ethylmalonic and methylsuccinic acids and glycine conjugates ([iso]valeryl-, butyryl-, and hexanoylglylcine).[19] Serum and urine samples from horses in North America with pasture myopathy were later found to also have a deficiency of MAD.[13] Thus, it seemed that the underlying mechanism for pasture myopathy was the same in Europe and North America.

4. The Toxin

Combining epidemiologic and pathophysiologic information, the responsible toxin would have to be seasonally associated with trees and capable of blocking MAD in muscle cells. In 2012, we determined that seeds from one particular tree in the midwestern U.S., *Acer negundo* (box elder, Manitoba maple) (Fig. 1A), were present in the fall on all inspected pastures where horses had succumbed to pasture myopathy.[2] The seeds of this tree (Fig. 1B) remain on the trees in the fall and are distributed onto the ground by high winds prior to an outbreak.[2] The topographical distribution of *Acer negundo* matched previous cases of pasture myopathy diagnosed in North America. A key connector was the fact that another tree within the same Sapinidae family, the Jamaican Ackee tree, produced a fruit that when unripe contained a nonproteogenic amino acid hypoglycin A, capable of causing an acquired deficiency of MAD when ingested by humans.[20] Subsequent studies found hypoglycin A in seeds from box elder trees growing in pastures where outbreaks of pasture myopathy had occurred.[2] In addition, the conjugated toxic metabolite of hypoglycin A, methylenecyclopropyl acetic acid (MCPA)-carnitine, was found in the bloodstream and urine of horses with pasture myopathy in the midwestern U.S.[2] Thus, hypoglycin A appeared to cause pasture myopathy in North America.

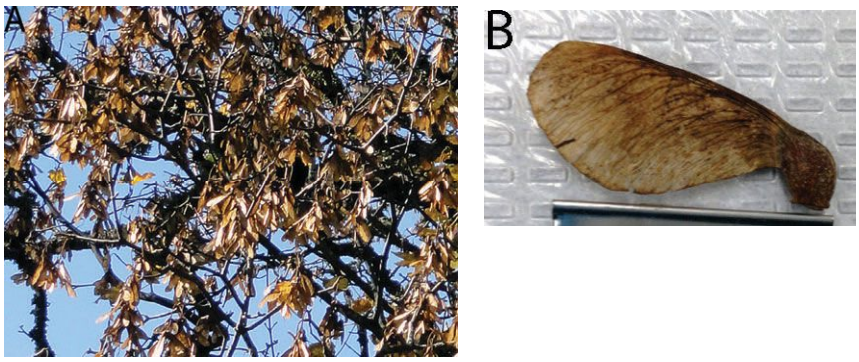

Fig. 2. A, Branches of the European sycamore maple (*Acer pseudoplatanus*) laden with seeds in the late fall. B, One samara (husk) from the sycamore tree with the seed (right) still present in the husk relative to the size of a razor blade (below).

Acer negundo was an unlikely source of toxin in Europe as this tree is not native to Europe and it was introduced largely as an ornamental varietal. A closely related tree, *Acer pseudoplatanus,* the sycamore maple (Fig. 2A), had previously been identified in European pastures where outbreaks occurred. Hypoglcyin A was analyzed and identified in seeds from Swiss sycamore trees (Fig. 2B) that were growing in pastures where horses had died from pasture myopathy and blood samples from affected European horses were also found to contain serum MCPA-carnitine.[21]

Thus, hypoglycin A present in seeds obtained in the fall from box elder or sycamore maples is the likely etiology for pasture myopathy in North America and Europe. Storms increase the number of seeds on pastures;[2,10,11] snow cover in the winter prevents access to seeds on the ground and ingestion of germinating seeds in the early spring and can lead to emergence of new cases. Seed burdens show annual variation, potentially explaining variations in outbreaks from year to year.[22] Toxin levels in seeds could vary from year to year as well, but this has not been studied. Disruption of lipid metabolism in muscle cells leads to degeneration of those muscle cells most active at rest, postural and respiratory muscles.

No studies have been performed in which hypoglycin A or *Acer* species seeds have been fed to horses to definitively prove the toxicity of *Acer* species seeds in horses. Thus, the number of seeds a horse would need to eat to become ill is not known. Further, quantitative assays of hypoglycin A have not been performed on seeds from other maple trees to determine their toxicity, nor have changes in hypoglycin A concentrations been reported over the course of a year.

5. Diagnosis

A presumptive diagnosis of hypoglycin A-induced pasture myopathy is based on the following:

1. Clinical signs of sudden rapidly progressive muscular weakness and stiffness, myoglobunuria, tachycardia, tachypnea, dyspnea, and recumbency, with death occurring in the majority of cases within 72 hours of the onset of signs. About 50% of horses will have cardiac damage. Most horses retain a normal appetite; occasionally horses have signs of choke, cardiac arrhythmia, colon impactions, and head swelling due to persistently low head posture.
2. Marked elevations in serum CK and aspartate transaminase activities, hyperglycemia, electrolyte derangements, and acidosis.
3. A history of grazing pastures containing or surrounded by maple trees with seeds in the fall or early spring.

A definitive diagnosis of hypoglycin A-induced myopathy is based on characteristic changes in serum acylcarnitines and urine organic acids. These tests are most readily available and are routinely performed by human metabolic disease laboratories such as the Baylor Institute of Metabolic Disease (http://www.baylorhealth.edu/Research/Institutes-Centers/IMD/Pages/Tests%20and%20Forms%2005–11.pdf). Normal equine values have been published for reference ranges.[13] Hypoglycin A and MCPA-carnitine assays are not commercially available.

6. Treatment

Early recognition of the disease is critical as the mortality rate likely improves with early treatment and prompt removal of horses from the source of toxin. Since this condition can present as a succession of cases, removal of all grazing animals from the pasture immediately is important along with checking serum CK activities. Horses should be rested in a warm dry environment and fed ad libitum hay and some grain to avoid a negative energy balance. Renal, respiratory, and cardiac function should be monitored by means of serum biochemistry profiles, blood gases, and cardiac troponin. Judicious use of nonsteroidal anti-inflammatory drugs is recommended, based on the status of dehydration

and renal function. Intravenous fluids need to be tailored to each horse's electrolyte derangements and severity of lactic acidosis at a rate that avoids fluid overload, especially if the cardiovascular system is compromised. Nasal oxygen may benefit horses with tachypnea. Intravenous antioxidants such as dimethyl sulfoxide, vitamin C, vitamin B complex (including riboflavin), and oral water dispersible natural vitamin E may be beneficial for the outcome.[1,6] The mortality rate is about 75%.[10] Horses that have recovered usually regain muscle mass over time and return to their former level of activity.

7. Prevention

Minimizing a horse's risk of exposure to box elder or European sycamore seeds in the fall is the best preventive strategy. Nevertheless, it is important to recognize that there are many older horses that have been housed on pastures with large numbers of *Acer* trees for years that have never become ill. Whether these horses avoid eating the seeds or have some form of immunity is unclear. Horses particularly at risk for pasture myopathy are those that are young or new to overgrazed pasture with *Acer* trees, those turned out more than 12 h/day, and those receiving little supplemental feed.[2,10] Ideally, trees with heavy seed burdens should be removed from pastures. If this is not possible, turnout of at-risk horses should be avoided in the fall when seed burdens are high or in the early spring.

8. Name Change for Atypical Myopathy and Seasonal Pasture Myopathy

There are other potential causes for pasture-associated myopathies in horses such as trematone toxin in white snakeroot and rayless goldenrod. With advances in defining the etiology of the most common pasture myopathy, the terms Seasonal Pasture Myopathy and Atypical Myopathy are now outdated and nonspecific. A change to the name Hypoglycin A Myopathy (H-AM) is proposed for those cases in which rhabdomyolysis develops in horses that are housed in proximity to seed-bearing *Acer negundo* or *Acer pseudoplatanus* trees, especially when elevated serum acylcarnitine or urine glycine conjugates concentrations are confirmed to be present.

Acknowledgments

This research represents a collaborative effort amongst researchers at the University of Bern, Switzerland, the University of Liege, Belgium, Institute of Metabolic Disease, Baylor Research Institute, Texas, Iowa State University, and the Department of Horticultural Sciences, University of Minnesota.

Conflict of Interest

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Effect of a Dietary Supplement on Gastric Ulcer Severity

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There is no statistically significant difference in the effect of omeprazole or a dietary supplement[a] in the treatment of squamous gastric ulceration in horses in active race training, with neither treatment resulting in improved ulcer scores following 90 days of treatment. Authors' address: University of Glasgow, Scotland, UK G61 1QH; e-mail: n.kerbyson.1@research.gla.ac.uk. *Corresponding and presenting author. © 2014 AAEP.

1. Introduction

The objective of this study was to assess the effect of a dietary supplement containing polar lipids (oat oil), soluble fiber (β-glucan), and amino acids (L-Glutamine and L-Threonine) on the development and treatment of squamous gastric ulceration in racing Thoroughbreds.

2. Materials and Methods

Sixty-seven Thoroughbreds in race training or pre-race training were recruited to the study on the basis of having ≥grade 1 gastric ulceration (The Equine Gastric Ulcer Council, 1999) on day 0 of the trial. Of this group, 39 horses completed the trial, which involved repeat gastroscopy on day 30, 60, and 90. Each horse was randomly assigned to one of two treatment groups; omeprazole[b] or the dietary supplement[a]; treatments were administered according to label dosage for the duration of the trial.

3. Results and Discussion

Chi-squared tests were used to assess the difference between treatment groups in terms of the proportion of horses improving by 2 or more grades: sixteen horses in each group improved by less than 2 grades or worsened ($P = 0.53$). McNemar's tests were applied to assess the effect of each treatment on gastric ulcer score. There was no significant improvement on gastric ulcer score with either omeprazole ($P = 0.38$) or the dietary supplement ($P = 0.50$) when a clinically significant gastric ulcer score of ≥2 was used.

Acknowledgments

Conflict of Interest

This study was funded by Freedom Health LLC.

Footnotes

[a]Succeed™, Freedom Health LLC, Aurora, OH 44202.
[b]GastroGard®, Merial Animal Health Ltd., Harlow, Essex, UK.

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Effect of a Supplement on Non-Glandular Gastric Ulcer Scores and Gastric Juice pH

Frank M. Andrews, DVM, MS, DACVIM-LA; Pilar Camacho, DVM; Giavanna Gaymon, DVM; Patrick Loftin, DVM; Frank Garza, Jr., MS; Michael L. Keowen, BS; and Michael T. Kearney, PhD

A supplement (SGU)[a] added to feed prevents the worsening of nonglandular gastric ulcers in stall-confined horses 2 weeks after discontinuation of omeprazole treatment and lessens the increase in gastric ulcer scores after intermittent feeding, without altering the gastric juice pH. Authors' addresses: Equine Health Studies Program, Louisiana State University, School of Veterinary Medicine, Baton Rouge, LA 70803 (Andrews, Camacho, Loftin, Garza, Keowen, Kearney); and Tuskegee University School of Veterinary Medicine, Tuskegee, AL 36088 (Gaymon); e-mail: fandrews@lsu.edu. *Corresponding and presenting author. © 2014 AAEP.

1. Introduction

SGU containing botanicals (herbs), coating agents, and natural antacids for horses with gastric ulcers has limited data available on its effectiveness. The purpose of this study was to evaluate the effect of SGU on nonglandular (NG) gastric ulcers scores and gastric juice pH after omeprazole treatment in stall-confined horses.

2. Materials and Methods

This study was performed in racing-age stall-confined Thoroughbreds (n = 8) as a 42-day 2-period crossover design in which all horses received no treatment (untreated controls) or treatment (SGU pellets, 40 g, twice daily). In addition, from days 1 to 14 all horses were treated with omeprazole paste[b], and then treatment was discontinued. From days 28 to 35, all horses underwent intermittent feed-deprivation. During feed-deprivation, all treated horses were fed SGU. From days 35 to 42, horses returned to their normal diet. Gastroscopy was performed on day −1, 14, 28, 35, and 42. Gastric juice pH was measured and NG gastric ulcer number (NGN) and severity (NGS) scores were assigned by a masked clinician (FMA).

3. Results

After 14 days, NGN and NGS scores significantly decreased ($P < 0.05$) in both groups compared to those on day −1. On days 28 and 35, NGN scores remained significantly lower in the SGU-treated horses when compared to the untreated controls. By Day 42, NGN and NGS scores were not significantly different in either group. Gastric juice pH was low and variable, except on day 14, when gastric juice pH was significantly higher in both groups due to omeprazole treatment.

4. Conclusions

SGU supplement fed to horses prevented gastric ulcers from increasing in horses after omeprazole

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treatment, without increasing gastric juice pH. Supplementation with SGU aids in the protection of the NG stomach from the rebound acid effects after omeprazole treatment is discontinued and in stall-confined horses undergoing intermittent feeding.

Acknowledgments

Conflict of Interest

This study was funded by Smart Pak Equine, LLC., Equine Health Studies Program Equine Funds and the LSU Foundation.

Footnotes

[a]SmartGut® Ultra, SmartPak Equine, LLC., Plymouth, MA 02360.

[b]GastroGard® paste, Merial Limited, Duluth, GA 30096.

Anatomy of a Practice Merger

John A. Chalk, Jr., CPA, J.D., CFP®, ChSNC

Mergers appear to be a growing trend among equine veterinary practices. Specifically, smaller practices are merging into larger practices, resulting in great opportunities for both the small and large practice owners. For many small practice owners, merging can provide them with growth opportunities, cost savings, future liquidity for their ownership, and a natural transition of their clients and employees into a larger organization when they are ready to retire. Author's address: 121 Countryside Court, Ste 140, Southlake, TX 76092; e-mail: jachalk@chalkcullum.com. © 2014 AAEP.

1. Introduction

A merger creates issues different from a simple acquisition or divestiture of a practice. Issues such as economic value, cultural fit, ownership structure, geography, real estate, and practice philosophy are just a few of the many issues that should be considered when an opportunity to merge is presented. Any potential party to a merger should begin by thinking about the following questions: "who am I?" "who are you and would you fit with me?" "how would we work together?" and "what is a potential structure?" This paper will briefly discuss these questions.

2. Who Am I?

Before a practice owner can begin to consider expansion they should begin with developing a clear understanding of who they are. This "identity check" involves several things such as financial history, client base, practice specialty(ies), practice type, and strategic objectives.

Examining the financial history involves analyzing complete financial statements over several years to understand the "financial story" of the practice. Historical revenue growth, ratio analyses, and owner distributions are just a sample of some of the things an owner should know about their practice before considering a merger. This type of financial analysis is critical for every business owner, even if you are not considering an expansion.

Examining the demographic profile of a practice involves understanding the market(s) served, market competitors, needs of the client base, type of services provided (ambulatory vs. hospital services), and core competencies. Studying these segments over an extended period of time can begin to tell the "story" of the practice. Defining strategic objectives help convert a vision into more specific plans. They set the major benchmarks and are designed to be measurable, specific, and realistic in order to guide decision-making.

It is only after you have a clear understanding of your practice that you can begin to explore whether or not other practices would be a possible merger candidate. Before you begin considering a specific practice, you should define the "perfect" merger candidate based on your identity and what you need to

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make the combined practice stronger than the two individual practices.

3. Who Are You and Would You Fit With Me?

Once you have identified what it is you are looking for in a merger partner, you can then begin investigating specific practices. Each possible candidate should be compared to the "perfect" merger candidate to determine just how close that individual practice is to your ideal. Obviously, we are not talking about comparing one bunch of bananas to another at the grocery store. There will be a very limited number of possible practice candidates but comparing any potential candidates against an ideal merger candidate is a very helpful exercise and should help to convince you to move forward, or not.

In order to determine if a merger partner is a match, you will need to examine information on the potential merger candidate. This information is very similar to the information you would use to understand your practice's identity. You will want to review the financial information as well as practice demographic information about the merger candidate. It is usual and customary to enter into a confidentiality agreement prior to examining the information of another practice. This due diligence investigation is concluded when the parties either agree to not proceed or agree to investigate a potential merger.

4. How Would We Work Together?

The next step to a practice merger is both parties determining if they want to be partners. This involves many aspects but is mostly about personality and practice habits of the owners and whether they can work together. We all know practitioners that we might respect professionally, but for one reason or another, we would never want to be their partner. This analysis takes some time and relationship building. One way to accomplish this is for each potential partner to spend time in the practice of the other potential partner. During this period, the two (or more) potential partners should work with and around each other. They should work with the staff of the other potential partners. By doing this you will get a "feel" for what it is like to work with this potential partner and whether your practice habits and styles are compatible.

5. What is a Potential Structure?

After determining that both parties would like to pursue a potential merger, it is then appropriate to begin to discuss the structure of a possible transaction. Typically, mergers involve one practice merging into another with the combined practice having combined ownership. The key to this process is determining the value of each practice and then using that value to determine the new ownership percentages in the new combined practice. For example, if two single owner practices wanted to merge and one practice was worth $400,000 and the other was worth $600,000, the ownership of the combined practice would be 40% and 60%, respectively.

This illustrates how important the practice value is when you are trying to determine the structure of a merger. Obviously, value is like beauty, it is in the eye of the beholder, and there are many ways to value a practice. It is important that the same method is used to value both practices so that both practices are treated as fairly as possible when you use those values to determine ownership of the merged practice.

Once the ownership of the new combined practice is determined, it is then important to put in place all of the documents necessary for the new practice partnership. These include partnership agreements, employment agreements (outlining how the combined practice will pay for the efforts of the partners), and buy-sell agreements that detail how the partners stop being partners. You should never enter into a merger without having a clear understanding, in writing, about virtually everything that has to do with money. Potential misunderstandings can be avoided with clear written communication involving all of the business aspects of the new practice.

6. Conclusion

Should you decide to explore the possibility of a merger, you must know who you are, have an extensive due diligence process to determine who they are, become comfortable with the structure of the transaction, and, last but not least, know your future partner(s) almost as well as you know your own family.

Acknowledgments

Conflict of Interest

The Author declares no conflicts of interest.

History of Colic Surgery and a Look to the Future

David E. Freeman, MVB, MRCVS, PhD, DACVS

An overview of colic surgery over the last 40 years suggests improvements in outcome, largely a result of early diagnosis and referral, allowing horses to arrive at referral hospitals in better overall systemic condition, as compared to years past. However, closer inspection of information from different sources suggests that postoperative complications are increasing and long-term survival rates could improve further. The growing emphasis on postoperative treatment could divert our attention from surgical steps that are critical to improving outcome. Author's address: University of Florida, College of Veterinary Medicine, Department of Large Animal Clinical Sciences, PO Box 100136, Gainesville, FL 32610-0136; e-mail: freemand@ufl.edu. © 2014 AAEP.

1. Introduction

The purpose of this presentation is to review progress made in the last 40 years of colic surgery, to determine where progress has been made, and with a look to the future, determine what further can be done to improve outcomes. Although most practitioners may feel that colic surgery is of little relevance to their daily activities, their role in improving survival and reducing complication rates is critical. They are the most important members of the team involved in saving the horse's life at critical steps in the process, the early stages of the disease process, when the need for referral should be recognized, and the later stages, during management of life-threatening postoperative complications. They also serve as a critical liaison with the owner, who will seek their guidance when the horse is hospitalized and after it returns home following surgery. Such guidance can only be provided by a practitioner who is well-informed about all the intermediate steps that pass from the time of referral to the day the horse leaves the hospital.

2. Short-Term Survival Rates—Early Studies

Although colic surgery was commonplace in university hospitals in the early 1970s, veterinarians and owners viewed it largely as a last resort, a prologue to an imminent death. A 1971 paper described a successful outcome in 10 of 38 horses (26%) with colic treated by surgery, and most survivors had a minor intestinal procedure.[1] Authors of this study concluded that, "although the overall recovery rate was low, the procedure is entirely justified if only as a means of diagnosis."[1] A review of cases from the University of California from 1958 to 1971 reported a mortality rate of 72.9% in 48 horses that had surgery for a small intestinal strangulation.[2] However, the mortality rate dropped to 46.6% in the last 14 years of the review period, which is evidence of improvement in management of these cases.[2]

In studies spanning a period from 1970 to 1984, Professor Barrie Edwards and Judith Hunt described complications of colic surgery and their impact on survival in 215 horses that recovered from general anesthesia.[3,4] Of the small intestinal cases, 55% survived compared with 68% in the large

NOTES

Table 1. Reported Median-Survival Times in Long-Term Studies on Small Intestinal Surgery

Authors	Period Covered	Type of Surgery	Median Survival Time
MacDonald et al. 1989[10]	1968–1986	Resection and anastomosis	27 days
	1968–1980	Resection and anastomosis	8 days
	1980–1986	Resection and anastomosis	219 days
Garcia-Seco et al. 2005[31]	1987–2002	Lipoma only	150 days
	1987–2002	Lipoma only—jejunocecostomy	90 days
Freeman and Schaeffer 2011[19]	1993–2002	Jejunojejunostomy (strangulated IL)	120 months
	1993–2002	Jejunojejunostomy (strangulated CL)	72 months
	1993–2002	Jejunojejunostomy (nonstrangulated IL)	10 months
Fogle et al. 2008[13]*	1994–2002	All lesions	18 days
Freeman and Schaeffer 2010[18]	1994–2005	Jejunocecostomy (HS)	97 months
	1994–2005	Jejunocecostomy (S)	96 months
Freeman et al. 2014[12]	1996–2011	No resection—strangulating lesions	120 months
Proudman et al. 2002[22]	1998–2000	Small intestine—EFE only	13 months
Proudman et al. 2005[11]	1998–2004	All lesions—not EFE	63 months
	1998–2004	Small intestine—EFE only	25 months
	1998–2004	Repeat celiotomy	17 months
Proudman et al. 2007[89]	1998–2006	Jejunojejunostomy	49 months
	1998–2006	Jejunocecostomy	23 months

Note: Median survival times were reported in some (MacDonald, Fogle, Freeman), calculated in others from data (Freeman), or determined from graphs (Freeman, Garcia-Seco, Proudman). IL = Interrupted Lembert; CL = Continuous Lembert; HS = Handsewn anastomosis; S = Stapled anastomosis; EFE = Epiploic foramen entrapment. *In this study, the follow-up period of 6 months was too short for 33 horses from 2003 to 2005 to reach their median survival time.

intestinal cases.[3,4] A report by Professor Huskamp from his private practice hospital in Germany reviewed 613 horses in which colic surgery was completed from 1979 to 1981.[5] He reported 87.7% short-term survival (alive at discharge from the hospital) for small and large intestinal lesions.[5]

3. Short-Term Survival Rates—Recent Studies

Recent retrospective studies on short-term survival rates in horses after colic surgery suggest that more than 80% of horses that recover from anesthesia after small intestinal surgery are discharged from the hospital.[6] This is the most challenging of surgeries that we do and traditionally is associated with a poorer prognosis than other procedures. These numbers appear to be superior to earlier publications and have been attributed to improvements in surgery and anesthesia. A more likely explanation is a more positive attitude from owners and referring veterinarians, leading to prompt referral that places the horse in the hospital when it is an excellent candidate for surgery and anesthesia. Another important contributing factor to this improved attitude towards colic surgery was an apparent increased prevalence of nonstrangulating displacements of the colon, which did not appear to be commonplace before 1977.[7–9] These cases did much to enhance the reputation of colic surgery because they have an excellent prognosis for complete recovery.[9]

Although it is difficult to measure the effect of early referral on outcome, the lower reflux volume, packed cell volume (PCV), and heart rate, and higher total leukocyte count at presentation in horses admitted to one hospital after 1980 compared with previous admissions provide evidence of a trend towards earlier referral.[10] In accordance with this, the study demonstrated a trend towards improved survival rates in horses that had surgery towards the end of the study period compared with the beginning[10] (Table 1). Also, there is recent evidence that some strangulated small intestinal segments can be viable and not require resection, presumably because surgery was performed early in the disease course (Fig. 1). These horses can have superior short-term and long-term survival rates compared with horses treated with resection and anastomosis.[11,12] Such cases could be the beneficiaries of early referral, allowing surgery to be performed before irreversible intestinal damage has developed. The risk of complications can also be reduced by early referral, as the risk for postoperative ileus (POI) after strangulating lesions and diarrhea after nonstrangulating lesions appears to increase with duration of colic.[13]

A comparison between present and past success rates with surgical treatment of colic suggests a considerable improvement, but this assessment needs to be tempered with caution. Firstly, survival rates reported by Professor Huskamp for small intestinal lesions treated between 1979 and 1981 were comparable to those reported today.[5] Secondly, most horses with strangulating lesions in the 1970s typically arrived at a veterinary hospital with more severe endotoxemic shock and more profuse reflux than many of our patients today.[2–4,10] Surgery was more complicated in the 1970s because the ischemic changes were more advanced and had progressed throughout a long segment of intestine. The ileum was involved in most and had more severe

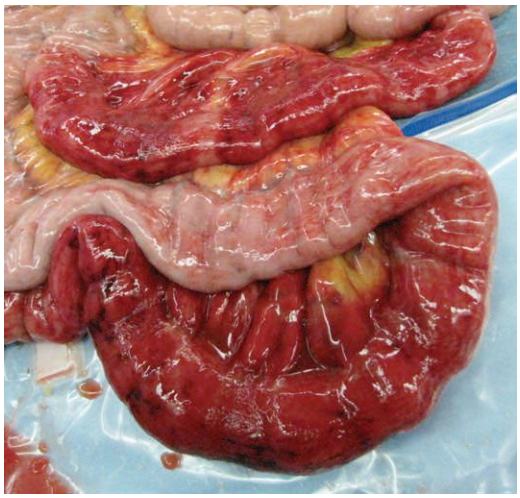

Fig. 1. Intraoperative photograph of two segments of strangulated small intestine that were judged to be viable and not needing resection. Assessing viability correctly can avoid unnecessary resection and improve survival rates. Early referral of such cases is a critical first step in getting these horses to surgery before resection is required.

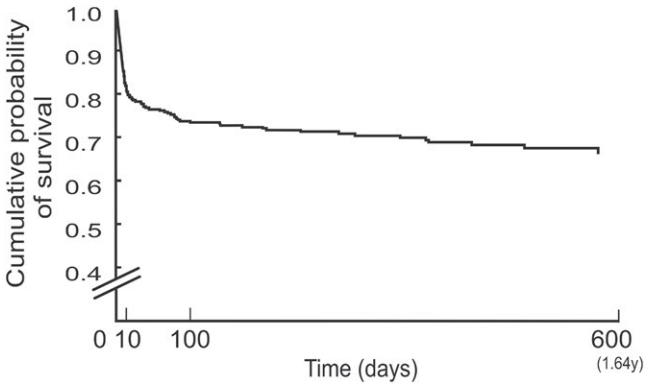

Fig. 2. Kaplan—Meier curve[22] showing the highest mortality is shortly after surgery, followed by a slower mortality rate over the next hundred days, eventually reaching a slower decline to the end of the follow-up period (n = 341 horses). The follow-up interval was not sufficiently long to demonstrate the median survival time. Reproduced with permission.

ischemic damage than is seen today, changes more likely to prompt an innovative approach to these surgeries[14,15] rather than discussions with the owner about terminating the case.[16] It could be argued that the surgeon should be selective in the decision to complete the surgery; however, any intraoperative selection process based on prognosis will only produce falsely elevated survival rates in those horses in which surgery is completed and thereby prevent improvements in surgery. On the other hand, the owner might select against resection for financial reasons or because of humane concerns.

4. Long-Term Survival

There is strong evidence that short-term survival rates provide an incomplete picture of the current status of colic surgery. Median survival time is a widely used measure of long-term survival and is obtained by Kaplan–Meier analysis of survival data (Fig. 2). It is the time at which half the horses that had completed surgery (fully recovered from general anesthesia) have died.[10,17–19] As evident in Table 1, median survival times from several studies differ considerably, which is in contrast to the consistently high survival rates reported for hospital discharge.[6]

When results of a study that spanned from 1968 to 1986[10] are compared with those from a more recent study (1994–2001),[20] the latter reports superior short-term survival after small intestinal resection and anastomosis (76.1%) than the earlier study (49%).[10] However, when the more recent group was included in another study on horses with all small intestinal diseases treated by surgery in the same time period and at the same institution, the median survival time was only 18 days[21] in that study, versus 27 days[10] in the older study (Table 1). These findings would suggest a lack of progress in long-term survival after colic surgery.

One of three consecutive papers published in 2002 from the University of Liverpool followed a large population of horses for a long period and could, therefore, identify a unique long-term pattern of mortality after surgery (Fig. 2).[22] Most deaths were shortly after surgery (87% probability of survival to 10 days), followed by a lower death rate during the next 100 days (82% probability of survival), with an even slower decline in mortality to the end of the follow-up period, possibly similar to the mortality rate in the general population (Fig. 2).[22] The highest risk period for first colic after discharge was also within 100 days after surgery.[22] In another report, the risk for death diminished markedly after 12 months in horses that had small intestinal surgery.[17] These findings suggest that potentially fatal complications arise in the early postoperative period, that later complications, such as adhesions, will be delayed until after discharge,[23,24] and that their effects or prevalence will then diminish over time.

Although postoperative complications, such as POI, postoperative colic, and postoperative endotoxemia can have adverse effects on short-term survival,[20,21,25] they could also adversely affect long-term survival.[21,23] Horses that recover from POI have poorer long-term survival rates than horses that did not develop this complication.[21,23] The reasons for this are unknown, although prolonged contact between amotile loops in horses with POI could favor adhesion formation, and this explanation has some basis in research findings in a pony study.[26]

Table 2. Criteria Used to Establish a Diagnosis of POI

Authors	Criteria	Comments
Blikslager et al. (1994)[32]	>2 L of reflux at any given intubation after surgery and all horses falling into this category also had heart rates >60 beats/min or signs of abdominal pain	Could include too many horses that are mildly affected or not affected at all, which could artificially improve the survival rate from this complication
Freeman et al. (2000)[12,17–19]	Any nasogastric reflux after surgery when clinical signs indicate the need to pass a stomach tube	Same limitations as the preceding and possibly worse
Roussel et al. (2001),[33] Cohen et al. (2004)[34]	Reflux volume >20 L during a 24-hour period after surgery or a volume >8 L on one occasion after surgery	Likely to exclude horses with mild POI and to artificially reduce the survival rate from this complication
Merritt and Blikslager (2008)[67]	a) Gastric reflux of ≥4 L upon any given intubation, or >2 L/h on repeated intubation, of gastric contents of pH >4.0. b) HR persistently >40 beats/min. c) Mild to severe signs of abdominal discomfort. d) Rectal and/or ultrasonic evidence of multiple loops of fluid distended small intestine	Proposed along with criteria for distinguishing between POI and a mechanical obstruction, none of which has been applied clinically

Note: Inclusion criteria based on the volume of reflux described by different authors for defining those cases that developed POI in their studies.

5. Are We Making Progress With Treatment of Postoperative Ileus?

Postoperative ileus is the most common complication after small intestinal surgery and is largely manifested by persistent reflux of large volumes of fluid through a nasogastric tube. Professor Barrie Edwards showed that it is possible to reduce the prevalence of POI over time with his cases, presumably through improvements in colic surgery and anesthesia and in the condition of his cases on arrival to the hospital. In 1985, he and colleagues reported POI in 19.5% of all colic surgeries (23% after small intestinal surgery),[3,4] which decreased to 9.6% in a more recent study.[22] Comparison of complication rates between studies is complicated by differences in inclusion criteria (Table 2). In a study that included all horses with any reflux, only 10% of horses were reported with POI after small intestinal surgery.[17] However, subsequent studies in other institutions reported a prevalence of 31% to 73%, despite use of larger reflux volumes in their inclusion criteria.[20,21,27–31] More importantly, when inclusion criteria are kept constant within studies, there is little evidence of reduction in the rate of POI over time[20,21,32–34] (Fig. 3). This is all the more remarkable, considering that horses are being presented earlier and in better overall condition and that other improvements have developed in colic treatment and anesthesia over those time periods.

6. Are We Making Progress With Treatment of Endotoxemia and SIRS?

Great progress has been made in the treatment of endotoxemia since the 1970s, largely through the benefits of early referral, aggressive use of fluid resuscitation, and use of the nonsteroidal anti-inflammatory drug (NSAID), flunixin meglumine. Endotoxin can be measured in the blood of horses before and after surgery for large and small intestinal causes of colic, and endotoxemia can be a devastating complication.[30,35–37] The term "systemic inflammatory response syndrome" (SIRS) and multiple organ dysfunction syndrome (MODS) were developed to classify syndromes largely of septic origin in human critical patients, and could be applicable to horses typically classified as endotoxemic.[38]

Endotoxemia or SIRS would seem more likely to develop in horses with colitis or recovering from

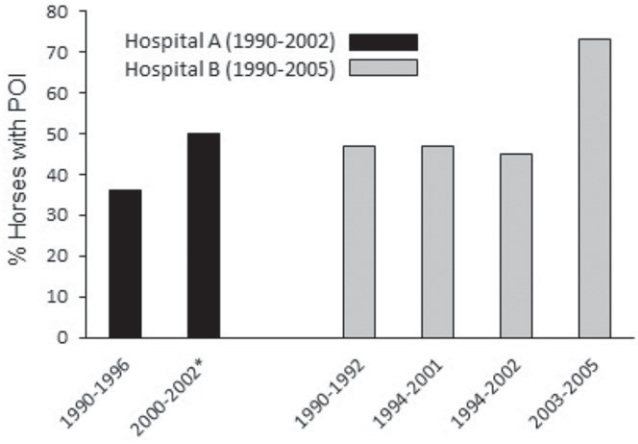

Fig. 3. Percentage of horses that developed POI after small intestinal surgery in two separate institutions identified by different letters on top of each bar graph, showing no improvement in POI over time.[20,21,32–34] Hospital A used inclusion criteria from reference 33 and Hospital B used inclusion criteria from reference 32 (Table 2). *Although this study included data from another institution, the prevalence of POI in that institution was lower than in Hospital A.

surgery for large colon volvulus, with or without large colon resection. Although endotoxemia was reported in 64% of horses in one study after small intestinal resection and anastomosis,[20] it should be very rare in horses after any type of small intestinal surgery. Firstly, the half-life of circulating endotoxin is short[36] so that it should be rapidly eliminated from the system once the primary cause is removed (by resection and anastomosis). Secondly, a functional gastrointestinal tract should be achieved in most horses after small intestinal surgery, thereby reducing production and eliminating absorption of any intraluminal endotoxin.

Although the small intestine that remains after surgery proximal to a strangulating obstruction can suffer sufficient preoperative distention to reduce transmural microcirculation, the resulting mucosal damage can range from none[39] to mild.[16,40] Even in horses in which strangulated small intestine was not resected, postoperative endotoxemia did not develop or was mild enough to escape clinical detection.[12] Therefore, any evidence of endotoxemia after surgery could indicate a failure of the surgery to establish a patent gastrointestinal tract, either through an obstructed anastomosis, a leaking anastomosis, or some form of mechanical or functional intestinal obstruction. The prevailing notion by some surgeons to accept endotoxemia as an expected complication following small intestinal resection and anastomosis is flawed and not supported by recent clinical studies.[12,17–19]

7. NSAIDS and Endotoxemia

The risk of postoperative endotoxemia after small intestinal surgery could be increased by use of a nonspecific COX inhibitor, such as flunixin meglumine, based on results of recent in vitro studies on ischemic and reperfused equine jejunum.[41] This has been attributed to inhibition of tight junction closure by flunixin meglumine, despite histological evidence of rapid mucosal repair.[41] The consequence of this is an increased transmucosal permeability to endotoxin and increased risk of postoperative endotoxemia. The COX-2 selective NSAID, firocoxib, did not have the same effect as flunixin meglumine. Also, this NSAID effect on mucosal repair seems unique to the small intestine because flunixin meglumine did not interfere with mucosal repair in a similar ischemia and reperfusion model in the equine colon.[42]

An important prerequisite for manifestation of this adverse effect of flunixin meglumine on mucosal repair is the existence of jejunal injury in the postoperative case that is of comparable severity to that induced in the research setting.[41] Such injury proximal to the site of resection and anastomosis is unlikely, based on research and clinical findings.[16,39,40] Also, when strangulated small intestine is judged viable and not resected, there is little if any clinical evidence of postoperative endotoxemia after twice daily full-dose flunixin meglumine, and such horses can have median survival times of 10 years (Table 1).[12] In a clinical study that measured lipopolysaccharide (LPS) in the plasma of horses after small intestinal surgery, flunixin meglumine did not appear to increase the risk of endotoxemia or POI compared with the COX-2 specific inhibitor, meloxicam.[30] More importantly, horses that received flunixin meglumine had superior pain scores than those that received meloxicam.[30] In in vivo studies, flunixin meglumine should block the systemic effects of any endotoxin that crosses the mucosa and escapes hepatic consumption. Therefore, the results of in vitro studies might not apply to the typical postoperative case.

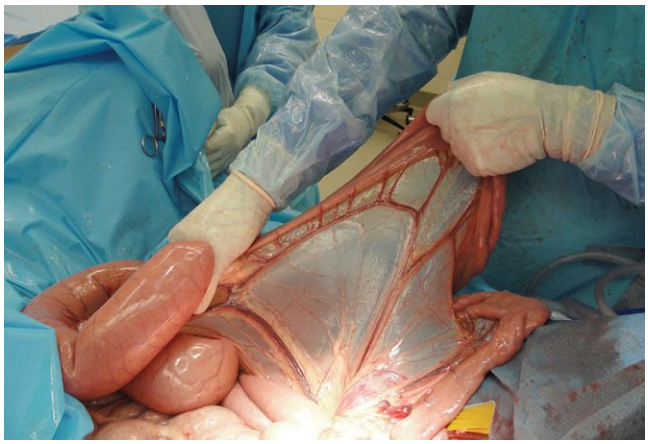

Fig. 4. Intraoperative decompression of distended small intestine before resection and anastomosis. Although this procedure can induce seromuscular inflammation in equine jejunum (Fig. 5), its benefits outweigh any adverse effects from this inflammation. However, it must be performed with careful manipulation and lubrication (saline or sodium carboxymethylcellulose) so as to minimize the inflammatory response.

8. What Causes Postoperative Ileus?

The current hypothesis for diminished motility that characterizes POI is an early neurogenic response followed by a later inflammatory effect on intestinal smooth muscle function.[43,44] The later phase is based on findings in laboratory animals that intestinal inflammation induced by surgical manipulation releases proinflammatory cytokines and prostaglandins from activated macrophages.[43] These inflammatory mediators then trigger an influx of inflammatory cells into the muscularis layers, followed by further release of inflammatory products that reduce muscle contractility.[43]

In horses, neutrophilic infiltration has been identified in the jejunum 18 hours after experimentally induced ischemia and intestinal manipulation (Fig. 4), which provides a temporal relationship between inflammation and onset of POI.[45] These findings have been validated by finding an association between POI in horses after small intestinal resection and the number of neutrophils in serosa and muscle

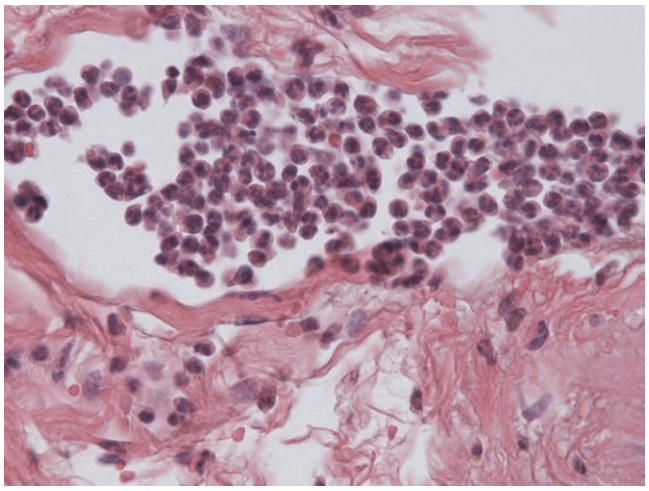

Fig. 5. Neutrophil infiltration in small intestinal serosa and longitudinal muscle after manipulation.

of the resection margins.[46] In our laboratory, we have demonstrated that any handling of large or small intestine induces an immediate and intense influx of neutrophils into the seromuscular layers in the manipulated intestine and even in distant segments, certainly producing a robust panenteric inflammation (Grosche et al, unpublished data; Fig. 5). However, connecting these findings to postoperative reflux in horses must be made with great caution for the following reasons:

- Surgical procedures strongly associated with POI in human patients can be performed in horses with a low risk for POI, such as abdominal surgery on sites other than the small intestine (e.g., urogenital tract surgery, large colon surgery) and laparoscopic surgery.[47] Therefore, if pathophysiology of POI in laboratory animals and human patients applies to horses, the magnitude of the functional response in horses seems to be considerably less than would be expected from the histological changes.
- The presence of inflammatory cells in a tissue is not always associated with collateral damage, possibly because of protective mechanisms generated by equine host cells.[48]
- The proposed role of inflammatory cells in intestinal smooth muscle as a cause of POI[45] has created a growing reluctance on the part of surgeons to fully decompress distended small intestine (Fig. 4). However, horses that underwent vigorous decompression of small intestine by the author during surgical treatments for small intestinal obstruction had a low prevalence of POI.[12,17–19] Failure to decompress distended small intestine could play a more important role in producing POI because fluid-filled loops of jejunum in the tight confines of the abdomen can create an obstructive "bent garden-hose effect."
- Horses that have a repeat celiotomy within 72 hours after the first surgery, and are subjected to the same intestinal handling as they had at the first surgery, do not seem to be prone to POI, despite the additional intestinal manipulation.[49] The inflammatory model would predict a different effect. In most of these cases, postoperative reflux is a major indicator of the need for a second surgery and can be effectively resolved by decompression of distended intestinal loops, in addition to other surgical procedures required to relieve the obstruction.[49]

9. Does Lidocaine Prevent Postoperative Ileus?

Although there is some evidence that the recommended regimen for using lidocaine to treat or prevent POI is effective for these purposes[25,50] and can improve short-term survival,[25] none of these studies examined long-term survival. Also, any assessment of lidocaine in management of POI and in improving survival in horses[25,50,51] should not ignore evidence that better results can be achieved without it.[12,17–19] The favorable outcomes reported without lidocaine cannot be attributed to intraoperative selection on the basis of prognosis or use of inclusion criterion that would reduce prevalence of POI (Table 2).[12,17–19]

In one study that demonstrated a favorable response to postoperative lidocaine when used in a prophylactic manner to prevent POI, 79% of the control group received lidocaine while under general anesthesia.[25] Because these control horses received lidocaine when its putative anti-inflammatory effects would be of most benefit,[34] when the intestine was being manipulated, their inclusion as a control group could be questioned. In a study that demonstrated a curative effect of lidocaine on POI, clinical inclusion criteria or methods of randomization were not well-defined, and horses with duodenitis/proximal jejunitis were also included.[50]

Despite the original claim that lidocaine could treat or prevent POI in horses through its putative prokinetic effects,[52] subsequent studies have demonstrated the absence of such an effect and that lidocaine might even delay intestinal transit.[53,54] Studies on equine small intestine subjected to ischemia and reperfusion demonstrated a positive response to lidocaine on smooth muscle contractility in vitro; however, this response could only be achieved with higher concentrations of lidocaine than can be safely achieved in plasma[55,56] and not with lower concentrations.[57]

The anti-inflammatory effects of lidocaine, which have been offered to explain its purported efficacy in treating POI, have been tested in different models of inflammation in horses, with mixed results. In horses that received intraperitoneal endotoxin (LPS), the clinical score and tumor necrosis factor-α

(TNF-α) activity were significantly lower in those treated with lidocaine than in a saline-treated group.[58] However, lidocaine had no effect on PMN cell count and total protein in peritoneal fluid and did not change IL-6 production.[58] Lidocaine CRI had no effect on mucosal inflammation induced in equine jejunum by ischemia and reperfusion, although it reduced inflammation caused by flunixin meglumine.[59] In the same model, lidocaine ameliorated the inhibitory effects of flunixin meglumine on recovery of the mucosal barrier from ischemic injury in equine jejunum.[60] In an ischemia and reperfusion model in equine colon, lidocaine improved repair of mucosa in the right dorsal colon but not the pelvic flexure.[61]

Lidocaine at therapeutic concentrations did not inhibit migration or adhesion of equine neutrophils in vitro but increased both measures of neutrophil function at higher concentrations than might be safe in vivo.[62] It also failed to reduce expression of proinflammatory genes in LPS-stimulated equine monocytes.[61] In the black walnut model of laminitis, lidocaine increased laminar E-selectin mRNA concentrations and did not inhibit inflammatory events in either the laminae or skin.[63] In horses with recurrent airway obstruction, lidocaine did not affect neutrophilic inflammation and did increase total cells in the bronchoalveolar lavage fluid.[64] Lidocaine also failed to protect against smooth muscle injury in intestine subjected to ischemia and reperfusion,[56] although a beneficial effect could be achieved at concentrations in excess of those that could be safely achieved in plasma.[65] In an experimental model of intestinal ischemia-reperfusion in horses, lidocaine significantly reduced a moderate pulmonary neutrophil influx without an apparent change in other inflammatory responses.[66]

Based on the preceding, lidocaine appears to be an ineffective prokinetic drug and might have little if any effect on neutrophil migration. If it does have a benefit, this might be through reducing tissue injury and improving intestinal repair, thereby reducing output of neutrophil chemoattractants from the site of injury.[61]

10. Postoperative Ileus as a Multifactorial Disease

POI is typically regarded as a functional disorder, with origins in pathophysiological events that interfere with normal gastrointestinal tract function. This has led to the proposal by Merritt and Blikslager that POI be defined as a "transient impairment of GI motility occurring shortly after abdominal surgery, especially where intestinal manipulation was involved, that is not caused by mechanical obstruction and where pharmacological (vs. surgical) intervention may be helpful in speeding resolution."[67] This definition is certainly consistent with the manner in which the disease is characterized in the veterinary literature.[32–34]

The role of mechanical events in POI was strongly suggested by results of a study that identified a strong association between POI and jejunocecostomy, whereas this complication was absent in horses that had a jejunojejunostomy.[17] This observation was underscored in a recent study, which showed that a higher reflux/colic rate could be induced by a minor change in the manner in which a jejunocecostomy is performed.[18] Also, repeat celiotomy has provided an opportunity to demonstrate the importance of apparently minor mechanical obstructions in the development of postoperative reflux in horses.[49] In one study, technical errors with the surgery accounted for most cases of POI, repeat celiotomies, and deaths.[17]

Based on the preceding, the best hope for preventing POI might well require that this complication be recognized as a multifactorial disease. Firstly, POI is diagnosed largely on the basis of a single clinical finding, a specific volume of gastric reflux through a stomach tube (Table 2), which provides no indication as to cause. Although a functional derangement could contribute to generating postoperative reflux, other contributing factors could include motility altered by the anastomosis, anastomotic stenosis caused by postoperative swelling (with a possible contribution from overhydration), failure to adequately decompress small intestine, shortening of a mesenteric closure, and other surgical errors that could interfere with the anatomy and function of an anastomosis. Currently, there is no reliable clinical feature or group of clinical features that can distinguish POI from a mechanical obstruction, and those proposed are not effective in the author's experience.[67]

11. Serosal Adhesions—A Success Story?

Available information would suggest that there is a trend towards a reduction in serosal adhesions in horses.[68] However, most adhesions after colic surgery probably go undetected or unrecorded, especially when horses are euthanized without necropsy.[68] Although some adhesions can be clinically silent,[68] these are probably rare in the small intestine, which has a very low tolerance for any restriction in mobility. In an epidemiological investigation on 1014 horses that had surgery for an acute abdominal crisis at University of Liverpool over a 16-year period, 27 (2.7%) horses had a repeat surgery at various postoperative periods to treat colic related to adhesions.[24] This study revealed a variety of other causes of postoperative colic other than adhesions, including recurrence of the original problem or development of a new and unrelated disease.[24]

A recent report demonstrated that horses treated with intraoperative intra-abdominal sodium carboxymethylcellulose (SCMC) had a better survival rate to 6 months after surgery than horses that were not treated with this drug.[21] Necropsy results were not described for horses that died, so the role of SCMC in preventing adhesions was not demonstrated.[21] Horses were not randomly assigned to

treatments, and a similar retrospective study did not demonstrate improved survival after intraperitoneal SCMC.[69] Although SCMC can reduce experimentally induced adhesions in horses,[70] there is some evidence that the commercially available product could increase adhesion formation in clinical cases.[71]

12. True Progress in Colic Surgery

Although many may regard this list as incomplete, these are improvements compared with what was available over many decades ago and constitute true progress in colic surgery over that interval.

- The most important advancement in the management of horses with life-threatening colic is early recognition and prompt referral to the veterinary hospital for further diagnostics and treatment.
- Modern suture material is superior to what preceded it for anastomosis and causes minimal tissue reaction, once responsible for local inflammation and adhesion formation.
- For abdominal wall closure, modern sutures replace nonabsorbable materials of the past that persisted and created sinus tracts, and replace surgical chromic catgut, which could not provide a long and durable closure of the linea alba. Consequently, the rate of incisional hernia formation has declined.
- Currently available NSAIDs provide greater analgesia and reduce signs of endotoxemia more effectively than their predecessors, although more specific COX-2 inhibitors might offer some advantages over less specific drugs.
- Fluid therapy, and an associated improved understanding of water and electrolyte balance, has done much to improve recovery of horses in severe shock.
- Modern anthelmintics have virtually eliminated large strongyles from the equine gastrointestinal tract, but the impact of their absence on colic is difficult to gauge.
- Ultrasonography plays a critical role in early diagnosis of intra-abdominal conditions that require surgical treatment and eliminates the guesswork in management of the colic case.
- Currently available analgesics can provide safe control of pain but for short periods that allow repeated assessment of the horse's condition.
- Improvements in anesthesia and pharmacological management of the cardiovascular system have improved our ability to anesthetize horses in severe shock.
- The growth of referral hospitals around the country has played a large role in improved survival rates after colic surgery. A horse with colic almost anywhere in the United States or Canada can be transported to any of a number of excellent facilities within a reasonable distance from its home.
- We can now manage nonstrangulating displacements of the large colon by nonsurgical methods, such as rolling or use of phenylephrine to treat entrapment of the large colon in the nephrosplenic space.[8,72,73]
- We can prevent recurrence of certain colics such as colonic displacements and volvulus by large colon resection[74] or colopexy,[75] entrapment of the colon over the nephrosplenic ligament by nephrosplenic space ablation,[76] and inguinal hernia by different laparoscopic methods of vaginal ring closure.[77]

13. Areas for Further Improvement

In 2009, the topic of a presentation at the annual meeting of the American Association of Equine Practitioners was *Advances in management of small intestinal diseases causing colic*.[78] In the Proceedings that followed, the statement was made that "the most dramatic improvements have arisen in intraoperative and postoperative medical treatment, which may in part explain changes in survival."[78] Clinical evidence does not support this statement because survival rates in studies that used these intraoperative and postoperative medical treatments[20,21,25,50] were inferior to those achieved in a contemporary study that did not use them.[17]

Many veterinarians place great emphasis on postoperative medical treatment.[78] Could this reflect a need for other improvements? To address this and other concerns, the following items are proposed to improve survival after colic surgery.

- Early and accurate diagnosis, referral, and surgery without delay are critical to prevent postoperative complications and death and to reduce cost of treatment. Improvements in diagnostic procedures will continue to achieve these goals.
- Short-term complications need to be reduced as much as possible because they reduce survival and predispose to long-term problems. Even incisional infections and hernia formation seem to be more likely in horses that develop severe endotoxemia[79] and POI.[34] Additionally laminitis can be significantly more likely in horses that develop POI.[34] Prospects of return to use or performance can be reduced by incisional hernia, diarrhea, or laminitis.[80,81]
- The concern has been raised in a recent study from a major veterinary hospital that survival rate after surgical intervention has not improved significantly during the years.[82] Although this is difficult to explain, it does need to be addressed and could be related to the high complication rate.[78]
- Postoperative fluid therapy should be goal-oriented instead of a rules-of-thumb approach

based on multiples of maintenance requirements. Whereas maintenance requirements were established on fed horses, most colic patients are not fed and the water needs of food-deprived horses are 13% of the needs for fed horses (Freeman, unpublished data). Overhydration can cause anastomotic and incisional edema and related complications,[83] salt-induced diuresis, and unnecessary expense. The consequences of overhydration are well recognized in human gastrointestinal surgery[83] and are more likely after prolonged anesthesia for colic surgery.[84]

- Horses should be administered flunixin meglumine (1.1 mg/kg) twice daily, for postoperative analgesia and inflammation[30] because potential adverse effects[41] of this drug have not been demonstrated in clinical use.[12,30]
- Common errors with time-dependent drugs (IV penicillin) should be avoided, such as giving too far in advance of surgery, failure to readminister at an appropriate interval during a long surgery, and continued use of antimicrobial drugs in the postoperative period when they might not be indicated.[85] Complications from such inappropriate use can include failure to prevent incisional infections, increased risk of antimicrobial-induced postoperative colitis, and increased risk of antimicrobial resistance.[85]
- Improved accuracy with clinical or adjunctive methods to correctly assess intestinal viability can identify when resection is needed and avoid unnecessary intestinal resection and the associated risk of complications and increased cost.
- Postoperative management should include early reintroduction to oral food intake, starting 18 to 24 hours after surgery (Fig. 6).[12,17–19] This approach is not intended to provide maintenance energy needs for an adult horse, but to restore normal gastrointestinal function and to improve attitude,[86] provided that surgery has produced a patent gastrointestinal tract. Healing of an anastomosis is enhanced by the physical presence of food material passing through it.[87]
- The value of repeat celiotomy as an effective means of dealing with postoperative complications should be recognized. Horses can tolerate the second surgery well, especially if done promptly.[49] Repeat celiotomy can be more cost-effective than protracted and ineffective medical treatments, but methods to reduce the associated high rate of incisional infections also need to be evaluated.
- Surgeons need to be sensitive to the cost of colic surgery. Overuse of intraoperative and postoperative medications that lack evidence-based support for their use in the colic patient can rapidly consume the owner's financial resources without improving survival.
- Both in the field and in the hospital, we should be selective with diagnostic procedures on occasions when they may not contribute to case management in a meaningful way or would not change our clinical approach to the case. If a veterinarian feels the need to do an abdominocentesis in the field, he or she must be aware of the limitations and risks, including any associated delays in referral. Referral might be a more efficacious alternative.
- Future clinical trials should place greater emphasis on long-term survival after particular treatments because effective treatments should allow horses to reach the end of their lifespans. These studies should also include age-matched controls, to control for attrition through a variety of reasons that could shorten lifespan or limit performance, other than colic.
- Owners and referring veterinarians should be aware that old horses (>16 years), even those in their late 20s to early 30s can handle general anesthesia and colic surgery well and have postoperative survival rates comparable

Fig. 6. Feeding hay or hand grazing in very small amounts at 18 to 24 hours after small intestinal surgery will stimulate motility and improve the horse's overall attitude. This is a critical step in hastening recovery.

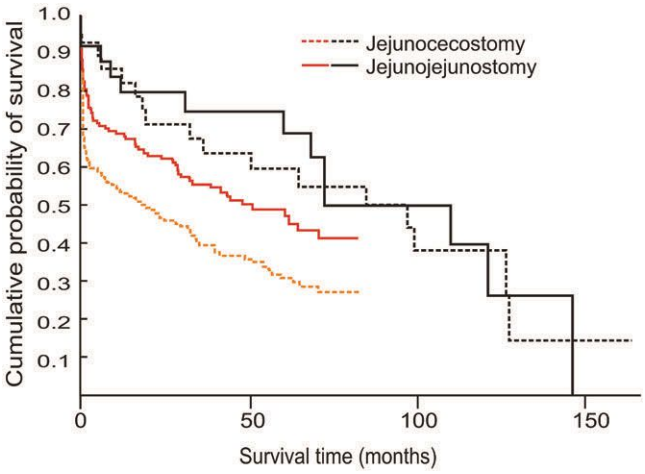

Fig. 7. These survival curves are from references 18,19 (black lines), and 89 (red lines). In reference 89, jejunojejunostomy had a superior survival rate than jejunocecostomy. The other curves were generated from two separate publications on these procedures in strangulated intestine[18,19] and show little difference between jejunojejunostomy and jejunocecostomy, both procedures performed by the same surgeon over the same timeframe. Solid lines are jejunojejunostomy and broken lines are jejunocecostomy.

to those in younger horses.[88] The decision to continue with treatment of older horses could be influenced by closeness to the end of their estimated life span and the presence of comorbidities (pituitary pars intermedia dysfunction, laminitis, and equine metabolic syndrome).

- Surgeons need to recognize the value of a jejunocecostomy, despite the lower survival rates and complications reported for this procedure,[89] because recent studies have demonstrated similar long-term survival rates as with jejunojejunostomy[18,19] (Fig. 7; Table 1).
- Owners and referring veterinarians should be aware that horses can return to the expected level of performance after colic surgery, and at the highest level in demanding sporting activities.[80,81,90,91]
- A multifactorial approach should be taken to treating the horse with postoperative reflux, placing more emphasis on surgical prevention and less on medical management.[78]
- The current level of funding through agencies that support research into colic needs to be augmented through more generous donations from horse owners and other supporters of equestrian activities. Those who derive enjoyment and wealth through horses need to contribute more to improving the health of the animal to whom they owe so much.

Acknowledgments

Conflict of Interest

The Author declares no conflicts of interest.

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Abdominal Ultrasound and Palpation *per Rectum* as Complementary Modalities in Diagnosing Equine Abdominal Pain

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1. Introduction

The main goals when performing diagnostics on a horse with abdominal pain are to determine a diagnosis, choose an appropriate therapeutic plan (which may involve surgery), and provide the owner with a prognosis. Palpation of the abdominal contents per rectum has long been considered a mainstay in the diagnostic evaluation of horses with acute or chronic abdominal pain. It was once held that ultrasonographic evaluation of the abdomen had no utility in the diagnosis of acute abdominal pain in horses,[1] but that view has changed dramatically over the last 20 years. The ready availability of portable sonographic equipment and better protocols for rapid transabdominal sonography has made percutaneous ultrasound an increasingly utilized modality in the diagnosis of acute colic. Sonographic evaluation is especially helpful in evaluating colicky horses in which rectal palpation is impossible or unacceptably risky. In horses amenable to palpation per rectum, ultrasonographic evaluation offers additional information about parts of the abdomen unavailable to palpation. Rather than viewing these diagnostic modalities strictly independently of each other, however, it is useful to think about how they each contribute to answering questions about the state of the anatomy within the abdomen. Each one is a powerful diagnostic tool, but together, they provide information that neither could alone.

2. Strengths and Limitations of Each

Abdominal palpation per rectum is useful for examination of the caudal aspect of the abdominal cavity. Indications include acute and chronic colic, fever of unknown origin, urogenital problems, reproductive evaluation, weight loss, inappetence, and other problems in which abdominal disease is suspected. Adequate restraint and the liberal use of lubricant are required minimums for performing a safe examination in any animal and the utility and safety of rectal palpation are often enhanced by the use of sedative, anesthetic, and antispasmodic medications.[2–4] No special equipment is required and skill is readily developed with practice and basic knowledge of the anatomy.

There are several limitations to the use of rectal palpation in horses. Palpation per rectum is not appropriate for very small or very fractious patients,

NOTES

in which circumstances of the safety of the patient and/or veterinarian is likely to be compromised. Very large horses may be also difficult to palpate, especially for smaller veterinarians. It is estimated that the caudal 1/3 of the abdominal contents are available for examination via palpation,[3] but veterinarians palpating large horses or veterinarians with short arms may find that only 1/4 of the abdominal contents can be reached. In addition, a late term fetus, bladder distention, or colonic and cecal gas distention may obstruct palpation of other structures.

The most dreaded complication of rectal palpation is rectal tear. Specific breeds, horses >9 years of age, and mares have been shown to be at increased risk for this complication.[4] The evaluation and treatment of rectal tears have been covered elsewhere in numerous excellent resources.[3–7] Although we as veterinarians are often more concerned about potential concerns to our patient's well-being, another potentially serious consequence of rectal palpation of a horse is injury to the veterinarian through kick or crush injury. Care must be taken in providing the safest environment for both patient and practitioner.

Ultrasound is a portable noninvasive imaging modality. It is estimated that the peripheral 2/3 of the abdomen of an average horse can be sonographically imaged with a percutaneous technique.[8,9] Most abdominal sonography is performed percutaneously, but scanning per rectum can also augment the information gathered during rectal palpation, especially when the left kidney, urinary bladder, ureters, reproductive tract, or caudal abdominal vasculature are suspected as causes of abdominal pain. A good working knowledge of abdominal anatomy and image optimization is necessary to obtain diagnostic sonographic images in colic cases; that said, with practice and guidance, abdominal sonography is not difficult to learn. Newer protocols for scanning acute colic cases, such as the fast localized abdominal sonography of horses exam,[10] provide guidance for performing rapid, consistent examinations that yield useful information in these cases.

As with any diagnostic modality, there are limitations to the application of sonographic evaluation to colic. Image quality can be affected by hydration, perfusion, and ambient temperature, as well as the horse's skin thickness and density, degree of adiposity, and haircoat. Patient preparation, which includes grooming, clipping the hair if needed, and the application of gel or isopropyl alcohol, is necessary for adequate image quality. Gas within the bowel prevents sound wave penetration and so the greater the gas distention of the viscera, the fewer structures that may be imaged. Not all ultrasound units have battery power, so access to a power source may be required. In addition, although more affordable units and probe options are becoming increasingly available, there is cost involved in obtaining optimum equipment. However, even using a standard rectal probe, some useful information may be obtained.

3. Evaluation of the Large Colon and Cecum

Diseases of the large colon are common causes of colic and include impaction, spasm, displacement, volvulus, intussusception, and colitis/typhlitis. Impaction of the large colon, particularly the pelvic flexure, is easily palpated per rectum, as is impaction of the cecum.[1,3–5] Impactions are less likely to be identified using ultrasound than via palpation. Large colon displacement and volvulus comprised 1/3 of all cases requiring surgical intervention in one study.[11] For conditions such as displacement or volvulus of the large colon, the band direction of the colons may be palpated, although severe gas distention may confuse the findings.

In normal horses, palpation per rectum of the nephrosplenic area reveals the left kidney, the spleen, and the nephrosplenic ligament. In horses with nephrosplenic entrapment, the colon is palpable dorsal to the ligament and the spleen may feel enlarged and/or displaced medially and ventrally by the colon. Sonographic evaluation reveals gas-filled bowel dorsal to the spleen, with the view of the kidney partially or wholly obscured. The spleen is displaced ventrally and the dorsal border of the spleen has a straight horizontal border that extends from the paralumbar fossa to the 10th to 12th intercostal space.[8]

Right displacement of the colon is characterized on rectal palpation as colonic bands coursing horizontally across the abdomen and the colon may be detected lateral to the cecum. However, severe gas distention in the colon can obscure the exam findings. The additional sonographic identification of colonic vessels on the right side of the abdomen is highly sensitive and specific for right dorsal displacement or 180° colon volvulus.[12]

In cases of vascular compromise and inflammation, sonographic assessment can add diagnostic and prognostic information, which is critical when providing information to clients leading to surgical decisions. Colonic wall thickness has been shown to be a predictor of colonic volvulus and may help to differentiate this condition from simple displacement.[13] Colon wall edema may be present in cases of vascular compromise and tissue inflammation, which may be seen in chronic severe impaction or colitis; the changes in the latter scenarios are generally much less severe than in a strangulating lesion. Having information about colon wall health or compromise is useful when trying to form a prognosis to advise clients and set expectations.

Colonic intussusception is rare, but ileocecal, cecocecal, and cecocolic intussusception have been reported in adult horses.[14] On rectal palpation, the intussusception may be occult or manifest as a mass in the right abdomen.[1,3,4,14] Sonographically, a classic "target lesion" appearance is noted in the right paralumbar region. The outer loop (intussus-

cipiens) appears thickened and edematous and may be separated from the incarcerated bowel (intussusceptum) by a small layer of fluid.[8] Although colitis is rarely a cause of acute colic without other suggestive signs, the ability of ultrasound to visualize fluid intestinal contents and measure colonic wall thickness can aid in the diagnostic and prognostic evaluation of these patients.

4. Evaluation of the Stomach

The stomach is not palpable on per rectum examination. An assessment of the size, position, and fluid content of the stomach may be helpful in making management decisions about when to manually evacuate the stomach in cases of small intestine obstruction. In addition, gastric impaction can be sonographically diagnosed by detecting a markedly enlarged stomach, which may encompass 5 or more intercostal spaces on the left and may even be visible in the right abdomen.[8] Although rarely a cause of acute colic, gastric masses can also be sonographically visualized if the mass is located along the greater curvature of the stomach, which lies along the left body wall, or if the stomach is filled with fluid contents to transmit the sound waves.

5. Evaluation of the Small Intestine

Disorders of the small intestine causing acute colic include simple luminal obstruction, which may be functional or physical, and strangulating lesions. The small intestine is not palpable in the normal horse; the discovery of distended or thickened small intestinal loops on rectal palpation is abnormal.[1,3,4] Percutaneous ultrasound of the normal equine abdomen may reveal several loops of small intestine, especially if the horse has been held off of feed[15,16] or has been administered an α-2 agonist sedative.[9,16,17] However, the presence of multiple loops of distended submotile or amotile small intestine on sonographic evaluation is abnormal. This finding is most commonly seen in the inguinal regions of the ventral abdomen but may be seen in various other locations. Motility assessment can be sonographically evaluated, as described by several authors,[17,18] but in the acute colic, so many factors can potentially influence motility that the clinical utility of this information is in question.

The appearance of the small intestine can offer valuable information about the nature and chronicity of small intestinal disease. In addition, sonographic assessment may be more sensitive than palpation per rectum at detecting abnormal small intestine, especially early in the course of the disease.[19] Distal obstructions may fail to produce nasogastric reflux or palpable loops of small intestine until ample time has elapsed to build fluid that fills the intestine in an orad direction.

It has been suggested that small intestinal wall thickness measurements may be a poor predictor of the etiology of small intestinal pathology because of the small area measured and the difficulty in accurate measurement of a curved line,[19] but the presence of edema in the wall of dilated small intestine in a horse with acute abdominal pain is suggestive of inflammation or vascular compromise in the visualized loops. Distended fluid-filled loops seen in conjunction with thickened amotile small intestine is consistent with a focal strangulation. Diffusely fluid distended small intestine, which appears as multiple circular loops stacked in multiple areas of the abdomen, may be seen with small intestinal mesenteric volvulus, global ileus, or proximal duodenitis/enteritis. Grossly thickened small intestinal wall seen focally may suggest the cause of an obstruction, as in the case of an intramural tumor or intramural abscess.

Sonographic visualization of abnormal small intestinal loops can also reflect the duration of an obstruction. Sedimented feedstuff in intestinal loops indicates complete stasis or obstruction of the loops for a period of time sufficient to allow settling of particulate matter out of suspension. The location of distended small intestinal loops can be suggestive of the etiology of the disorder. Several authors[19,20] have noted that epiploic foramen entrapment is associated with finding distended small intestinal loops in the right abdomen and, particularly, in the dorsal right abdomen. Rarely, distended, atonic, and/or edematous loops of small intestine are identified in the nephrosplenic and gastrosplenic spaces. Although the presence of small intestine in these spaces is generally considered a normal finding, the small intestine can become incarcerated in these ligaments and thus abnormal bowel in these locations should alert the practitioner to the possibility of entrapment.[19,21]

Small intestinal intussusception is rare in adult horses, but has been reported in horses from 6 months to 3 years of age. The most common site for small intestinal intussusception is the ileum, but jejunojejunal intussusception has also been described.[14] These lesions may be palpated per rectum, depending on their location in the abdomen. Sonographically, they have the same general appearance as intussusception of the large bowel.[8,19]

6. Evaluation of the Peritoneal Space

Palpation per rectum of the caudal abdomen yields information about the caudal peritoneal and retroperitoneal spaces. Pneumoperitoneum secondary to bowel rupture can be palpated as a dorsal emptiness in the abdomen, with the rectum snug against the examiner's arm.[4] Sometimes, emphysema of the serosal surfaces can be detected as crepitus and free ingesta within the abdomen gives the peritoneal surfaces a gritty texture.[3] Pneumoperitoneum is readily detected sonographically, and with leakage or rupture of the gastrointestinal tract, increased peritoneal fluid containing free gas echoes and particulate matter is ventrally visualized.

Increased peritoneal fluid is very readily identified sonographically and the fluid can be character-

ized by its sonographic appearance. Anechoic fluid indicates lower cellularity, while turbid fluid is consistent with highly cellular fluid. Cellular fluid with evidence of fibrin and/or adhesions is consistent with peritonitis. Swirling homogeneous cellular fluid is seen with hemo-abdomen; the kidneys, spleen, liver, ovaries, and broad ligament (mares) should be interrogated to determine the source of the bleeding. Fluid volumes of less than 10 L are generally not detectable on palpation per rectum.[4]

7. Evaluation of the Solid Organs

Other than the caudal spleen and the left kidney, the solid organs of the abdomen are not typically palpable per rectum. Although uncommonly implicated as the cause of acute abdominal pain, sonographic evaluation of these organs can provide important information about them that can help determine a diagnosis. Kidney or ureteral stones, renal hemorrhage, choleliths causing main bile duct obstruction, hepatomegaly from diffuse hepatic disease or hepatic abscess, or splenic hematoma are all solid organ problems that can manifest as acute abdominal pain.

8. Summary

Ultimately, in evaluating a horse with colic, the results of all diagnostics, including physical exam, evaluation of hydration status, nasogastric intubation, palpation per rectum, ultrasonographic scanning, and abdominocentesis fit together to form a data set that gives the practitioner diagnostic and prognostic information that hopefully leads to a successful therapeutic plan. Performed together, palpation per rectum and sonographic examination provide complementary information about lesion location, etiology, and severity.

Acknowledgments

Conflict of Interest

The Author declares no conflicts of interest.

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Value of Clinicopathology in Diagnosing and Managing Acute Abdominal Crisis in the Horse

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1. Introduction

The value of laboratory analysis is often overlooked in diagnosing and managing horses suffering from acute abdominal crisis. The following is a review of different laboratory tests that are commonly available and offer important insight into either the diagnosis or the management of a case. A description of the tests, when they are appropriate, how to interpret, and the value to the practitioner is provided.

2. Materials and Methods

A Pubmed search was performed on various search terms related to clinicopathology of the horse or equine and colic.

3. Results

The search returned 230,912 papers and these were reviewed for relevance to the subject, of which 27 were selected for discussion.

4. Discussion

The desire to improve the accuracy of the diagnosis when evaluating a horse with acute abdominal pain has led to multiple studies of physical exam and laboratory parameters. Some of the earliest publications focused on blood lactate[1] and blood pressure.[2] Although much has improved in the management of colic in the last 40 years, clinically important results continue to focus on the parameters of oxygen delivery and perfusion.

Hematology

A complete blood count is frequently performed in the workup of a horse suffering from colic. It can be a very useful parameter to help understand the current state of the disease process. Increased white blood cell (WBC) values can be seen with intestinal inflammation, chronic infection or abscess, and chronic peritonitis. Leukopenia is a common finding if there is any compromise to the mucosal barrier as the horse neutrophil is very responsive to endotoxin. As the leukocytes marginate and/or undergo apoptosis, the WBC can rapidly decrease. The severity of the reduction in the neutrophil count is related to the severity of the endotoxemia. A recent study by Salciccia et al showed that serial WBC counts offer value in horses after colic surgery because nonsurvivors are more likely to have a WBC $<$3900 cell/mm between 28 and 60 hours postsurgery.[3]

Hematocrit or packed cell volume and hemoglobin are two parameters that can provide useful insight into the volume status of a patient. A value can be obtained by centrifugation or by allowing blood to

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settle. On the farm, the author frequently pulls up 10 ml of whole blood into a syringe and allows it to settle over the next 15 min. After the red blood cells settle, the gradations on the syringe can help approximate a percent of red blood cells in the blood. Increased values, more so than decreased values, are of primary concern in colic cases. Increases can occur with splenic contraction and dehydration. The author has also seen cases of persistent polycythemia related to chronic liver dysfunction. Hematocrit is frequently cited as an important predictor of outcome. However, in a study of 44 horses with neorickettsiosis, hemoglobin concentration at admission was independently associated with survival.[4]

Chemistry

Similarly, in this same population of previously cited horses, elevations in serum creatinine and urea nitrogen were higher in nonsurvivors.[4] Elevations in creatinine can occur secondary to reduced perfusion of the kidneys with hypovolemia, which is termed prerenal azotemia. With prerenal azotemia, the urine should be concentrated and the horse responds to fluid therapy with a reduction in the azotemia. When there is true renal damage, the urine specific gravity may be concentrated or it may be isosthenuric. The renal values do not respond as quickly to fluid therapy. Groover et al studied 167 horses suffering from colic or colitis and found that horses with persistent azotemia (>72 h) were 3 times more likely to die or be euthanized.[5]

Blood glucose is another value in which persistent elevations are associated with poor outcomes.[6,7] Glucose homeostasis is frequently dysregulated in critical illness in other species. In a study of 228 horses, Hassell et al. found an association of nonsurvival with hyperglycemia. In this study, glucose was elevated above the reference range in 45% of the population at admission. Similarly, Hollis et al found that 50.2% of horses had blood glucose values above the reference range. Additionally, horses with hyperglycemia at 24, 36, and 48 h after admission had a worse prognosis.

Glucose dynamics are not the only system affected during colic. In a study of 32 horses undergoing colic surgery, alterations in metabolism and liver function were identified.[8] Increases in total bilirubin can occur secondary to true biliary damage, but much more frequently are associated with reduced caloric intake. In horses suffering from colic with increased total bilirubin, there is commonly an increase in triglycerides. This supports the observation that increased total bilirubin is a frequent finding in horses off feed or not consuming enough calories. In a study by Underwood et al an increased triglyceride concentration was associated with poor outcome, underscoring the need to provide adequate calories in the immediate postoperative period. Additionally, the study identified a group of horses with increased bile acid concentrations and sorbitol dehydrogenase activity that was negatively associated with survival. Interestingly, there was no increase in gamma glutamyl transferase (GGT) activity or blood ammonia concentrations in this pilot study.

As a membrane bound enzyme, GGT activity can increase without death of the cell. The GGT activity does increase with displacement of the large colon.[9] While not associated with outcome, horses with a right colon displacement more frequently had increased GGT activity (49%) compared to horses suffering from a left colon displacement (2%). It was hypothesized that the position of the colon when displaced to the right causes compression of the bile duct, leading to obstruction and increased GGT activity. Similarly, horses with duodenitis proximal jejunitis frequently have increased serum GGT activity compared to horses suffering from strangulating small intestinal lesions.[10]

Alkaline phosphatase is another enzyme found in the liver. It is also found in intestinal mucosa, among other locations in the body. Higher values were found in the blood and peritoneal fluid in horses suffering from colic.[11] There is significant overlap between inflammatory lesions, strangulating lesions, and peritonitis, preventing this test from specifically separating surgical from medical cases. However, the relative increase in alkaline phosphatase activity is greater in lesions with more severe intestinal damage.

Lactate values have been studied more than any other parameter relating to the outcome of colic in the past 10 years.[12–19] As a byproduct of glycolysis, increased lactate is an indication of increased anaerobic metabolism. There are multiple causes for increased lactate, but the most clinically important is a reduction in oxygen delivery.[14] It is possible for lactate to be elevated with normal oxygen delivery both secondary to reduced clearance with liver dysfunction and secondary to poor tissue oxygen uptake related to cellular dysfunction with sepsis or inflammation. Lactate measurement has been validated for horses by point-of-care monitors.[18,20] While whole blood is clinically useful, better agreement is found when plasma is used. Initial research with lactate was on single measurements, but as more clinicians have begun measuring this value, it has been recognized that serial measurements are more appropriate.[16] Because there is important overlap in initial values at presentation in survivors and nonsurvivors, no single cut point is used to try and classify horses. Johnston et al found a strong association of lactate values at admission and colonic viability.[17]

Similar to lactate, cardiac troponin I (CTnI) has recently been validated on a point-of-care monitor in the horse.[21] Additionally, alterations in CTnI values have been reported in horses suffering from colic or endotoxemia[22–24] Radcliffe et al found that moderate increased CTnI values postoperatively carried a poor prognosis in horses post colic surgery.[23]

Additionally, Nath el al found elevations in CTnI on presentation in 36% of horses with strangulating lesions and 47% of horses with inflammatory lesions.[22] Of the non-survivors, 50% had elevated CTnI concentrations compared with only 22% of survivors. Because endotoxin can lead to an increase in CTnI concentrations, it is likely that the horses with elevated CTnI were those with higher levels of endotoxin exposure. Additionally, CTnI has a very short half-life and quickly returns to normal once the initial insult is resolved.[24]

Abdominocentesis

An analysis of peritoneal fluid can add critical information for cases that are not straightforward. Peritoneal fluid analysis has been described in colic multiple times in the last 30 years. Classically, the evaluation is focused on markers of inflammation. Matthews et al identified color and protein values as the two most important variables distinguishing surgical cases from medical cases.[25] Serosanguinous fluid had a 98% positive predictive value for the need of surgery, while a normal color had an 89% negative predictive value for the need of surgery. Total protein was less predictive but still better than total nucleated cell counts. When considered as a whole, any sample with one abnormality had a 79% positive predictive value for surgery while a completely normal value had an 89% negative predictive value for surgery. Freden et al found less value in single variables with the exception of abdominal fluid color.[26] A study of 100 abdominal fluid samples also found less value in the total nucleated cell count, although the count was higher in nonsurvivors.[27] The author suggests a differential of the cells may be more useful in predicting outcome. More recently, Peloso and Cohen found that increasing peritoneal lactate values over time were consistent with strangulating lesions.[12]

5. Conclusion

Investigations continue to improve our understanding of clinicopathologic changes associated with acute abdominal crisis and in the hope of identifying that single measurement that may clearly divide surgical from medical cases and survivors from nonsurvivors. Because of the complexities associated with the response to inflammation, abnormalities associated with poor oxygen delivery, and regional differences in diseases, a single value is unlikely to be found. However, the use of serial measurements shows promise in improving the prognostic value for cases, monitoring the effects of therapy, and identifying surgical lesions more quickly.

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Conflict of Interest

The Author declares no conflicts of interest.

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Updates in the Medical Management of Colic: Moving Beyond Mineral Oil

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1. Introduction

Colic remains one of the leading reasons for which horse owners consult their veterinarians. While exact numbers are difficult to obtain and vary geographically, reported risks have ranged from 3 to 10 episodes of colic per 100 horse-years.[1] Fortunately, less than 5% of horses with colic have a surgical lesion. Therefore, medical treatment is indicated for the vast majority of horses with colic. While there is unfortunately no new "silver bullet" treatment for colic, this paper will review the main medical treatments currently in use: fluid therapy, laxatives, and analgesics.

2. Fluid Therapy

Fluid therapy is used in horses for a variety of reasons, but the basic goals are to replace losses, support the cardiovascular system, and maintain hydration in the face of ongoing losses. In horses with colic due to intestinal impactions, fluids are commonly given in an effort to promote hydration of the colonic contents. Fluids may be given either intravenously or enterally.

Intravenous fluids are more expensive, require an indwelling intravenous catheter with the associated risk of thrombophlebitis, and may be impractical on the farm. However, horses with diarrhea, signs of hypovolemic shock, or nasogastric reflux greater than 2 to 3 L will generally necessitate IV fluids to maintain hydration. In hospital settings, high volume intravenous fluids (two to three times maintenance rate or 100–150 mL/kg/d) are often given under the premise that there will be net flux of fluid into the gut lumen resulting in softening of impacted material. However, there is conflicting evidence as to the effect of IV fluid volumes on fecal hydration. In one study of normal horses, 5 L/h of IV fluids for 12 hours (approximately 100 mL/kg/d or twice maintenance) did not show any effect on hydration of right dorsal colon contents or feces.[2] In another study comparing fluid rates and means of administration to dehydrated horses, fecal and systemic hydration were restored at twice maintenance IV fluids; however, there was no additional benefit seen when increasing the rate to three times maintenance. In fact, horses given three times maintenance IV fluids had increased urine production and sodium loss, which could contribute to electrolyte abnormalities as well as rebound dehydration when fluids are discontinued.[3] There is in fact too much of a good thing!

Rapid transit of fluids through the small intestine makes enteral fluids a logical option in treatment of large colon impactions. Fluids delivered via naso-

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gastric tube will exit the stomach within 15 minutes and reach the cecum and large intestine within 1 to 2 hours in most normal horses. It is also thought that intermittent bolus delivery may result in more fluid being delivered to the colon, as it will overwhelm the small intestine's capacity for absorption.[2] Nasogastric administration may also stimulate the gastrocolic reflex, thus, aiding with overall gastrointestinal motility. There is evidence that leaving an indwelling nasogastric tube in place for 72 hours does delay gastric emptying, so repeat intubation may be better despite its inconvenience and behavioral effects.[4] Enteral fluids are overwhelmingly less expensive and easier to prepare than IV fluids, as they do not need to be sterile. Enteral fluid therapy is also slightly more forgiving than intravenous fluids in terms of rate and electrolyte composition. However, large volumes of plain water or hypotonic solutions may cause marked electrolyte abnormalities including hyponatremia, hypokalemia, and hypocalcemia.[2,5] Isotonic fluids, however, are generally well tolerated with no significant effects on plasma biochemistry values except for a mild hemodilution when using a potassium rich solution with 6 g NaCl and 3 g KCl per liter of water.[5] Most average size horses can tolerate 6 to 10 L/h of intragastric fluids; however, there does seem to be some individual variation. Horses with a significant amount of ingesta may show signs of discomfort when large volumes (5 L) of fluid are administered. Fortunately, this generally resolves with time, walking, or decompression via the nasogastric tube. Abdominal distension can also be seen with high volume enteral fluids but is generally well tolerated by most horses.[6] Many horses treated with enteral fluids for an impaction will develop self-limiting diarrhea due to excretion of fluids as the impaction resolves, and cecal rupture has also been reported.[7]

Administration of enteral fluids has been evaluated in several studies in normal horses. In one study of adult horses, plain water given at once (50 mL/kg/day), twice (100 mL/kg/day), and three times (150 mL/kg/day) maintenance administered via nasogastric tube over four treatment periods every 6 hours has been shown to be safe and effective at restoring intestinal hydration with a volume-dependent effect on fecal volume.[3] In another study, a balanced electrolyte solution given continuously at a rate of 10 L/h via nasogastric tube was shown to be more effective in hydrating ingesta that an identical rate of fluids given intravenously along with one intragastric dose of magnesium sulfate (1 g/kg in 1 L water). There was also a trend of more fecal production in the horses treated with enteral fluids, and there were less systemic effects.[6] This balanced electrolyte solution contained 5.27 g NaCl, 0.37 g KCl, and 3.78 g NaHCO3 per 1 L of water resulting in a solution with 135 mmol Na/L, 5 mmol K/L, 95 mmol Cl/L, and 45 mmol HOC3/L. In normal horses, hydration was measured in the feces and right dorsal colon of horses with indwelling fistulas in a crossover design of six treatments. The balanced electrolyte solution and sodium sulfate resulted in the best hydration of right dorsal colon (RDC) contents, while sodium sulfate, magnesium sulfate, and balanced electrolyte solution resulted in the most hydrated feces. Sodium sulfate caused hypocalcemia and hypernatremia while plain water caused hyponatremia leaving the balanced electrolyte solution as the safest and most effective option.[2]

Enteral fluids have also been shown to be effective in management of clinical cases of nonstrangulating large colon lesions. In a retrospective analysis of 147 horses with large colon impactions where all were treated with IV fluids (8.2–14.3 mL/kg/h) and only 49 (33.3%) received any enteral treatment, the mean time to resolution of impaction was 48 hours with a range from 1 to 6 days. Additionally, 24 horses (16.3%) required surgical intervention.[8] This contrasts with another study of 108 horses with large colon impactions or displacements where horses were given either enteral fluids alone (8–10 L of a potassium-rich isotonic electrolyte solution every 2 hours) or enteral fluids simultaneously with intravenous fluids (2 mL/kg/h lactated Ringer's solution). Both groups had a mean time to resolution of approximately 24 hours for impactions and 14 hours for displacements with no significant electrolyte abnormalities. The overall success rate was 99% for impactions and 83% for displacements.[5] Another retrospective study of 53 horses found that impactions treated with enteral fluids resolved faster, had shorter hospitalizations (4 vs 7 days), and lower mean hospital bills (£483 vs £2006) than horses treated with intravenous fluids.[9] The bulk of the evidence supports treatment of colic with enteral fluids whenever possible.

Enteral fluids can also be used to correct mild electrolyte abnormalities. A potassium-rich balanced electrolyte solution safely corrected mild hypokalemia in horses with large colon impactions and displacements.[5] Administration of 1 g/kg body weight of NaHCO3 has been shown to increase cecal pH, which may be useful in the treatment of horses with grain overload.[10]

3. Cathartics/Laxatives

Despite its routine use, there is little literature to support the use of mineral oil in horses. Mineral oil will lubricate ingesta but does not treat dehydration. It is most useful as a marker of transit time, as it should be seen in the feces of a normal horse 12 to 24 hours after administration. Mineral oil has also been advocated for use as a cathartic in cases of intoxication. Recent work, however, suggests that its use in cases of cantharidin toxicity may be contraindicated. Rats treated with mineral oil had increased absorption of cantharidin with increased morbidity and mortality relative to negative controls or those treated with other adsorbents.[11] Careful attention must also always be paid to placement of the nasogastric tube prior to delivery, as deposition

of mineral oil in the lungs can cause a fatal lipoid pneumonia.[12,13]

Psyllium is a bulk laxative frequently used in the treatment and prevention of sand colic. There is conflicting evidence as to its efficacy, however. Of twelve ponies with sand surgically placed into the cecum, six were treated with 1 g/kg psyllium and six were untreated. All were euthanized 11 days later with no significant difference in the amount of sand retrieved between the treatment and control groups.[14] Another trial evaluating psyllium (0.5 g/kg), wheat bran (1 g/kg), and mineral oil (8 g/kg) also failed to prove increased sand evacuation relative to untreated controls.[15] More recent work showed that horses given psyllium (0.5 kg q12h) in addition to mineral oil (2 L q24h) had increased sand clearance relative to those administered mineral oil alone (51.0% vs 26.1% total sand removal)[16] suggesting that psyllium may improve sand clearance. There is some concern that colonic flora will degrade psyllium after chronic exposure, thus decreasing any laxative properties. This has led to the common practice of only feeding psyllium for 1 week out of every month for sand prevention in endemic areas. The benefit, however, is that when fed routinely (50–100 g daily), fermentation of psyllium by intestinal bacteria results in the production of short-chain fatty acids that may benefit healing of the colonic mucosa in cases of right dorsal colitis.[17]

Magnesium sulfate is frequently dosed at 0.5 to 1.0 g/kg enterally for treatment of impactions. Systemic absorption of MgSO4 is believed to be limited; however, slight increases in plasma magnesium have been noted.[6] Renal excretion should prevent hypermagnesemia; however, magnesium toxicosis has been reported in dehydrated horses.[18] Simultaneous administration of enteral magnesium sulfate with intravenous fluids is thought to promote secretion of fluids into the intestinal lumen by increasing the intraluminal osmolality. However, as previously discussed, there is work to support that a balanced electrolyte solution given enterally is superior to IV fluids plus MgSO4 in promoting hydration of ingesta.[2] Enteral MgSO4 (1 g/kg in 1 L water) is superior to either plain enteral water or IV fluid therapy at 5 L/h for 12 h in improving fecal water content.[6]

Dioctyl sodium sulfosuccinate (DSS) is an anionic surfactant, which decreases surface tension, thus, facilitating water penetration. Historically, it has been used to treat impactions at 4 to 8 oz in 8 L of water; however, it is not superior to the use of magnesium sulfate and water.[19] Toxicity (colic, diarrhea) has also been reported at a dosage of 50 mg/kg, and DSS is, therefore, of questionable use when there are other safer alternatives.

Activated charcoal and/or di-tri-octahedral smectite (DTOS) may be used when the colic is thought to be due to ingestion of a toxin. Di-tri-octahedral smectite is a natural hydrated aluminomagnesium silicate that adsorbs positively charged organic cations in the intestinal tract. It is used to treat and prevent enteritis in a variety of species and is now frequently used for the prevention and treatment of colitis in horses. It was first reported to prevent lincomycin-induced colitis in four horses, speculatively due to absorption of clostridial toxins.[20] It has since been shown that DTOS effectively absorbs *Clostridium difficile* toxins A and B, *C perfringens* enterotoxin and exotoxins, *Bacteroides fragilis* toxin, and gram-negative endotoxin in vitro.[21–23] DTOS does not inhibit the effect of metronidazole, which is fortunate as both drugs may be used simultaneously for treatment of clostridial infections. Horses treated with DTOS after surgery for large intestinal disease had a significant reduction in the prevalence of postoperative diarrhea (10.8%) compared with negative controls (41.4%).[24] In the previously discussed research on cantharidin, rats administered activated charcoal or smectite had survival times similar to the negative controls, suggesting that these treatments may be preferred over mineral oil in cases of blister beetle toxicity.[11] Horses may also be exposed to toxins iatrogenically due to misreading of labels on gallon sized jugs. Propylene glycol has been accidentally administered to horses due to the similarity of the label to mineral oil. Propylene glycol toxicity can result in D-lactic acidosis with severe depression and ataxia or death.[25,26] Isopropyl alcohol has also been accidentally administered resulting in central nervous system depression and colic. Supportive medical therapy, gastric lavage, and repeated administration of activated charcoal is advocated in these cases as significant amounts of isopropyl alcohol and acetone are adsorbed by charcoal in vitro.[27]

Gastric and enteric phytobezoars due to persimmon ingestion may also be treated successfully with medical treatment. Intragastric or oral administration of carbonated cola or diet cola has been reported successfully.[28] The mechanism of dissolution is not well understood but is likely due to a combination of mucolysis, dissolution from carbon dioxide bubbles, and acidification.

4. Pain Management

The signs of pain seen with colic can be due to distension of a viscous, tension of the root of the mesentery, or inflammation. Pain management is an important part of managing colic as pain itself can cause motility inhibition. While many of the drugs used to treat colic may cause transient decreased motility, the beneficial effects typically outweigh those consequences. Multiple analgesic agents are often used for the treatment of colic: non-steroidal anti-inflammatory drugs (NSAIDs), opioids, and α2-adrenergic agonists (Table 1). As distension is a frequent source of pain, nasogastric intubation to relieve gastric distension may also be an important part of pain control. Cecal trocharization may be performed in horses with marked large colon or cecal gas distension when

Table 1. Frequently Used Analgesics and Their Recommended Doses

Drug	Dosage
Nonselective NSAIDs	
Flunixin meglumine	0.25–1.1 mg/kg IV or PO q8–24h
Phenylbutazone	2.2–4.4 mg/kg IV or PO q12–24h
Ketoprofen	2.2 mg/kg IV q24h
COX-2 Preferential NSAIDs	
Meloxicam	0.6 mg/kg IV q12h
Firocoxib	0.27 mg/kg loading dose then 0.09 mg/kg IV q24h
Carprofen	0.7 mg/kg IV, PO q24h
Etodolac	23 mg/kg IV, PO q24h
α2-Adrenergic Agonists	
Xylazine	0.2–1.1 mg/kg IV, IM
Detomidine	10–40 μg/kg IV, IM
Opioids	
Butorphanol	0.02–0.1 mg/kg IV, IM
Other	
N-butylscopolammonium bromide	0.3 mg/kg IV
Lidocaine	1.3 mg/kg over 5–10 minutes, then 0.05 mg/kg/min IV CRI

surgery is not an option; however, there is a risk of causing peritonitis.

NSAIDs inhibit cyclooxygenase, which has two isoforms: COX-1 and COX-2. COX-1 is constitutively expressed and is important for physiologic activities such as renal perfusion and mucosal blood flow; therefore, most of the detrimental side effects of NSAIDs are due to its inhibition. COX-2 is inducible and causes the release of pro-inflammatory prostanoids, making it the suggested preferred target of action to minimize toxic effects. Toxicity is unlikely when NSAIDs are given for short duration at appropriate doses, but repeated administration to dehydrated horses may result in serious side effects, such as acute kidney injury (medullary crest necrosis), right dorsal colitis, and gastric ulceration. Using multiple NSAIDs concurrently is also contraindicated as it increases the risk of toxicity. Flunixin meglumine, phenylbutazone, and ketoprofen are nonselective NSAIDs. Carprofen, meloxicam, firocoxib, and etodolac are preferential COX-2 inhibitors but have limited evaluation for their use in the medical treatment of colic. There is some evidence that they have less effect on mucosal repair and will likely continue to increase in use.

Flunixin meglumine is the most frequently used analgesic in cases of colic. Dosed at 1.1 mg/kg IV, onset of action should occur in approximately 20 minutes and last for 8 to 12 hours. This dose should be repeated no more frequently than every 12 hours. While labeled for use either intravenously or intramuscularly, potentially fatal clostridial myositis has been reported secondary to intramuscular flunixin injection,[29] so IV or oral use is recommended. Flunixin can also be used to treat endotoxemia at a dose of 0.25 mg/kg IV q8h. Phenylbutazone and ketoprofen are used predominantly to treat musculoskeletal pain but can provide fair analgesia for visceral pain.

The COX-2 preferential NSAIDs have been evaluated more extensively for musculoskeletal use and in models of intestinal ischemia, which are beyond the scope of this presentation. However, several studies have included evaluation of their effects on visceral pain. With ischemic-injured jejunum, firocoxib was found to provide equivalent analgesia to flunixin meglumine based on postoperative pain scores.[30] It is worth noting that firocoxib cannot be administered in an aqueous solution, so if given through an intravenous catheter, patient blood should be drawn back into the catheter and extension set first or it should be given via direct venipuncture. Another study evaluating horses after small intestinal surgery found that those treated with meloxicam showed more signs of gross pain than those treated with flunixin suggesting less potential visceral analgesia with meloxicam.[31]

Alpha2-adrenergic agents are frequently used for both their sedative and analgesic properties. Their onset of action is more rapid and analgesia is more potent than NSAIDs. Xylazine has a short duration of action of 20 to 60 minutes. Detomidine is 100 times more potent than xylazine and lasts for 40 to 120 minutes. One multicenter clinical trial found that detomidine at either 20 μg/kg or 40 μg/kg provided better subjective analgesia than flunixin or butorphanol.[32] Side effects of the α2-agonists include transient hypertension followed by hypotension and bradycardia. They also cause transient ileus that lasts longer than the sedative and analgesic effects. Detomidine may mask surgical lesions, so judicious use is recommended.

Butorphanol is the most commonly used opioid and is a mixed agonist-antagonist. It provides adequate analgesia falling between NSAIDs and α2-agonists. While butorphanol is less likely to cause excitement than other opioids, it should be administered concurrently with an α2-adrenergic agonist to minimize those effects. Butorphanol will also cause some ileus, which can be decreased by administering it as a continuous rate infusion (23.7 μg/kg/h). Horses given a 24-hour infusion of butorphanol after colic surgery had a significant reduction in pain scores.[33]

N-butylscopolammonium bromide[a] blocks the muscarinic acetylcholine receptors of the gastrointestinal tract. It is frequently used for its spasmolytic effects in horses with gas colic and impactions. It can also be useful for facilitating rectal examinations. In a duodenal and colorectal distension model, there was no effect on duodenal distension or motility; however, the colorectal distension thresh-

old did increase.[34] Effects last 20 to 30 minutes; however, tachycardia may persist for 50 minutes.

Lidocaine is a local anesthetic that binds to voltage-gated sodium channels, thus, preventing propagation of action potentials. It is frequently used as an intravenous continuous rate infusion in horses for its potential analgesic, anti-inflammatory, and prokinetic effects; however, there is a large amount of conflicting evidence. Lidocaine at 5 mg/kg loading, followed by 100 μg/kg/min CRI was shown to be a visceral analgesic using EEG changes as a measure of nociception in anesthetized ponies undergoing castration.[35] However at a dose of 2 mg/kg loading followed by 50 μg/kg/min CRI, lidocaine had no significant effects on duodenal distension pressures and minimal effect on colorectal distension pressures suggesting minimal visceral analgesia.[36] Lidocaine has also been shown to improve mucosal healing in experimentally induced small intestinal ischemia due to a presumptive decrease in the production of inflammatory cytokines.[37] While lidocaine has not been shown to have direct prokinetic properties, it is used commonly by many practitioners as such for postoperative ileus. In normal horses, however, lidocaine administration has been seen to decrease mean fecal output and increase fecal transit time.[38] Therefore, practitioners using lidocaine for its analgesic effects need to be aware that it may prolong transit time, and this may be exacerbated when combined with other drugs that decrease motility (opioids, alpha-2 agonists). In addition, lidocaine toxicity may occur at serum concentrations between 1.65 and 4.53 μg/mL,[39] and these concentrations can be affected by other drugs, liver disease, and general anesthesia. Signs of toxicity include muscle fasciculations, anxiety, ataxia, collapse, and electrocardiographic changes. Fortunately, the half-life of lidocaine is short, so discontinuing the infusion generally results in resolution of clinical signs.

5. Conclusion

Medical therapy continues to be an appropriate and effective treatment for most cases of colic. It is important to remember, however, that any horse that remains painful despite appropriate medical therapy and analgesia should be referred for possible surgical intervention.

Acknowledgments

Conflict of Interest

The Author declares no conflicts of interest.

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[a]Buscopan®, Boehringer Ingelheim, St. Joseph, MO 64506.

Evidence-Based Hand Hygiene in Equine Practice Where "Clean Hands Save Lives" Becomes "Clean Hands Save Horses"

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Guidelines for hand asepsis have changed. Well-formulated alcoholic rubs have several advantages and are more efficient than traditional medicated soaps. Hand hygiene protocols are crucial in our fight against hospital acquired infections, and acquired resistance to chlorhexidine is an increasing problem. Authors' addresses: Department of Large Animal Sciences, University of Copenhagen, Denmark (Verwilghen, van Galen); Department of Pathobiology, (Weese); and Department of Clinical Studies (Singh), Ontario Veterinary College, Guelph, ON, Canada N1G 2W1; e-mail: denis@equinespecialists.eu. *Corresponding and presenting author. © 2014 AAEP.

1. Introduction

Over the past few decades, the World Health Organisation (WHO) has put increased focus on hand hygiene campaigns under the slogan "Clean Hands–Save Lives" following the global recognition of hand hygiene being a key factor in reducing surgical site infections (SSI) and other hospital associated infections (HAIs). These campaigns have tackled both hand hygiene as part of routine patient care as well as improved pre-surgical hand preparation protocols.

As equine medicine progresses, we must adapt our decision-making in light of new scientific evidence. As much as possible, recommended protocols should now be evidence-based,[1] recognizing that there are varying strengths of evidence and that equine-specific evidence may be limited. Regardless, increasing information from the human field and general understanding of infectious diseases indicate a need for changes in perceptions and practices in diagnostic methods and treatments in equine veterinary medicine,[2] as has occurred (and continues to occur) in human medicine. Despite this paradigm shift, in the field of hand hygiene, things have slowly evolved. Surely, the field has advanced since the 1800s, when Semmelweis and Lister were making seminal (yet controversial) discoveries of the benefits of hand hygiene. They laid the foundations for the concepts of asepsis and vigorously fought the disbelief they were facing, yet while basic concepts of hand hygiene are now widely recognized, parallel actions in veterinary medicine are often lacking. Are we open-heartedly embracing the evidence today?

According to a survey performed in 2009 amongst ECVS and ACVS diplomates, only 6.7% of respon-

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dents indicated that they were following WHO guidelines for pre-surgical hand asepsis.[3] A subsequent survey in 2013 showed that, while practices had improved, 66% of the 218 respondents[4] still did not follow the current WHO guidelines. *So what do current guidelines and evidence say? Additionally, what is right: current guidelines or current practices?*

2. Hand Preparation Today

The goal of surgical hand hygiene is not to sterilize the skin. Rather, it is to reduce or eliminate the burden of potentially pathogenic transient skin organisms and to reduce resident microbiota for the duration of a surgical procedure in order to reduce the risk of an SSI. These goals should be achieved while minimizing damage to the skin that might promote rebound bacterial overgrowth or compromise future hand asepsis attempts. The transient microbiota is acquired by contact with people, animals, and contaminated surfaces. It colonizes the superficial layers of the skin and is the most common cause of SSIs. The resident microbiota is a more established deeper component of the microbiota that is regarded as less pathogenic. Elimination of this component may be undesirable because its presence is a means of defense against the growth of opportunistic transient pathogens. Techniques involving aggressive cleaning of the skin with alkaline medicated soaps, like chlorhexidine gluconate (CHX) scrubs will have deleterious effects on the skin's local defense mechanisms.[5] Moreover, while brushing and scrubbing does not necessarily have any beneficial impact on bacterial reduction,[6] they can cause small excoriations and, therefore, damage to the skin, further increasing the risk of skin colonization by pathogenic species.[7] Despite the suboptimal features of the surgical scrub, 44% of the respondents to a survey of veterinary surgeons stated that they always use brushes. Current recommendations are to use soft sponges, if any, for hands and forearms, only if visibly soiled and brushes for fingertips.[8]

Ideally, the chosen method must balance antimicrobial activity, skin compatibility and ease of use to be optimally effective and used. A variety of methods and products for surgical hand preparation are available. Aqueous solutions containing either povidone-iodine (PVP) or CHX have been widely used for many decades. Alcohol-based hand rubs (AHRs) have been described for surgical hand preparation for more than a century,[9] although only recently have they become widely recommended for surgical hand preparation. Although no randomized controlled trials have been conducted showing any significant differences in SSI rates between the aforementioned methods, the AHR method is considered superior for a number of reasons.[10,11] The antibacterial efficacy of products containing high concentrations of alcohol was shown to significantly surpass that of any medicated soap currently available.[12] The initial reduction of the resident skin flora (microbiota) is so rapid and effective with AHRs that bacterial regrowth to baseline values on the gloved hand takes more than 6 hours.[13] These observations were also confirmed in a veterinary trial that compared the activity of an AHR solution to CHX and PVP soap.[14] In that study, a 1.5 min application of an AHR solution was performed with the 3 h residual effect on colony forming units (CFU) reduction of AHRs significantly better than for traditional hand scrubbing with PVP and CHX. Another potential benefit of AHRs is the lack of any known (or plausible) mechanism of alcohol resistance in bacteria. Therefore, there is no concern that repeated alcohol application could select for alcohol-resistance or co-select for resistance to antimicrobials. It should be noted that when choosing an AHR solution, the product should meet the EN12791 or equivalent standard required for pre-surgical hand-rub formulations.[15] Many available gel products will not meet these surgical standards and are therefore not recommended.

About 17% of respondents of one survey[4] used a medicated scrub technique combined with an AHR, in clear contradiction to published evidence and recommendations that do not advocate the use of both methods together. Medicated soaps are either less effective or have a similar efficacy to the hand rub. An initial scrub with medicated soaps will not result in ultimately increased reduction of microbiota after AHR application.[14] On the contrary, long-term use of medicated soaps increases the risk of dermatitis, making the skin more difficult to decontaminate and the combination of medicated soaps and AHR likely to increase the risk of skin damage, while providing no demonstrable positive effect. Furthermore, prior hand washing can alter the effectiveness of AHR solutions,[16] particularly if hands are not completely dried before AHR application. Hand washing also increases preparation time, cost, carbon footprint, and water usage.[17,18] It is estimated that 20 L of water is used per hand when preparing with medicated soaps. Faucets and basins can also be sources of *Pseudomonas* spp. and other Gram-negative bacteria, creating a potential for recontamination of the hands prior to gloving.[8] Considering the aforementioned concerns, it has been suggested in human medicine there is no reason to include a hand wash before AHR solutions are applied[16,19] and that hands should only be washed if they are visibly soiled. However, compared with human hospitals, the bacterial burden in veterinary settings may be higher, especially for equine surgeons. Until further objective data are available, it is reasonable to recommend a short hand-wash with a gentle soap (*p*H neutral) prior to the AHR application when the surgeon has handled animals or their environments. In this way, bacterial spores and organic material that might interfere with alcohol antisepsis can be eliminated.

3. Resistance

A new and more worrying aspect supporting the reduced use of medicated soaps is the emergence of increased acquired resistance towards antiseptics.[20,21] In particular, the prevalence of *Staphylococcus aureus* carrying the chlorhexidine resistance gene *qac*A/B has been shown to increase in the hospital environment.[22] This is a concern not just because of resistance to chlorhexidine, but also because chlorhexidine resistance can potentially coselect for resistance to clinically-relevant antibiotics. The overuse of CHX scrubbing soaps, which results in the spreading of large quantities of diluted active substance that is more likely to be bacteriostatic than bactericidal, could be a contributing factor in antibiotic resistance among pathogens on the hands or in the environment. Due to the fast killing activity of alcohol and the lack of any known (or plausible) genetic mechanisms that would allow for transmission of inherent alcohol resistance, acquired resistance to AHRs has not been shown to date, nor is it likely to be encountered, providing another major reason to move towards these products.

4. Outside Surgery

Outside the closed environment of the surgery theater, the hands of healthcare workers are thought to play a crucial role in the occurrence of SSI and other HAIs. Hand hygiene is therefore regarded as one of the most effective measures to prevent HAIs. Several reports have shown a temporal association between interventions to improve hand hygiene measures or compliance rates and reduced infection rates. A recent British report evaluating the "Clean your hands" campaign showed that in the 4-year study period, the AHR use per bed day raised more than a twofold simultaneously with a twofold decrease in MRSA (methicillin resistant *Staphylococcus aureus*) bacteremia and *Clostridium difficile* infections,[23] strongly emphasizing the importance of these measures.

While studies on the impact of hand hygiene on HAIs are often done as part of multi-modal interventions and the specific role of hand hygiene improvements can be difficult to discern, it is widely accepted that hand hygiene compliance is a key component of infection prevention and an indicator of patient care. While there are clear differences between equine and human healthcare, there is no reason to think that the impact of hand hygiene in equine medicine and surgery would be any less than in human medicine. This, combined with evidence of deficiencies in hand hygiene in equine medicine, indicates a need for improvement. Compliance with hand hygiene measures should be improved. The weakest link is typically compliance by clinicians, as the practices of nursing staff are often superior.[24,25] Increasing the ease of performing hand hygiene is likely a critical factor and the introduction of AHRs and improving the accessibility of materials has been an important factor to increase hand hygiene compliance rates.[26] New technologies are underway to electronically monitor the compliance in human healthcare, with financial incentives for health care staff. Ultimately, however, increased awareness and personal responsibility by all personnel is critical for improving hand hygiene rates.

5. Skin Health

It is clear that the veterinary occupation also has a real impact on the health of our skin. Not only are human medical staff known to acquire more a pathogenic resident microbiota over time,[27] a high proportion of healthcare workers develop occupational dermatitis, mostly related to repeated washing with chlorhexidine soaps.[28,29] However, one of the most important determinants of healthcare workers' resistance to change to AHRs is the unfounded belief that AHRs are more harmful to their skin than soap and water.[30,31] In reality, each hand wash detrimentally alters the lipid layer of the superficial skin, creating the loss of protective agents such as amino acids and natural antimicrobial factors. Prolonged and repeated washing leads to a damaged barrier function of the *stratum corneum,* resulting in the skin becoming more permeable for toxic agents and bacteria. On the contrary, AHR solutions have been shown to have less detrimental effects on skin than soaps in both human healthcare[32] and veterinary settings.[10] This corroborates the results from the aforementioned survey[4] that identified surgeons using antimicrobial soaps as pre-surgical hand preparation methods as having lower overall hand health scores compared to those using AHRs. This was particularly true for skin moisture content, with surgeons using antimicrobial soaps reporting significantly dryer hands than those using AHR solutions (Table 1). This is mainly due to the absence of the aforementioned deleterious effects of soap washing combined with the fact that AHR products contain humectants and need shorter application times.[30] Furthermore, although lipid layers are also dissolved with AHRs, they are not washed away but rather reincorporated by the action of rubbing and emollients that are included in virtually all AHR products can facilitate restoration of a normal barrier. As mentioned before, unnecessary washing should be avoided, particularly with hot water.[28]

6. Compliance Issues

In our latest survey,[4] 89 respondents stated they believe AHRs are superior to traditional hand scrubbing techniques for obtaining hand asepsis. Nevertheless, over 42% of these respondents report the use of antimicrobial soaps in their protocol, clearly indicating a lack of compliance with their own beliefs. Reasons for this have not been specifically evaluated, but it may be rooted in the fact that many

Table 1. Hand Health Scores From 2013 Surgical Preparation Survey[4]

	Protocol	Appearance	Intactness	Moisture Content	Sensation	Overall Score
A	Antimicrobial scrub (n = 91)	7	6	5	7	6.25
B	Antimicrobial scrub followed with AHR (n = 45)	7	6[a]	5	7	5.75
C	Neutral soap followed by AHR (n = 35)	7	6	6[b]	7	6.75[c]
D	AHR only (n = 15)	7	7	7[c]	7	6.75[c]
E	Alcohol chlorhexidine combination (n = 24)	7	6	5	7	6

Based on "hand and skin self-assessment tool" WHO guidelines Appendix 3.[8] Seven point based scale with 1 being abnormal and 7 normal. The overall score is based on the mean of the 4 individual assessments. Scores are reported as medians.

[a]Intactness: significantly different from C and D.

[b]Moisture content: C and D significantly different from A, B, and E but not from each other.

[c]Overall scores: significantly different from A, B, and E

surgeons consider the act of scrubbing as an "ancestral ritual" towards preparation to surgery, something that may prevent them from shifting to more efficient protocols.[25] In addition, this may highlight the fact that involvement of the hospital management in setting up changes in hand hygiene policies is essential.[33] The use of campaigns and internal training sessions has been shown to be of great benefit[24,25] in increasing hand hygiene compliance amongst staff in human medicine. Similar initiatives should be made in veterinary medicine in order to obtain better compliance with the application of current knowledge on hand hygiene. Considering the application technique of AHRs is important and the use of hand scrubs is probably less prone to errors as all parts of the hands and arms get wet under the tap, good education is mandatory when introducing AHRs.

7. Conclusions

There have been significant changes made in the basic science of hand hygiene in the past decades, though the veterinary community does not yet seem to have embraced the changes.

In order to comply with current medical standards, the veterinary community should aim at introducing AHR solutions in both the pre-surgical and hygienic surgical hand disinfection protocols. The WHO statement "Clean Hands–Save Lives," in an AAEP format: "Clean Hands–Save Horses!"

It is in our hands!

Acknowledgments

Conflict of Interest

The Authors declare no conflicts of interest.

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How to Prepare a Biosecurity Program for an Equine Veterinary Hospital

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1. Introduction

Biosecurity is essential for veterinary practices and maintaining trust and clientele. Whenever horses are transported and comingled with new animals, treated with antibiotics, eating an altered diet, or undergoing other stressors, there is a chance for a perfect storm where there is possible shedding and increased susceptibility to a contagious infectious agent. There is always a risk of acquiring an infectious disease in an equine hospital, whether it is *Salmonella spp*., an infection at the intravenous catheter site with a multidrug resistant (MDR) bacteria, or influenza. A biosecurity program needs to be in place to reduce these risks and is becoming a necessary part of standard-of-care expectations of our equine clients. The goals of a biosecurity program at a veterinary practice are to reduce the risk of nosocomial infections in hospitalized horses and to protect human health.[1–4]

2. Materials and Methods

The development of a biosecurity program begins with someone who is in charge and accountable. In most veterinary practices it would be best to have a veterinarian and a technician involved in the oversight of a biosecurity program. However, the entire practice needs to be engaged in proper biosecurity practices, as the program is only as good as the weakest link. *If the veterinarians are practicing good biosecurity and lead by example, then the rest of the staff will follow suit.* The training of personnel and keeping everyone informed of the protocols is a continuous activity. Preparing written biosecurity protocols using everyone's input helps educate and inform the practice as a whole and is a very healthy process for the group. Once the documents are finalized, they should be easily accessible to everyone and referenced when needed. Having these documents in an easy-to-read format and providing it to visitors, such as visiting student externs before they arrive, sets the stage for a practice that cares about biosecurity and helps protect your clientele.

Formalizing a biosecurity program begins with putting protocols into place, then monitoring patients and assessing for problems, and training personnel. Continuous updating of the program is essential to its success.

Assessment and Critical Evaluation of Existing Protocols and Structure

If you are designing a new facility or wanting to formalize an infectious disease program at your

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practice, these are the broad topics that you will want to investigate.

- Facility layout
- Traffic (people, horses, dogs, cleaning equipment, vehicles)
- Horse stabling
- Stall sanitation
- Manure, soiled bedding, and hay disposal
- Medical equipment sanitation
- Operating room sanitation
- Medical waste disposal
- Water source and disposal
- Feed and hay storage
- Vector and wildlife control (to include rodents, mosquitoes, ticks, and flies)
- Existing written infection control policies

An assessment of existing work-flow patterns and biosecurity protocols is a good place to start. Knowing your limitations will help you make critical decisions that will help protect your patients. For instance, deciding whether your clinic should admit a 'fever of unknown origin' patient. This initial assessment allows for a list of actionable items, interventions, and a long-term plan for improvements to your overall program.

This presentation will focus on general hygiene and effectively isolating patients to contain any infectious diseases.

General hygiene and cleaning and disinfection of the hospital and medical equipment is paramount to cleanliness and reducing pathogens in the environment. Some guidelines for cleaning the facilities and medical equipment are briefly summarized.

Stalls, Treatment Rooms, Animal Facilities

Remove all equipment, bedding, and organic material; then use a scrub brush to clean with soap and water getting into all corners and crevices, rinse with water, and then apply a disinfectant mix (Fig. 1). The disinfectant must be mixed as the label describes and appropriate protective clothing worn by the staff. The disinfectant should be allowed the amount of contact time specified on the label and then rinsed and the area allowed to dry. The disinfectant used in a hospital depends on safety, historical problems, cost, wear-and-tear on the facilities.[a] For some pathogens, such as *Cryptosporidium sp.*, *Clostridium difficile*, and Rotavirus that are hardest to eliminate from the environment, a specific protocol may be set up for cleaning stalls and the surrounding area after the affected patient leaves. For example, 1:10 bleach solution is commonly used for eliminating *Clostridium difficile* spores. Rotavirus, a nonenveloped virus, requires an accelerated hydrogen peroxide, oxidizing agent, or phenolic compound.

Fig. 1. The Cornell University Barn Crew are cleaning and disinfecting a stall in the main hospital (noninfectious). Removal of all organic material, scrubbing the surfaces with soap and water, and then applying a disinfectant as described on the product label are essential. The foamer used here allows for good dispersal of the disinfectant on all surfaces. When cleaning and disinfecting a stall that housed an animal with an infectious disease, appropriate personal protective equipment (PPE; full body suit, gloves, and overboots) should be worn.

Medical Equipment Such as Ultrasound and Radiological Equipment

This equipment should be spot cleaned after each use with a disinfectant wipe (Fig. 2). A regular schedule (every week, two-weeks, monthly) should be adhered to for a more aggressive cleaning of the entire piece of equipment and the cart upon which it is housed. In order to keep up with the regularly scheduled full-cleaning, have a sign-up sheet on the equipment with the date and signature of the person performing the cleaning.

Other Equipment

There are many items such as twitches, nasogastric tubes, brushes, and much more that come into contact with individual animals and should be cleaned between animals. If possible these items could be assigned for use in one particular patient and then cleaned after the patient is discharged.

Hand Hygiene

Hand hygiene is a very important part of a biosecurity program and cannot be stressed enough. Hands should be washed before and after handling each patient. Similar to human healthcare workers, one should wash hands when entering an examination room in front of clients. This sets a good example and shows that your practice cares about hygiene. Hands should be rinsed with water, soap applied, and then washed for about 20 s (sing "Happy Birthday"). All hand surfaces should be washed covering the top, bottom, between each fin-

Fig. 2. Medical equipment should be cleaned after each use and between patients. Disinfectant wipes are convenient and can be kept on the equipment cart.

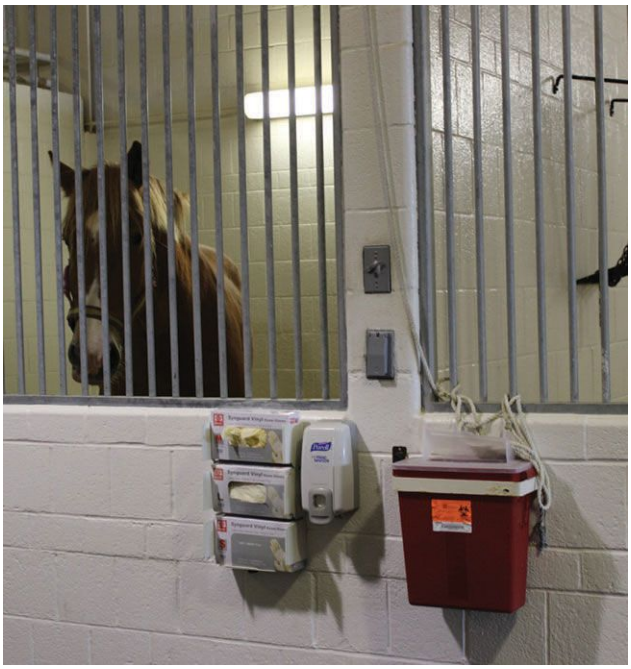

Fig. 3. Hand sanitizers and examination gloves should be very accessible in barn and treatment areas where sinks are not available.

ger, and sides. Following which, hands should be completely dried. Washing hands has the advantage of removing dirt and debris, as well as "rinsing pathogens" down the sink. Hand sanitizers are a good alternative in areas where a sink is not available, but do not replace the physical washing of the hands. In addition, most hand sanitizers consist of alcohol, which is not effective against *Cryptosporidium spp.* or *Clostridial* spores. Wearing examination gloves for certain situations such as changing bandages, assessing incisions and wounds, evaluating catheters (IV, urinary, etc.), treating eyes, when handling horses with infectious diseases, and when handling critical and immune suppressed patients can prevent personnel from infecting patients and vice versa. The examination gloves should be worn when handling the patient or the affected site and then gloves removed and hands washed and/or sanitized. Sometimes people become nonchalant when wearing examination gloves and contact other surfaces, for example, they evaluate the abdominal incision of a post-operative colic and then pick up their cell phone wearing the same gloves. One should always be cognizant of their actions and potential for contamination of other equipment surfaces and then indirect transmission to other patients. In order for staff to maintain good hand hygiene, sinks and hand sanitizers need to be easily accessible, stocked with soap and hand towels, and clean (Fig. 3).

Cleaning Personal Items

Personal items such as cell phones, stethoscopes, and pen-lights is dependent on the individual and is often overlooked. In surveys of hospitals for various pathogenic bacteria, cell phones have been positive in many instances.[5] Disinfectant wipes are convenient and can be used to clean cell phones and other items. This should be done on a daily basis. The cell phone, in particular, goes from patient to patient and from hand to mouth and is extremely important to clean. Reminding staff to do so, or having daily sessions where everyone cleans together, can improve hygiene.

Eating and Drinking in the Hospital and Stable Areas

The fast pace of veterinary practice leads to eating lunch on the run. In general, a veterinarian and their staff should not be eating or drinking while handling a patient, especially in front of clients. This portrays a lack of professionalism, cleanliness, and disregard for personal health and the health of the animal. Eating and drinking should be limited to the office areas and a designated break room. In addition, hands should be washed before and

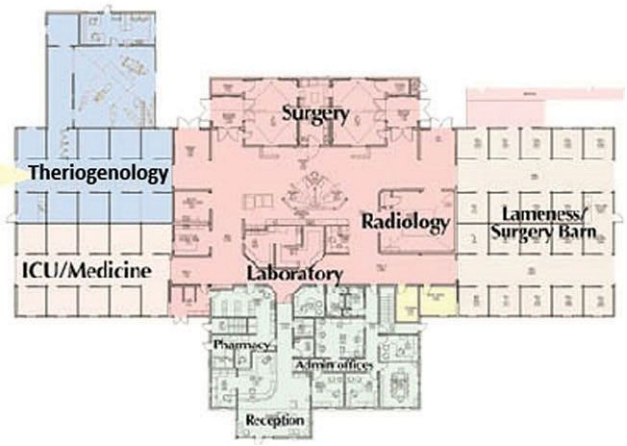

Fig. 4. Housing patients in low- and high-risk zones for infectious disease reduces the risk of making a healthy horse sick. This hospital floor plan has colics and foals admitted into the medicine ward away from the outpatients, as well as lameness and surgical patients.

after eating. For compliance, a convenient location for eating and drinking needs to be provided to the employees.

Grouping of Patients Based on Risk

The housing and management of patients should be from low risk of shedding a contagious disease to high risk, with the least traffic taking place through the high risk patient areas. The order from least to highest risk is outpatients < elective surgical patients < colics and foals < infectious disease patients and should be housed separately (Fig. 4). For example, outpatient lameness and reproductive evaluations should be evaluated and treated away from the inpatient colics and neonatal foals. The elective surgical patients should also be housed separately—these patients are generally healthy but when undergoing surgery are often treated with perioperative antimicrobials, have undergone anaesthesia, have been held off feed, etc., putting them at risk. The colics should be kept separate from other populations of animals because they are at highest risk of shedding *Salmonella spp*. A separate area for foals is ideal to protect them because they are highly susceptible and also because they often present with diarrhea and are an infectious disease risk. It is ideal to also group personnel to work in each separate area on a given day. Ideally, there is a separate isolation facility for managing infectious or suspect infectious disease cases. Alternatively, a semi-isolation situation can be implemented in the hospital.

Preparing for and Handling the Contagious Infectious Disease Patients

Monitoring Patients for Infectious Diseases on Admission

In some instances, where a particular infectious disease is high in prevalence in the area, a veterinary hospital may decide to monitor for that infectious disease in all horses on arrival. For instance, the University of Guelph cultured nasal swabs of horses on arrival and throughout hospitalization for methicillin-resistant *Staphylococcus aureus* (MRSA).[6] This allowed them to take action and isolate any positive patients and, hopefully, reduce in-hospital transmission of MRSA. Many veterinary teaching hospitals perform fecal *Salmonella spp.* cultures when admitting patients.[4,7] The cost-benefit of such an approach needs to be considered and the time it takes for the diagnostic test results to be reported often limits the usefulness of this type of screening.

Contagious Infectious Diseases of Concern to Equine Veterinary Hospitals

Determining what infectious agents are likely to represent a risk to your hospital is important. Table 1 includes a list of agents that are communicable from horse to horse and are likely to result in nosocomial infections and impact the hospital's ability to

Table 1. Infectious Diseases of Most Concern for Horse-to-Horse Transmission in a Veterinary Hospital

Respiratory (aerosol, direct contact, indirect contact)	Gastrointestinal (fecal-oral, indirect contact)	Neurological	Surgical site, wound and catheter infections (direct, indirect contact)
Influenza	*Salmonella spp.*	EHV-1 (EHM)	MRSA[b]
EHV-1 and EHV-4[a]	*Clostridium difficile*		*Resistant Enterococcus spp.*
Rhinitis A&B virus[c]	Coronavirus		Other MDR bacteria
Strangles	Rotavirus		
EVA[a]	*Cryptosporidium sp.*		

Note: EHV-1 = equine herpes virus-1; EVA = equine viral arteritis; EHM = equine herpes myeloencephalopathy; MRSA = methicillin resistant *Staphylococcus aureus*; MDR = multidrug resistant.

[a]EHV-1 and EVA transmission is through respiratory secretions and direct nose-to-nose contact or exposure to an aborted fetus and placenta.

[b]Horses can be nasally colonized with MRSA and their stall can be transiently infected.[5]

[c]Rhinitis virus has been found in horse urine 37 days post-infection.[8]

admit patients. These agents have been listed by body system, which also represents their major mode of transmission, with most hospitals being concerned with fecal-oral, aerosolization, and direct and indirect contact transmission through fomites. Interfering in critical steps in the transmission cycle will help reduce disease in other hospitalized horses. For example, foot baths could be helpful in preventing spread of fecal pathogens but is limited in efficacy against respiratory pathogens.

Identifying Patients With Infectious Diseases Prior to Admission

Once your hospital has identified pathogenic agents of risk, the next step is identifying clinical signs that would alert you and your front office staff to take special precautions to protect the health of others in the hospital. The front office staff, technicians, and interns should be coached to have the clients or referring DVMs speak directly with the Biosecurity Officer prior to shipment of any horse with a possible contagious disease. Fever, diarrhea, nasal discharge, and multiple animals affected on a farm are syndromic signs that alert the clinicians of a possible infectious disease. Time and time again, some key element is lost in the history (no matter how thorough) prior to admission that later reveals itself during the initial diagnostic evaluation; therefore, err on the side of caution and isolate the patient until more information is gathered. Sometimes an initial assessment can be made in the horse trailer—taking the temperature, palpating mandibular lymph nodes, etc. and determining if the patient needs to be isolated or not. In addition, the horse could be worked up in a more separate area of the hospital and then it can be decided whether it requires isolation or not. This happens at Cornell University, not uncommonly with horses with colic that end up having a fever and leukopenia, where other reasons for colic are excluded and colitis becomes the primary differential. The horse then is admitted to the isolation unit and the colic evaluation area is closed and rigorously cleaned and disinfected.

Criteria for Placing a Horse in Isolation

Criteria should be set for placing a horse in isolation. Fever of unknown origin, leukopenia, diarrhea, respiratory signs, lymphadenopathy, multiple animals on a farm affected, and any acute onset ataxia with difficulty urinating and defecating could be clinical signs that warrant placement of a horse in isolation. The hospital should also have a protocol to handle patients with nonhealing wounds and possible MDR infections. Methicillin-resistant *Staphylococcus aureus* patients should also have special consideration because nasal colonization can allow for dispersal of the pathogen and lead to a heavily contaminated stall and surrounding area during hospitalization. Therefore, when entering the stall and handling the horse the veterinarian's and staff's clothing may become contaminated and allow for fomite transmission.[5] Fortunately, MRSA does not persist in the environment and is not a long-term inhabitant of hospital surfaces, while other MDR pathogens such as *Enterococcus spp*. can survive for long periods in a hospital environment and cause nosocomial infections. Often, providing barrier precautions and semi-isolation within the hospital for these patients and bandaging the wounds can be sufficient to prevent high levels of environmental contamination. However, there are always scenarios that seem somewhat blurred and the person in charge of biosecurity should be contacted at any time of day to discuss the patient history, the current status of the hospital, and make a decision on where that particular horse should be housed. The convenience factor often comes into play and, generally, people regard an isolation situation as more work. Therefore, the biosecurity person in charge needs to hold their ground and handle push-back from colleagues in order to keep the hospital safe. Placing an animal in isolation versus doing clean-up of the entire hospital once an animal is diagnosed with something infectious is much easier for everyone in the long run. The team becomes much more vigilant when they have gone through an infectious disease scare together.

Setting Up an Isolation Facility: Separate Facility or Semi-Isolation Within the Hospital

Separate Isolation Facility

Having an isolation facility as a stand-alone building from the rest of the hospital has many advantages; however, this is somewhat costly. In general, the building should be separate but still easily accessible. The isolation area should have designated equipment and supplies. If planning to handle diseases spread by respiratory transmission, separate ventilation for each stall should be provided. An anteroom with space that provides the ability to dress in appropriate personal protective equipment (PPE) and prepare the necessary medications and supplies before entering the stall is a key element to an isolation unit. Since a separate isolation facility may not be cost effective for smaller haul-in equine facilities, this presentation will focus more on setting up perimeters to do semi-isolation within the hospital.

How to Set Up Semi-Isolation Within a Hospital

When a separate isolation facility is not available, animals can be housed in a hospital using a perimeter and barrier precautions upon entry into the area. This is not advised for respiratory viruses that can be aerosolized throughout a barn such as influenza and equine herpesvirus. In fact, nosocomial infections of equine herpesvirus-1 have been reported on numerous occasions when in-hospital semi-isolation attempts were made.[8,9] Often the shared airspace is blamed, however, breaches in biosecurity protocols should not be overlooked and when the isolation is being done in the hospital it is

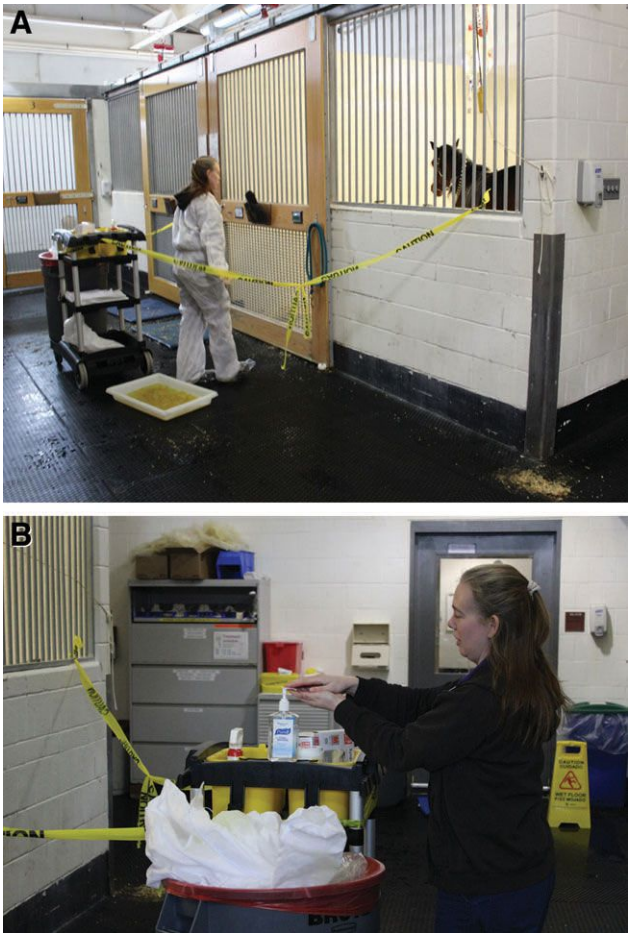

Fig. 5. A, Semi-isolation within the hospital involves an obvious perimeter (yellow caution tape) with a footbath or mat at the entry, a cart that holds PPE (in this instance, full body covers, plastic disposable boots, and examination gloves), and biohazard waste disposal. B, When exiting the stall, the PPE should be removed and placed in the medical waste container and hand sanitizer applied.

more likely that these breaches impact other hospitalized patients.

The stall utilized for hospital semi-isolation would be best if it were at the end of a barn or away from major human and animal traffic in order to minimize exposure and perhaps a stall next-to and/or across from the proposed semi-isolation stall can remain vacant to allow for a "buffer zone." The further away the suspect infectious case is away from the rest of your hospitalized patients, the better. A perimeter can be set up around the stall using ropes, caution tape, or small construction fencing. In order to enter the area one needs to don personal protective equipment (PPE) and step through a footbath or footmat. The PPE can be on a cart that bridges the perimeter (Fig. 5A). Since most pathogens are either fecal-oral or respiratory transmission, PPE should consist of a covering for the body (for example coveralls, Tyvek type suit), in addition to foot covers and gloves.

Personal Protective Equipment (PPE)

In general, the PPE should be one-time use items that are disposable. There is no way to get into a coverall that has been worn into a stall and is contaminated on the outside without contaminating oneself. Gowns where a person's limbs are not covered are not as ideal as a full-body suit, especially for management of neonates where you are often straddling the foal to restrain or perform a procedure. A full-body suit is advised and one that is the least permeable to water. Cost can be a big consideration when deciding what type of PPE since these suits can be costly and the number of suits used per day can be very high in critical care patients. In general, it is best to pick one protocol for the PPE and use that in all infectious disease patients—otherwise, the staff becomes confused as to what to use and when.

The perimeter line should be crossed only once the PPE is on, similar to entering and exiting a surgical suite. A footbath may also be placed at this site. All personnel entering the site should follow these guidelines from clinicians to clients to stable hands. Upon exiting the site, the PPE is removed and placed in the medical waste basket that is also conveniently located. Hand sanitizers should be used and then the personnel should immediately go and wash their hands (Fig. 5B). The staff should be organized with their physical examinations and treatments, making every attempt to limit the number of times they enter the stall. The medical record should remain outside of the stall. The stall should have a designated stethoscope and thermometer in addition to cleaning equipment. Any items necessary for treatments should be taken into the stall each time. Any hospital equipment, such as the ultrasound, must be cleaned after use in the isolated area, taking care to disinfect the wheels (even roll through the footmat, if possible, or spray the wheels with a disinfectant). In addition, the patient must remain in the stall at all times and not be walked throughout the hospital. Once an animal is discharged, the area should remain isolated until all of the items are picked up and the stall is cleaned and disinfected. If the patient is positive for an infectious disease, for example, *Salmonella spp*., environmental cultures of the stall, surrounding area, and areas of high human traffic after the cleaning and disinfection could be performed prior to opening this area to new patients.

Perform Diagnostic Testing on Infectious Disease Patients

A definitive diagnosis is imperative when dealing with infectious diseases and essential to a successful biosecurity program. It is always best to be "in the know" when it comes to managing infectious diseases in an equine hospital, so that you can disrupt the transmission cycle. Developing a good rapport with your veterinary diagnostic laboratory in order to expedite samples in urgent situations is also of

major importance. While many diseases are handled with the same good biosecurity practices, there are specifics of each infectious disease and durations of quarantine, etc. that can help in the overall management.

Active Surveillance and Monitoring

The biosecurity officer should be proactively monitoring for adverse events such as surgical site infections or diarrhea in post-operative patients and decide when a problem is occurring. As a problem arises, the biosecurity officer and staff may reinvestigate existing protocols, evaluate how patients are prepared for surgery, antimicrobial drug use, etc. Once a limitation of the program is determined, new strategies that could break the transmission cycle and limit disease in future equine patients could be implemented.

3. Results and Conclusions

Biosecurity and infection control are essential for equine hospitals where horses with enhanced susceptibility to infection are concentrated in a small area, thus increasing the risk of transmission of pathogens from one horse to another. Nosocomial infections are an inherent risk of hospitalization and attempts should be made to limit their occurrence through a well formulated biosecurity program. Prevention is far better than costly treatments, and sometimes, poor outcomes, when it comes to nosocomial infections.

Acknowledgments

Conflict of Interest

The Authors declare no conflicts of interest.

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For more information on disinfectants go to http://www.cfsph.iastate.edu/pdf/antimicrobial-spectrum-of-disinfectants and http://www.cfsph.iastate.edu/Disinfection/Assets/CharacteristicsSelectedDisinfectants.pdf.

Frequency of *Corynebacterium pseudotuberculosis* Infection in Horses Across the United States During a 10-Year Period

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Corynebacterium pseudotuberculosis infection in horses is increasing and occurs throughout the United States. Further studies to determine changes in annual incidence and to identify potential changing climatic conditions or vector populations associated with transmission of infection are warranted. Authors' addresses: The Department of Medicine and Epidemiology (Spier), the William R. Pritchard Veterinary Medical Teaching Hospital (Kilcoyne), University of California, Davis, CA 95616; the Veterinary Diagnostic Laboratory, University of Kentucky, Lexington, KY 40511 (Carter, Smith); and the Department of Veterinary Pathobiology (Swinford) and the Equine Infectious Disease Laboratory, Department of Large Animal Clinical Sciences (Cohen), College of Veterinary Medicine and Biomedical Sciences, Texas A&M University, College Station, TX 77843; e-mail: sjspier@ucdavis.edu. *Corresponding and presenting author. © 2014 AAEP.

1. Introduction

Corynebacterium pseudotuberculosis causes various clinical forms of infection, including external abscesses in the pectoral or ventral abdominal area, internal abscessation, or infection of the limbs. The purpose of this study was to examine the occurrence of *C. pseudotuberculosis* infection throughout the United States over a 10-year period.

2. Materials and Methods

State veterinary diagnostic laboratories in the United States were solicited to participate in a cross-sectional study. Data from 2237 horses culture-positive for *C. pseudotuberculosis* identified by 15 veterinary diagnostic laboratories were collected. Data requested included number of cases of *C. pseudotuberculosis* infection in horses seen per year, geographical regions from which the sample was obtained, month and year of sample submission, breed and age of horses, and category of clinical presentation (i.e., internal infection, external infection, or ulcerative lymphangitis).

3. Results and Discussion

Fifteen laboratories provided data from 23 states. The proportion of cases during 2011 and 2012 (54%; 1213/2237) was significantly ($P < 0.0001$) greater than that for period from 2003 to 2010

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(46%; 1024/2237). *C. pseudotuberculosis* was recovered from horses in states where disease has not been previously recognized as endemic. Horses were identified year-round. No significant association between the clinical form of disease and age or breed was observed.

Acknowledgments

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Conflict of Interest

The Authors would like to thank Zoetis for funding and declare no conflicts of interest.

Minimum Inhibitory Concentration Determination of Equine *Corynebacterium Pseudotuberculosis* Isolates from 1996 to 2012

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Equine *Corynebacterium pseudotuberculosis* has not developed appreciable changes to minimum inhibitory concentrations (MIC) patterns over time. Authors' addresses: William R. Pritchard Veterinary Medical Teaching Hospital, (Rhodes); Department of Medicine and Epidemiology, (Magdesian, Spier, Edman); Department of Clinical Pathology, Microbiology and Immunology, (Byrne); and Department of Population Health and Reproduction (Kass), School of Veterinary Medicine, University of California, Davis, One Shields Ave., Davis, CA 95616; e-mail: sjspier@ucdavis.edu. *Corresponding author; †Presenting author. © 2014 AAEP.

1. Introduction

Few reports exist regarding the antimicrobial susceptibility patterns of *C. pseudotuberculosis*. The purpose of this study was to evaluate for temporal trends in the in vitro antimicrobial activities of 23 antimicrobials against equine *C. pseudotuberculosis* isolates. A secondary aim was to determine if a relationship exists between MIC and abscess location.

2. Materials and Methods

The study was retrospective and cross-sectional. Medical records were reviewed to obtain clinical and MIC data. Two hundred and seven *C. pseudotuberculosis* isolates from 196 horses were included. Minimum inhibitory concentrations were determined by the microdilution technique for isolates with missing MIC data. MIC values over three periods were compared (1996–2001, 2002–2006, 2007–2012).

3. Results

There were 140 horses with external abscesses, 20 with internal abscesses, 23 horses with both external and internal abscesses, and 4 with ulcerative lymphangitis. Commonly used antimicrobials with MIC_{50} and MIC_{90} values below the susceptibility break points used in this study included the aminoglycosides, doxycycline, erythromycin, azithromycin, chloramphenicol, enrofloxacin, rifampin, tetracycline, and trimethoprim-sulfamethoxazole. Ceftiofur, clarithromycin, ampicillin, and penicillin have variable break points depending on the micro-

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organism used, complicating interpretation. There were no significant changes in MIC values over the study period, and there was no relationship between MIC patterns and abscess location.

4. Discussion

C. pseudotuberculosis shows susceptibility to many antimicrobials and has not developed resistance over time; however, it is important to consider pharmacokinetics and pharmacodynamic properties of antimicrobials when formulating a treatment plan.

Acknowledgments

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Conflict of Interest

The Authors declare no conflicts of interest.

Voluntary Surveillance Program for Equine Influenza Virus in the United States From 2010 to 2013

Nicola Pusterla, DVM, PhD, DACVIM*; Philip Kass, DVM, MPVM, MS, PhD; Samantha Mapes, MS; Cara Wademann; Nina Akana; Craig Barnett, DVM; Cynthia MacKenzie, DVM; and Wendy Vaala, DVM, DACVIM

This study provides valuable and contemporary information on the frequency of equine influenza virus (EIV) detected by real-time polymerase chain reaction (qPCR) in the U.S. The results also point to the fact that older and previously vaccinated horses were susceptible to EIV. Author's addresses: Department of Medicine and Epidemiology (Pusterla, Mapes, Wademan, Akana) and Department of Population Health and Reproduction (Kass), School of Veterinary Medicine, University of California, Davis, CA 95616; and Merck Animal Health (Barnett, MacKenzie, Vaala), Summit, NJ 07901; email: npusterla@ucdavis.edu. *Corresponding and presenting author. © 2014 AAEP.

1. Introduction

Recent surveillance studies for equine respiratory viruses have shown that EIV continues to be a prevalent viral respiratory pathogen of horses throughout the U.S. and Europe. Due to the highly contagious nature and rapid spread of EIV, this virus has severe financial implications for the horse industry. The objective of this study was to gain a better understanding of the prevalence and epidemiology of EIV shed by horses presented to veterinarians with an upper respiratory tract infection from March 2010 to November 2013.

2. Materials and Methods

Nasal secretions from 2375 equids with acute onset of respiratory disease were tested by qPCR for EIV. Categorical analyses were performed to determine the association between observations and EIV. Furthermore, observations from EIV positive study horses were compared to previous data from March 2008 to February 2010. Sequencing analysis of the HA1 gene of EIV was performed on 29 EIV isolates.

3. Results and Discussion

A total of 230 (9.7%) equids tested qPCR positive for EIV. The EIV positive equids in the 1 to 5 and 6 to 10 year age group, as well as Quarter Horses, were over-represented. Fever, nasal discharge, and coughing were more commonly observed in EIV positive horses than the entire study population. The EIV qPCR positive study cases were significantly older and more often vaccinated against EIV compared to EIV qPCR positive animals from the 2008 to 2010 study period. All of the sequenced EIV isolates belonged to the clade 1 Florida sublineage.

Acknowledgments

Conflict of Interest

This study was supported by Merck Animal Health.

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Detection of Modified-Live Equine Intranasal Vaccine Pathogens in Adult Horses Using Quantitative Polymerase Chain Reaction

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The duration of detection of modified-live intranasal vaccine pathogens in nasal secretions by qPCR appears to be dependent on vaccine schedule and ambient temperature. Authors' address: Department of Veterinary Medicine and Epidemiology, School of Veterinary Medicine, University of California, Davis, CA 95616; e-mail: charms@ucdavis.edu. *Corresponding and presenting author. © 2014 AAEP.

1. Introduction

Horses displaying signs of respiratory disease are routinely tested using real-time polymerase chain reaction (qPCR) analysis of nasal secretions. However, qPCR testing from a nasal swab does not distinguish between modified-live vaccine and wild-type pathogens. Thus, if a horse recently vaccinated intranasally subsequently develops signs of respiratory disease it would be unclear whether the qPCR detection of that specific pathogen is from the recently administered vaccine or represents true infection. Therefore, the objective of this study was to provide a timeline for how long after intranasal vaccination a horse will continue to shed modified-live *S. equi* subsp. *equi* (*S. equi*) and equine influenza virus (EIV).

2. Materials and Methods

Twenty-three adult horses were randomly assigned to one of two vaccine groups (*S. equi* = 12 horses; EIV = 11 horses), with two of the horses in each group to remain unvaccinated. All horses to be vaccinated received the vaccine in the left nostril at different periods according to the manufacturers' recommendations. After each vaccination, both nostrils of every horse were swabbed daily for 5 days. The swabs were processed and analyzed via qPCR for the presence of *S. equi* and EIV.

3. Results and Discussion

For both vaccine pathogens, qPCR detection was limited to 2 days following first vaccination (*S. equi* and EIV) and 1 day at second vaccination 3 weeks later (*S. equi*). Following a boost vaccination at 6 months, qPCR detection lasted up to 5 days for both vaccine pathogens. The authors hypothesize that lower environmental temperatures during the 6 month revaccination period caused prolonged pathogen shedding times, possibly due to enhanced replication within the upper respiratory airways and impaired mucociliary clearance.

Acknowledgments

Conflict of Interest

This study was supported by Merial and a grant from The Center for Equine Health, University of California, School of Veterinary Medicine at Davis.

The vaccines were kindly provided by Zoetis and Merck Animal Health.

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How to Use and Interpret Quantitative Polymerase Chain Reaction for the Laboratory Diagnosis of Equine Herpesvirus-1 Infection

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1. Introduction

Rapid laboratory tests for detection of equine herpesvirus-1 (EHV-1) are most useful in potential epidemic situations because immediate identification of the causative agent is often critical for guiding management strategies. Real-time polymerase chain reaction (qPCR) has become the diagnostic test of choice due to its quick turn-around-time and high analytical sensitivity and specificity. The qPCR is an enzymatic exponential DNA amplification technique that, under optimal conditions, is capable of detecting a small number of target viral genes. The PCR detection of EHV-1 is routinely performed in respiratory secretions from a nasal or nasopharyngeal swab and uncoagulated blood samples collected into Ethylenediaminetetraacetic acid (EDTA) tubes. Many conventional PCR protocols (single or nested PCR) targeting specific genes of EHV-1 have been published for the molecular detection of EHV-1.[1–6]

The increasing application of PCR for the molecular detection of EHV-1 in practice settings has presented new dilemmas with regard to how test results are interpreted and used by both equine practitioners and regulatory veterinarians since routine PCR assays are unable to differentiate between replicating (lytic), nonreplicating, or latent virus. Advances in technology and the use of novel PCR platforms, such as qPCR, allow calculation of viral loads for equine herpesviruses.[7–13] Although considerable progress has been made in developing PCR assays, the lack of protocol standardization between laboratories and the variability in the use of contamination and quality assurance controls remain an ongoing challenge.

Viral load testing, although not routinely offered by many veterinary laboratories, represents a major improvement in the interpretation of PCR results in the EHV-1 field, allowing better characterization of disease stage, better assessment of risk of exposure to other horses, and better monitoring of response to treatment. The objective of this paper is to discuss the use and interpretation of quantitative PCR for the laboratory diagnosis of EHV-1 infection and to illustrate viral kinetics using field cases.

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2. Materials and Methods

Quantitative PCR for EHV-1

Biological samples (whole blood and nasal secretions) were collected from symptomatic and asymptomatic horses naturally infected with EHV-1. The horses either presented to the William R. Pritchard Veterinary Medical Teaching Hospital, University of California, School of Veterinary Medicine at Davis or were part of recent outbreaks of EHV-1. Blood samples and nasal swabs were processed for nucleic acid purification using an automated nucleic acid extraction system,[a] according to the manufacturer's recommendations. All samples were assayed for the presence of the equine glyceraldehyde-3-phosphate dehydrogenase (eGAPDH) and the glycoprotein B (gB) gene of EHV-1 using previously reported TaqMan PCR assays.[11] Absolute quantitation of EHV-1 target molecules was performed using standard curves for EHV-1 and eGAPDH and expressed as EHV-1 gene copies per million cells.[11]

Molecular Surrogate for Lytic EHV-1

The lytic cycle of infection for EHV-1 results in the release of new virus particles from the infected cells. During lytic infection, viral gene transcription and regulation is sequentially regulated into three distinct phases: immediate early, early, and late phase. The detection of transcripts for late genes has been successfully used as a surrogate for lytic infection of equine gammaherpes viruses.[11,14] One hundred and thirty dual blood and nasal secretion samples from naturally infected horses were used to correlate the presence or absence of late gene transcripts, by measure of messenger RNA (mRNA) of the gB gene of EHV-1, with absolute quantitation of EHV-1 target genes. Total RNA purification and transcription of RNA to complementary DNA (cDNA) was performed according to previously reported protocols.[11]

Characterization of Disease Stage

To determine viral load signature profiles of horses in different stages of EHV-1 infection, blood and nasal secretions were processed and quantitation was performed as mentioned earlier. The study population was comprised of 27 horses with neurological signs (EHM group), 28 horses with fever and no neurological signs (EHV-1 group), and 52 exposed but asymptomatic horses (asymptomatic group). The statistical difference ($P < 0.05$) in EHV-1 viral loads between the groups was determined using the Mann-Whitney Test.

Monitoring of Response to Treatment

Twelve horses presenting to the William R. Pritchard Veterinary Medical Teaching Hospital at Davis from 2006 to 2013 with acute onset of neurological deficits and laboratory confirmation of EHV-1 infection were sampled daily during their hospitalization period to determine the viral kinetics of EHV-1 in blood and nasal secretions. All horses received similar medical treatment, which included the administration of dexamethasone (0.05 mg/kg, 0.1 mg/kg IV q 24 h for 2–3 days), valacyclovir (30 mg/kg PO q 8 h for 6 treatments followed by 20 mg/kg PO q 12 h for up to a total of 10 days), flunixin meglumine (0.5–1.1 mg/kg IV q 12 h), α-tocopherol (10,000 IU PO q 24 h), di-methyl sulfoxide (1 g/kg IV diluted in fluids q 24 h for 3 days) and pentoxifylline (10 mg/kg PO q 8 h). Duration of EHV-1 detection in blood and nasal secretions was determined for each horse.

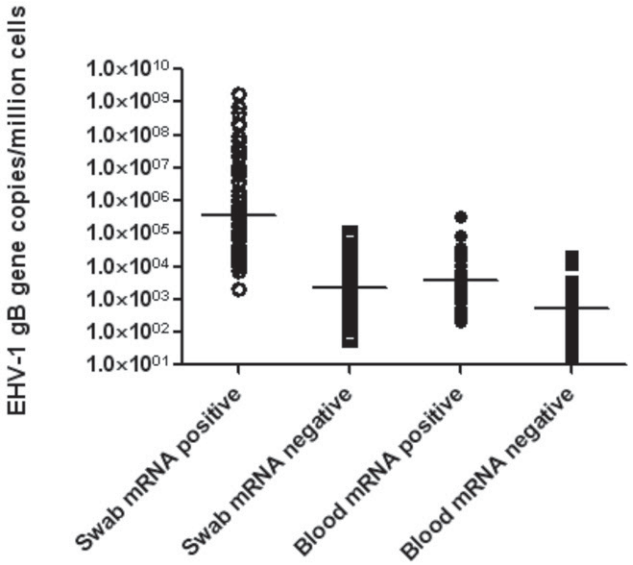

Fig. 1. Absolute quantitation of EHV-1 in nasal secretions and blood from 130 horses with clinical EHV-1 infection. Sample type is grouped by presence or absence of mRNA transcripts for the gB gene of EHV-1. Horizontal bars represent medians.

Indicator for Survival

Absolute quantitation for EHV-1 was performed on admission for 22 EHM horses presenting to the William R. Pritchard Veterinary Medical Teaching Hospital, University of California at Davis. The horses were grouped into survivors[15] and non-survivors.[4] Non-survivors were horses euthanized because of progression of disease despite intensive treatment and did not include horses euthanized because of financial reasons. Statistical difference ($P < 0.05$) in EHV-1 viral loads between the two groups was determined using the Mann-Whitney Test.

3. Results

The presence or absence of mRNA transcripts for the gB gene directly related to the absolute quantitation of this gene at the genomic DNA level (Fig. 1). Nasal secretions with the presence of lytic EHV-1 showed an average of 170 times higher viral load than nasal secretions with no lytic EHV-1 ($P = 0.0001$). Differences also occurred at the blood

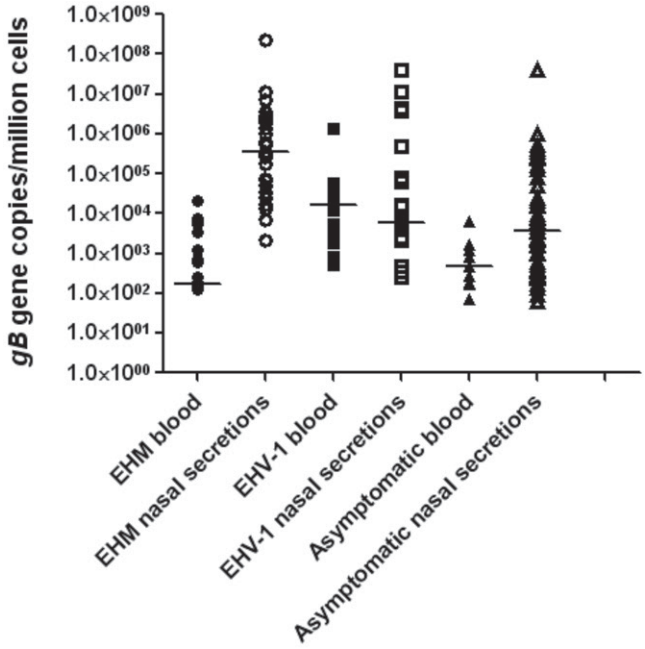

Fig. 2. Viral loads expressed as gB gene copies/million cells in blood and nasal secretions of 27 horses with EHM, 28 horses with EHV-1 infection but no neurological signs, and 52 asymptomatic exposed horses. Horizontal bars represent medians.

level, with a 7-fold difference in viral load between lytic and nonlytic EHV-1 ($P = 0.0001$).

Only 17 of the 27 horses with EHM tested PCR positive in blood samples, whereas EHV-1 was detected in the nasal secretions of all horses with EHM (Fig. 2). When comparing only the samples from the 17 neurological horses with dual positive results, viral loads in nasal secretions (median = 5.16×10^5 gB gene copies/million cells) were significantly higher ($P = 0.0001$) than in blood samples (250 gB gene copies/million cells). Equine herpesvirus-1 was detected in samples of both blood and nasal secretions from all 28 EHV-1 group horses. Viral loads were similar ($P = 0.76$) between blood samples (1.6×10^4 gB gene copies/million cells) and nasal secretions (6.0×10^3 gB gene copies/million cells). Only 13 asymptomatically infected horses had positive PCR results in blood (459 gB gene copies/million cells), while 51 of the 52 asymptomatic horses tested PCR positive in nasal secretions (3.7×10^3 gB gene copies/million cells). When viral loads in blood were compared amongst the three infected groups, the EHV-1 group horses had significantly higher viral loads ($P = 0.0001$) than EHM and asymptomatic horses. Significant differences were also found for viral loads in nasal secretions between the EHM horses and EHV-1 horses ($P = 0.003$) and between the EHM horses and asymptomatic horses ($P = 0.0001$). No significant difference was found in the nasal viral loads between EHV-1 horses and asymptomatic horses ($P = 0.26$).

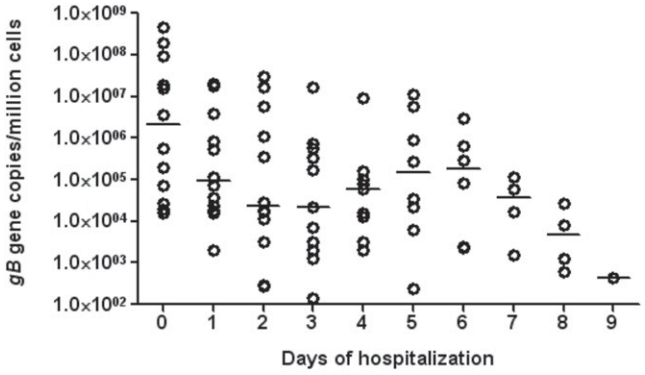

Fig. 3. Temporal viral loads in nasal secretions from 12 horses with EHM. Day 0 represents the day of admission to the hospital. The results are expressed as gB gene copies/million cells. Horizontal bars represent medians.

All 12 horses treated at the William R. Pritchard Veterinary Medical Teaching Hospital at Davis for EHM tested PCR positive for EHV-1 in blood and nasal secretions on admission. The detection period of EHV-1 in nasal secretions ranged from 3 to 9 days (median 6.5 days) with steadily decreasing viral loads (Fig. 3). The EHV-1 in blood was detected for 2 to 5 days (3 days) with rapidly declining viral loads (Fig. 4).

Viral loads from EHM horses on admission were a reliable indicator of outcome. The viral loads in nasal secretions and blood from non-survivors were an average of 300 and 10 times higher, respectively, when compared to the viral loads in survivors (Fig. 5; $P = 0.0025$).

4. Discussion

In recent years, qPCR has supplanted conventional cell culture for the detection of EHV-1 based on its quick turn-around-time, high sensitivity/specificity, and cost-effectiveness. Furthermore, quantitative PCR represents a major improvement in the detec-

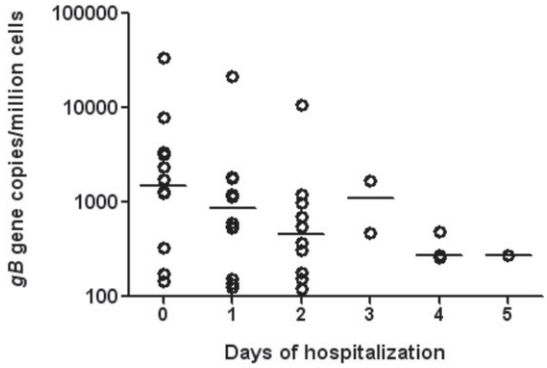

Fig. 4. Temporal viral loads in the blood from 12 horses with EHM. Day 0 represents the day of admission to the hospital. The results are expressed as gB gene copies/million cells. Horizontal bars represent medians.

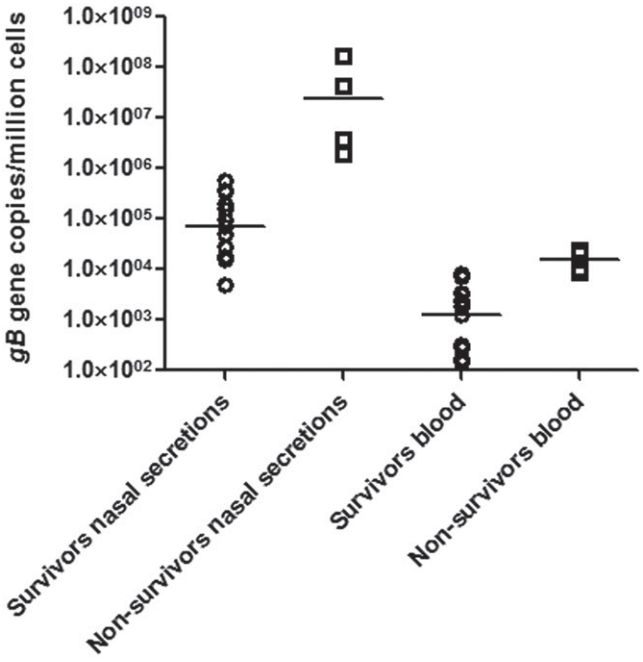

Fig. 5. Absolute quantitation of EHV-1 in nasal secretions and blood from 11 EHM survivors and 4 EHM non-survivors. Horizontal bars represent medians.

tion of infectious pathogens by allowing characterization of disease stage, assessment of risk of exposure to other animals, and monitoring of response to therapy. This approach has long been used in the human medical field, where the molecular quantitation of specific pathogens, such as HIV or hepatitis C virus, is considered standard of care.[15–19] In recent years, novel molecular platforms, such as qPCR, have enabled the study of viral kinetics of EHV-1 in respiratory secretions, blood samples, and tissues after experimental or natural infection.[7–12] However, research and diagnostic laboratories should use quantitative methods with the least evidence of variability between samples and extraction protocols. In order to improve standardization of quantitative PCR, a recent study compared viral load in nasal secretions using 4 different absolute quantitation methods.[20] Protocols normalizing results against a preselected volume (i.e., eluted DNA or volume of nasal secretions) were more prone to interlaboratory variations than protocols standardizing viral load to the entire swab, a house-keeping gene, or an arbitrarily chosen amount of extracted DNA. It is important that diagnostic laboratories use an internal quality control system to increase the reliability of results and minimize the risk of reporting false-negative results.

The use of qPCR for the detection of the gB gene of EHV-1 and its transcripts allowed the characterization of the viral state in 130 blood samples and nasal secretions. The molecular approach used in this study represents a crude measure of very complex biological processes that are still not fully understood. Furthermore, one must bear in mind that during an ongoing EHV-1 infection, the viral state rarely fulfills the "all or none" rule, but rather progressively transitions from one state to the next. The lytic or replicating state is molecularly easy to assess based on the detection of transcripts from essential genes, such as the gB gene, that are absolutely required for virus replication. Detection of virus in the lytic state is a strong indicator of active infection and potential for viral transmission and environmental contamination. Lytic infection in nasal secretions was characterized by the presence of transcriptional activity of the gB gene and, as expected, was associated with high viral loads at the DNA level (range 2×10^3–1.7×10^9 gB gene copies/million cells), while nonreplicating virus was routinely seen with low viral loads (62–1.0×10^5 gB gene copies/million cells). Because of the greater difference between lytic and non-lytic EHV-1 in nasal secretions, a threshold of 10^5 gB gene copies/million nasal cells was able to differentiate between lytic and nonlytic virus in all samples. The difference between lytic (200–3.3×10^5 gB gene copies/million cells) and nonlytic (18–2.0×10^4 gB gene copies/million cells) EHV-1 in blood samples was narrower, making a specific threshold more difficult to establish. However, one must bear in mind that the detection of EHV-1 gB gene DNA with no detection of transcriptional activity of the same gene may have other possible biological (i.e., abortive EHV-1 infection without the expression of late genes) and technical (i.e., differences in analytical sensitivity of the assays, sample processing) explanations. From an infectious disease control standpoint, one can assume nasal secretions to contain infectious EHV-1 based on a high viral load ($\geq 1 \times 10^5$ gB gene copies/million cells) and/or evidence of transcripts for the gB gene.

The study showed differences in viral loads determined by qPCR between disease stages in adult horses as well as between clinically and subclinically infected horses. Neurological deficits associated with EHV-1 infection appear during the viremic phase of infection and the interval between infection and subsequent onset of neurological disease is usually between 6 and 10 days.[21] The 27 neurological horses showed low viral loads in blood and up to one million-fold higher viral loads in nasal secretions. Anecdotally, many veterinarians mistakenly believed that horses with neurological manifestations of EHV-1 infection were no longer shedding virus. The results of this study clearly demonstrate that horses with EHM shed high loads of replicating EHV-1 in nasal secretions and, therefore, pose a substantial risk to other horses. From a biosecurity standpoint, horses affected by EHM should be isolated and confined away from other horses in order to decrease the risk of exposure to susceptible individuals. Although the risk of transmission of EHV-1 from neurologically affected horses has not

been thoroughly evaluated, indirect spread by fomites and personnel is the more likely reason for the rapid spread of infection during outbreaks of EHM. Recent outbreaks at racetracks, riding schools, and veterinary hospitals provide ample documentation of the contagiousness of neurologically infected horses.[22–25] Viral loads in blood and nasal secretions of horses with EHM were different from the viral loads encountered in febrile and subclinical horses. All 28 horses presenting for fever without neurological deficits had detectable EHV-1 in both blood samples and nasal secretions with similar viral loads between the two sample types. Our results are in agreement with experimental studies showing that viremia commonly coincides with the pyrexia peak in EHV-1 infection.[10] While the minority of the subclinically infected horses had detectable EHV-1 in blood, almost all had detectable EHV-1 in nasal secretions, with 21% of them displaying viral loads $\geq 1 \times 10^5$ gB gene copies/million cells. These results highlight the infectious nature of exposed but asymptomatic horses and the need to test both symptomatic and asymptomatic horses during an outbreak in order to help guide infectious disease control measures.

Equine herpesvirus type-1 in 12 horses with EHM remained detectable in blood and nasal secretions up to day 5 and day 9 of hospitalization, respectively. Viral loads slowly decreased in the blood and nasal secretions of all horses over the hospitalization period. The results are in agreement with a recent study determining nasal shedding in 16 horses with EHM.[24] In that study, the last day of EHV-1 shedding in horses with EHM was disease day 9. The effect of specific treatment could not be determined since all diseased horses received a similar treatment.

The evaluation of viral loads in blood and nasal secretions in horses with EHM on admission showed remarkable differences between survivors and nonsurvivors. However, based on the small number of horses per group, one must interpret the results carefully and always use clinical findings and response to treatment to determine prognosis, rather than single laboratory values. Non-survival in 4 horses with EHM was associated with the development of clinical signs of encephalopathy. The biological basis for the variable virus burdens in the different equine patients remains elusive. One recent study on severe acute respiratory syndrome-associated coronavirus in human beings determined that the viral load was a determinant of clinical outcome and was associated with polymorphism of genes involved in innate immunity.[26] It remains to be determined if a similar mechanism applies to horses with EHM.

In conclusion, the present study showed that the EHV-1 viral load and viral state can be determined using real-time PCR on field samples collected during various EHV-1 outbreaks. This molecular approach can help assess the risk of exposure to other horses and permits determination of viral kinetics in infected animals. The results of this study represent the first attempt in the development of guidelines for the use and interpretation of qPCR results from EHV-1 cases. This information is important to determining the risk of exposure to other horses and to helping practitioners and regulatory officials make appropriate decisions regarding the management of horses that test positive for EHV-1 by PCR.

Acknowledgments

Conflict of Interest

The Authors declare no conflicts of interest.

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[a]CAS-1820 X-tractor Gene, Corbett Life Science, Sydney, Australia.

Exercise-Induced Changes in Back Dimensions: The Influence of Saddle-Fit, Rider-Skill, and Work-Quality

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The back muscles of horses working correctly enlarge transiently with work. If a saddle does not fit properly, this increase in size does not occur, which may impair back function. Saddle-fit should be assessed before and after exercise. Authors' address: Centre for Equine Studies, Animal Health Trust, Lanwades Park, Kentford, Newmarket, Suffolk, CB8 7UU, UK; e-mail: sue.dyson@aht.org.uk. *Corresponding and presenting author. © 2014 AAEP.

1. Introduction

Recommendations concerning saddle-fit are empirical and based on anecdotal information. We know that the saddle needs to fit the horse in motion, but there has been no investigation of whether the thoracolumbar region changes in shape in association with exercise or how improper saddle-fit may influence potential changes. The objectives were to quantify exercise-induced back dimension changes and to describe the association with work-quality, saddle-fit, and rider-skill.

2. Materials and Methods

Sixty-three sports horses in normal work were prospectively assessed. Thoracolumbar shape/symmetry were measured at predetermined sites before and immediately after a 30 min exercise period; width ratios for two levels at each site were calculated. The work-quality and rider-skill were graded; the presence of lameness and saddle-fit were recorded. Descriptive statistics, univariable and multivariable mixed-effect linear regression were performed to assess the relationship between horse-saddle-rider factors and changes in back dimensions.

3. Results

The mean back-shape ratio immediately after ridden exercise was greater compared with before work for all sites. Mean changes in back-shape were greater in horses working correctly versus not working correctly and in horses with correctly-fitting vs ill-fitting saddles at each site. Mean changes were greater in horses ridden by good > moderately > poorly-skilled riders. Mean changes were less in lame horses compared with sound horses.

Acknowledgments

Conflict of Interest

The Authors declare no conflicts of interest.

Research Abstract

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How to Look for Sacroiliac Disease During Lameness Examination: Some Simple Clinical Indicators

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1. Introduction

Back pain, and more specifically sacroiliac pain, has become a commonly recognized cause of reduced performance in the horse in the last decade.[1–6] Many improvements in diagnostics and therapy have been made, and treating sacroiliac problems in horses is now part of many sport medicine practices.[3]

In general, complaints about sacroiliac pain in horses are diverse.[1–4] Coming to a diagnosis can be a daunting task and often involves several diagnostic tools to exclude other sources of reduced performance.[4–6] When a clinician, confronted with a case of suspected sacroiliac disease, has a "checklist" of clinical symptoms or indicators related to sacroiliac disease to rely on, the likelihood of recognizing the dysfunction is improved.[7,8] The more "iliosacral related" symptoms that are present at initial clinical examination, the more likely it is that the horse is suffering from sacroiliac disease.[7–11]

Some of these parameters are excellent indicators for spinal dysfunction or pathology, and incorporating an evaluation of these parameters into the standard clinical exam for lameness or performance related complaints can be very helpful to the lameness clinician, as they may otherwise be easily overlooked.

2. Materials and Methods

When sacroiliac pathology is present, the normal movement of the sacroiliac region of the horse will be compromised.[1,3,5,6] Altered sacroiliac mobility can be associated with several very specific alterations in the normal gait patterns in the horse.[7,8] A short description of these gait alterations and how to evaluate them during a clinical exam is listed below.

Tracking Narrow Behind

One of the first alterations of gait due to sacroiliac dysfunction, tracking narrow behind, is often visible in walk and even more obvious in trot. In walk, there are always at least 2 limbs on the ground in the support phase, whereas in trot there is a suspension phase with no limbs on the ground and then a diagonal support phase with 2 limbs on the ground,[12] making loading and rotational forces on the sacroiliac region more pronounced. When sacral rotation is avoided, the horse will place the hind feet close together, what is called narrow be-

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hind. It can look like the horse is walking or trotting on a cord as the hind feet are placed on the same line in front of each other.[7,8]

Lateral Walk

When observing the walk on a straight line, the walk should be a clear 4 beat motion.[11] When spinal dysfunction is present and the horse is asked to perform a serpentine in hand, the walk becomes more lateral, similar to a pace (hind limb and front limb on the same side advance at the same time). This finding has been associated with cervical spinal cord compression ("Wobbler's") but is not well described with other spinal dysfunction. In the author's opinion, the loss of a 4 beat walk is a clear indicator of tension (pain?) and decreased mobility in the spine.[8,9]

The best way to evaluate for the symptoms of lateral walk and narrow behind is to first have the horse walk and trot in hand to and from the examiner/clinician, as in any lameness exam, and then to have the horse walk again but in a serpentine pattern. Observing the horse from the side when it passes by on the straight line can also be very helpful.

Haunches In/Out

When observing a horse going away and coming at the clinician, the front limbs and the hind limbs should be on 2 tracks, the hind limbs follow the front limbs in the same plane. With sacral dysfunction, one hip is often kept slightly lower (may be subtle), resulting in a slight bending to one side. When the haunches are brought to one side, the observer notes that the horse is on 3 or more tracks. The hind limbs do not follow the front limbs in the same plane but are placed laterally, away from the central axis. This is easily observed when the horse is lunged on a circle. If the haunches are more in, one sees the front limbs making a circle with a slightly larger diameter than the hind limbs. Conversely, when the haunches are out, the hind limbs travel on a larger diameter. In canter, younger/untrained horses often have a slight haunches in at the canter, but nearly every "normal moving" horse should be tracking straight ("true") in walk and trot on a 25 to 30 foot diameter circle.

Asymmetric Tail Position

The best way to evaluate tail position is to observe the walk in a straight line and in a serpentine away from the clinician.[7,8] When observed from behind, a horse's tail should be in a relatively central position, and when walking serpentine, the tail should remain in a midposition with slight movements toward the side of the bending in the serpentine. When sacral dysfunction is present, the tail may be held asymmetrically. When the tail is held to one side, and stays to one side in the serpentine, there is a clear indication of sacroiliac dysfunction.[7,8]

Bunny Hop Canter

The fourth gait alteration often associated with sacroiliac dysfunction is a deterioration of the quality of the canter. This may even be a clear complaint of the client! A normal canter is 3 beats, and the motion of the left lead and right lead should be comparable and relatively symmetric.[11] There is a clear moment of suspension, and when the footfalls are observed as the feet land in their specific pattern, there is a clear separation of the landing moments between the two hind feet.[11]

When sacroiliac dysfunction is present, this clear 3 beat is less obvious or will completely disappear. The hind feet may land nearly at the same moment and very close together.[7–10] A good way to describe this phenomenon is "bunny hop."[7–10]

Observing the horse in a relaxed canter (tension in the horse can mimic the bunny hop canter without indicating true spinal dysfunction) in both leads on a lunge or ridden facilitates the evaluation of the canter. The author would advise having the horse do some canter for several minutes in each direction, so it is possible to analyze the quality of the canter over time and not be distracted by initial behavior such as excitement, bucking, etc.

Reduced Flexibility of the Lumbosacral Region

The last part of the exam for achieving information about the sacroiliac region is "hands-on." After observing the flexibility of the lumbosacral region in walk, trot, and canter, now the clinician evaluates the flexibility by manipulating it.

With one hand on the tuber coxae and one hand on the tail, lateral flexion in both directions is tested by simultaneously pulling on the tail and pushing slightly on the tuber coxae.[7,8] Ventral flexion is evaluated by making the horse "tuck under" by scratching it with a pointy object on the hamstrings on each side.[7,8] Lateral and ventral flexibility should be symmetrical. Clear reduction of lateral and/or ventral flexibility is a good indicator for sacroiliac dysfunction.[8]

3. Results

In the author's practice, we have included the serpentine in walk, lunging at all 3 gaits, and the manipulation of the lumbosacral region as a standard protocol in every lameness exam. During 9 years of practice (2005–2014), the author has examined 811 cases with a complaint of reduced performance or subtle hind limb lameness. Of these, 327 (40%) were diagnosed with sacroiliac disease based on clinical exam, imaging data (ultrasonography and scintigraphy), and improvement in symptoms or abnormal diagnostic findings at 2, 4, and 6 month recheck intervals after initial treatment and rehab. Of these 327 cases of diagnosed and clinically substantiated sacroiliac disease, 322 had a positive score ("present") for at least 3 indicators mentioned above. Of the 489 cases with a final diagnosis dif-

ferent from sacroiliac disease, less than 2% (7 horses) had more than 3 indicators positive.

4. Conclusion

Results in our practice show a clear value of using these specific indicators for diagnosing sacroiliac dysfunction. With a small extension to the standard procedure for a lameness exam, much more specific information about the condition of the sacroiliac region can be obtained with little extra effort

Acknowledgments

Conflict of Interest

The Author declares no conflicts of interest.

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Influence of Aquatic Exercise on Postural Sway Characteristics in a Model of Equine Carpal Osteoarthritis

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Underwater treadmill exercise significantly improved balance control in horses with experimentally induced carpal osteoarthritis under varying stance conditions. Authors' addresses: Orthopaedic Research Center, Department of Clinical Sciences (King, Haussler, Kawcak, McIlwraith, Frisbie) and Department of Health and Exercise Science (Reiser), Colorado State University, Fort Collins, CO 80523; e-mail: Melissa.King@colostate.edu. *Corresponding and presenting author. © 2014 AAEP.

1. Introduction

Exercising in water provides an effective medium for increasing joint mobility, increasing muscle activation, promoting normal motor patterns, and reducing the incidence of secondary musculoskeletal injuries. The improved muscle strength and function associated with aquatic exercise significantly improve proprioception and motor control and reduce the abnormal postural sway characteristics typically reported in osteoarthritic adults.

2. Materials and Methods

Osteoarthritis was induced in one middle carpal joint of 16 horses. Horses were assigned to either underwater or overground (without water) treadmill exercise. Force platforms were used to collect postural sway data from each horse at four different time points. Horses were made to stand stationary on the force platforms under 3 stance conditions: normal square stance, base-narrow placement of the thoracic limbs, and removal of visual cues (blindfolded).

3. Results

Displacement of the center of pressure differed significantly depending on the stance condition. Among horses exercised on the underwater treadmill, postural stability in both the base-narrow and blindfolded stance conditions improved in comparison to the overground treadmill exercised horses. Horses exercised on the overground treadmill were only effective at maintaining a stable center of pressure during the normal stance position.

4. Discussion

Variations in stance position had profound effects on the mechanics of standing balance in horses with

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experimentally induced carpal joint osteoarthritis. Underwater treadmill exercise significantly improved the horses' postural stability, which is fundamental to providing evidence-based support for equine aquatic therapy.

Acknowledgments

Conflict of Interest

The Authors declare no conflicts of interest.

Short- and Long-Term Outcomes of Pelvic Fractures in 136 Thoroughbreds (2000–2010)

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Pelvic fractures in Thoroughbreds have a good prognosis. Acetabular involvement, comminution, and articular involvement negatively affect the short-term outcome, but those discharged from the hospital could go on to be successful. Authors' addresses: High Plains Veterinary Clinic, 996 10th Avenue SE, Sidney, MT 59270 (Peters); and Rood and Riddle Equine Hospital, PO Box 12070, Lexington, KY 40580-2070 (Ruggles, Bramlage, Hopper, Embertson, Woodie); e-mail: petersvmd@gmail.com. *Corresponding and presenting author. © 2014 AAEP.

1. Introduction

Pelvic fractures are a cause of poor performance in horses. Our objectives are to describe the current epidemiology of pelvic fractures in a Thoroughbred population and determine the short-and long-term outcomes.

2. Materials and Methods

The records of Thoroughbreds diagnosed with a pelvic fracture during the period 2000–2010 were reviewed. The short-term outcome (discharged from hospital) and long-term success (racing or live offspring) were evaluated with respect to different factors.

3. Results

There were 136 horses included, of which 72 (62%) raced after their fracture and 42 (36%) were subsequently able to have at least one foal. Acetabular involvement ($p < 0.001$), articular involvement ($p = 0.008$), and comminution ($p < 0.001$) were significantly associated with a negative short-term outcome. Of horses with acetabular involvement, 60% had a good short-term outcome, 75% of which were successful long-term. Racehorses with fractures only involving the ileum ($p = 0.014$), as well as young horses and racehorses ($p = 0.0002$) were more successful. Overall, 86% of horses had a good short-term outcome, 77% of which were successful long-term.

4. Discussion

This study found a better short-term prognosis for pelvic fractures than previously reported. While acetabular involvement was significantly associated with a negative short-term outcome, over half of them were still successful long-term. Comminution and articular involvement were significantly associated with a negative short-term outcome but did not impact the long-term success.

Acknowledgments

Conflict of Interest

The Authors declare no conflicts of interest.

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Caudal Lumbar Vertebral Fractures in California Quarter Horse and Thoroughbred Racehorses

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Lumbar vertebral fractures occur in horses with pre-existing pathology at the 5th and 6th lumbar vertebral junction (L5–L6) that likely predisposes horses to catastrophic fracture. Authors' addresses: J.D. Wheat Veterinary Orthopedic Research Laboratory (Collar, Zavodovskaya, Hitchens, Wisner, Stover); and Surgical and Radiological Sciences (Spriet), University of California, School of Veterinary Medicine, Davis, CA 95616; and Pathology, California Animal Health and Food Safety Laboratory, University of California Davis, School of Veterinary Medicine, San Bernardino, CA, 92408 (Uzal); e-mail: emcollar@ucdavis.edu. *Corresponding and presenting author. © 2014 AAEP.

1. Introduction

Repetitive overuse injuries often predispose to catastrophic injuries in racehorses. Lumbar vertebral fractures are not typically related to external trauma and may occur secondary to pre-existing abnormalities.

2. Materials and Methods

California racehorse postmortem reports and associated jockey injury reports were retrospectively reviewed. Vertebral specimens from 6 racehorses with lumbar vertebral fractures and 4 control racehorses euthanized for non-spinal fracture were assessed using visual, radiographic, computed tomography, and histologic examinations.

3. Results

Lumbar vertebral fractures occurred in 38 Quarter Horse (QH) and 29 Thoroughbred racehorses over a 22-year period, primarily involving L5 and/or L6 (87% of QHs, 48% of Thoroughbreds). Lumbar vertebral fractures were the third most common musculoskeletal cause of death in QHs and commonly involved a jockey injury. Quarter Horse specimens contained anatomic variations in the number of vertebrae, dorsal spinous processes, and transverse articulations. The examined lumbar vertebral fractures (5 QHs, 1 Thoroughbred) coursed obliquely (craniventral to caudodorsal) across the adjacent L5-L6 vertebral endplates and intervertebral disk, although one case involved only one endplate. All cases had evidence of ventral vertebral body abnormalities consistent with pre-existing maladaptive pathology.

4. Discussion

Lumbar vertebral fractures had a common configuration and evidence of anomalous bone modeling

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and remodeling at the site of fracture. Pathology of the caudal lumbar vertebral region appears to precede catastrophic lumbar fracture and may be more common than recognized.

Acknowledgments

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Conflict of Interest

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Comparison of Training-Related Injury Rates Between Juvenile Thoroughbreds Trained for Two-Year-Old Sales and Those Trained Solely for Racing

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No significant differences in injury rates or ability to start a race were observed between populations: the intensive training required for 2-year-old sales may not be detrimental to racehorses. Authors' address: Infield Equine Diagnostics/ Florida Equine Veterinary Associates, Ocala FL 34482; e-mail: infieldems@hotmail.com. *Corresponding and presenting author. © 2014 AAEP.

1. Introduction

Limited information exists comparing rates of injuries between juveniles trained for 2-year-old resale and those trained exclusively to race.

2. Materials and Methods

Training records for juveniles at a single training center were reviewed. The information included miles galloped and breezed, injury type and date, and age at first start. A ratio of breezed to galloped miles was calculated for each horse. Miles at risk were compared using survival plots and age at first start was compared using T tests. The incidence rates of injury were compared between groups. The number of horses which started a race was compared using Fisher's exact test. Significance was set at $p = 0.05$.

3. Results

Forty-nine percent of the 45 "race" horses demonstrated injury requiring time out of training compared to 42% of the 57 "sales" horses. Injuries were classified as sore shins, fetlock injuries, suspensory and flexor injuries, and miscellaneous. No significant difference was observed in injury incidence rates between the groups, although the timing of injury differed between groups. An analysis of the breeze/gallop ratio highlighted significant differences in training methods between groups ($p = 0.04$) with greater breeze miles in the "sales" group. "Sales" horses were significantly younger at first start ($p = 0.004$), although no difference in ability to start was observed ($p = 0.76$).

4. Discussion

The distribution and rates of injury did not differ between groups even though the training methods and timing of injury differed. Further investigation is warranted to evaluate this finding in the general population.

Acknowledgments

Conflict of Interest

The Authors declare no conflicts of interest.

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Clearance of Corticosteroids Following Intra-Articular Administration of Clinical Doses to Racehorses

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Association of Racing Commissioners International (ARCI) regulatory recommendations are appropriate for administration of maximum intra-articular doses of 18 mg of triamcinolone acetonide (TCA), 600 mg methylprednisolone acetate (MPA), 18 mg isoflupredone, and betamethasone doses less than 30 mg. Authors' addresses: K.L. Maddy Equine Analytical Chemistry Laboratory (Knych), School of Veterinary Medicine (Arthur), University of California, Davis, CA 95616; and Von Bluecher, Blea, Hunkin, Inc., PO Box 970, Sierra Madre, CA 91024 (Blea); e-mail: hkknych@ucdavis.edu. *Corresponding and presenting author. © 2014 AAEP.

1. Introduction

Over the last several years, there has been a nationwide cooperative effort to establish threshold concentrations and withdrawal times for corticosteroid use in racehorses. Withdrawal time guidelines are based on pharmacokinetic studies of single intra-articular corticosteroid injections.[1,2] As dosing regimens are specific to individual horses and highly variable, it is not possible to establish regulatory guidelines for every dosing scenario. The goal of the study described here was to assess the applicability of current regulatory recommendations for corticosteroids based on clinical protocols used by practitioners.

2. Materials and Methods

Fifty-eight Thoroughbred racehorses received varying doses of TCA,[a,b] MPA,[c] isoflupredone,[d] or betamethasone[e] intra-articularly in various joints by the treating practitioner. Blood samples were collected at 0, 7, 10, 14, 21, 28, and 35 days post drug administration. Serum samples were analyzed by liquid chromatography mass spectrometry for quantitation of drug concentrations.

3. Results and Discussion

Serum elimination varied depending upon the dose and the number and specific joints treated. Serum concentrations fell below the ARCI recommended threshold by days 7 (100 pg/mL) for both TCA (9–18 mg dose) and isoflupredone (4–18 mg dose) and day 21 (100 pg/mL) for MPA (40–600 mg dose). Betamethasone fell below the recommended threshold (10 pg/mL) by 7 days for doses less than 30 mg but not until 10 days for doses of 30–60 mg. It is important to note, however, that results reported here may differ if compounded formulations are used.

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Acknowledgments

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Conflict of Interest

The Authors declare no conflicts of interest.

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[a]Vetalog®, Boehringer Ingelheim Vetmedica, Saint Joseph, MO 64506.
[b]Kenalog®, Bristol-Meyers Squibb Company, Princeton, NJ 08540.
[c]Depo Medrol®, Zoetis, Florham Park, NJ 07932.
[d]Predef 2X®, Zoetis, Florham Park, NJ 07932.
[e]Celestone®, Merck & Co, Inc., Whitehouse Station, NJ 08889.

Infectious Arthritis Following Intra-Articular Injection in Horses Not Receiving Prophylactic Antibiotics: A Retrospective Cohort Study of 2833 Medical Records

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Infectious arthritis (IA) following intra-articular injection is rare and prophylactic antibiotics are likely unnecessary. The treating veterinarian and combined corticosteroids (CS) and polysulphated glycosaminoglycans (PSGAG) treatment were significant risk factors. Combined intra-articular diagnostic anesthesia and treatment did not increase the risk of infection. Authors' addresses: Evidensia Equine Hospital Helsingborg, Bergavägen 3, SE-25023 Helsingborg, Sweden (Bohlin, Kristoffersen); and National Veterinary Institute, Technical University of Denmark, Section for Epidemiology, Bülowsvej 27, DK-1870 Fredriksberg C, Denmark (Toft); e-mail: Anna.Bohlin@djursjukhus.com. *Corresponding and presenting author. © 2014 AAEP.

1. Introduction

Intra-articular injection is commonly performed in lameness diagnosis and treatment; the most feared complication is infectious arthritis, and to some veterinarians this justifies routine use of prophylactic antibiotics.

2. Materials and Methods

A retrospective cohort study of medical records from a private equine hospital was done. Records of horses undergoing lameness diagnostics and treatment including intra-articular injections were identified. Risk factors were investigated using Fisher's exact test with a level of significance $P < 0.05$.

3. Results

Medical records of 2833 horses, 5139 visits, and 14124 intra-articular injections were identified. The incidences of infectious arthritis at injection level were: all substances 9.2/10000 (0.092%), local anesthetic 9.1/10000 (0.091%), overall treatment 17.0/10000 (0.17%), CS 16.1/10000 (0.161%), hyaluronan 15.9/10000 (0.159%), and PSGAG 39.4/10000 (0.394%). No horse undergoing diagnostic anesthesia alone experienced infectious arthritis; performing combined diagnostic anesthesia and treatment was not a risk factor. No specific joint had an increased risk for infection. At horse level, the significant risk factors identified were the treating veterinarian, relative risk (RR) = 4.1 ($P = 0.04$),

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and combined PSGAG and CS treatment RR = 2.7 ($P = 0.05$) compared with CS used alone.

4. Discussion

Infectious arthritis was a rare event following intra-articular injection. Approximately 1000 horses should be treated with prophylactic antibiotics to prevent one infection following intra-articular injection. The use of PSGAG-CS combination and the treating veterinarian were the only identified risk factors.

Acknowledgments

Conflict of Interest

The Authors declare no conflicts of interest.

Spontaneous Rupture of the Proximal Superficial Digital Flexor Tendon: A Clinical Syndrome in Aged Equids

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Acute rupture of the proximal superficial digital flexor tendon (SDFT) can occur spontaneously in older horses resulting in severe pain and prolonged lameness. Pasture soundness is achievable but requires long term rest and rehabilitation. Authors' addresses: Department of Surgical & Radiological Sciences (Vaughan, Whitcomb, Galuppo) and William R. Pritchard Veterinary Medical Teaching Hospital (Shields), School of Veterinary Medicine, University of California, One Shields Avenue, Davis, CA 95616; e-mail: mevaughan@ucdavis.edu. *Corresponding and presenting author. © 2014 AAEP.

1. Introduction

Injury to the SDFT is a well-documented cause of lameness in horses of all ages and disciplines; however, acute rupture of the SDFT has been infrequently described.

2. Materials and Methods

Records were reviewed for equids presenting for metacarpal ultrasound from August 2003 to July 2013. Cases were included if complete rupture of the SDFT was detected at any location within the carpal canal or metacarpal region.

3. Results

One mule and 13 horses were identified, ranging in age from 15 to 30 years. Affected equids were retired or lightly ridden (10), show jumpers (3), or breeding stock (1). All presented for concurrent swelling and lameness of the affected limb, graded as severe in 12 of 14 cases. SDFT rupture was unilateral (13) or bilateral (1 mule). All ruptures occurred in the carpal canal and proximal metacarpal region. Concurrent carpal sheath effusion (5), superior check ligament desmitis (2), and carpal flexor retinaculum rupture (1) were also noted. Recheck ultrasound revealed substantial improvement in 4 horses. The mule was euthanized after diagnosis. Six horses attained at least pasture soundness, including 3 used for light riding, 1 breeding stallion, and 2 that were retired. Four were retired due to lameness, including 2 that ruptured the contralateral SDFT. One remains in rehabilitation, and 2 were lost to follow-up.

4. Conclusion

Spontaneous rupture of the proximal SDFT should be considered in older horses or mules with severe acute lameness and SDFT swelling. Affected equids have a fair prognosis for pasture soundness after prolonged rehabilitation.

Acknowledgments

Conflicts of Interest

The Authors declare no conflicts of interest.

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People, Places, and Prices: What is the Grade Point Average of Your Practice?

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1. Introduction

The country roads of West Texas are almost completely void of humanity at 2 in the morning. It was an hour and a half drive back to the house from an "emergency" horse call that had turned out to be a dermatological condition. On the way home, I would drive past 3 veterinary clinics that had obviously chosen not to attack a midnight skin rash.

My mind was occupied with veterinary clinics. . . . What makes one thrive and another in the same geographical area wither up and die? I am no CPA, but 16 years of practice has given me time to watch clinics be born, come to life, and then either thrive or just exist.

What is it that makes a person choose the veterinarian that they are going to trust with the health and well-being of their animal? My mind drifted back to 1990 and my first job as a veterinarian in Clarendon, Texas. Dr. Chuck Deyhle was my mentor, and at that time had been in practice for 40 years. To this day, I consider him to be one of the most successful practitioners I have ever seen.

His words came to me from all those years of weeding out the adjectives. (It seems that people who really understand a subject can tell you about it in a few simple sentences while covering all that is relevant.)

Some of his last words to me as I left for Lamesa to open my own practice were, "Bo, there are three reasons people choose a veterinarian: They offer a better service, they have a better price, or they just like the doctor and the staff." I left Clarendon with that on my mind, and actually took it to heart.

Since then I have been to countless practice management seminars and paid people to come by and evaluate what we need to do to be better, and no matter how they put it, it always comes back to those three things.

Why do some practices thrive and others just exist? People. Places. Prices. If you really look at a practice as an unbiased observer, you will find that thriving practices are in tune with these three factors. If you look at struggling practices, you will find that at least one of these things is greatly lacking.

As I drove along those dark roads in the middle of the night, I considered these things a little more closely. We are subliminally conditioned because of the way we are educated to seek rewards. For example, we start school in August, we are given a syllabus that tells what all is about to happen, we are given a series of tests, and we know how well we did. All veterinarians become masters of this lifestyle, or we would have never gotten into veterinary school. So what happens to us when we get out and

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go to work? There are no defined rewards; we have no scale to tell how we are doing.

I was so enslaved to this way of thinking that I couldn't get away from it. I never knew if I was making an A, B, C, D, or F. So I decided to make a grade scale and use it with each client. I would actually write it on the medical records at the end of an encounter with a client…A,B,C,D, or F. Here was the scale:

If the client leaves and actively tells people you are a good vet = A
If the client is asked, they will say you are a good vet = B
If the client is asked, they will say that they simply use you = C
If the client is asked, they will say you are not very good = D
If the client leaves and actively tells people you stink = F

By the end of most client encounters, I found that I could tell what they thought of what I had done. I decided the ultimate challenge was to get an A with a client that had an animal die or if a surgery went bad.

I drove along and decided to grade our clinic in the three areas that Dr. Deyhle had determined were essential for a healthy practice:

People: It is an attitude that goes beyond the veterinarians in a practice. It encompasses the entire staff and reflects what the priorities of the practice are. If you own a practice, you know this is the tough one. It is hard to be happy all the time. It is hard to tell the story of heart worm infection 12 times a day. It is hard to work 60 to 80 hours in a week and still be fun to be around on Saturday. In fact, I don't think it is even possible. We need people around us that can be happy when we are not. We need people that are proud of the practice they work for and can't wait to brag about it when the chance arises.

The more staff there are, the harder it is. It is a constant struggle that evolves with new people and old personalities. No veterinary clinic is truly successful without employees that are wonderful; the doctor cannot do it alone. A successful veterinarian should never take complete credit for the merits of the practice. It is a team effort and all should be praised if things are good.

How does your team respond to success, or more importantly, failure? It is persistence in the face of failure that measures the character of the people in a practice. It is sometimes hard to remember that employees are just that—employees. If you own a practice, you know what I mean. Owners get the mindset that everyone thinks the same way they do. This is not so. Employees need to be included in the evolution of the practice whether it is going well or not. People will often choose a veterinary clinic simply based on the attitude and personality of the doctors and the employees.

I analyzed our people: doctors, techs, and office staff. Look at your practice. What grade would you give them on each client that came through the door today? If you wrote it on the medical records, would you get a 3.5, 2.7, or 4.0?

Places: Dr. Deyhle said that one component of practice growth was the services offered. I think this area includes the technical merit and personality of the practice. What is the place like? Is it new? Does it offer more innovative methods and up to date skills? Is it clean and modern? Is the animal well cared for? Is there a sense of compassion? Do they go beyond what is expected to see to it that the client understands what is happening? Are the doctors perceived as being really smart and good at their trade? Do they have neat toys that are state of the art? How much time does each client and each animal get? How many follow up phone calls are made?

It boils down to how much the practice cares and how technically advanced they are. Prospective clients are looking closely at these things when considering where to go for veterinary care. If you looked through the eyes of each client that came in the door, what grade would your clinic get?

Prices: For some reason, we don't want to admit that this makes any difference. We recently were wondering how we fit into the price situation of our geographical area, so we decided to just call around and get a feel for what a few procedures cost. We got a price range from $69.00 to $312.00 to spay a 20 pound dog. Now whether we believe it or not, great service will not always overcome exorbitant prices. There are people out there that are on a budget. I have seen more veterinarians go out of business from high prices than from any other thing.

Pricing is a practice philosophy. Do you want to see a huge volume at low prices or a small volume at higher prices? Do you want to cater to the upper crust or the middle class masses? Do you feel that if you aren't charging enough you are insulting your ability and level of education? Do you find yourself continually comparing the cost of similar human procedures done by medical doctors?

People often make a choice simply based on price. This is especially true when it comes to elective procedures and drug sales. Is your practice healthy on the pricing side of things? This is the area that "practice analyzers" have a field day with. They are money-oriented and want you to analyze everything as a return on investment.

It is a natural thought progression to charge more for something as we get better at it or tired of doing it. If you don't like doing a C-section on a cow that has had a dead calf in it for 8 days, charge more. The theory here is that almost every job can be fun if you are paid enough to do it. The problem with this is that eventually almost every task becomes

routine and boring, and the next thing you know, no one can afford you.

It is a fine line between charging enough to make a happy living, becoming greedy, and being able to sleep at night because you feel right about what you have been paid for what you have done.

What grade would you give yourself on the clients you saw today and the perceived value they attributed to your services? Are they going to go out and actively tell others that it was a good experience?

Now, going back to my story… I am happy to say that by the time I got home I was feeling a bit better about the day. Our clinic is healthy and growing and making a difference in the little part of Texas that is our practice environment. We sure don't have a 4.0 grade point average, but we are studying hard and trying our best to get it there.

I thought about the three clinics I passed to get to that horse. I wondered what their grade point average would be. I wondered if they even cared what it was or if they were beyond caring.

A veterinary clinic is not easy to understand. There are too many variables to make any absolutes. This is why we need to step back on occasion and truly evaluate what is happening. It is also why we need to have a personal scale to look at and find areas we need to work on. Have a look at what you do each day and see if business doesn't pick up a bit as your grade point average increases.

Acknowledgments

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The Author declares no conflicts of interest.

Getting What You Want—It's All About Negotiation

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1. Introduction

When most veterinarians think about negotiation, they think of bargaining with a salesman over the price of a new digital radiology unit. In truth, negotiations take place continually in life: between business owners and employees, veterinarians and horse owners, and parents and children. Anytime a decision requires input from more than one person, negotiation is involved. Because these types of decisions are very common, developing skill in negotiation is an essential element of effective communication.

Negotiation is defined as a process during which two or more parties attempt to resolve their differing interests. There are several characteristics common to all negotiations, whether they are between warring countries or simply between a parent and child[1]:

1. Negotiation occurs between two or more parties.
2. The parties have a conflict of needs, desires, or interests that need resolution.
3. Parties negotiate by choice, voluntarily, because they feel they can gain a better outcome than by simply accepting what the opposing party is offering.
4. Negotiations require both parties to move from their opening positions in order to reach an agreement.
5. The parties prefer to negotiate rather than fight, sever a relationship, have one dominate and the other capitulate, or take their dispute to a higher authority for resolution. In addition, when there are no rules or laws to guide resolution, or when parties choose to bypass those rules, negotiations will often occur.
6. Successful negotiation manages tangibles as well as intangibles.

2. Types of Negotiation

Some negotiations are zero-sum or *distributive* and some are mutual-gains or *integrative*. Distributive bargaining occurs when the goals of one party are in direct conflict with the goals of the other. This type of bargaining is essentially a competition over who will get more of a limited resource. Integrative negotiation is a collaborative cooperative activity that aims to allow the needs of all parties to be met.[2]

Distributive

Generally, distributive bargaining is centered on haggling about a price and is competitive; both parties' interests are in direct conflict. There is a fixed resource (often money) and both parties are seeking to maximize their gain. Because these negotiations result in a winner and a loser, using distributive tactics should be reserved for situations where a single simple deal is being made and a future rela-

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tionship with the other party is not important, or an integrative negotiation has progressed to the point of each side "claiming value." An equine veterinarian purchasing a used truck from a car salesman would do well to use distributive bargaining techniques.

In distributive bargaining, each party has in mind a target point: their optimal goal of the point at which negotiations will conclude. They also have a resistance point, beyond which they will break off negotiations and walk away. The opening offer will serve as an anchor for the negotiation and is very important because all moves will take place around this initial stake in the ground. If opening offers (whether from seller or buyer) are too far from the target point, it is possible that no negotiation will even occur. For instance, if the used truck salesman states that the truck's price is $35,000 but the target point of the prospective buyer is $15,000, it is quite unlikely that a negotiation will occur; the buyer will assume she cannot afford the vehicle.

The spread between the buyer's and seller's resistance points is known as the bargaining range, because any offer outside of this range will be immediately rejected by the other party. If the truck salesman's lowest acceptable price (his resistance point) for the used truck is $20,000, he will summarily dismiss an offer of $15,000. Through the process of making offers and counteroffers: each party begins to reveal their resistance point. Consider a seller with an opening price of $30,000, a target of $25,000, and a resistance point of $20,000 bargaining with a buyer with a target of $17,500 and a resistance point of $22,500. One can see that the bargaining range is between $20,000 and $22,500. Within a few minutes of conversation, both parties will understand the others' position better and a deal may be made that satisfies both parties. A positive bargaining range occurs when the buyer's resistance point is above the seller's. In the case of a negative bargaining range, the seller has a minimum price higher than the maximum the buyer is willing to pay. Either the negotiation will end or the parties will re-evaluate their resistance points. Or the buyer will pursue an alternative.

The best alternative to a negotiated agreement (BATNA) is a critical concept for all negotiators. Alternatives are important because they give negotiators the power to walk away from a deal that is not attractive. Knowing the best alternative to an agreement before entering into a negotiation is critical to making good decisions. Because of their awareness of their true "bottom line," negotiators with a firm understanding of their BATNA have power and confidence and, generally, are more successful in achieving their goals.[3] If the veterinarian has identified another used truck at another dealership with an asking price of $22,500 (her BATNA) she will be much more likely to press the salesman for a sale price on the subject truck closer to her target and less likely to agree to a price close to her resistance point.

The basic strategies in distributive bargaining are to push for a price very close to the seller's resistance point by making extreme offers and small concessions and/or convince the seller to reconsider his resistance point by influencing his beliefs about the value of what he is selling. Because during negotiations concessions are essential, opening offers should be sufficiently distant from the resistance point to allow room for an exchange of offers. Research shows that parties are more satisfied with agreements if there is a series of concessions rather than if their first offer is accepted, perhaps because they feel they could have done better.[4] When making concessions, one can determine when a counterpart's resistance point is being reached as successive concessions become smaller.

Although a few people approach all discussions as though they were distributive bargaining sessions, reserving this approach for those situations that are truly one-time transactional agreements with no relationship components is the most rewarding approach.

Integrative

The goals of the negotiating parties in integrative negotiation are not mutually exclusive: this is win-win bargaining. In this type of negotiation, there is a focus on what is found in common rather than on differences, on interests rather than positions, on meeting the needs of all parties, on an exchange of information between sides, and on an enlargement of the pie through innovative ideas. In order to be a successful integrative negotiator one must build trust through honesty and integrity, have a positive outlook that sees abundance rather than scarcity, recognizes that others' interests have equal validity as yours, be able to see the big picture, and have strong listening skills.[5]

By facilitating a reciprocal flow of information, both sides gain understanding of the needs and concerns of their counterparts, leading to less extreme resistance points. Identification of the others' true objectives and desired outcomes can lead to recognition of commonalities and areas of alignment. This makes searching for solutions that meet both sides' goals more successful and satisfying. By generating multiple alternatives, innovative solutions may arise that increase overall value.

As an example, an associate may want to pursue acupuncture training but the tuition and time away needed exceed her contractual continuing education budget and allotted days off. An active dialogue is needed, focusing on understanding the costs, benefits, goals, and threats of this desire. By defining all of the parameters of this "problem" collectively, both the associate's and the practice's needs and priorities can be accurately identified. The practice may have concerns about the tuition fees, travel costs, and lost time in production/emergency duty,

as well as concerns about future loss of this employee after paying for the acquisition of these skills. The associate desires to add an additional profit center to the practice to increase her value as an employee and increase her potential earnings. She also desires to add a skill that she could pursue in the future in her own practice if she decides that emergency duty is a poor lifestyle once she becomes a parent. A collaborative approach to this situation where all concerns as well as aspirations are voiced has a high likelihood of producing an agreement that honors the needs of all parties. One creative solution would have the practice pay the associated expenses in return for an agreement that a certain number of subsequent years of service were required or repayment of prorated expenses would be stipulated if the associate departed prematurely. One can easily imagine the associate's poor morale if the request was simply denied, or the practice owner's anger and feelings of betrayal if an associate promptly departed after training financed by the practice without a repayment clause. Both parties must think of what their BATNA is in this situation. The associate's BATNA might be to pay for the training herself and utilize vacation time in order to attend. The practice's BATNA could be to give the associate unpaid leave to attend the course, with her assuming all expenses in excess of her continuing education budget. When assessing these BATNAs, it is clear that a negotiated settlement is likely to be more satisfactory for all parties.

All successful negotiations require careful preparation, including determination of the BATNA, the target point, the resistance point, and the opening offer. Integrative bargaining requires the additional steps of defining the problem, identifying both parties' needs and interests, collaboratively generating alternative solutions, and selecting a solution after careful evaluation of the value created by the choices generated.[6]

3. Negotiation Styles

Different individuals have varied innate preferred negotiating styles. These include avoidance, compromise, accommodation, competitive, and collaborative. By understanding one's own preferences and being aware of the potential for others to have differing styles, a person can become more comfortable with experimenting with other negotiating styles. A cooperative approach is often more effective than a competitive one and an awareness of personal preferences can lead to better outcomes. Simple assessment exercises are available that reveal preferred negotiating styles. Gender and culture also play a part in negotiating styles.[7]

Dr. G. Richard Snell, a professor at the Wharton School of Business, created an assessment tool for negotiation style, printed as an Appendix in his book *Bargaining for Advantage*.[8] The results of this assessment can reveal a preferred style of negotiation. Those with strongly *accommodating* styles have strong skills in relationship building and enjoy helping solve others' problems. They can excel in many customer service roles and integrative bargaining situations, but can be vulnerable to competitive counterparts. They may sometimes place more value on relationships than warranted by the situation. Those with weak *accommodation* often focus on being "right" and have difficulty seeing other perspectives. Others may see them as stubborn and unreasonable, or uncaring about others' feelings.

Negotiators that are predisposed to *compromising* are eager to find an agreement that will close the negotiation. Seen as friendly and reasonable, compromisers often grasp the first fair solution that presents itself and are vulnerable to choices made without adequate fact-finding. Those low on *compromising* often have strong principles and passion, but are subject to standing on principle when common sense dictates otherwise; they can be seen as stubborn and irritating.

People that favor *avoiding* dislike confrontation and will dodge all situations that lead to disagreement. This can manifest as diplomacy and tact and can be very helpful in tense negotiations. Well-handled conflict avoidance can bring difficult groups to agreement. However, important information is often not brought into the open due to fear of difficult conversations. Those who have low *avoiding* preferences have a high tolerance for assertive or even aggressive conversation. They are often seen as lacking tact or being overly confrontational. However, they can be valuable in some bargaining situations.

Those predisposed to *collaborating* enjoy solving tough problems through negotiation and work hard to find a best solution. When negotiations reach claiming value stages, these people may fail to gain their share of the resources due to their wish to build consensus and are vulnerable to competitive negotiators. Those low in *collaborating* prefer a more controlled detail oriented process and may lose clarity and focus in the seeming chaos of a group setting.

Negotiators that prefer *competing* like to win and they enjoy negotiating because it provides a contest. Although highly skilled in the processes of negotiation, their style is dominating and can damage relationships. Because intangibles are difficult to assign a value to, they often focus on the tangible aspects of bargaining, leaving value on the table. Those low on *competing* value fairness and trust, but because they are seen as non-threatening, in some situations they will be at a disadvantage.

Although people have preferred styles, becoming comfortable with recognizing, working with, and practicing other styles is important, since your counterparts in negotiation may have a different preferred style. Depending on the bargaining situation in which you find yourself, your style may help you be more successful or be a source of weakness. Self-awareness can help you mitigate the negative effects and capitalize on the positive.

Table 1. Strategies for Negotiations

Importance of Future Relationship	Degree of conflict: High	Degree of conflict: Low
High	1. Balanced (business partnership, joint venture, committee) *Best strategies:* Collaboration Compromise	2. Relationships (marriage, friendship, work team) *Best strategies:* Accommodation Collaboration Compromise
Low	3. Transactions (market transaction, practice sale, house sale, divorce) *Best strategies:* Competition Collaboration Compromise	4. Tacit conflict avoidance through cooperation (roadway intersection, airplane seating) *Best strategies:* Avoidance Accommodation Compromise

4. Steps for Integrative Negotiation

A sequence of steps for an integrative negotiation can be followed as a collaborative activity:

1. What is the problem?
   a. Depersonalize it.
   b. State the problem as a specific goal to be attained.
   c. Explore all possible aspects of the problem.
   d. Explore all related issues.
   e. What is most important? What is least important?
2. What obstacles must be overcome to achieve the goal?
3. What interests are present? Interests are underlying concerns, needs, desires and fears that motivate a negotiator to take a particular position.
   a. Are they substantive? (related to the focus of the negotiation)
   b. Are they process-oriented? (related to how the negotiation unfolds)
   c. Are they relationship-based? (related to value placed on relationships)
   d. Are they interests "in principle"? (related to fairness or values)
4. Explore why certain solutions are wanted by certain parties: why do they want what they want?
5. What criteria will be used to judge proposed solutions? Agree on criteria before generating solutions.
6. What are possible solutions to solve the problem?
   a. Generate multiple alternatives.
   b. Avoid judging or evaluating solutions while brainstorming.
   c. Avoid ownership of solutions.
   d. Ask outsiders.
   e. Attempt to expand the pie, creating additional value.
7. Evaluate solutions on the basis of the criteria previously determined.
   a. Narrow choices by rank ordering.
   b. Judge solutions on how good they are and how they will be accepted by those implementing them.
   c. Consider combining options into packages that please multiple interests.
   d. Try to reach consensus rather than voting in order to have full commitment by all parties for implementation of negotiated settlement.
8. Formalize the agreement,

5. Strategies for Negotiations

Strategies for negotiations can be chosen from a model that balances the importance of future relationships with the perceived degree of conflict. Used along with self-awareness of preferred style, one can confidently take an approach to resolve differences using recommended strategies (Table 1).[9]

You will notice that the strategies listed mirror the styles discussed earlier. In the broader categories of cooperative and competitive strategies, both have strengths and weaknesses and can be effective in different circumstances. However, multiple studies have shown that the most successful negotiators, even in professional settings such as union negotiations, are cooperative rather than competitive.[10] This is reflected in the preference for cooperative strategies given in the rubric.

6. Conclusion

Negotiation is unavoidable and takes place regularly during the ordinary events of our work and personal lives. Understanding the difference between distributive and integrative negotiations, knowing our preferred negotiation styles, being fa-

miliar with the process of an effective negotiation, and understanding the best strategies for different situations all can contribute to negotiation with more satisfying outcomes.

Acknowledgments

Conflict of Interest

The Author declares no conflicts of interest.

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Clinical Communication Skills That Optimize Collaboration Between Equine Referring Veterinarians and Specialists

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1. Introduction

Coordinated veterinary care amongst all providers is critical to positive client and patient outcomes. Referring veterinarians (rDVMs) are the primary providers of veterinary care for horses and determine if and when referral to a specialist or referral center is necessary. As such, the influence of rDVMs on the caseload of the referral centers is significant. Given the importance of the rDVM to the ongoing health of the horse and the success of the referral center, purposeful attention should be paid to the relationship between rDVMs and the specialists to whom they refer.

2. Relevance

The needs and expectations of equine referring veterinarians have remained largely unexplored. Michigan State University (MSU) conducted an investigation into the satisfaction of rDVMs with the MSU Large Animal Veterinary Teaching Hospital.[1] The study revealed scheduling difficulty and poor communication as challenges that rDVMs faced with the service provided to them. In response to a question about how the teaching hospital could improve, 16.8% of rDVMs responded "improving communication" and 6.3% responded "respecting referring veterinarians."[1] Similarly, the results of a study investigating the opinions of small animal rDVMs regarding communication and nutritional product recommendations made by small animal clinicians at veterinary teaching hospitals found that there were notable differences between how Veterinary Teaching Hospital (VTH) veterinarians and rDVMs perceived many elements of their interactions and communications.[2] Specifically, differences with regard to the desire of rDVMs to receive recommendations regarding nutrition and the timing of the receipt of discharge information and were found; eight percent of the veterinary teaching hospital veterinarians indicated that they communicated with the rDVM at discharge, while 50% of the rDVMs indicated that they felt this occurred.[2]

More is known in the field of human medicine, where the factors that influence to whom primary care physicians refer, as well as primary care physician-specialist interactions, have been investigated.[3–8] There is strong evidence that the relationship between the referring physician and specialist impacts the likelihood of referral. Specifically, competence and good communication between

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the referring physician and specialist were found to be primary considerations when referring physicians cited reasons for specialist selection.[3] Furthermore, previous experience with or knowledge of the specialist, quality of prior feedback, mutual respect, and likelihood of return of the patient to the referring physician for primary care were also found to be important factors.[3,5–8] Physicians also cited patient-related factors, such as the referring practitioner's belief that the patient would have a good experience and patient management, as considerations in their decisions of where to refer.[8] These findings support the importance of rDVM-specialist interactions and relationship.

3. The Need for Collaboration and Communication

A commentary based on the work of the Northeast Veterinary Liaison Committee, comprised of large and small animal rDVMs and board-certified specialists, academicians, and administrators, suggested guidelines for rDVM-board-certified specialist interactions regarding referral care, based on the premise that there is a greater need for communication, professional respect, and collegiality in the field of veterinary medicine.[9] The need for communication between the rDVM and specialist prior to admission, at the time of first examination, and for the duration of the time the patient is under the care of the specialist is underscored. Emphasis is also placed on the need for communication between the specialist and rDVM in the event that problems other than the initial complaint that prompted referral arise.[9]

The needs of rDVMs when they engage a specialist to provide care for a patient are not necessarily all that different from those of a lay client seeking the services of a veterinarian; both the rDVM and specialist have information critical to a positive client and patient outcome. As such, collaboration, defined as "to work with another person or group in order to achieve or do something,"[10] is an appropriate descriptor and model of referral interactions to use as a guide. Effective working relationships require mutual respect and trust. Timely and frequent communication between the rDVM and specialist facilitate collaboration and contribute to the formation of a respectful and trusting relationship.

4. Facilitating Effective Collaboration Using Clinical Communication Skills

The skills discussed in the following text can be used during all stages of the referral experience; however, particular attention should be given to the initial conversation between the rDVM and specialist regarding the case since it sets the stage for the referral experience. The following points should always be addressed: the rDVM's goal for the care to be provided, the services and expertise the specialist can offer, the cost of care being discussed, and each party's expectations of the other's involvement or role. When the patient is discharged and returned to the care of the rDVM, it is important that there be clear communication about the care that the patient received while at the referral center, the care the patient needs going forward, and how that care is to be provided. Both rDVMs and specialists can use communication skills as a framework for the effective exchange of information, goal setting, and plan formation during their interactions; these skills also serve to build and enhance relationships.

Open-Ended Inquiry

Open-ended questions or inquiries are those that cannot be answered with a simple "yes" or "no"; they necessitate the provision of a thoughtful response to the question asked.[11] Ascertaining the reason for referral and understanding the goals of referral care are critical in setting the groundwork for a successful referral experience; both of these can be accomplished through the use of open-ended questions. Furthermore, as the course of referral continues, open-ended questions can be used to gain information about what the other party is thinking and invite his/her participation into planning. Using open-ended questions also demonstrates respect and how one values another's opinion. Examples of open-ended inquiries are, "Tell me how you think I can be of help in this case?" and "What do you think would be the best course of treatment in this case?"

Attentive Listening

The ability to listen well is essential to successful communication and is not as simple as merely hearing what the other person has to say. Attentive listening is comprised of several skills: the use of pausing after the question has been asked to provide time for the other party to respond, paraphrasing the responder's answer, and nonverbal listening cues.[11] Pausing allows the other party time to formulate a thoughtful response and demonstrates commitment to two-way communication. Paraphrasing statements can begin with, "What I'm hearing you say is…" or "If I understand you correctly…" Many nonverbal cues are not apparent when communication occurs over the telephone, as most referral conversations do; as such, the cues provided by vocal tone and speed of speech have increased importance. The use of an even tone and unhurried pace indicate that one is listening and giving the conversation his/her full attention. During face-to-face conversations, head nodding, eye contact, and turning one's body in the direction of the speaker are nonverbal cues that indicate attentive listening. One cannot be an attentive listener if one is focused on planning one's response; one must direct his/her full focus to the speaker. Listening attentively demonstrates respect, facilitates the accurate exchange of information, and enhances understanding of the other's perspective.

Empathy

Empathy is a two-step skill; the first step involves one's understanding and appreciation of another's

experience or situation. The second step is an outward, verbal or nonverbal, demonstration of that understanding back to the other party.[11] Empathy necessitates an attitude of curiosity and respect. It is important to recognize that the use of empathy does not indicate agreement with another's perspective or endorsement of their beliefs. The value of empathy in rDVM-specialist interactions is that it creates an increased awareness of the other party's position, which can allow for more accurate communication, as well as facilitating collaboration and teamwork. For example, "I can understand your hesitation about working with this client again" and "I can imagine this has been a frustrating case for you to try to manage in the field."

Contracting

Contracting, from a communication skill perspective, is the negotiation of what roles each party will play in the course of care.[11] In the case of rDVM-specialist interactions, it can be used to determine what roles and responsibilities each party will take for a given referral prior to the transfer of care occurring, whether that be at admission or discharge. The benefit of contracting is that a clear, mutually acceptable, and agreed upon plan is formed. Furthermore, involving both parties in the creation of the plan respects the knowledge and experience of the rDVM and specialist alike. Contracting can also be used to set out the frequency and type of communication that will occur between the rDVM and specialist during the course of referral care. Doing so will facilitate ongoing collaboration and increase the likelihood that communication will occur that satisfies both parties. The formality of the contract can vary significantly, from a mutually constructed written document to an informal verbal agreement, based on the needs of the case and of the rDVM and specialist involved. The endpoint of a verbal contracting conversation could be: *rDVM:* "Alright, so you're going to do the arthroscopy with your resident, Dr. K. She is then going to be in charge of the case until the horse goes home. Dr. K will call me each day with an update and I'll speak with the client about how things are going. Do I have that right?" *Specialist:* "Sounds good to me."

Safety Netting

Safety netting is the creation of a back-up plan in the event that the initial plan for care is no longer suitable,[11] and should take place at the same time as determining the initial plan for care. It should also occur at the time that the patient's care is returned to the rDVM. The discussion of contingencies in advance of the need to change the plan provides an opportunity for a comprehensive plan to be negotiated and decreases the potential for conflict in the event that unforeseen circumstances do occur. A safety net can be as simple as, "If anything other than what we've discussed arises, I'll give you a call."

5. Overcoming Resistance

When resistance is encountered, it can be due to a misalignment of goals or an incomplete understanding of the other party's position, needs, or expectations. The use of open-ended inquiry, attentive listening, and empathy facilitate the development of common ground, which, once found, becomes the foundation for the conversation and often decreases or eliminates resistance. Finding similarity is a powerful step towards partnership, collaboration, and effective communication; in the case of referral care, both the rDVM and specialist can likely agree that their ultimate goal is to provide the best possible care to the patient. The use of signposting, a communication skill that introduces the next phase of the interaction,[11] can also be beneficial. Using a signpost statement can serve to provide a rationale for the topic of conversation one is introducing; by providing the other party with a purpose for a particular thread of the discussion, it increases their willingness to participate. For example, "This client has been with me a long time. I'm hoping we can discuss what's going to happen when this horse is at the hospital so I can prepare her for it" and "I'm hoping we can chat about how to treat this case. While he's been here, we've noticed some things that weren't noted in the history you sent." Sharing thinking is another communication skill that can help facilitate a collaborative interaction[11] and decrease resistance. It provides the other party with insight into one's reasoning and can serve to share information in a nonconfrontational and nonjudgmental fashion. An example of how one might share their thinking behind a request for frequent communication is, "I'm thinking that this client is going to be a bit high maintenance and will likely need some hand-holding. If you and I can talk daily about what's going on, then I'm happy to talk to him about the horse. I think doing it that way will make your life a bit easier."

6. Conclusion

- The relationship between the rDVM and specialist impacts the quality of care provided to the patient, as well as the experience of the client.
- Research has shown that there is room for improvement in the communication between rDVMs and specialists.
- The use of clinical communication skills, including open-ended questions, attentive listening, and empathy, facilitates effective communication regarding referral care.
- Before you end a conversation where referral care is being arranged, ensure that a contract for care has been created and safety net measures have been discussed.

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Conflict of Interest

The Author declares no conflicts of interest.

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Breaking the Silence: Disclosing Medical Errors

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In circumstances where a medical error results in an adverse outcome, a thoughtful response on the part of the veterinarian, staff, and practice is required. The insurance carrier should be contacted as soon as possible. The fear of formal complaints and potential malpractice suits can cloud judgment when considering the best course of action. This presentation will review communication techniques for constructively responding to these difficult situations. Author's address: University of Florida, College of Veterinary Medicine, 2015 SW 16th Ave., Gainesville, FL 32610; e-mail: housea@ufl.edu. © 2014 AAEP.

1. Introduction

There has been increasing public awareness of patient safety in human healthcare and attention on a disturbing rise in medical errors. Further, research on the relationship between communication and malpractice risk has shown that the majority of malpractice litigation in human medicine is related to poor communication between the patient and the physician.[1–3] In addition, the importance of having open and frank discussions when medical errors occur in healthcare has been highlighted by several professional organizations and institutions and published in the scientific and news media.[4–10]

In human medicine, there have been numerous changes that have taken place to address the complex problem related to the rise in medical errors and patient safety through accreditation standards, state legislation, and professional expectations. One notable change has included human hospital requirements implemented in 2002 requiring that healthcare providers have open discussions with patients or their representatives whenever outcomes differ significantly from the anticipated. Many organizations and states have come forward to encourage forthrightness when there has been a disappointing outcome due to medical error. These requirements have fueled the call for specific training for healthcare providers in meeting the challenge of engaging in such honest yet difficult conversations. While many healthcare providers are adept at sharing bad news with their patients in a sensitive and empathic manner, when the bad news is an adverse outcome due to a medical error it requires additional tools, insight, and practice.

In veterinary medicine, it is also evident that veterinarians and healthcare staff must possess the necessary skills and understanding to communicate effectively when clients and families are disappointed with the outcome of care. In order to address this constructively, clinicians must understand what caused the disappointing outcome, how the client and family feel and think about it, and then respond in a timely and empathic manner. When the adverse outcome is the result of medical error, it requires a particularly thoughtful response on the part of the veterinarian, staff, and practice. To be clear, an adverse outcome is unanticipated or unexpected harm caused by medical care. An example of an adverse outcome would be a horse

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breaking its leg during anesthetic recovery. As we know, this is a potential complication of anesthesia that can occur without any veterinary error. A veterinary error is failure to complete a plan as intended or the use of a wrong plan to achieve a clinical result. Examples of a veterinary error would include administering the wrong medication or wrong dose to a patient.

In addition to their sensitivity to the client and family's experience of disappointment when there have been adverse outcomes in care, clinicians and healthcare organizations are deeply fearful of formal complaints and potential malpractice suits. As a result, simply telling clinicians that they "ought" to disclose an unanticipated outcome or error is ineffective. Recognizing this, Breaking the Silence: Disclosing Medical Errors, was developed in conjunction with the American Veterinary Medical Association Professional Liability Insurance Trust (AVMA PLIT) to enable veterinarians to identify, appreciate, and practice using a model and techniques that are essential in responding to clients constructively when there has been an adverse outcome due to error.

The model recommended for disclosure discussions is the TEAM model:

- Truth, teamwork, transparency
- Empathy
- Apology and accountability
- Management through to resolution

2. Discussion: The Team Model

Truth, Teamwork, Transparency

Truth

The client is entitled to an accurate understanding of what happened and its consequences. The key to that understanding is the practice's candid investigation to sort through factors until reaching the most probable explanation. Reluctance to accept responsibility unless 100% certain is setting a much higher standard of certainty than was used to recommend and undertake the treatment in the first place and will likely seem very self-serving to the client.

Transparency

Trust is rebuilt most effectively when the client senses that nothing is being hidden from them. The practice willingly shares relevant information and elicits and answers clients' questions without the client needing to do her own investigation and come up with the "right questions."

Teamwork

The practice team must feel empowered to recognize and report problems that could potentially compromise patient safety so that the practice may take action to correct the problem. Team members are most likely to feel that they can do this when the practice team has shared values, adopts a nonpunitive approach to errors, and a commitment to honesty with each other and with clients. The ultimate resolution of the harm with the client may involve a number of staff members working together to recognize, elicit, and then follow through on the steps needed to resolve the situations as well as possible with the client.

Empathy

Empathy involves the ability to share one's appreciation for the client's perspective, feelings, and experience. When clients experience disappointing outcomes related to the health of their animal, they may express a range of emotions that require effective and sensitive communication skills by the veterinarian and staff. When the disappointment is related to a medical error on the part of the practice, the emotions may be heightened particularly if the practice gives any suggestions that they are withholding information or are insensitive to the client's feelings and needs. Core skills such as reflective listening and empathy are critical in such discussions, for example, "I know you are angry and that is perfectly understandable."

The veterinarian must be willing to acknowledge and act based on an appreciation of the consequences of the error to the patient and the client. Rationalization and minimization can undermine our ability and willingness to empathize with the full range of impacts. The veterinarian and practice team must be willing to appreciate the impact that recognizing and reporting an error may have on the staff involved. Their fears, heartbreak, perhaps defensiveness, and concern for consequences for themselves are all normal and need to be acknowledged. Creating a nonpunitive approach to errors that focuses on understanding and addressing latent vulnerabilities to error rather than punishing individuals is a key to empathizing with staff.

Apology and Accountability

When error has caused harm, a sincere and complete apology is necessary for resolution.[11] "I am so sorry for the error that we made that caused this to happen to Sandy." Studies of apology reveal 4 critical components:

- Acknowledging the error
- Explaining how it occurred
- Holding oneself accountable to prevent its recurrence
- Offering reparation for the harm the other has experienced

The client wants to know that some good will come from the harm for other animals treated by the practice through correcting the procedures that led to the error. In order to convince the client that this will happen, the practice (see T above) must

demonstrate that it has made its best effort to understand what actually happened and its causes and is willing to reveal that to the client. Finally, after recognizing the error caused unnecessary harm to the animal and the client, an offer to do what one can to "repair" the damage and "make the client whole" as much as possible is essential to create resolution.

Management

Each of the steps above serves only as a promise until they have been successfully enacted to the client's satisfaction. That means managing a number of elements through to their final resolution in the client's mind:

- Take the best clinical care of the animal in accordance with the client's wishes. The practice is likely to be continuing to provide and advise the client about the animal's medical care. This includes honest and sensitive shared decision making about treatment options, possibility of transfer to another practice, and how costs of care will be handled.
- Psychologically and perhaps legally, the client is likely to feel taken advantage of if the practice appears to be profiting in any way from providing care that caused harm or that is in response to harm it has caused. If an error in care has essentially undone the benefit of effective treatment immediately preceding it, the practice must see that from the client's perspective, being charged even for the effective care probably feels inequitable. As a result, financial responsibility must be sensitively negotiated, and a third party mediator may help to establish a standard of fairness if there is disagreement. This is particularly important if the client chooses advanced and costly treatments at another practice.
- Managing the situation through to resolution can take a number of conversations over an extended period of time as new questions, choice points, and practical and financial consequences of the error and harm become more evident. The practice should plan to keep in regular contact with the client until full resolution appears to have been reached.
- Your malpractice liability insurance carrier will obviously have an interest and want to have a say in how disclosure and resolution of harm due to error is managed. It could be asked to make payments to your client on your behalf. Include your insurance carrier's claims department early on in this process. Be ready to discuss with them your investigation, your practice's values around disclosure and resolution, as well as being open to their procedures for accepting liability and offering compensation.

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How to Determine Fetal Gender in Early and Advanced Gestation

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1. Introduction

A wealth of information on equine fetal anatomy and physiology has been acquired in recent years. Ultrasonographic fetal monitoring techniques have documented, in great detail, fetal growth and organ development at various stages of gestation. Ultrasonographic anatomy of fetal sex organs, from the genital tubercle to fully developed organs, has been extensively described in its entire progression.[1–7] Fetal gender determination in the mare provides a useful management tool to breeders by allowing a predelivery estimation of the value of offspring. Knowing fetal gender in advance of delivery allows for commercial strategies to be implemented, as the value of stock at sales time is often determined by the gender of the offspring. Furthermore, it is well established that some stallions have a greater proportion of quality female versus male offspring or just the opposite. Finally, culling of broodmares is easier when fetal gender is known.

2. Diagnostic Windows for Fetal Gender Determination

First Stage Diagnosis: Early Gestation

There are two different stages when the diagnosis of fetal sex can be determined by ultrasonography. The first stage is between 57 and 70 days gestation and involves the identification of the genital tubercle (Fig. 1) by transrectal ultrasonography.[1] The genital tubercle, the precursor of the penis in the male and the clitoris in the female, appears around day 55 of gestation as a hyperechoic equal sign (=), located between the fetal hindlimbs, at an approximately equal distance between the tail and the umbilicus. As gestation progresses, the genital tubercle migrates towards the tail in the female fetus (Fig. 2) and towards the umbilical cord in the male (Fig. 3). The shape of the genital tubercle may change over time, appearing trilobed (Fig. 4) or conical (Fig. 3).

The early stage technique requires good equipment and considerable expertise, allowing consistent fetal sex determination within a small diagnostic window (optimal time days 59–68) and by a single diagnostic parameter. Best imaging will be accomplished when the fetus engages high up into a uterine horn (Fig. 5) and the fetus to transducer distance is greatly reduced. After day 70, the fetus tends to reside more deeply in the mare's uterus/abdomen and is not consistently accessible for imaging by transrectal ultrasonography due to the disproportionate increase in fetal fluid volume compared to fetal body mass, typical of this stage of gestation. After day 100, the fetus can be reliably found within the mare's pelvis.

NOTES

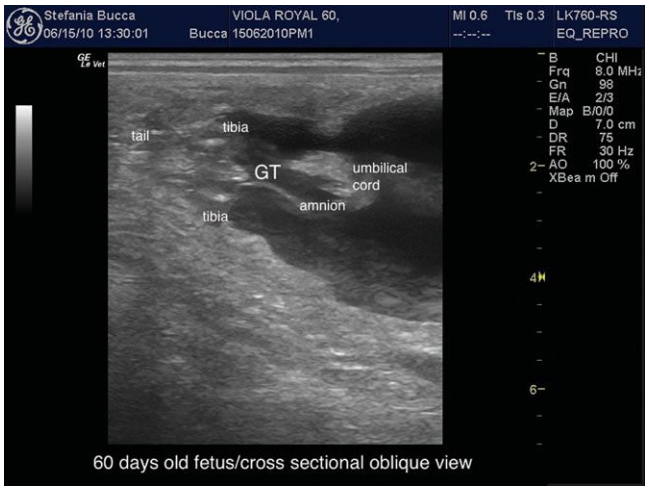

Fig. 1. Transrectal, cross-sectional oblique view of a 60 day-old male fetus (8 MHz linear transducer). The genital tubercle (GT) is seen emerging between the hind limbs and caudal to the umbilical cord.

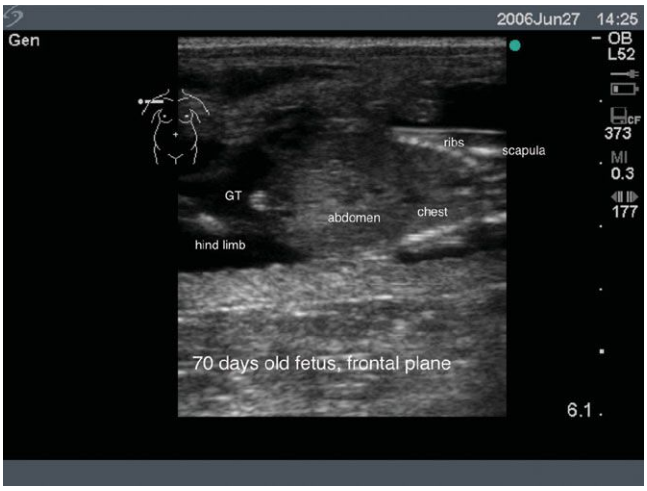

Fig. 3. Frontal plane view of a 70 day-old male fetus by US per rectum (7.5 MHz linear transducer); the GT presents with a conical shape. The chest and scapula are identified cranially (to the right of the sonogram), and the outline of the diaphragm can be barely detected between chest and abdomen.

Second Stage Diagnosis: Advanced Gestation

The second stage for fetal sex diagnosis avails of a much wider diagnostic window between 100 and 260 days gestation. Multiple parameters can be used to validate diagnosis (fetal primary sex organs) at this stage, but a combination of transrectal and transabdominal ultrasound scanning may be required.

Fetal presentation plays a substantial role in the diagnostic approach in advanced gestation. Establishing fetal orientation should be readily accomplished in the course of the examination, knowing that in the fetus in posterior presentation the hindquarters will be easily accessible per rectum and expedite transrectal diagnosis. The active nature of the equine fetus determines frequent changes in presentation up to 9 months gestation, making transrectal examination potentially diagnostic even in advanced pregnancy. The ability to change presentation decreases as gestation advances,[6,7] as fetal size and the encasement of the hindquarters within the fetal horn prevent further rotations along the short axis. After 9 months, fetal sex can still be determined transrectally in grossly undersized fetuses and when a posterior or transverse presentation persists. A transabdominal approach will be required for diagnosis when the fetal hindquarters are out of reach of the operator's hand per rectum, as it commonly occurs from around 5 months gestation and when the fetus is in anterior presentation (Fig. 6).

Fig. 2. Cross sectional oblique view of a 60 day-old female fetus, by US per rectum (8 MHz linear transducer). The GT is seen emerging from the buttocks.

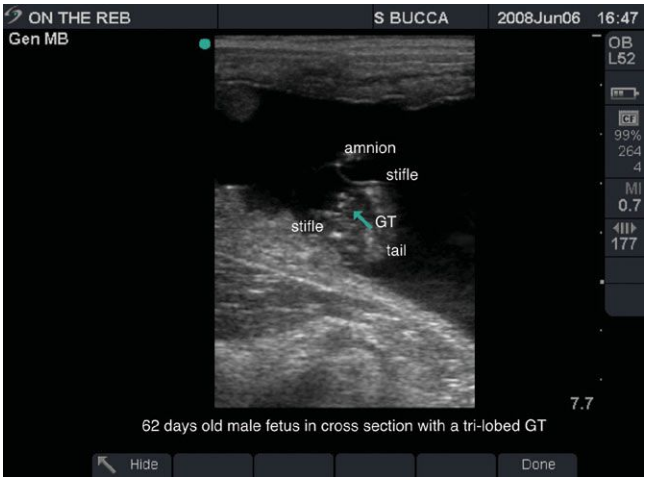

Fig. 4. Cross sectional view of a 62 day-old male fetus with a trilobed GT, US per rectum (7.5 MHz linear transducer).

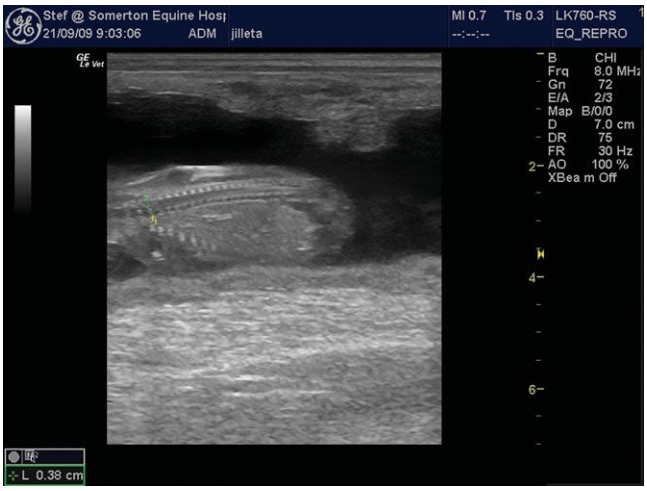

Fig. 5. Detailed sonographic image of a 75 day-old fetus engaged within the uterine horn, in close proximity to the transducer (linear 8 MHz).

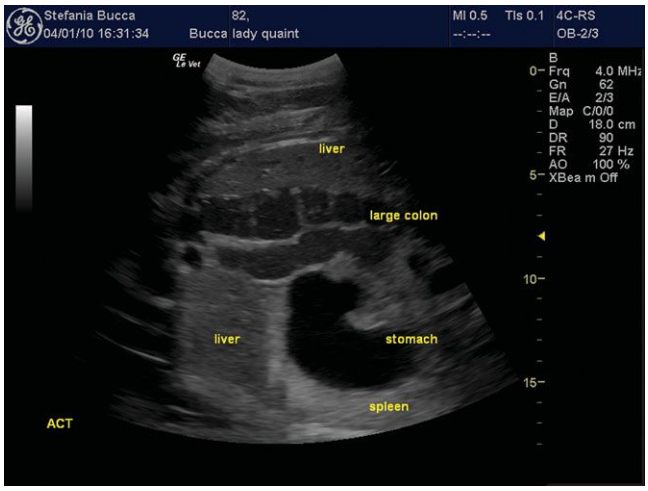

Fig. 7. Transabdominal scan of the abdominal content of a 7 month-old fetus (4 MHz convex transducer).

Finally, fetal gender determination in advanced gestation can be carried out during summer, fall, or early winter, at a more convenient time of the year for the busy equine reproduction clinician.

3. Equipment

B-mode, real-time portable scanners, equipped with 5 to 7.5 MHz linear-array transducers are commonly employed for assessment per rectum. Linear, sector, or convex 3.5 to 6 MHz transducers can be used transabdominally up to day 260, depending on the size of mare and fetus, the thickness of the mare's ventral abdomen, and the stage of gestation. Occasionally, a 2.5 MHz transducer may be required for adequate visualization of the relevant fetal structures. Doppler technology offers a valuable additional tool in the evaluation of the fetal gonad, both in transrectal and transabdominal scans.[8]

4. Techniques

Transrectal sonographic viewing of the equine fetus requires standard rectal palpation skills as per routine ultrasound (US) examination of the mare's reproductive tract. Thorough cleansing of the mare's abdomen is necessary for diagnostic percutaneous US evaluation. Mares are best examined in stocks and although not usually required, sedation of the mare in advanced gestation reduces fetal activity and lowers the fetus towards the ventral abdomen, enhancing transabdominal imaging. Sedation is contraindicated when a trans-rectal approach is adopted. Clipping, shaving, alcohol, and/or coupling gel application can optimize percutaneous imaging. During the summer months, excellent imaging can be obtained by simply sponging alcohol over the unclipped hair of the ventral abdomen.

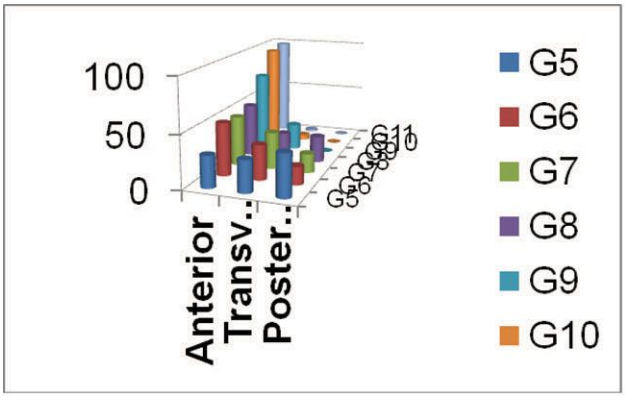

Fig. 6. Incidence of fetal presentation throughout gestation; on x-axis month of gestation, and on y-axis percentage of observed fetal presentation.[7]

Fig. 8. Transrectal view of a 108 day-old female fetus in posterior presentation: right of the sonogram mare's caudal (8 MHz linear transducer).

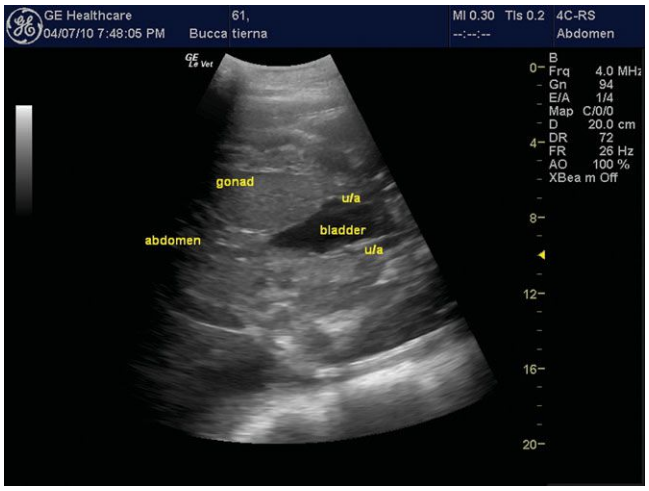

Fig. 9. Transabdominal frontal view of a 7 month-old fetus, showing the gonad in close proximity to the bladder and the associated umbilical artery (4 MHz convex transducer).

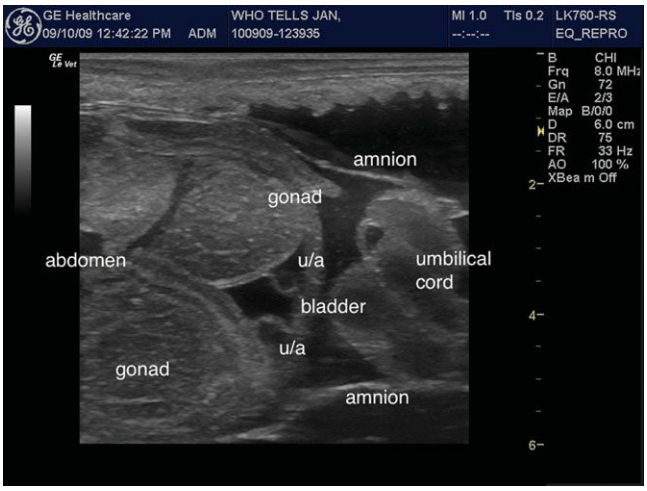

Fig. 11. Transrectal scan of a 110 day-old filly fetus in transverse presentation, where both gonads can be seen in cross sectional view (8 MHz linear transducer).

Orientation of the sonogram should be initially established in order to identify the scanning direction on the screen of the ultrasound unit. The transducer is then advanced along the sagittal plane within the rectum or over the ventral abdominal wall until fetal parts are recognized. The fetal heart and chest will be located first, as they are easily identified; cranial and caudal orientation of the fetus will be determined next. Fetal head and neck indicate cranial while the bean-shaped, echolucent stomach silhouette is used as a landmark for caudal orientation (Fig. 7). Dorsal and ventral orientation is established by identifying the spinal cord and, opposite to that, the umbilicus. Fetal presentation and position will subsequently be defined. As the equine fetus tends to be a rather dynamic entity, temporary obstruction of the view will intermittently occur due to superimposing umbilical cord loops or fetal bony structures (mostly pelvic limbs) casting acoustic shadows over the areas of interest. Quite often, during early stage diagnosis, the fetus will just disappear from the acoustic field. Considerable time and patience are required during the learning process curve, but an experienced operator will accomplish diagnosis in a short frame of time, ranging between 30 s and 5 min.

Gender determination is made by scanning of the caudal fetal abdomen, hindquarters, and buttocks to identify the position of the genital tubercle or the anatomical structure of primary sex organs. Frontal, cross-sectional and oblique scanning planes may all be required to obtain adequate visualization of

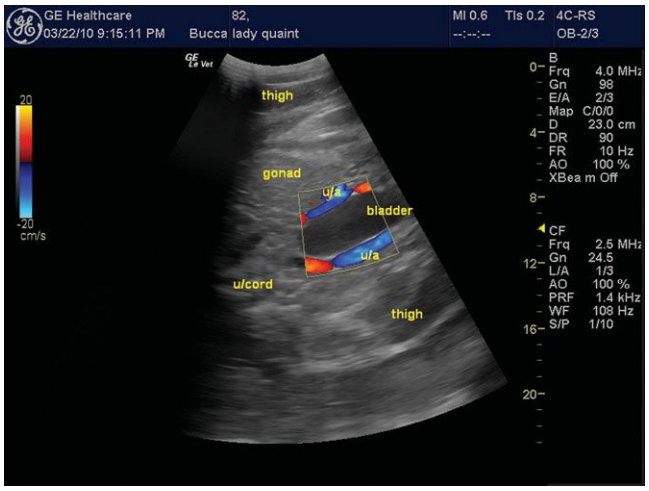

Fig. 10. Transabdominal frontal view of the caudal end of a 7 month-old filly fetus (4 MHz convex transducer). Doppler color flow identifies the two umbilical arteries adjacent to the bladder.

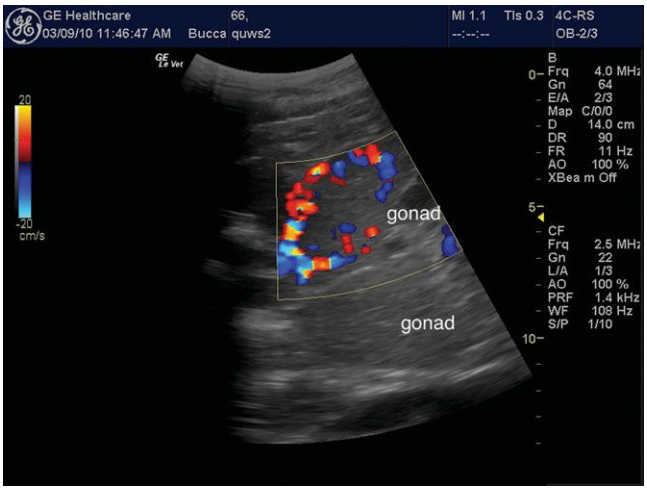

Fig. 12. Strong color Doppler signal over the peripheral layer (ovarian medulla) of an 8 month-old female fetus (transabdominal examination, 4 MHz convex transducer).

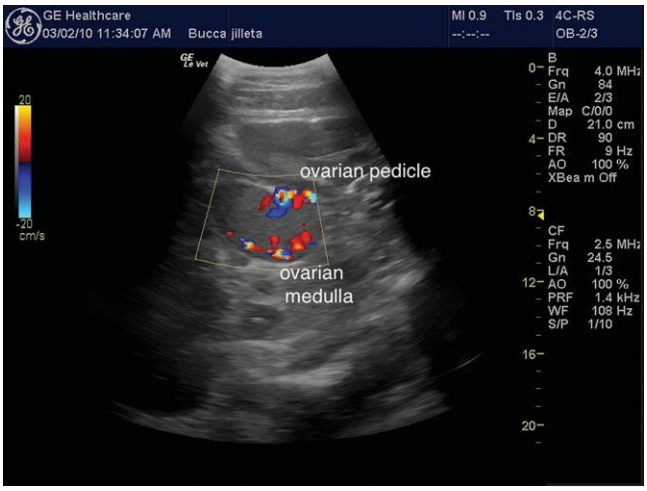

Fig. 13. A strong color Doppler signal is detected over the ovarian pedicle and partially on the peripheral layer of this 7 month-old filly fetus (transabdominal examination, 4 MHz convex transducer).

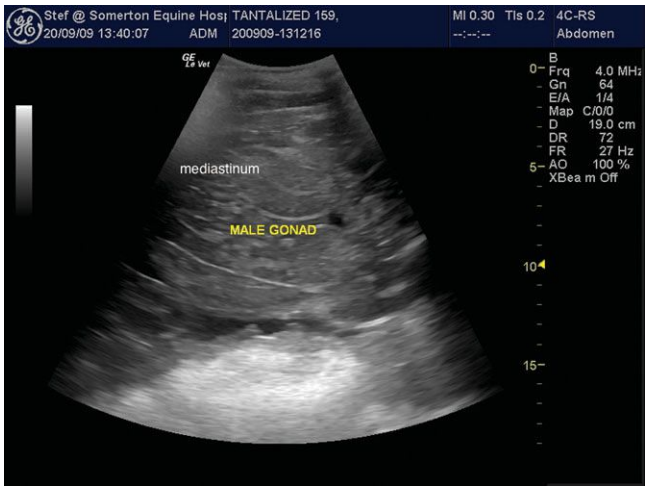

Fig. 15. Transabdominal scan of a 5 month-old fetus. The gonad shows a typical male structure, with a hyper-echoic, longitudinal, central line (mediastinum) (4 MHz convex transducer).

diagnostic parameters, particularly during early fetal gender determination.

5. Diagnostic Parameters in Advanced Gestation

Fetal gonads are easily identified within the caudal abdomen as two symmetrical oval structures, ventral to the kidneys, with an oblique orientation of their long axis, converging caudally towards the pelvic inlet (Fig. 8). The caudal poles of the gonads are adjacent to the bladder and in close contact with the abdominal tract of the umbilical arteries (Figs. 9 and 10). The fetal gonads represent an excellent landmark within the caudal abdomen and show a distinctive echotexture that differs from male to female,[8] adding great diagnostic value when assessed during the course of a fetal sexing examination. A marked diversity in echotexture can be appreciated in the female gonad between cortex and medulla (Fig. 11), with intense color Doppler signal over the outer band of tissue (Fig. 12), demonstrating high vascularization of the peripheral area. An additional area of intense color Doppler signal can be visualized on the outer lateral portion of the female gonad, corresponding to the ovarian pedicle (Fig. 13). Male gonads appear uniformly echodense, with a small outer dotted area (Fig. 14) and a hyperechoic longitudinal, central line (mediastinum) (Fig. 15). Intense color Doppler signal is detected in these two areas, as they correspond re-

Fig. 14. Transabdominal scan of an 8 month-old male fetus. An outer dotted area can be identified over the lateral aspect of the gonad, corresponding to the pampiniform plexus (4 MHz convex transducer).

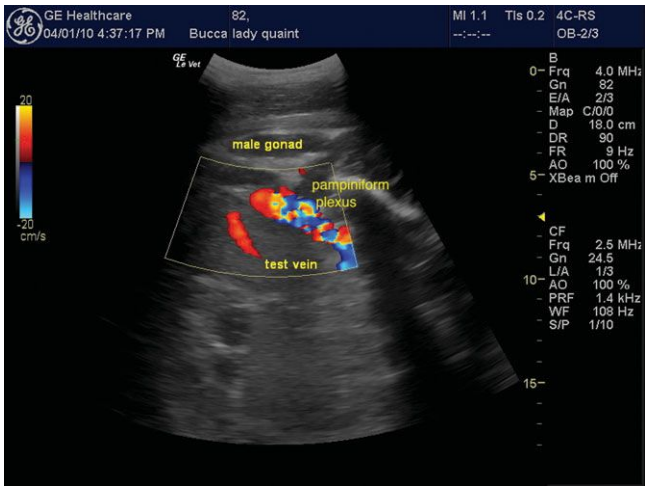

Fig. 16. Same fetus as in Fig. 14 when color Doppler technology was applied to the ventral pelvic area. A strong color signal identifies the location of the pampiniform plexus and, within the gonad, the testicular vein (4 MHz convex transducer).

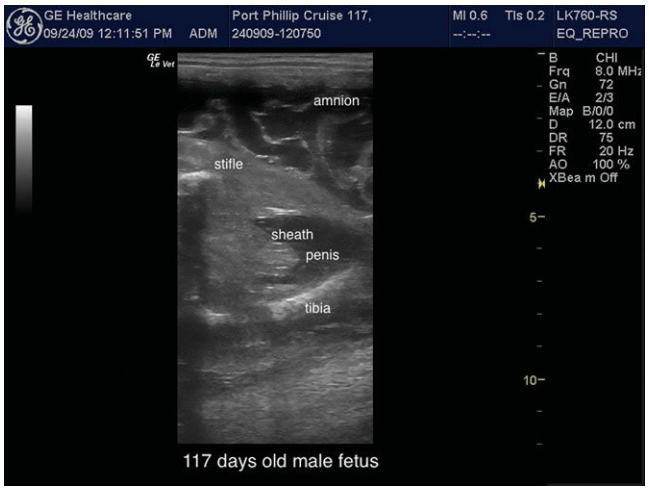

Fig. 17. Transrectal scan of the pelvic area of a 117 day-old male fetus in transverse presentation (8 MHz, linear transducer). The penis appears encased within the sheath.

Fig. 19. Transabdominal view of the pelvic area of an 8 month-old male fetus. The urethra can be seen running over the full length of the flaccid penis, as a hyperechoic, linear structure (4 MHz convex transducer).

spectively to the pampiniform plexus and the testicular vein (Fig. 16). The pampiniform plexus appears larger than the ovarian pedicle and displays a more intense color Doppler signal.

The fetal primary sex organs may be clearly identified on ultrasound as early as 100 days gestation. In the male fetus, a fully comprehensive gender diagnosis will include the identification of penis and prepuce (Figs. 17–19), scrotum/testicular compartments (Figs. 20 and 21), urethra (Fig. 22), and gonads. The penis is visualized in the ventro-caudal abdomen, just behind the umbilicus. Sometimes the umbilical cord's strong pulsatile activity causes a passive bouncing motion to the penile shaft that is resting over the umbilical arteries (Fig. 23). The penis may be partially or completely encased within the prepuce but can often appear fully extended and occasionally erect. In the erect penis, the urethra is visualized in cross section as a distinct circular hyperechoic structure (Fig. 24). The urethra can be easily visualized along the ventral shaft of the flaccid or erect penis as a double hyperechoic line. Longitudinal and cross-sectional images of the urethra can be obtained over the male perineum. The fetal scrotum displays a composite echodensity, as the scrotal compartments appear as two symmetrical, oval, less echodense areas (Fig. 20). The hypoechoic appearance of each scrotal compartment relates to the presence of the adjacent gubernaculum testis. The male fetal gonads appear uniformly echodense with a hyperechoic central line,

Fig. 18. Transabdominal view of the pelvic area of a 7 month-old male fetus. Note the close association of the erected penis in cross section over the umbilical cord (4 MHz convex transducer).

Fig. 20. Transabdominal view of the pelvic area of a 6 month-old male fetus. The scrotum bulges out ventrally with a prominent midline raphe (4 MHz convex transducer).

Fig. 21. Transabdominal view of the pelvic area of a 5 month-old male fetus. The perineal urethra merges into the testicular compartments (4 MHz convex transducer).

Fig. 23. Transabdominal view of the pelvic area of a 7 month-old male fetus. The penis rests over the umbilical cord (4 MHz convex transducer).

correspondent to the testicular vein, as described above.

In the female fetus, the primary sex organs to be visualized to reach diagnosis include mammary gland, nipples, vulva/clitoris, and gonads. The fetal mammary gland can be visualized in the pubic region and appears triangular (Figs. 25 and 26) or trapezoidal (Fig. 27) in shape and uniformly echodense. The nipples emerge from the ventral border of the mammary gland as relatively large hyper-echodense areas (Fig. 28). No relevant structures can be visualized over the ventral perineum (Fig. 26), as opposed to the male fetus, where the urethra runs the entire length up to the anus (Fig. 22). The fetal clitoris is a hyperechoic structure that bulges out of the buttocks (Fig. 29). It is positioned high up in the perineum but should not be confused with the anus, adjacent to the tailhead. The vulvar commissure can be seen coursing between the anus and the clitoris in a cross-oblique section of the fetal buttocks (Fig. 30).

6. Diagnosis

Diagnosis by a single exam per rectum is rapidly attained when the fetus is in posterior presentation, even up to 8 months gestation. The rate of positive diagnosis per rectum reaches 100% between 110 and 130 days gestation, with an estimated time of less than 150 s.[9] In transverse presentation, gender determination per rectum is easily accomplished

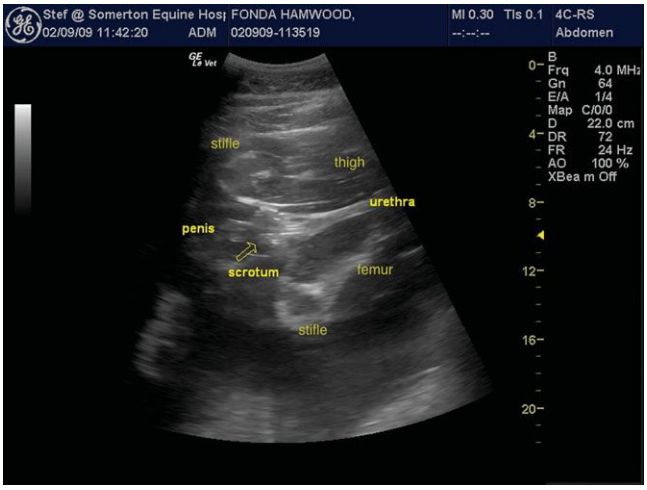

Fig. 22. Transabdominal view of the perineum of a 5 month-old male fetus. The urethra fills out the perineal area as a double line structure, in longitudinal section (4 MHz convex transducer).

Fig. 24. Transrectal scan of a 170 day-old male fetus in transverse presentation. The urethra can be viewed as a hyperechoic circle in a cross-sectional view of the erected penis (7.5 MHz linear transducer).

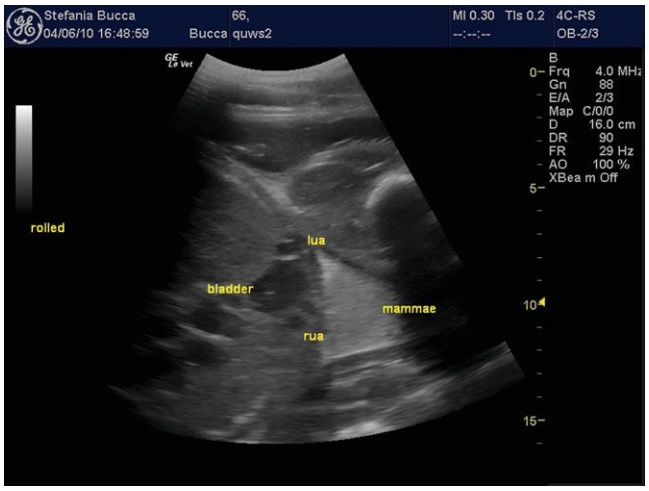

Fig. 25. Transabdominal view of the pelvic area of an 8.5 month-old female fetus. The mammary gland appears as a hyperechoic triangular structure (4 MHz convex transducer).

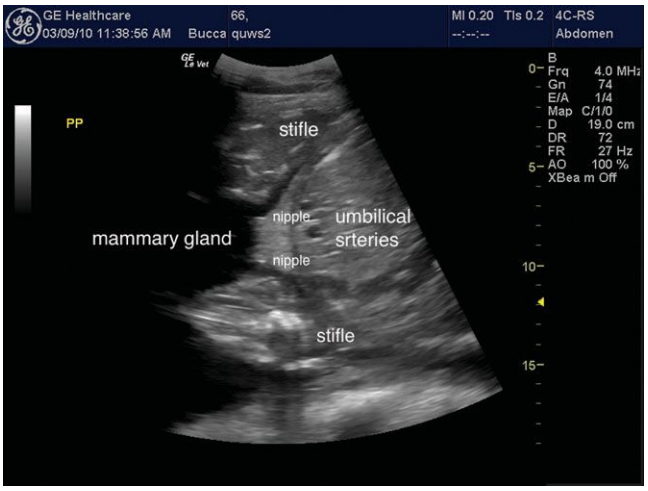

Fig. 27. Transabdominal view of the pelvic area of an 6.5 month-old female fetus. The mammary gland looks like a trapezoidal shaped structure and the nipples emerge as hyperechoic projections of its ventral border (4 MHz convex transducer).

when the fetus assumes a ventrocaudal position within the mare's pelvis. In anterior presentation, the fetal hindquarters can be visualized transrectally up to 5 months gestation according to fetal size and location within the uterus. Rotation of the fetus over the long and short axis is commonly observed up to 8 months of pregnancy, and frequent changes of presentation occur around 5 to 6 months. At this time, repeating the exam 5 to 10 min later may find the fetus in a more advantageous position for diagnosis. A transabdominal approach is usually necessary for gender determination over 5 months of gestation, when the fetus lies in anterior presentation.

Good knowledge of fetal anatomy and rapid identification of fetal parts are essential for ease of diagnosis. Diagnosis in advanced gestation should be based on at least three identified parameters. The time required to conduct a diagnostic examination is 1 to 5 min for the experienced examiner. Variability depends on the difficulty encountered in visualizing the area of diagnostic interest, and a very active fetus generally makes a poor candidate for a rapid diagnosis. Video recording of scans provides opportunities for further studies and detailed evaluations.

Finally, proper identification of the mare at the time of examination and the provision of a signed certificate of fetal gender diagnosis should be an integral part of the service offered.

Fig. 26. Transabdominal view of the pelvic area of an 198 day-old female fetus, showing the mammary gland and the perineum (4 MHz convex transducer).

Fig. 28. Transabdominal view of the pelvic area of a 6 month-old female fetus showing the mammary gland and one nipple (4 MHz convex transducer).

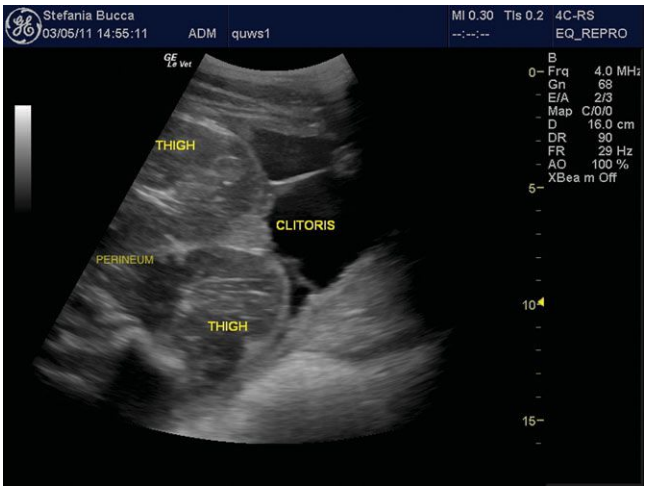

Fig. 29. Transabdominal view of the perineal area of an 5.5 month-old female fetus. The clitoris bulges out of the buttocks (4 MHz convex transducer).

Fig. 30. Transabdominal view of the perineal area of a 7 month-old female fetus, displaying the vulvar lips and the clitoris (4 MHz convex transducer).

Acknowledgments

Conflict of Interest

The Author declares no conflicts of interest.

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How to Assess the Equine Pregnancy by Ultrasonography

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1. Introduction

Multiple routine and emergency circumstances in equine practice may require an in-depth evaluation of the pregnancy. Ultrasonography (US) offers an effective and noninvasive method to assess fetoplacental health throughout gestation. Proper fetal imaging is crucial in fetal gender determination and in the diagnosis and management of twin pregnancies, two diagnostic techniques that practitioners in the field should strive to perfect.[1–5]

Pregnant mares presenting with a vaginal discharge and/or premature lactation should be thoroughly evaluated for fetoplacental health using ultrasonography. In addition, significant derangement in feto-maternal exchange mechanisms and placental function, triggered by maternal medical or surgical conditions, may compromise fetal well-being and ultimately endanger fetal survival. Under these circumstances, the need for a comprehensive assessment and monitoring of fetal well-being is warranted. Regular monitoring of fetal well-being should continue once weekly for several weeks, even after maternal recovery from the original complaint. Finally, routine scanning of term mares is carried out on some breeding farms to assess presentation and identify other factors that may complicate birth, suggesting the need for assistance to the mare at delivery or to the neonate shortly after birth.

Ultrasound offers safe and continuous viewing of fetal life in the mare, from completion of fetal organogenesis (day 40) to term. The combination of transrectal and transabdominal scanning techniques provides extensive investigation of fetal growth and development and monitoring of the fetal environment.[6–13] Doppler ultrasonography represents an additional tool to assess fetal viability by characterizing blood flow through maternal, fetal, and placental circulations.[14] Clinical applications of Doppler blood flow velocimetry in the pregnant mare are currently being investigated, showing great promise as novel diagnostic instruments in the evaluation of fetal health.

Equipment and Technique

Linear, convex, and sector ultrasound technologies can all be successfully used to image the equine pregnancy. Complete US fetoplacental assessment is based on the acquisition of a series of biophysical parameters that requires both transrectal and transabdominal imaging. Transrectal US scanning provides access to the caudal aspects of the gravid uterus and should always be performed at any stage of gestation.

NOTES

Fig. 1. Transrectal scan of the orbital area of a fetus in anterior presentation at 275 days gestation; mare's tail: right of sonogram.

Fig. 2. Transabdominal scan of the cranial chest of a term fetus; measurement of the aortic diameter is taken at the aortic arch as it emerges from the heart in the left hemithorax, ventral to the spinal cord.

Transrectal US usually employs linear technology with frequencies ranging from 5 to 10 MHz. Convex and sector transducers better adapt to the curvilinear contour of the mare's abdomen and are preferred for percutaneous ultrasonography of the gravid uterus. The equine fetus becomes visible by this route from day 100 of gestation onward. A wide range of frequencies, spanning from 2 to 8 MHz, is needed by this route to reach scanning depths of up to 30 cm and to visualize the growing fetus from midgestation to term. Optimal skin preparation greatly enhances image quality, as per standard percutaneous ultrasonography.

Sonographic Profile of the Equine Fetus

The "sonographic" profile of the equine fetoplacental unit requires the establishment of a minimum database to ensure adequate fetal growth and development and demonstrate appropriate levels of activity and responsiveness within an adequate environment.

Fetal Growth and Development

Several parameters can be measured to estimate fetal size. Orbital diameters/eye volume[11,13,15,16] (Fig. 1), aortic diameter[11,13] (Fig. 2), bi-parietal diameter (Fig. 3), and to a lesser extent fetal chest and femur length[17] have all been reported as useful indicators of fetal growth. The aortic diameter correlates to fetal size more efficiently than any other anatomical structure and measurement should be taken in systole, on a longitudinal scan of the dorsal fetal left hemithorax, in close proximity to the spinal cord (Fig. 4). US provides excellent anatomical detail of the entire fetus in mid to advanced gestation (Figs. 4–8), when fetal sexing diagnosis can be easily accomplished and congenital abnormalities identified. Some of the developmental abnormalities detected during late gestational scans include microphthalmus, hydrocephalus, small and large intestinal segmental atresia, and renal abnormalities. After 9 months gestation, the quality of the image may decline due to fetal size and positioning within the mare's abdomen. Ossified remnants of the vitelline sac can be visualized from early to late gestation (Fig. 9), usually with no need for concern.[18]

Fetal Activity and Responsive Patterns

Fetal activity and tone reflect central nervous system (CNS) function and development, with decreased activity and declining muscular strength resulting from depressed CNS function. Activity is required to ensure satisfactory muscular development and skeletal joint function, allowing for successful postnatal adaptation. Dormant (inactive) phases are observed at all stages of pregnancy but

Fig. 3. Transrectal scan of a 110 days-old fetus in anterior presentation, with measurement of the bi-parietal diameter. Mare's tail: right of sonogram.

Fig. 4. Transabdominal scan of a 6 month-old fetus, displaying the anatomical features of the dorsal chest and cranial abdomen.

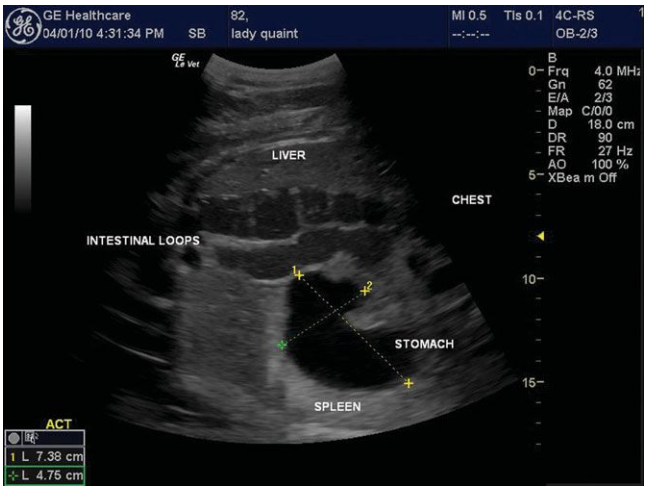

Fig. 6. Transabdominal scan of an 8 month-old fetus, displaying the anatomical features of the abdomen.

are more common and prolonged in late gestation, where they can last up to 60 minutes or longer on occasion. Lack of fetal movements and sudden bouts of excessive activity followed by abrupt cessation have both been associated with a negative outcome.[5] Rhythmical breathing movements may be observed in all fetuses in advanced gestation (from 7 months), when the diaphragm is visualized (Fig. 5). Nevertheless, fetal breathing is intermittent in nature and cannot be consistently evaluated.

Fetal Heart Rate

Fetal heart rate (FHR) and FHR reactivity represent the most sensitive indicators of fetal well-being. Cardiac frequency, obtained by M-mode echocardiography and automatically estimated by the cardiac calculation software (Fig. 10), declines as gestation progresses and increases during activity, with accelerations of 25 to 40 beats per minute of approximately 30 seconds duration. Sustained tachycardia[12,13] or a large range of FHRs may indicate fetal distress but could be brought on by painful maternal systemic problems or excitement. Sustained bradycardia,[12,13] inappropriate FHR for gestational age, or lack of heart rate reactivity suggests CNS depression, probably attributable to hypoxia and may indicate impending fetal demise. Fetal cardiac rhythm is usually regular, and cardiac arrhythmias[19] are commonly associated with a negative outcome. Cardiac activity may also be estimated by assessment of peripheral pulses, particularly by the fetal carotid pulse, easily accessible by US per rectum in the fetus in anterior presentation.[20]

Fig. 5. Transabdominal scan of an 8 month-old fetus, displaying the anatomical features of the ventral chest, the cranial abdomen, and the diaphragm.

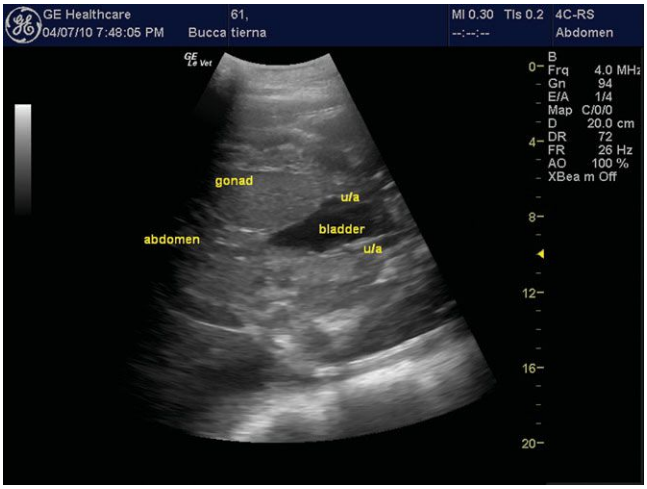

Fig. 7. Transabdominal scan of a 7 month-old fetus, displaying the anatomical features of the ventrocaudal abdomen.

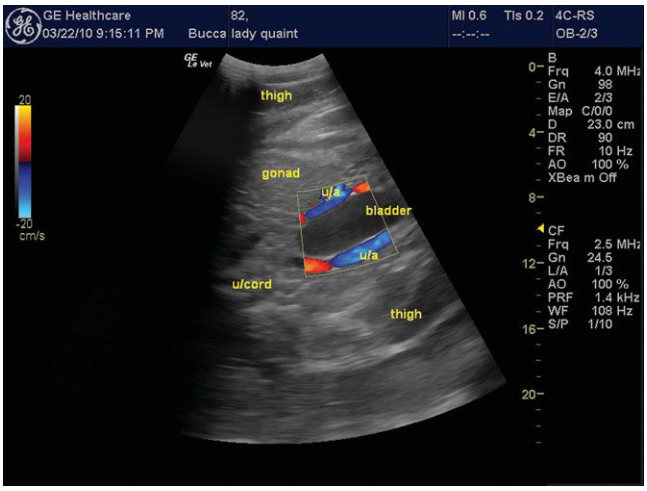

Fig. 8. Same scan as Fig. 7 with additional color Doppler signal, identifying the umbilical arteries adjacent to the bladder wall.

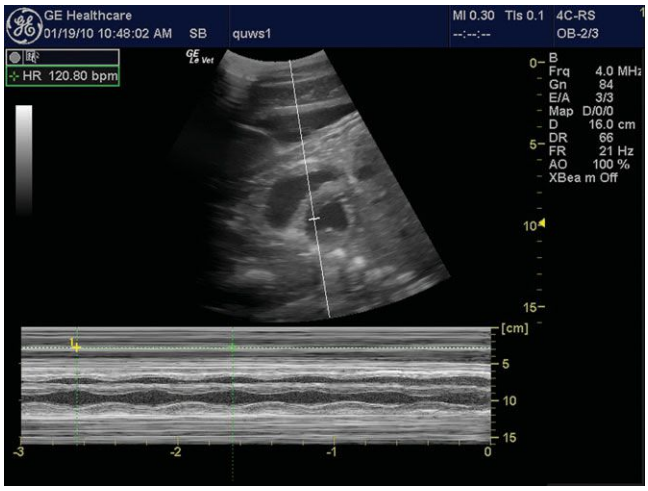

Fig. 10. Fetal heart rate assessment in a 6 month-old fetus, by transabdominal, M-mode ultrasonography.

Adequate Environment

Evaluation of fetal environment includes assessment of fetal orientation, volume and quality of fetal fluids, combined thickness and contiguity of the utero-placental unit, cervical relaxation, and should confirm the presence of a single fetus.

Fetal Orientation: Presentation

Abnormal presentation causes dystocia, and early detection may prevent a serious perinatal crisis by implementation of specific strategies at delivery. Under normal circumstances, fetal mobility gradually declines as gestation advances, and after 9 month rotation along the short axis is restricted by fetal body size and the encasing of the fetal hindlimbs within the gravid uterine horn.[21,22] Detection of an abnormal presentation after 9 months gestation should raise concern and be investigated as term approaches to formulate an appropriate plan of action.

Volume and Quality of Fetal Fluids

The equine pregnancy includes an allantoic and an amniotic compartment. The distribution of allantoic fluid is directly related to fetal dynamics and uterine tone, with no preferential area of maximal fluid depth detectable. Amniotic fluid tends to collect more frequently around the cranioventral half of the fetus (Fig. 11). Minimal and maximal allantoic and amniotic fluid depth values are reported in the literature.[10–13,23]

Free-floating particles (vernix) are commonly observed swirling within the fetal fluids and become more visible during episodes of fetal activity, particularly in the amniotic sac. The hippomane is de-

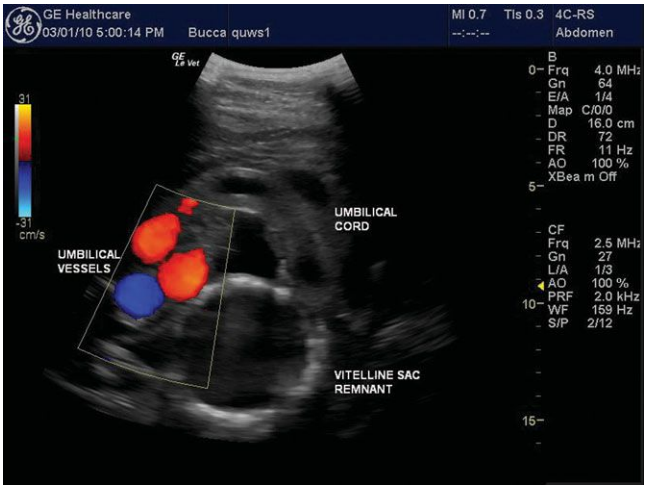

Fig. 9. Transabdominal sonogram of an 8 month-old fetal umbilical cord, with an attached ossified remnant of the vitelline sac.

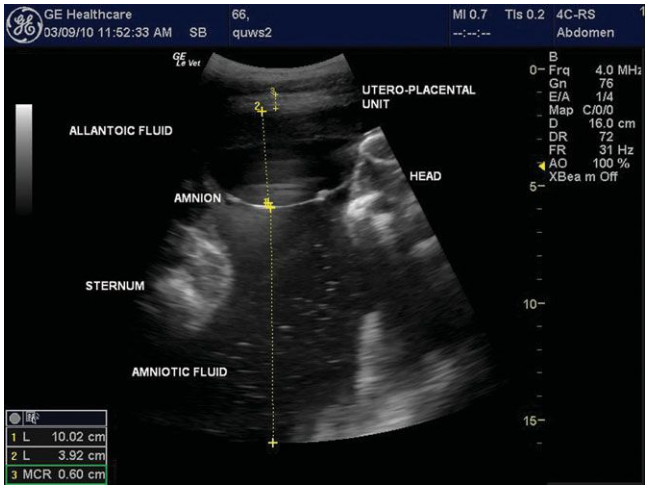

Fig. 11. Transabdominal sonogram of a 6 month-old fetus, showing measurement of fetal fluid depths.

Fig. 12. Transabdominal scan of a term fetus, where a hippomane can be visualized between the fetal chest and the ventral uterine wall.

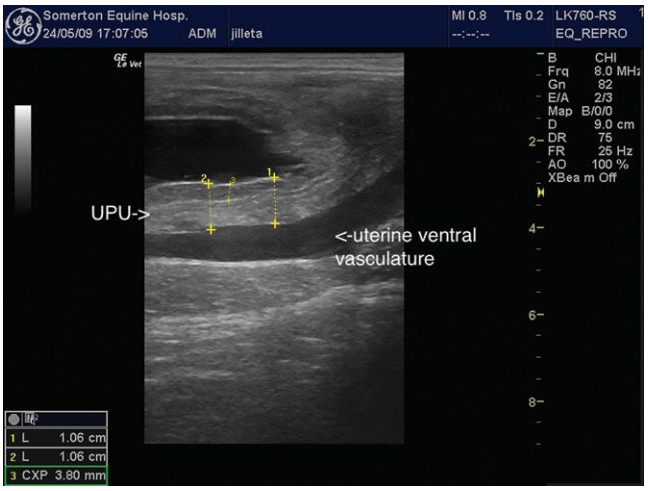

Fig. 13. Transrectal scan of the cervical pole in a term pregnancy and measurement of the CTUP.

fined as an allantoic calculus and can often be visualized floating within the allantoic fluid, with a typical oval shape and onion like, concentrical structure (Fig. 12). Sudden release of meconium (fetal diarrhea) in the amniotic compartment may sometimes be observed in highly distressed fetuses just prior to birth, stillbirth, or abortion. Pathological increases in fetal fluids have been reported (hydramnion and hydroallantois).[23–29] Markedly reduced volumes of amniotic fluid (olygohydroallantois) may be observed in mares suffering from severe systemic illness. An association of the condition with a poor fetal outcome has been reported.[30] Objective assessment of fetal fluid depth requires extensive scanning of the mare's abdomen and is best carried out during phases of fetal quiescence.

Combined Thickness and Continuity of the Utero-Placental Unit

The literature reports reference values for the combined measurement of the utero-placental unit at different stages of gestation[31–36] (Fig. 13). Both uterus and placenta should present with similar echotexture up until term, when diffuse sonolucency of the allantoic layers of the placenta may be observed. Adequate utero-placental contact should also be maintained throughout gestation. However, small areas of separation of the placental membranes and uterus are commonly observed in normal pregnancies without any apparent effect on the health of the fetus. An average combined thickness of the utero-placental unit of 1.26 ± 0.33 cm has been reported in mares with normal term pregnancies. Measurements should be taken avoiding areas of compression of utero-placental thickness by the fetus, using the ventral uterine vasculature as landmark. The utero-placental thickness is affected by numerous factors, which may reduce the efficiency of placental function. Such conditions include inflammatory, degenerative, and vascular changes with/without associated edema of fetal membranes, potentially resulting in premature placental separation. Large and/or progressively enlarging areas of placental separation may lead to inefficient exchanges, adversely affecting fetal growth and well-being and resulting in red bag delivery at parturition and decreased neonatal viability. The non-pregnant horn presents with a folded, thickened appearance and, although normal, could be mistakenly interpreted as utero-placental thickening (Fig. 14). The lumen of the non-pregnant horn is relatively small but may suddenly increase to accommodate larger volumes of fetal fluids in response to uterine dynamics and fetal shifting; a marked reduction in CTUP of the non-pregnant horn can be observed under these circumstances (author personal observations).

Fig. 14. Transabdominal scan of the non-pregnant horn in late gestation.

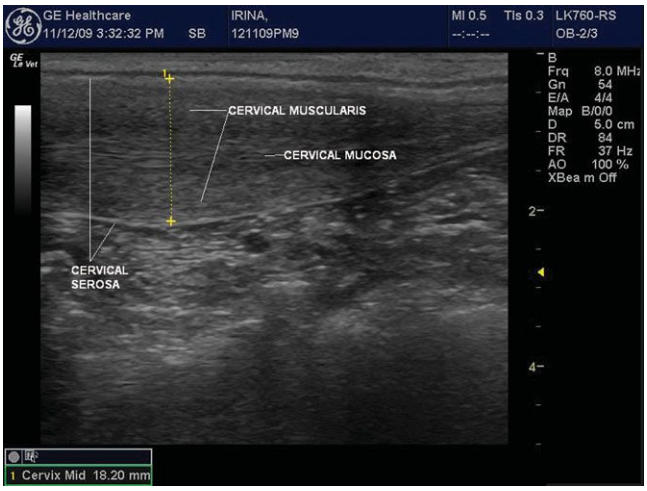

Fig. 15. Transrectal scan of the caudal third of a Grade 2[37] cervix at 6 months gestation.

Fig. 17. Color and spectral Doppler flow evaluation of the left uterine artery at 8 months gestation.

Cervical Parameters

Recent data on cervical size and echotexture in the pregnant mare suggest a high degree of cervical tone maintained up to 9 months gestation, followed by progressive cervical relaxation until delivery.[37] A high degree of correlation between cervical size and sonographic appearance was also demonstrated (Figs. 15 and 16).

Doppler Ultrasonography

Doppler ultrasonography represents an additional diagnostic instrument to characterize blood flow in the pregnant mare and provides an insight on fetal (umbilical and carotid arteries), maternal (uterine arteries) (Fig. 17), and placental circulations (intraplacental vessels). In addition, two distinctive color Doppler signal patterns differentiate male from female fetal gonads, offering an auxiliary tool in the diagnosis of fetal gender.[38]

Doppler ultrasonography has become an important clinical instrument for the assessment of placental performance in healthy and high risk human pregnancies, but applications to the equine pregnancies are still limited due to the lack of reference values. In normal pregnancies, haemodynamic changes in the uterine arteries progress from a high resistance/low flow pattern during the first half of gestation to a low resistance/high flow system in the second half.[39] The transition correlates closely with the onset of placental angiogenesis in response to fetal growth and the development of the placental microcirculation. Ousey et al (2012) reported a threefold total blood flow volume increment during late gestation (210 days to term) when fetal body mass seemingly increases three to fourfold.[39]

Doppler velocimetry indices of the umbilical vasculature and carotid artery are currently being investigated in order to establish fetal hemodynamic patterns throughout gestation. Signs of circulatory derangement indicating fetal hypoxia and intrauterine-growth-restriction could then be identified as routinely done in the US evaluation of the human pregnancy.

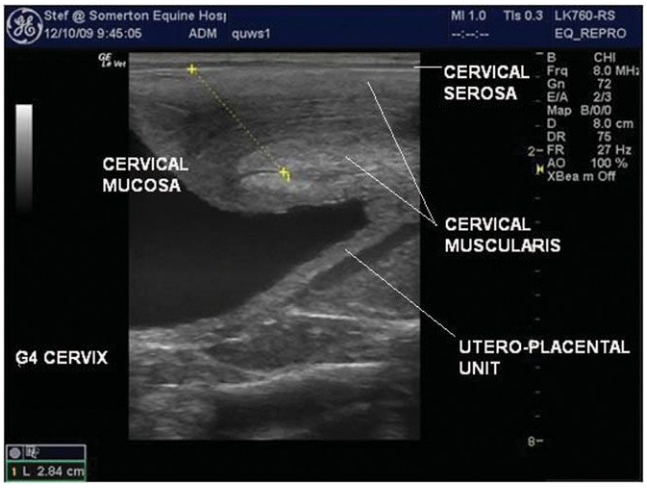

Fig. 16. Transrectal scan of the cranial third of a Grade 4[37] cervix at 10 months gestation.

Acknowledgments

Conflict of Interest

The Author declares no conflicts of interest.

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How to Perform Hysteroscopy in the Mare

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1. Introduction

Hysteroscopy is not a common procedure in broodmare practice. A thorough breeding soundness examination that includes transrectal palpation and ultrasonography, endometrial cytology, endometrial biopsy, endometrial culture, and vaginal/cervical speculum examination will detect many reproductive maladies frequently encountered in the mare. However, in some circumstances, these more commonly employed techniques are insufficient to clearly identify or ameliorate the problem, and hysteroscopy has been used to achieve this end. In an early study, 17 of 40 mares admitted to a veterinary teaching hospital with a history of infertility had reproductive tracts that were considered normal on palpation, yet hysteroscopy revealed abnormalities in 26 of these 40 mares.[1] Since that time, abnormalities observed through the use of transrectal ultrasonography have either obviated the need for or alternatively prompted the use of hysteroscopy to render a more definitive diagnosis or treatment.

The use of hysteroscopy in the mare was described as early as the 1960s.[2] Subsequently, many authors have published articles on the use of hysteroscopy in the mare for a variety of purposes.[1,3–9] While the term hysteroscopy will be used throughout this manuscript, it is also called uteroscopy and has been referred to as examination of the uterus via fibroscopy, endoscopy, and videoendoscopy in other publications.

2. The Equipment

A flexible videoendoscope, 100 cm in length and 1 to 1.4 cm in diameter, is commonly used for hysteroscopy. The unit should have sufficient light intensity and image quality to optimize visualization of the uterine lumen. Most modern units are equipped with a video monitor, which enhances the examination. The use of recording equipment to capture still and video images is highly recommended to enable review of the procedure and to provide a record of the examination. Ideally, the insertion tube should have a working channel that accommodates ancillary equipment such as catheters, grasping forceps, biopsy forceps, electrocautery loops, and laser fibers.

Since endoscopy equipment is used for examination of a variety of body cavities, it is important that it be thoroughly cleaned and sterilized according to the manufacturer's instructions between uses. Particular attention should be paid to areas where moisture may accumulate, as these sites provide an environment that facilitates the growth of *Pseudomonas aeruginosa*. Persistent endometritis with *Pseudomonas* as well as growth of other organisms has been reported following hysteroscopic proce-

NOTES

dures.[10] If the equipment has undergone chemical sterilization, it is also imperative that the insertion tube, including all of its channels and the water reservoir, be thoroughly rinsed to remove the disinfecting solutions as these chemicals can be very caustic to mucosal tissues.

3. Preparation of the Mare

The mare should be placed in stocks with the tail wrapped and deviated away from the perineal area. Feces should be evacuated from the rectum and the perineum of the mare should be cleansed in an aseptic manner. Sedation and analgesia should be administered. Most practitioners have a favorite sedation protocol, and the following is the author's preferred method. Depending on the size and temperament of the mare, she is administered detomidine HCL[a] (0.006–0.01 mg/kg, IV), and a catheter is placed in the jugular vein. Depending on the anticipated length of the procedure, 5 or 10 mg of detomidine HCL is added to a 500 or 1000 mL bag of saline, respectively, which is then connected to the catheter in a routine manner. The detomidine/saline mixture is then administered by slow drip until the desired level of sedation is achieved. If necessary, more can be administered during the procedure as needed. The author prefers this method because it maintains a more even level of sedation and avoids the wide fluctuations in sedation that can occur over time with repeated larger boluses of sedative. After the mare is sedated and just prior to the start of the procedure, butorphanol tartrate[b] (0.01–0.02 mg/kg) is administered intravenously thought the catheter.

4. Basic Procedure

Under ideal circumstances, at least three people should be present to perform the hysteroscopic procedure; one person remains at the head of the mare for restraint as well as monitoring and administering sedation, one person inserts and maintains the position of the insertion tube through the cervix, and one person operates the steering controls of the insertion tube and directs the overall procedure.

After the insertion tube has been rinsed thoroughly, the image should be "white balanced" to optimize visualization. It is easy to become disoriented once the insertion tube enters the uterus, especially if rotation of the tube has occurred. Therefore it is helpful, prior to inserting the instrument into the mare, for the operator to select a point of reference and become familiar with the directional controls of the steering tube.

A small amount of sterile lubricant is applied to the end of the insertion tube, avoiding contact with the lens, and the assistant wearing a sterile glove and sleeve carries the insertion tube through the vulva and vestibule into the vagina so that the cervix can be visualized (Fig. 1). The cervix is then penetrated and slightly dilated with the index finger, and the insertion tube is passed through the cervix into the uterus. Once inside the uterus, the lumen needs to be distended to allow visualization. While the hysteroscopic procedure can be performed at any time in the mare's cycle, diestrus is preferable because, at that time, the cervix is of sufficient length to be grasped around the insertion tube and maintain uterine distention. Administering progestogens to estrous or anestrous mares for 5 to 7 days prior to the procedure can also accomplish this. In periovulatory mares, the cervix may be sufficiently relaxed that the entire hand carrying the insertion tube can be placed though the cervix into the uterine body and then the hand pulled back against the internal cervical os to act as a plug to maintain distention.

Fig. 1. Endoscopic view of the cervix.

Distention of the uterine lumen can be accomplished by insufflation with filtered air administered via the endoscopic equipment or by a sterile, isotonic fluid such as saline or lactated Ringer's solution (LRS). Note that insufflation with air can cause endometrial hyperemia, which should not be misinterpreted as inflammation. The method of distention depends on clinician preference and the purpose of the procedure. Regardless of which method is chosen, over distention of the uterus should be avoided so as not to cause significant discomfort to the patient or damage to the uterus. One study determined that a hysteroscopy can be performed safely and efficiently within a pressure range of 12.8 to 28.6 mm Hg and that clinical or cardiac signs of discomfort or effects on the circulatory system were only registered after pressures significantly exceeded 100 mm Hg.[11] Precise monitoring of intraluminal pressures is not practical in most clinical environments, so it is recommended to use only the amount of distention necessary to optimize visualization and to continuously monitor the mare for signs of discomfort.

Insufflation allows visualization of normal structures as well as the relative amount and location of

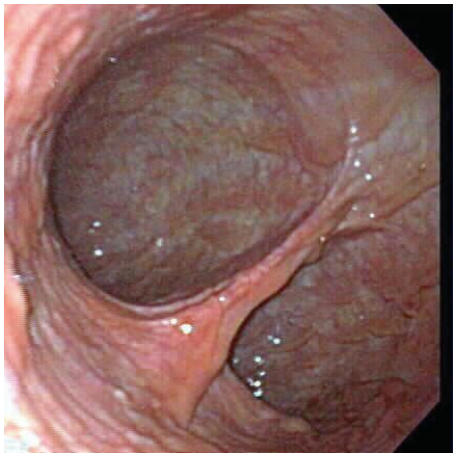

Fig. 2. Bifurcation of the uterine horns viewed after insufflation.

Fig. 3. Uterotubal papilla at the uterotubal junction.

foreign materials such as purulent exudate or blood, etc. Samples of exudate, foreign material, or other abnormalities can be obtained via instruments introduced through the working channel. However, when using insufflation, the lens of the insertion tube may require repeated rinsing to remove mucus or other debris during the procedure. Distention with fluid tends to keep the insertion tube lens free of direct contaminants, but visualization can be compromised if significant amounts of exudate, blood, or other materials become suspended in the distention fluid. If this occurs, drainage or aspiration of the cloudy fluid followed by redistention is recommended prior to proceeding with the procedure. Fluid distention of the uterine lumen can be accomplished using the fluid reservoir or the working channel of the endoscope, but this can take an inordinate amount of time. The author prefers to attach a stallion catheter to the insertion tube with waterproof tape so that the two can be passed through the cervix simultaneously and maintained in proximity. The distal end of a typical IV extension set is then inserted into the belled end of the stallion catheter, and this system is connected to a 5 L bag of LRS or other sterile, isotonic fluid. If drainage of the distention fluid becomes necessary, the extension set can be disconnected and the fluid allowed to escape via gravity flow, or a suction device can be attached to the stallion catheter to facilitate drainage.

Once the uterine lumen is sufficiently dilated, the uterine body and bifurcation of the uterine horns should be visualized (Fig. 2). The insertion tube is directed toward the uterine horn of choice and slowly advanced proximally through the entire length of the horn until the uterotubal papilla at the uterotubal junction (UTJ) is observed. This structure can vary somewhat in appearance being relatively flat and somewhat difficult to visualize in some mares to very prominent in others (Fig. 3). The instrument is then slowly withdrawn to the level of the bifurcation and then the opposite uterine horn is similarly examined. The character and color of the endometrium is observed throughout the examination; it should be relatively uniform in its entirety. The color is typically a pale pink, but the extent of endometrial folds observed can vary with the degree of luminal distention. If the examination is performed during estrus, the surface of the endometrium will typically glisten with mucus, and the endometrial folds will be visibly edematous (Fig. 4).[9] Any abnormalities such as discoloration, ulceration, plaque-like lesions, biofilm, cystic structures, luminal protuberances, adhesions, foreign bodies, or accumulations of blood or exudate should be digitally recorded and sampled as necessary. When the examination is completed and the insertion tube is being removed, the majority of air or fluid used for luminal distention should be removed either passively or by gentle suction. If the fluid distention

Fig. 4. Endometrial folds of the uterus prior to optimal distension.

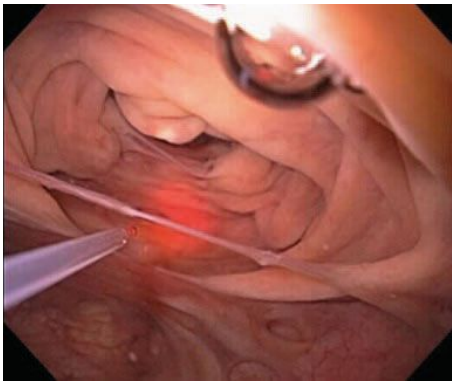

Fig. 5. Laser fiber approaching a thin transluminal adhesion prior to photoablation.

Fig. 7. Multiple transluminal adhesions viewed by hysteroscopy after multilocular fluid filled structures were detected via transrectal ultrasound.

technique was employed, fluid remaining in the bag can then be used to further lavage the uterus.

5. Common Applications

The most common use of hysteroscopy in our practice is for laser ablation of endometrial lymphatic cysts using an Nd:YAG or diode laser. Various methods of cyst ablation using hysteroscopy have been previously described in more detail and improvements in pregnancy rates have been reported following these procedures.[12–16] The cysts are first identified using transrectal ultrasonography, and their size and location are recorded in the mare's record. The mare is prepared as described above, and the entire uterus is examined noting the location of any visible cysts prior to initiating cyst ablation. Either air insufflation or fluid distention can be used. The author prefers to use fluid when possible because, in addition to keeping the lens clean, no smoke is produced while ablating the cyst in the fluid environment as can occur with insufflation, which results in temporary obscurement of the visual field. Another advantage of fluid distention is the beneficial effect of concurrent uterine lavage. Laser ablation can also be used for removal of small transluminal adhesions (Fig. 5). Note, however, that when fluid is used for luminal distention, more wattage will be necessary to achieve the same degree of cauterization as would occur if air insufflation were used.

Hysteroscopy has also been used for direct examination of the endometrium when suspicious, nondefinitive findings are observed via transrectal ultrasonography. Examples include foreign bodies appearing as hyperechoic structures, differentiating of large multiloculated structures as cysts or transluminal adhesions (Figs. 6–8), or suspected uterine tumors.[17–20] Retrieval of foreign bodies and endometrial biopsy procurement via hysteroscopy has recently been described.[8] Attempts to suppress estrus with marbles has been attempted by

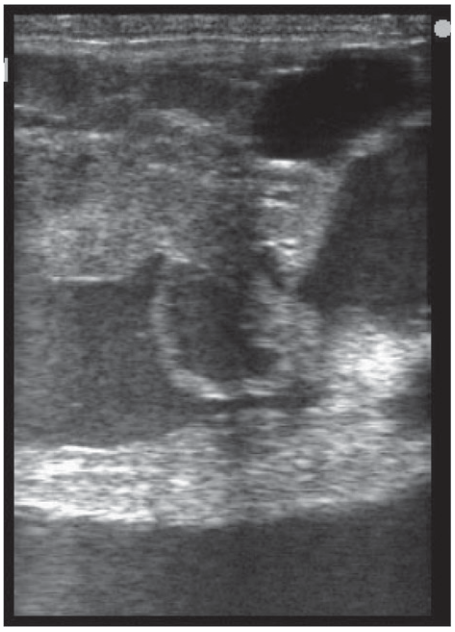

Fig. 6. Ultrasonographic image of the uterus of a mare with large multilocular fluid filled structures.

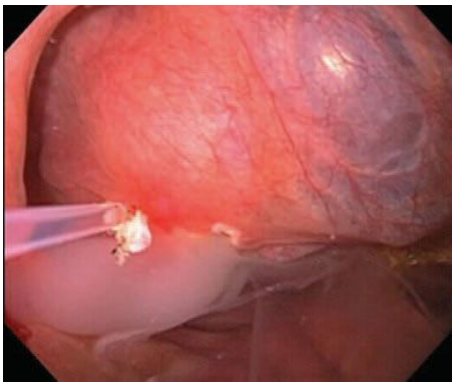

Fig. 8. Purulent fluid being released during laser photoablation of a large transluminal adhesion.

a number of practitioners, sometimes with disastrous results, when multicolored marbles are used and they disintegrate within the uterine lumen causing significant damage. Although large fragments could be retrieved via hysteroscopy, the disintegration often results in numerous minute glass fragments that become embedded in the endometrium and are virtually impossible to remove. While biopsies can be obtained via flexible instruments passed through the working channel, samples obtained are typically small and shallow whereas using a standard biopsy instrument passed along the side of the insertion tube will provide superior samples.[8,21]

Two manuscripts describing direct hysteroscopic insemination of the UTJ with low numbers of sperm were presented at the AAEP meeting in 1998.[22,23] However, achieving direct cannulation of the UTJ is too difficult for routine use and, subsequently, deposition of semen on or near the UTJ was shown to be an effective method of low dose, deep horn insemination.[24] Comprehensive summaries of hysteroscopic insemination studies have been published.[25–27] While hysteroscopy insemination allows direct visualization of the UTJ, semen deposition at the tip of the uterine horn using a manual, transrectally guided technique has been shown to be equally effective and is more economical and efficient for most practitioners.[28]

Recently, Inoue described direct cannulation of the UTJ and deposition of dye into the oviduct as a method of determining tubal patency.[29] He proposed that the difficulty others have experienced in cannulating the UTJ was a result of over insufflation of the uterus, which makes it difficult to center the UTJ in the center of the endoscopic image. Therefore, it was recommended that the endoscope be passed up the uterine horn to the level of the UTJ with minimal insufflation of the uterus.

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[a]Dormosedan®, Zoetis Inc., 333 Portage Street, Kalamazoo, MI, 49007.

[b]Torbugesic®, Zoetis Inc., 333 Portage Street, Kalamazoo, MI, 49007.

How to Perform an Examination of the Internal Reproductive Tract of the Stallion

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1. Introduction

Palpation and ultrasonographic examination of the internal genital tract of the stallion can be included as part of the routine breeding soundness examination. Kenney et al. described palpation of the internal inguinal rings and accessory sex glands per rectum as a routine part of the breeding soundness examination.[1] Examination of the terminal aorta/proximal iliac arteries is a simple addition to this transrectal examination and can provide valuable additional information on the reproductive soundness of a stallion. In stallions with reproductive abnormalities such as azoospermia, oligospermia, pyospermia, hemospermia, suspected cryptorchidism, weakness of the hindlimbs during mounting, enlargement of the scrotum, and even hematuria, examination of the internal inguinal rings, accessory sex glands, and terminal aorta/proximal iliac arteries can be a critical part of a complete examination that allows the clinician to determine a definitive cause for the pathology.

2. Methods and Results

Anatomy

The internal inguinal rings are located at approximately 5 and 7 o'clock on the ventral floor of the caudal abdomen, approximately 10 cm cranial to the pelvic floor. Although referred to as 'rings,' these structures are more like 'slits' that are delineated cranially by the fibers of the internal abdominal oblique muscle and caudally by the inguinal ligament. In the normal stallion, the only structure that should descend from the abdomen through this slit is the spermatic cord. Transrectally, the spermatic cord can be palpated as a thin string with a palpable pulse originating from the testicular artery. The spermatic cords extend from the cranial end of each ampulla towards the ipsilateral inguinal ring.

The paired ampullae are the most cranial of the accessory sex glands. The ampullae are glandular thickenings of the ducti deferentia and consist of numerous branching crypts in which sperm can be stored prior to ejaculation. Immediately caudal and lateral to the ampullae are the paired seminal vesicles. The seminal vesicles extend outward from the midline at an approximate 40° angle to the urethra. When empty, the seminal vesicles become flattened and when distended with fluid they become roughly oval.[2] The ampullae and seminal vesicles narrow caudally and extend under the isthmus of the prostate to open through a common excurrent duct into the urethra at the seminal colliculus. The isthmus of the prostate is located on the ventral

NOTES

Table 1. Abnormalities Associated With the Internal Inguinal Rings

Pathology	Clinical signs	Abnormal findings on transrectal palpation	Abnormal findings on transrectal ultrasonographic examination	Comments
Acquired inguinal/scrotal hernia	· Enlarged scrotum · Colic	· Bowel loops descending through affected ring · Distended small intestine	· Transcutaneous ultrasonographic examination of scrotal contents is typically performed	· Ultrasonographic examination typically performed transcutaneously · Bowel in inguinal canal/scrotum · Hydrocele · Viability of bowel can be assessed ultrasonographically
Spermatic cord torsion	· Enlarged testis/scrotum · Colic · Hydrocele?	· Decreased pulses in spermatic cord if torsion occurs proximal to inguinal ring	· Absence of blood flow in cord distal to torsion (Doppler ultrasonography) · (Transcutaneous ultrasonographic examination of scrotal contents is more typically performed. The testicular parenchyma is typically very heterogeneous with evidence of vascular stasis and edema.)	· Can be asymptomatic and clinically insignificant if blood flow is not impaired

midline caudal to the bulk of the seminal vesicles and dorsal to the trigone region of the bladder. The right and left lobes of the prostate are roughly wing-shaped and extend laterally from the isthmus. The prostate gland empties into the dorsal urethra through numerous prostatic ducts. The paired bulbourethral glands are roughly spherical and are located on either side of the urethra just cranial to the anal sphincter at the ischial arch. The function of the accessory sex glands is to add fluid volume, enzymes, amino acids, and buffers to the ejaculate.[3,4]

The caudal aorta terminates just under the sacrum along the dorsal midline. It is readily palpable due to its large size and strong pulse. The proximal internal and external iliac arteries are also readily palpable as they branch from the terminal aorta.

Examination Technique

Examination of the internal reproductive tract of the stallion is performed per rectum. Stallions can be restrained and prepared for this examination similarly to mares. Stocks are preferable, since most stallions are not accustomed to this procedure. However, tractable animals can also be safely examined without stocks, using the same routine safety precautions that one would use with a mare (e.g., examination around a stall door). Sedation is usually not needed, but can be used for nervous or difficult animals. Internal structures should be identified by palpation (when possible) prior to ultrasonographic examination.

Once palpation is completed, ultrasonography can be performed. Due to the superficial location of the accessory glands, a 7.5 MHz or higher frequency linear array transducer is preferred. If a 7.5 MHz transducer is not available, a 5 MHz transducer can be used, although some detail will be lost. The transducer is held, introduced, and advanced in the rectum as it is for ultrasonography of the mare. If a linear array transducer is not available, a microconvex transducer or a sector scanner with an off-line beam may suffice. The larger size of the sector scanner can make examination per rectum difficult and may preclude transrectal ultrasonographic examination in ponies or miniature horse stallions.

Internal Inguinal Rings

The internal inguinal rings can be palpated by sweeping one's hand along the caudoventral abdominal floor just cranial to the pelvis until the 'slit' of each internal ring is encountered. The ring can more readily be identified as described by Kenney et al, "…by folding one's fingers against the palm of the hand and then unfolding the fingers while pushing the finger tips against the abdominal wall."[1] In a normal animal, it should be possible to follow the entire caudoventral body wall in an uninterrupted fashion from left to right and across the midline. If the veterinarian is unfamiliar with the normal feel of the inguinal rings, tractable stallions may allow palpation of the internal inguinal ring with a hand inserted in the rectum while simultaneously following the spermatic cord up to the external inguinal ring with the contralateral hand by reaching forward and upward around the stallion's hindlimb. For example, to identify the right inguinal ring and canal, insert the left hand into the rectum while reaching forward with the right hand around the stallion's right hindlimb. Identify the spermatic cord and follow it dorsally. By attempting to touch the fingers of your left hand to the fingers of your right hand through the body wall, you will familiar-

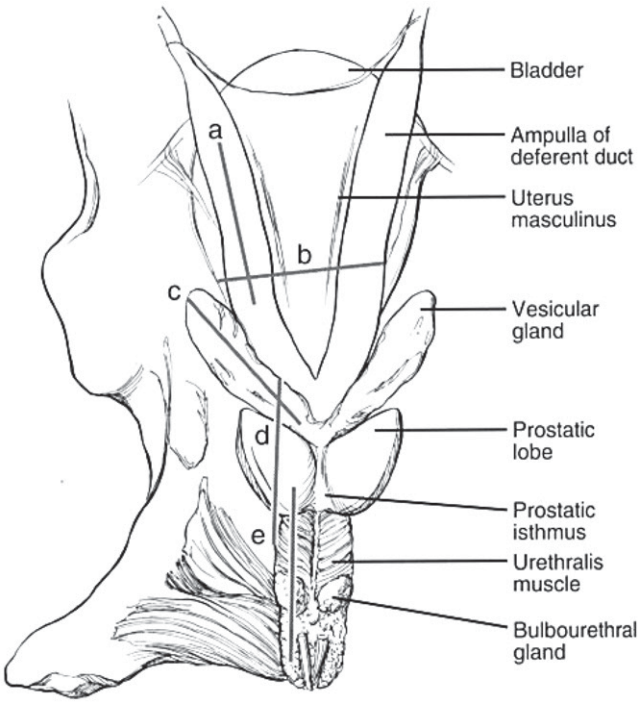

Fig. 1. Dorsal view of the anatomy of the accessory sex glands of the stallion. Solid lines indicate the proper positions of the linear array transducer: A, for imaging the ampulla in sagittal section, B, for imaging the ampullae in cross-section, C, for imaging the seminal vesicle in sagittal section, D, for imaging the lobe of the prostate in sagittal section, and E, for imaging the bulbourethral gland in sagittal section. Figure reprinted from: *Equine Diagnostic Ultrasound*, Reef VB, Sertich PL, Turner RM. V.B. Reef (ed.), Philadelphia: W.B. Saunders, 1998.

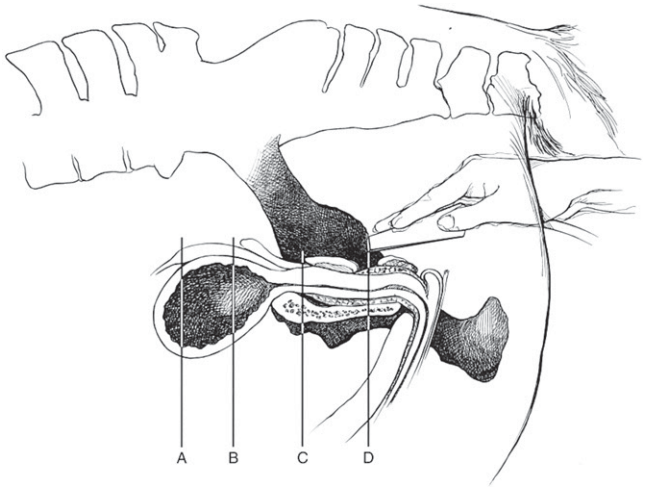

Fig. 2. Lateral view of the anatomy of the accessory sex glands of the stallion. This figure illustrates both proper handling and the appropriate depth penetration for proper placement of the linear array transducer for imaging of the accessory glands. Solid lines indicate proper location of the cranial most aspect of the transducer: A, for imaging the ampullae, B, for imaging the seminal vesicles, C, for imaging the prostate, and D, for imaging the bulbourethral glands. The caudal location of the bulbourethral glands requires that the palm of the veterinarian's hand be outside the rectum. Figure reprinted from: *Equine Diagnostic Ultrasound*, Reef VB, Sertich PL, Turner RM. V.B. Reef (ed.), Philadelphia: W.B. Saunders, 1998.

ize yourself with the location and feel of the right inguinal ring and canal. After identifying the internal inguinal rings, each spermatic cord can be palpated as it enters the internal inguinal ring and then the cord can be traced caudally to the ipsilateral ampulla (Table 1).

Ampullae

The ampullae palpate as paired soft tissue tubular structures running on top of the pelvic floor and converging at their caudal ends towards the midline. The ampullae can be imaged ultrasonographically in sagittal sections with the linear array transducer placed directly over and parallel to the long axis of each gland (Figs. 1 and 2). With the transducer in this position, the ampullae are imaged as soft tissue bands that extend from left to right across the upper edge of the ultrasound screen (Fig. 3). The lumen of each ampulla may be visible as a black (fluid) or white (no fluid present) line through the approximate center of the gland. In normal stallions, the lumen and total diameter of the ampulla will increase after sexual stimulation and decrease following ejaculation.[5] The mean total diameter of the ampullae in light horse stallions is 11 mm with a range of 4 to 18 mm.[6]

The ampullae can also be visualized in short axis cross section by turning the linear array transducer at a 90° angle to the long axis of the glands (Fig. 1). This view can be difficult or impossible to obtain with larger transducers or in smaller stallions as the pelvic diameter may limit proper positioning of the probe. In short-axis cross section, both ampullae appear as circular soft tissue structures that can be followed caudally as they converge upon the midline (Fig. 4).[2] Higher frequency transducers will provide sufficient image quality to image the seminal colliculus. The pelvic urethra becomes visible in short axis cross section ventral and medial to the ampullae as the glands are followed caudally and approach the midline.[7]

When a higher resolution transducer is employed, it may also be possible to image the uterus masculinus in some animals. This structure is a remnant of the embryologic paramesonephric (Mullerian) ducts and, when present, is found medial to the ampullae just cranial to the prostatic isthmus.[2] The size of the uterus masculinus varies, but it typically appears as a thin-walled oblong structure filled with anechoic fluid. It is common to find this embryologic remnant in stallions and it is not typically associated with clinical pathology (Table 2).

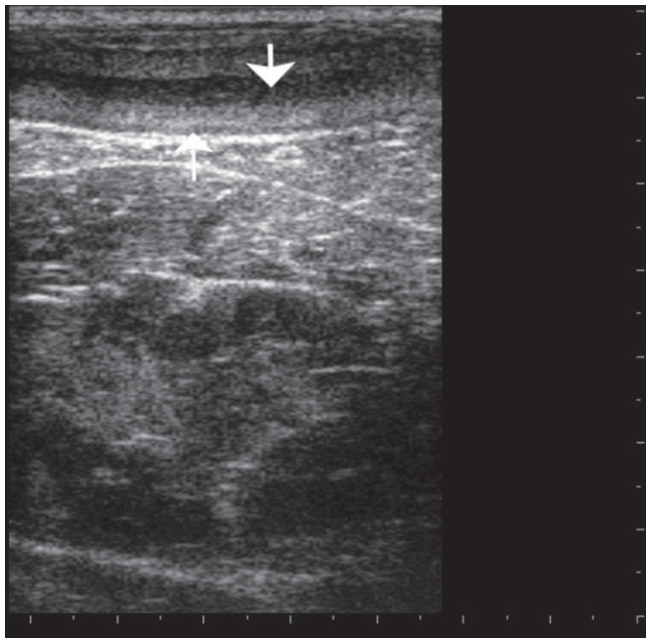

Fig. 3. Normal ampulla of a stallion in sagittal section. The ampulla extends from left to right across the top of the image. The upward facing arrow is pointing to the ventral edge of the ventral wall of the gland. The downward facing arrow is pointing to the anechoic lumen of the gland. This image is from a sexually stimulated stallion. However, it also can be normal for the lumen to be much smaller or even not visible at all if the gland is empty.

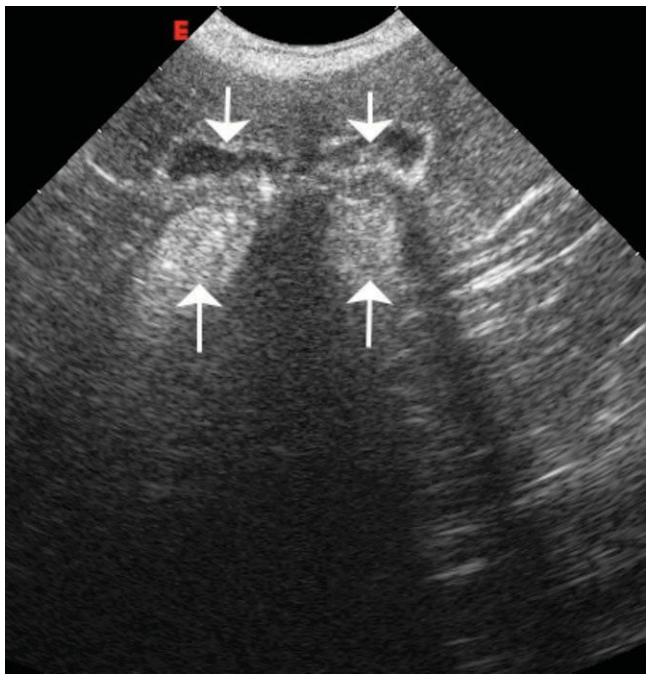

Fig. 4. Normal ampullae and seminal vesicles in short-axis cross section. Upward facing arrows point to the ampullae. Downward facing arrows point to the seminal vesicles.

Seminal Vesicles

The seminal vesicles may be palpable just lateral to the caudal half of the ampullae, but only if they are well-distended with fluid. When empty, the seminal vesicles typically are difficult to palpate. To image the seminal vesicles ultrasonographically in sagittal sections, the transducer is moved slightly laterally and caudally with respect to each ampulla and positioned so that the long axis of the transducer parallels the long axis of each seminal vesicle (Fig. 5). By rotating the transducer 90°, the seminal vesicles can also be imaged in short axis cross section dorsal and slightly lateral to the ampullae (Fig. 4). When the glands are empty, they can be difficult to identify. The empty seminal vesicles appear as elongated, flattened, and somewhat irregular soft tissue structures. Varying amounts of fluid may be present within the glandular lumen. The ultrasonographic character of the fluid in normal glands varies from anechoic to that of mixed echogenicity, sometimes with echogenic flecks throughout. With sexual stimulation, the amount of fluid within each gland will increase and the gland will become easier to identify and more bladder-like in shape. With prolonged sexual stimulation, the volume of intralumenal fluid can become quite large and the glands will appear more thin-walled. This fluid is usually, but not always, expressed as gel during ejaculation.[5] The mean total diameter of the seminal vesicles in light horse stallions is 12 mm with a range of 4 to 20 mm (Table 3).[6]

Prostatic Isthmus and Prostatic Lobes

The prostatic isthmus is located on the ventral midline of the pelvis, just caudal to the bulk of the ampullae and seminal vesicles. The lobes of the prostate are aliform and extend outward to the right and left of the isthmus. The prostatic isthmus may be palpable as a ridge on the midline, caudal to the trigone of the bladder, and is imaged on the animal's midline by moving the transducer caudally from the seminal vesicles, parallel to the long axis of the stallion's body. The prostatic lobes are viewed by moving the transducer laterally to the left and right of the midline (Fig. 6). In the absence of sexual stimulation, the prostatic parenchyma appears gray-white and heterogeneous, containing numerous small anechoic fluid spaces. The overall size of the prostate and size and number of the fluid-filled spaces increase following sexual stimulation.[5] In a highly teased animal, the appearance of the gland can be quite dramatic with large radiating anechoic fluid spokes extending throughout the parenchyma. The mean thickness of the lobe of the prostate in light horse stallions is 25 mm with a range of 10 to 40 mm. The mean isthmus thickness is 10 mm with a range of 4 to 25 mm.[6]

Prostatic neoplasia has rarely been reported in geldings as a cause of hematuria[8] and it therefore can be helpful to familiarize oneself with the characteristics of the gelding prostate. Because the

Table 2. Abnormalities of the Ampullae

Pathology	Clinical signs	Abnormal findings on transrectal palpation	Abnormal findings on transrectal ultrasonographic examination	Comments
Sperm accumulation	· Poor sperm motility · Highly variable numbers of sperm · High percentage of detached heads	· None	· Heterogeneous, "moth eaten" appearance of the glandular tissue · Hyperechoic debris within lumen and glandular tissue	· U/S abnormalities not consistently found and not pathognomonic
Blocked ampullae	· Oligo- or azoospermia initially. Supra physiologic sperm numbers with poor sperm motility and detached heads after relief of blockage · Low seminal plasma alkaline phosphatase if blockage is bilateral	· Ampullae may be palpably enlarged and feel tense	· Dilated ampullary lumen distal to blockage · Hyperechoic debris within ampullary lumen and crypts · Heterogeneous glandular parenchyma	· U/S abnormalities not found in all cases but can be diagnostic when present

prostate is an androgen-sensitive organ, the gelding prostate is smaller and less well-defined than that of the stallion. Unlike the distinct prostate of the stallion, in the normal gelding the prostate can be difficult to discern ultrasonographically (Table 4).[9]

Bulbourethral Glands

The bulbourethral glands are not palpable due to their location beneath the urethralis and bulboglandularis muscles. However, the glands can be imaged caudal to the prostate to the right and left of the ventral midline just inside the anus (Fig. 1). Because of their caudal location, to properly place the transducer over these glands, the wrist and palm of the veterinarian's hand must be withdrawn from the rectum leaving only the fingers holding the ultrasound transducer just within the anal sphincter (Fig. 2). The bulbourethral glands are circular to oval soft tissue structures (Fig. 7). After sexual stimulation, the glands enlarge and small anechoic fluid-filled spaces may be visible within the parenchyma.[5] The mean length of the bulbourethral glands in light horse stallions is 38 mm with a range of 17 to 59 mm. The mean height is 24 mm with a range of 12 to 39 mm (Table 5).[6]

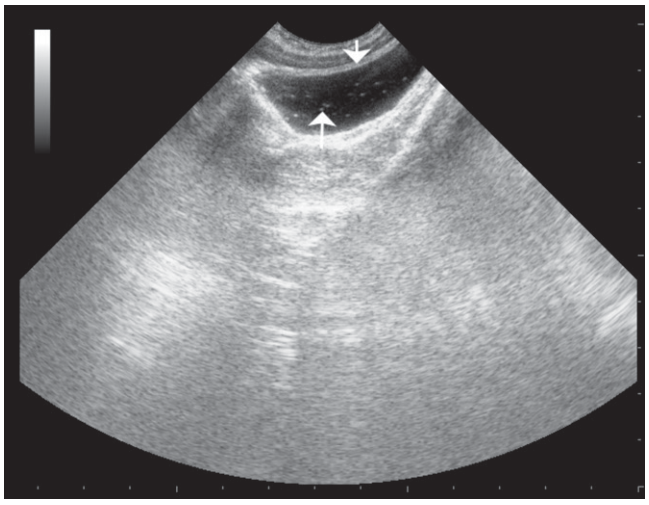

Fig. 5. Normal seminal vesicle in sagittal section. This gland is moderately filled with fluid that is typically expressed during ejaculation as gel. The downward facing arrow points to the thinned dorsal wall of the gland. The upward arrow points to the fluid in the lumen of the gland. Note that the seminal vesicles can vary greatly in size and shape depending on the amount of fluid within the lumen.

Terminal Aorta and Iliac Arteries

By advancing your arm typically past the elbow and turning the palm dorsally, you will be able to palpate the terminal aorta on the dorsal midline and follow it caudally to the branching of the iliac arteries. The terminal aorta is visualized ultrasonographically by advancing the transducer cranially until it is positioned approximately under the sacrum. The linear array transducer is held so that the long axis of the transducer parallels the horse's spinal column and the beam of the transducer is directly dorsally. The aorta appears as a large vessel in sagittal section. Normally, the walls of the artery are smooth and blood flows steadily and smoothly within the lumen. As the aorta is followed caudally, one can identify the internal and external iliac arteries branching at the termination of the aorta. The iliac vessels are followed dorsolaterally along the body wall until they are lost in the hindlimb musculature (Table 6).

3. Miscellaneous Abnormalities

Midline Cysts of the Seminal Colliculus

Midline cysts of the seminal colliculus have been implicated as a cause of ejaculatory dysfunction and

Table 3. Abnormalities of the Seminal Vesicles

Pathology	Clinical signs	Abnormal findings on transrectal palpation	Abnormal findings on transrectal ultrasonographic examination	Comments
Seminal vesiculitis	· Hemospermia · Pyospermia · Debris in ejaculate · Poor sperm motility and/or longevity of motility · Pain during ejaculation · Colic/Pain? · Subfertility	· Affected gland may be enlarged, painful and fluid-filled	· Echogenic/ flocculent fluid in lumen, sometimes with visible fibrin tags · Irregular lumen · Thickened, wavy, gland wall	· Seminal vesicles of normal stallions can become dramatically enlarged with fluid during sexual stimulation. Don't confuse this with pathology. When necessary, the glands also can be examined and even cannulated and treated using urethroscopy.

variable semen quality in stallions.[10] It has been suggested that, to be clinically significant, the cysts must be of an appropriate size and located immediately adjacent to the excurrent ducts of the ampullae and seminal vesicles, very close to the opening of the ducts at the seminal colliculus (caudal to the prostate). Ultrasonographically, these cysts are oval, teardrop-shaped, rectangular- or spindle-shaped, and contain anechoic to slightly echogenic material.

Fig. 6. Wing of the prostate gland. Note the aliform shape. Black arrows point to the lateral edge of the gland. This gland is moderately stimulated, as shown by the distinct anechoic fluid pockets scattered throughout the parenchyma. Highly stimulated stallions will have even more dramatic fluid accumulation.

Cryptorchidism

A cryptorchid horse is one in which one or both testicles have not descended into the scrotum. Retained testicles can be accurately identified, located, and measured ultasonographically using either a 5 or 7.5 MHz linear array transducer transrectally or a lower frequency (e.g., 3.5 MHz) sector scanner transducer transabdominally for abdominal testicles or by scanning externally over the external inguinal ring for inguinal testicles.[11,12] Scanning over the external inguinal ring is easiest to accomplish using either a sector scanner or a microconvex transducer, due to their smaller footprints. If these are not available, a linear array transducer will suffice.

If a suspected cryptorchid testis is not identified near the external inguinal ring or within the inguinal canal, then transrectal examination of the caudal abdomen can be performed to determine if the testicle is within the abdomen. Transcutaneous ultrasonographic examination of the caudal abdomen can also be used to identify retained testes. Abdominal testicles can be located anywhere from the caudal pole of the kidney to within the internal

Table 4. Abnormalities of the Prostate

Pathology	Clinical signs	Abnormal findings on transrectal palpation	Abnormal findings on transrectal ultrasonographic examination	Comments
Prostatic masses/ neoplasia	· Dysuria · Hematuria · Stranguria	· Enlarged, asymmetric prostate gland	· Loss of normal architecture of prostate · Heterogeneous, more solid appearance to glandular tissue · Large cystic spaces in some cases	· Rare · Can occur in geldings or stallions

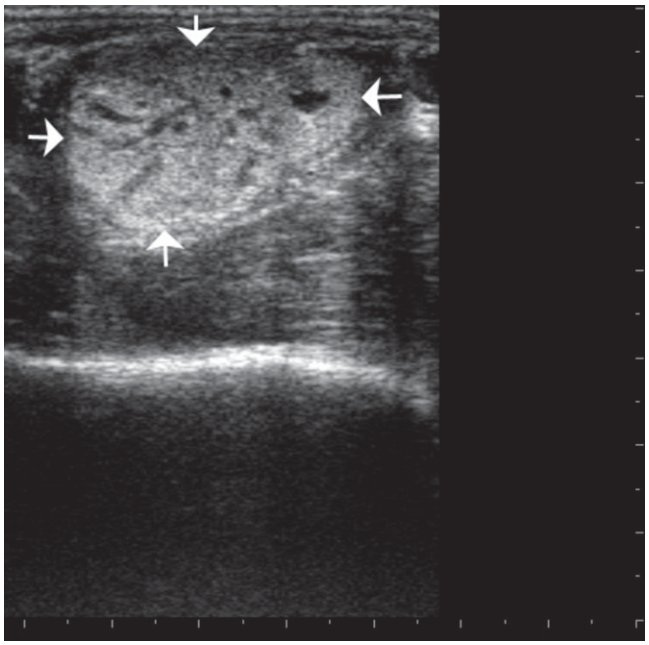

Fig. 7. Sagittal section view of a bulbourethral gland. White arrows delineate the edges of the gland. The sizes of the anechoic fluid pockets within the gland will vary with sexual stimulation.

inguinal ring (i.e., anywhere along the path that the testis should follow during its normal descent into the scrotum). However, the majority of retained testes migrate to the internal ring, but fail to enter the ring (the point of greatest resistant to the descent of the testis). Therefore, most cryptorchid testes are found immediately adjacent to the internal inguinal ring or within the inguinal canal. As a result, when searching for a retained testicle by palpation or ultrasonography, it is usually most efficient to first examine the inguinal ring and the areas immediately adjacent to it. If the testicle is not identified, then the search can be widened to include the larger area between the inguinal ring and the caudal pole of the kidney (the site of its embryonic origin). Starting at the inguinal ring, palpate and then sweep the transducer back and forth slowly, gradually advancing towards the caudal pole of the kidney.

An abdominal testis sometimes can be identified by palpation without the aid of ultrasonography. However, the small size of the retained testis, together with its softer texture, can make the tissue difficult to identify. Ultrasonographic examination often improves one's ability to identify the presence of an abdominal testis. The parenchyma of a cryptorchid testicle is generally hypoechoic relative to that of a descended testicle, but nonetheless remains distinctive. In some cases, the spermatic cord may be identified and, in fact, the cord may be more readily identifiable than the testicular parenchyma itself. With some practice, this technique can be highly sensitive for the identification of abdominal testes.[11]

4. Discussion

The ability to evaluate the internal reproductive tract of the stallion is a skill that is important to any veterinarian performing breeding soundness evaluations or even semen evaluations on stallions.[13] However, because pathology of the internal reproductive of the stallion is relatively uncommon, examination of these structures is something with which most veterinarians are unfamiliar. Familiarity with the normal palpable and ultrasonographic features of the structures of the caudal abdomen of the

Table 5. Abnormalities of the Bulbourethral Glands

Pathology	Clinical signs	Abnormal findings on transrectal palpation	Abnormal findings on transrectal ultrasonographic examination	Comments
Cysts	· None	· None	· Anechoic, circular, well-delineated, single or multiple intraglandular cysts	· Clinically insignificant

Table 6. Abnormalities of the Terminal Aorta/Iliac Arteries

Pathology	Clinical signs	Abnormal findings on transrectal palpation	Abnormal findings on transrectal ultrasonographic examination	Comments
Aortic-iliac thrombosis	· Ejaculatory failure · Hindlimb weakness in association with exercise/exertion · Disturbances of hindlimb innervation · Hindlimb pain · Affected limb is palpably cooler than contralateral limb	· Reduced or absent pulses	· Soft tissue thrombus visualized extending into lumen from vessel wall · Blood flow disturbed by presence of thrombus	· U/S diagnostic

stallion can allow the clinician to readily identify pathology when it is present. Even if you do not work with many breeding stallions in your practice, this skill can be acutely and critically important when presented, for example, with a stallion with an enlarged scrotum for which spermatic cord torsion and inguinal herniation are high on the differential list. The ability to identify an abdominal testicle can also provide an invaluable service to a client, while also providing the surgeon information on the exact location of the testis prior to entering the abdomen, thus allowing the surgeon to select the most appropriate surgical approach. For these reasons, practitioners are encouraged to take the opportunity to examine normal stallions and geldings whenever the opportunity arises.

Acknowledgments

Conflict of Interest

The Author declares no conflicts of interest.

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How to Measure Testes Size and Evaluate Scrotal Contents in the Stallion

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1. Introduction

Ultrasonographic (US) evaluation of the scrotal contents is used to evaluate the normalcy of the spermatic cord, testes, and scrotum. In addition, it is the most accurate and simple method for measuring testes size (i.e., volume), which is used to determine the efficiency of sperm production in the testes. Historically, stallion testes are measured using calipers. Calipers have the disadvantage of requiring the testis to be positioned so as to completely fill the scrotum. Often this requires manipulation of the testis by grabbing the scrotal neck to force the testis into a scrotal position. This type of manipulation can aggravate the stallion (i.e., kick). In contrast, the ultrasound can image and measure the testis in situ thereby minimizing excessive scrotal manipulation. Ultrasonographic testis measures are also more accurate since the parenchyma can be directly visualized and specific landmarks for measurement can be identified. Finally, US can be simpler and faster than the caliper method.

2. Materials and Methods

Ultrasonographic evaluation of the scrotal contents in the stallion includes the spermatic cord (the spermatic artery, ductus deferens, and spermatic venous network (pampiniform plexus)), the epididymis (head, body, and tail), the testis, vaginal cavity, and tunics and scrotum (skin, dermis). The clinician should be able to identify position, location, and the normal echoic pattern of these structures. The examination of the scrotal contents, in addition to determining normalcy, should also include measurement of the length, width, and height of each testis. These measures are then used to calculate testis volume and determine the efficiency of sperm production.

Equipment

In general, linear array scanners with a 5.0-mHz probe can be used for evaluation of the scrotal contents and provide greater depth when measuring the length. Higher frequency probes provide greater detail but can be limited by the depth of penetration. Ideally, small sector scanner probes (Fig. 1) provide better access due to their smaller size and more specific identification of scrotal structures. The length measure of large testis may be greater than the limit of the US (e.g., 10–12 cm), and therefore that measure may have to be estimated. The T-type linear probe allows ease of handling and placement in the scrotal area.

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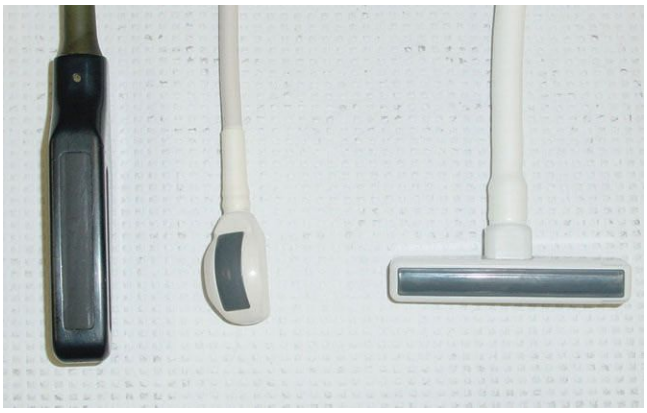

Fig. 1. Ultrasound probes available for evaluation of scrotal contents; left: linear array; middle: "finger" sector scanner; right: T-type linear array.

Evaluation of the Testes and Scrotal Contents: Stallion Position and Location

1. Examine the stallion in a stall or quiet breeding shed area. Most stallions can be restrained in stocks if there are no other distractions to excite them. Administration of a light dose of detomidine hydrochloride[a] (e.g., 0.007 mg/kgIV) may facilitate the evaluation.
2. An experienced stallion handler should be at the head of the stallion during the whole evaluation (Fig. 2).
3. Examine the stallion from the left flank area with the ultrasound positioned (Fig. 2) close to the evaluator but a safe distance from the stallion.
4. An initial cursory manual evaluation of the testes and scrotum should be performed to determine the degree of tractability and resistance that the stallion might exhibit (Fig. 3). The intent of this evaluation is to briefly evaluate size, position, and location of the testes and scrotal contents but not perform deep palpation since this may aggravate the stallion. Deep palpation should be performed as the final step in the evaluation.
5. Cover hands with disposable latex examination glove.
6. Apply a small amount (i.e., enough to allow probe-skin contact) of lubricant to the US probe surface. Gel can also be applied directly to the scrotal skin.
7. Once the clinician is comfortable with the stallion's temperament towards the procedure, a position closer to the flank and scrotum can be taken.
8. Individual testis measures in centimeters (length, width, and height) can now be taken. Since the width and height are usually of similar size and easiest to measure, it is convenient to take these first. It is recommended to evaluate the near testis (left) first and take duplicate values for each measure.
   a. Height: The height is measured by placing the probe ventrally and directing the beam dorsally so that the central vein (Figs. 4 and 5) is approximately two-thirds of the distance from the surface and the spermatic artery can be visualized dorsally. There is no need to grasp or manipulate the testis for this measure. Note that the testis does not lie in a horizontal position but rather is tipped so that the cranial testis is higher than the caudal testis. This will require

Fig. 2. Stallion positioned in a stock showing the location of the stallion handler, clinician, and the position of the ultrasound.

Fig. 3. The stallion is positioned in a corner with a padded wall. The clinician approaches the flank area to determine the stallion's receptivity to the procedure.

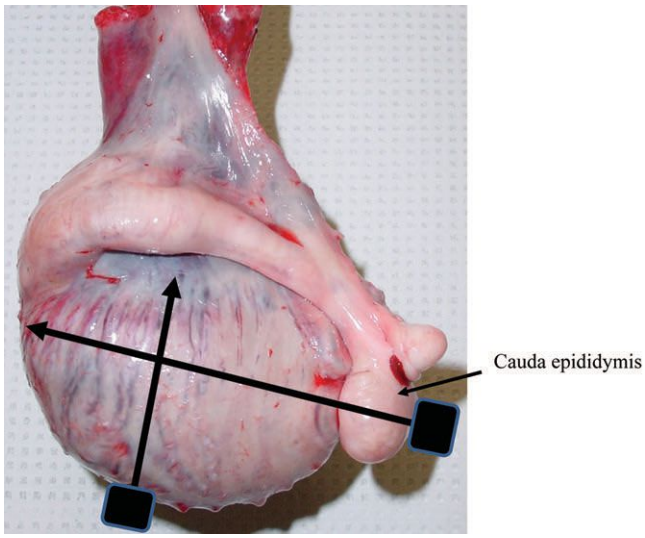

Fig. 4. Cranial-caudal view of the left testis. The height of the testis is measured by placing the ultrasound probe ventrally with the beam directed dorsally. The length is measured by placing the probe caudally and directing the beam cranially. Notice the slight rotation of the testis to an upward position and the position of the cauda epididymis.

Fig. 5. Lateral-medial view of the left testis. The width of the testis is measured by placing the ultrasound probe laterally at the widest point of the testis with the beam directed medially.

the clinician to angle the probe caudally so that the cross-section of the testis appears round rather than elliptical.

b. Width: The opposite testis should be pushed dorsally so that the testis of interest is allowed to occupy the scrotum without being compressed or distorted, which will alter (i.e., reduce) the width measure (Figs. 6 and 7). Avoid grabbing the neck of the scrotum to force the testis into the scrotum similar to the technique recommended for measuring the circumference of the bull testes. This can cause strong contractions of the cremaster muscle making the testis even harder to manipulate. The probe should be positioned laterally on the scrotum, approximately one-third of the distance from the cranial scrotum. This is usually the widest portion of the testis. The shape of the testis should be approximately round and oblique sections should be avoided.

c. Length: Similar to the height measure the testis should hang in situ without being manipulated. The US probe is placed on the most caudal aspect of the scrotum and directed cranially to include the greatest caudal-cranial distance. If the tail of the epididymis is included in the image (Fig. 4) it should not be included as part of the measure (Figs. 4 and 8). To avoid "off center" images, the probe should be rotated vertically approximately 30° to visualize the greatest length.

d. A minimum of two measurements/dimension are taken and repeated until they are within 3 to 5 mm of each other.

9. After both testes have been measured, the clinician should do a thorough manual as well as

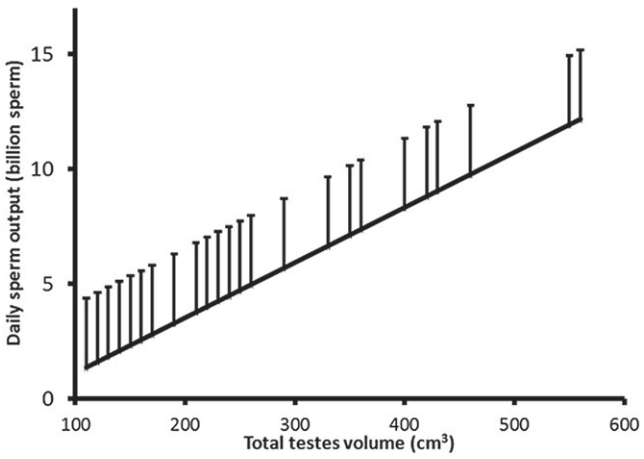

Fig. 6. Shown is the relationship between total testicular volume (TTV) and daily sperm output (DSO, mean ± SD).

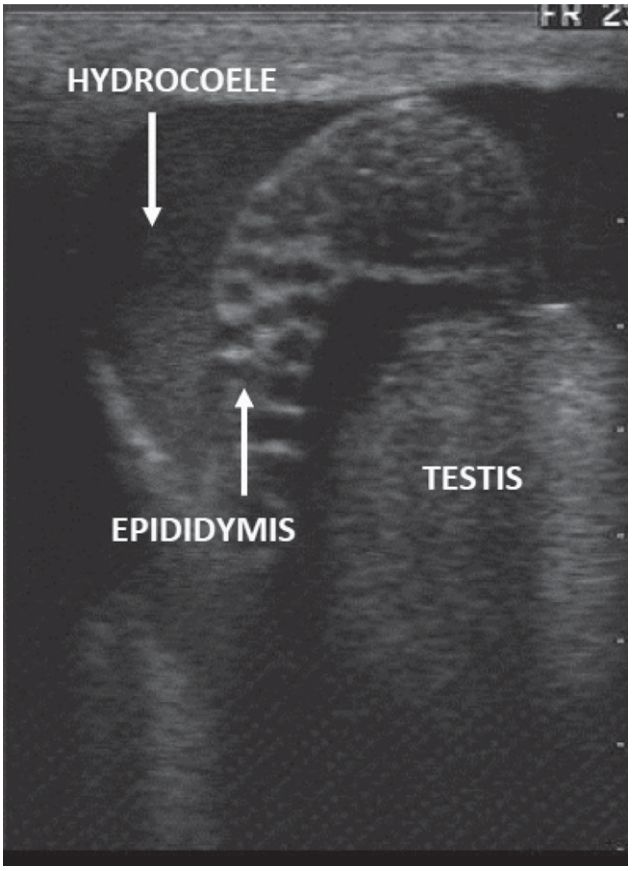

Fig. 7. Hydrocoele surrounds the epididymal tail. Notice the prominent lumen in the epididymis, often a result of accumulated sperm.

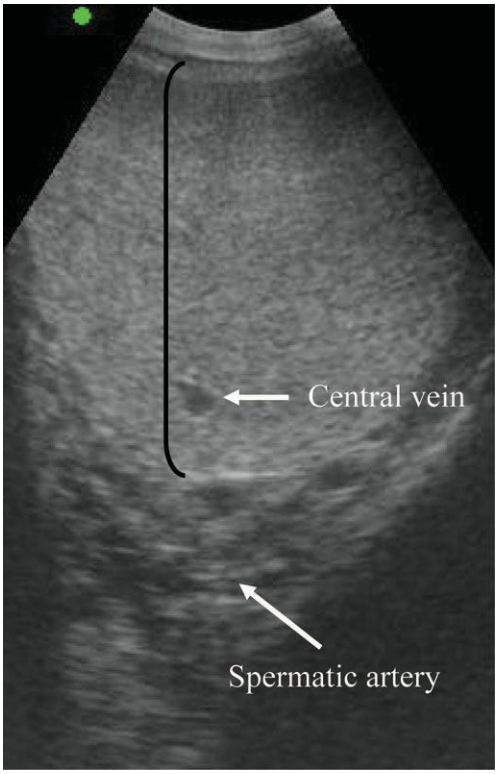

Fig. 8. Image of the height measure with the probe placed on the ventral scrotum and directed dorsally. Notice the central vein and spermatic artery. The bracket shows the correct measure which is taken from the ventral to the dorsal testis. The dorsal testis is identified by the hyperechoic line. The overall image should be approximately round.

ultrasonic evaluation of the scrotal contents. The manual examination should start at the neck of the scrotum and evaluate each testis structure (head, body, and tail of the epididymis) individually as well as determine that the testes are able to move freely within the vaginal cavity.

Qualitative Evaluation of the Scrotal Contents: The Ultrasound Examination

Following the evaluation of testes size, the scrotal contents should be evaluated qualitatively (i.e., circulation, echotexture, shape, and anatomic orientation).

1. Orienting the US horizontally, evaluate the neck of the scrotum, which should visualize the cross-section of the spermatic artery. Blood flow should be confirmed either through grey-scale movement of the blood or more accurately through color Doppler imaging.
2. As you proceed, ventrally from the scrotal neck to the craniodorsal area of the testis, the probe will be rotated to a vertical position similar to the position for the width measure. This will allow visualization of the termination of the central vein, as it empties into the pampiniform plexus. The probe is passed caudally allowing visualization of the whole testis in cross-section. At the caudal end of the testis, the tail of the epididymis is visualized. Fluid accumulation (hydrocele), if present, will vary in the volume present. Small amounts will accumulate in the area around the tail of the epididymis resulting in easy identification of the tail. As the hydrocele increases, fluid will expand ventrally and eventually surround the whole testis.

The clinician is required to perform the examination in the vicinity of the flank area of the stallion. For obvious reasons, this is a risky position for the clinician. If semen collection is performed in conjunction with the evaluation, stallions tend to be more tractable following semen collection. Regardless, it is recommended to sedate the stallion to facilitate a thorough and complete evaluation. The ultrasound evaluation of the scrotal contents should be considered a stand-alone primary procedure, and therefore sufficient time and patience should be al-

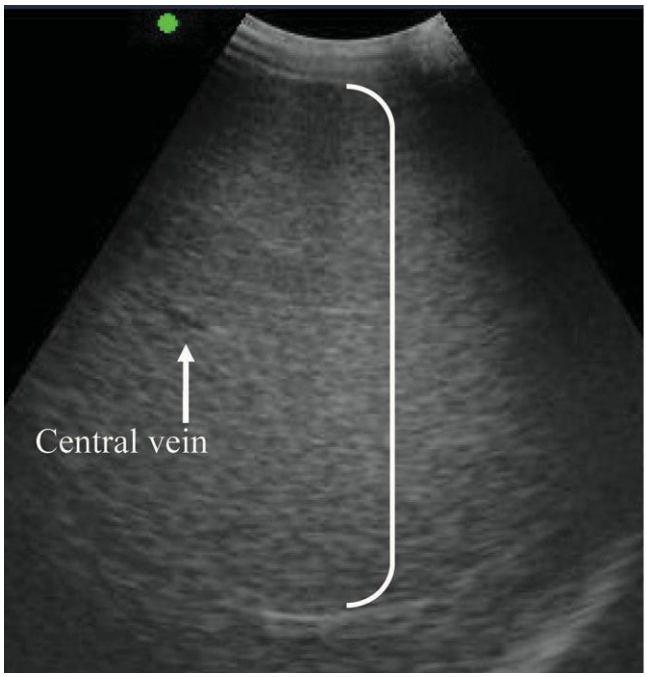

Fig. 9. Image of the width measure with the probe placed on the lateral scrotum and directed medially. The bracket shows the correct measure with the medial point identified as a hyperechoic line. Similar to the height measure the image should be round.

lotted to allow for a thorough examination of the scrotal contents as well as accurate measurement of the testes dimensions. An inadequate examination can result in erroneous testis measures leading to an incorrect clinical interpretation.

Structures to Evaluate

1. Spermatic cord: The primary structure visualized is the tortuous spermatic artery as it winds to the testis (Fig. 3).
   Pathologic changes in spermatic cord torsion, varicocele (dilation of the pampiniform plexus), generalized edema resulting from inflammation (orchitis or epididymitis).
2. Testis: Examination of the testis parenchyma, which should be homogenous in echotexture.
   Pathology: Neoplasia (seminoma is the most common tumor of the descended adult testis). Benign cysts on the periphery of the testis. Hematomas from trauma.
3. Epididymis: The head, body, and tail regions are located craniolateral, dorsolateral, and caudal, respectively, on the horizontally oriented testis.
   Pathology: Displacement due to spermatic cord torsion. Small or absent in hypoplasia or dysplasia. Prominent/enlarged due to sperm accumulation or inflammation (Fig. 9).
4. Vaginal cavity: The vaginal cavity is a potential space that communicates with the peritoneal cavity through the inguinal canal.
   Pathology: Peritoneal content leakage (e.g., peritoneal fluid, hydrocele, intestines). Circulatory (artery, vein, lymphatic) compromise to the spermatic cord can result in free fluid accumulation; trauma/hematocele; infection/pyocele.
5. Scrotum: Consists of the skin, dermis, and parietal vaginal tunic.
   Pathology: Edema or hyperplasia due to trauma or hypoproteinemia.

3. Discussion

Interpretation of Testes Volume

Measurement of testes size is an important part of the stallion breeding soundness evaluation since testes size is associated with sperm production. The number of sperm produced by the stallion may impact fertility and, thus, the number of mares that a stallion can breed. Historically, testes size was determined using the linear measure, total scrotal width, in which calipers were used to measure the combined testes width, the mediastinum testes, and the scrotum. This technique, however, does not determine the three-dimensional shape (i.e., volume) of each testis. While the relationship of length, width, and height tends to be proportional, there are instances where they are not, and the determination of each dimension will more accurately determine volume.

Subsequently, ultrasonography has been introduced as a technique to measure and evaluate the testes. This technique has the advantage of being specific and accurate since the clinician can visualize the testis parenchyma and identify landmarks that assure measurement of the length, width, and height.

Measurements (length, width, and height) from each testis are performed as described above. The measures from each testis are then inserted in a formula that approximates the volume of an ellipsoid[1]

$$4/3\pi \text{ (length-cm) (width-cm) (height-cm)} = \text{volume of the testis in cm}^3$$

or

$$0.5233 \text{ (length-cm) (width-cm) (height-cm)} = \text{volume of the testis in cm}^3$$

The volumes from each testis are combined to give the total testicular volume (TTV).

The TTV is then inserted into the regression formula to calculate the expected daily sperm output (DSO) (Fig. 10). There are two formulas that have been previously published.[2,3] It is recommended to use both formulas to obtain a range of values.

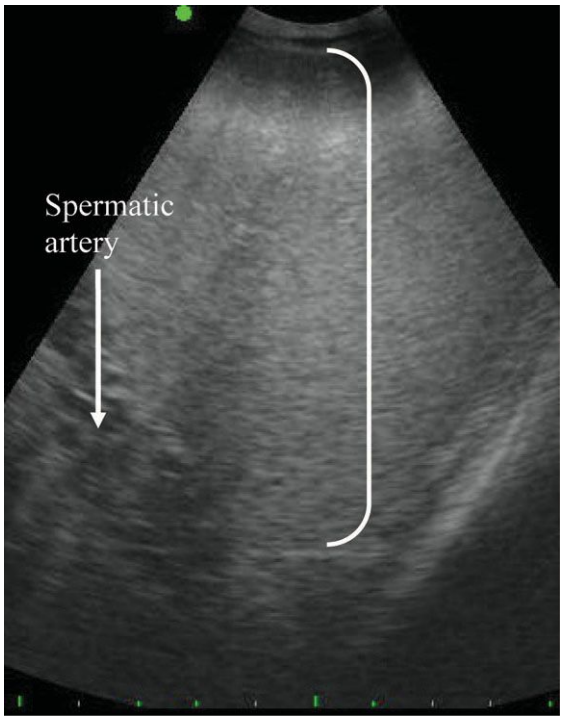

Fig. 10. Image of the length measure with the probe placed on the caudal scrotum (top) and directed cranially. The bracket shows the correct measure with the cranial point identified as a hyperechoic line. Notice the spermatic artery located on the dorsal surface of the testis.

Predicted DSO (billions)

$$= 0.024\ (\text{TTV}) - 1.26^{2}$$

$$= 0.024\ (\text{TTV}) - 0.76^{3}$$

An example of how to use the formula is given below. Measures for each testis dimension are taken, and the volume is calculated for each testis and then summed to determine the TTV. In this case, the TTV is 191 cm^3. This value would then be inserted in the two formulas above to give a range of expected DSO from 3.3 to 3.8 billion sperm.
Example:

	Left testis	Right testis	
Length (cm)	7.8	8.0	
Width (cm)	4.0	4.9	
Height (cm)	5.0	5.3	
Volume (cm^3)	82	109	191 cm^3 – total testes volume

Interpretation: The intent of measuring the TTV is to:

1. Determine the *absolute volume measure*. The average total testes volume for the population of stallions measured in the two studies was approximately 250 cm^3. Based on this TTV the *average* stallion should produce approximately 4.74 to 5.24 billion sperm when at DSO. This translates to a spermatogenic efficiency of approximately 18 million sperm/cc testis parenchyma/day. The absolute value becomes particularly important when measuring sexually immature stallions retired to stud following performance careers. The clinician is often asked to render an opinion on a stallion's testes size in relation to his potential as a breeding stallion. Recognizing that a 2 to 4 year stallion has a TTV similar to the average sexually mature stallion is useful when rendering an opinion. The clinician must also recognize that TTV alone does not "qualify" a stallion as fertile, but a "normal" testes size does reduce the risk of "subfertility."
2. *Spermatogenic efficiency* is the number of sperm produced per gram of testis parenchyma. This value can be determined by dividing the DSO value by the TTV. Efficiency of sperm production in the stallion ranges from 16 to 19 $\times$ 10^6 sperm/gram of testis parenchyma.[4] While the TTV is an important measure, it does not describe testis function (i.e., efficiency). Therefore, it is possible to have "normal" size testes that are not producing a "normal" amount of sperm (i.e., inefficient). Inefficiency will also be noticed when the *actual* DSO value is less than the *expected* DSO value.
3. Compare the *expected* DSO value to the *actual* number of sperm collected from the stallion. Ideally the clinician would like to collect a stallion once daily for 5 to 7 days to determine DSO. Oftentimes, this is impractical due to time and monetary constraints and a lesser collection frequency is more practical. Stich et al reported that testes volume will affect the number of days required to reach DSO with a smaller TTV (148–245 mL) reaching DSO sooner (day 5) than medium (253–274 mL; day 6) or large testes (292–466 mL; day 6–7).[5] In lieu of collecting a stallion for 5 to 7 days, collecting two ejaculates 1 hour apart and using the second ejaculate as an approximation of DSO, is a useful compromise. In most cases, the clinician is not interested in whether the stallion is producing *too much* sperm but rather whether he is producing *too little* when compared to the expected DSO value.

Acknowledgments

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Conflict of Interest

The Author declares no conflicts of interest.

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[a]Dormosedan, Pfizer Animal Health, New York, NY 10017.

How to Perform Urethral Endoscopy in the Stallion for Diagnostic Purposes

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1. Initial Considerations

While the procedure of urethral endoscopy might appear to be relatively unobtrusive, the operator should be aware that the examination can lead to complications. As such, precautions should be taken to avoid such unintended consequences. A key safeguard is to ensure that instrumentation and associated fluid that gain entry into the urethra are free of microbial contaminants or chemical irritants. It is important that the operator specifically follow the prescribed sterilization protocol(s) recommended by the manufacturer of the equipment, as deviations in cleaning may damage the equipment. If the protocol dictates that the instrument be submerged for disinfection, it is of paramount importance to perform a leak test on the endoscope prior to submersion in a solution, as accidental fluid invasion into some compartments of the endoscope can result in damage to sensitive internal components, resulting in costly repairs. A leak testing instrument, which may come as an accessory for the endoscope or be purchased separately, creates positive pressure (e.g., 150 mm Hg) within the endoscope such that bubbles will be detected at the site(s) of leakage upon submersion in the water bath. Semi-automated cleaning kits are available to aide in properly disinfecting endoscopes.

As endoscope insertion tubes are generally passed into a variety of body cavities and used by an assortment of personnel, it is critical that all operators are trained in endoscope care, including initial cleaning techniques for the endoscope immediately after each use so that organic matter is not permitted to dry upon extended storage. The suction/biopsy channel of the instrument is most prone to accumulation of debris when a patient is examined. Cleaning the endoscope with an enzyme-detergent solution prior to a disinfectant solution may be indicated. The water reservoir is a likely site for growth for the opportunist bacteria, *Pseudomonas aeruginosa*, as standing water is particularly supportive of growth of this microorganism. As such, one must not disregard disinfection of the water reservoir and the associated channel to the light-guide lens.

While one requires assurance that the instrument is sterile prior to use, it is equally important that disinfectants are properly rinsed from the endoscope; otherwise, the disinfectant(s) can be profound contact irritants that lead to destruction of mucosal surfaces. Of particular note, one must be assured that disinfectant solution is removed from the water

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reservoir and associated channel for rinsing the lens.

2. Reasons for Performing a Diagnostic Urethral Endoscopy in the Stallion

Endoscopic examination of the stallion urethra can be a valuable diagnostic approach for stallions with specific clinical manifestations. The most common ailments which justify urethral endoscopy as a diagnostic tool are seminal vesiculitis and rents in the penile urethra. These conditions generally present with pyospermia or hemospermia/hematuria, respectively. Occasionally, seminal vesiculitis can lead to visually apparent red blood cells, as well as excessive neutrophils, in ejaculated semen. These disorders are generally not associated with outward signs of pain at the time of ejaculation or urination. If no lesions are observed on the penile integument or urethral process, than an internal source of bleeding or infection is to be suspected. Urinary incontinence, stranguria, or urine discoloration may lead one to suspect bladder lesions such as cystitis, uroliths, or bladder tumors. Uncommonly, stranguria may be associated with lesions of the pelvic or penile urethra, such as urethral calculi or transluminal adhesions. Hematuria may also be indicative of kidney disease, such as might occur with renal carcinoma or pyelonephritis. With the exception of the seminal vesicles, primary infections of the remaining accessory genital glands are quite rare or unreported. While this communication is technique-based rather than disease-based, the essence of this section is to demonstrate that numerous different disorders might exist that justify use of endoscopy as a diagnostic tool. In cases of idiopathic hemospermia, pyospermia, urine discoloration, urinary incontinence, or stranguria, urethral endoscopy/cystoscopy is likely indicated for direct viewing of these sites.

3. The Equipment

A flexible endoscope is required for urethral endoscopy. While fiberoptic endoscopes can be used for this purpose, video endoscopes generally provide superior image quality. It is preferable to have a video monitor for easy visualization of the field of view by both operators and other attendees for the procedure. Recording devices for video and still captures are also recommended. The working length of the insertion tube should be a minimum of 100 cm, and the diameter should be a maximum of 1 cm. A smaller tube diameter may be necessary for smaller or younger stallions or for navigation through the openings of the seminal colliculus into the seminal vesicle (vesicular gland) luminae. Accessory instruments that may be valuable during the procedure are aspiration catheters, culture swabs, grasping forceps, and biopsy forceps. While this communication is directed toward use of the endoscope as a diagnostic instrument, if one envisions using the instrument for laser, electrocautery, or cryosurgical applications, it is advisable to discuss with the manufacturer(s) appropriately designed endoscopes for such purposes.

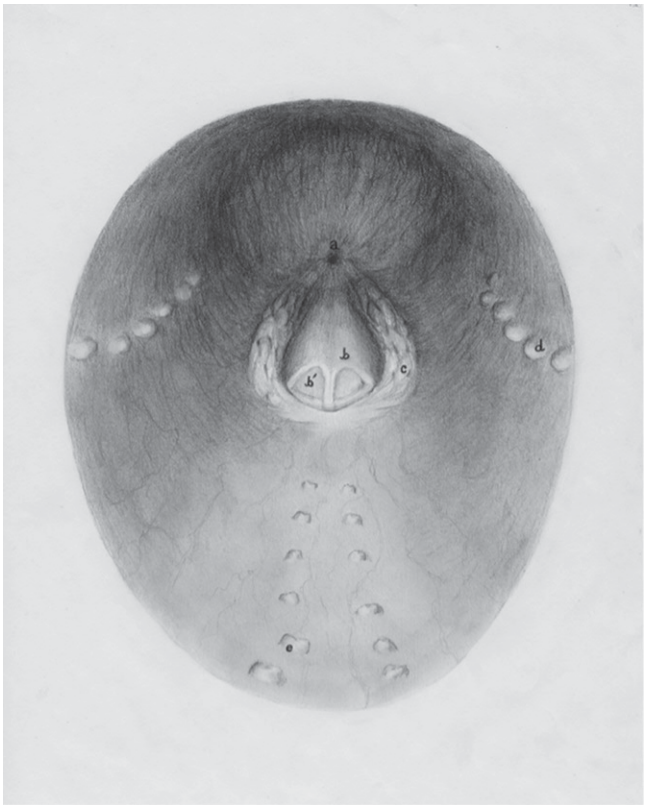

Fig. 1. Illustration of the pelvic urethra in a male horse, as viewed by urethral endoscopy: (a) bladder orifice, (b) seminal colliculus (or colliculus seminalis), (b′) ejaculatory duct (common opening for ductus deferens and excretory duct of seminal vesicle (vesicular gland), (c) openings of ducts of prostate gland, (d) openings of ducts of urethral glands, and (e) opening of ducts of bulbourethral glands.

4. Anatomical Considerations

An illustration of an endoscopic view of the stallion urethra reveals the openings to an assortment of glands as well as the ejaculatory ducts and the bladder orifice (Fig. 1). The insertion tube bends approximately 180° when passing over the pelvic brim so all of the structures in the pelvic urethra are "upside down" when viewed through the endoscope. The openings to the bulbourethral glands are located posteriorly in the pelvic urethra in two linearly-arranged rows, as viewed endoscopically. The openings to the urethral glands are located slightly more proximally and laterally in single rows. The seminal colliculus (colliculus seminalis) is a small protuberance on the ventral midline (as viewed endoscopically) immediately posterior to the bladder orifice. The seminal colliculus contains two ejaculatory duct openings. Each opening is typically a common exit point for secretions of the corresponding ductus deferens and ampulla, as well as the

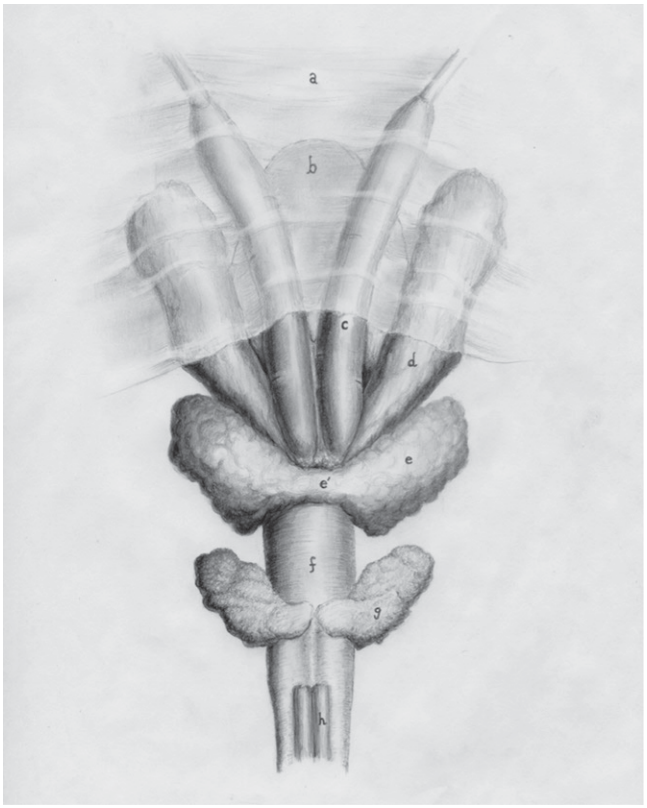

Fig. 2. Illustration of the accessory genital glands of the stallion (dorsal view): (a) genital fold, (b) bladder, (c) ampulla, (d) seminal vesicle (vesicular gland), (e) lobe of prostate gland, (é) isthmus of the prostate gland, (f) pelvic urethra (enclosed by urethralis muscle), (g) bulbourethral gland, and (h) retractor penis muscle.

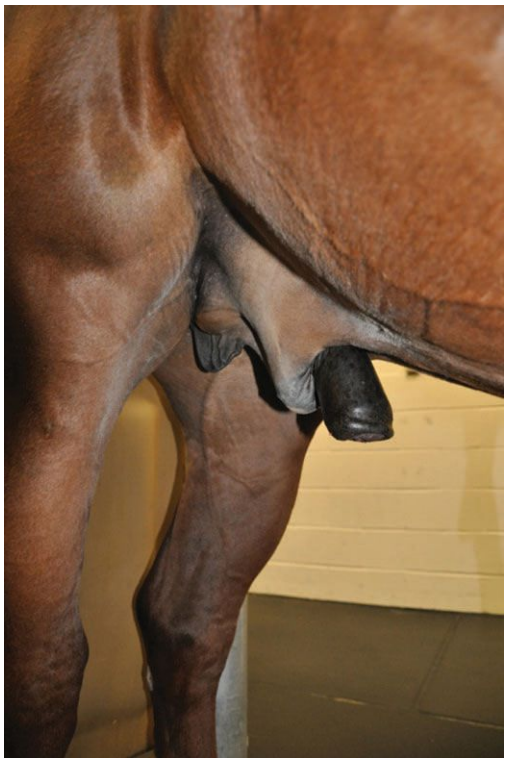

Fig. 3. Partially exteriorized penis at three minutes following intravenous administration of detomidine hydrochloride (6 μg/kg). The prepuce can be reflected at this time to grasp the free portion of the penis for cleaning. Additional sedation will allow more lengthening of the penis but the additional length of the penis can result in inadequate exposure of the proximal urethra and bladder with a 100-cm insertion tube in larger stallions.

ipsilateral seminal vesicle (vesicular gland). Infrequently, the ducts of the seminal vesicles have openings separate of the ejaculatory ducts on the seminal colliculus. The seminal colliculus is enveloped laterally by small tissue mounds, which contain numerous slit-like openings of the prostate gland. The accessory genital glands are illustrated in Fig. 2 and are generally discernible transrectally by palpation and/or ultrasound.

The Examination

Sedation of the stallion with detomidine hydrochloride[a] (6–10 μg/kg, intravenously) aids in exteriorization of the penis and provides limited analgesia during the procedure (Fig. 3). The examination can be initiated 3 to 5 minutes following drug administration and should be performed with the patient restrained in a set of stocks to minimize movement. Use of acepromazine maleate (or other derivatives of phenothiazine) for tranquilization is contraindicated in stallions because of the propensity for drug-induced paralysis of the retractor penis muscle, which can lead to irreversible penile prolapse.

Once the penis is exteriorized by sedation of the stallion, the penis should be subjected to gentle traction and surgical scrubbing, using povidone iodine[b]- or chlorhexadine[c]-based soap products. As the point of entry of the endoscope insertion tube is the ure-

Fig. 4. A sterile scrubbing technique should be applied to the distal penis prior to insertion of the endoscope. Care should be taken to include adequate cleansing of the fossa glandis and glans penis. It is important to remove all soap residue from the penis, and this can be particularly difficult to complete with the urethral sinuses of the fossa glandis.

Fig. 5. A 60-mL syringe with a catheter tip can be used to lavage the urethral sinuses with sterile water or saline to ensure that soap residue is removed from these sites.

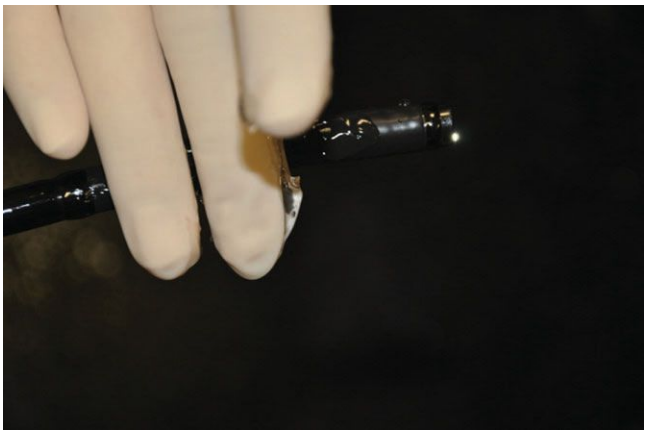

Fig. 7. A small amount of sterile lubricant can be applied to the distal end of the insertion tube, using care not to contaminate the lens with the lubricant, as this could impair good visibility of the urethral lumen.

thral orifice, cleansing should focus on the distal penis (Fig. 4), being careful not to allow contamination of the site by microbes of the proximal penile and preputial integuments. It is important to thoroughly rinse all residual soap from the penis, as it can result in contact dermatitis. The recesses of the fossa glandis are the most vulnerable sites for accumulation of soap residue, so this area should be flushed with sterile saline after the surgical scrub is completed (Fig. 5). Following sterile preparation of the site, sterile gauze can be used to encircle the distal penis with overlying digital pressure to stabilize the penis for the procedure (Fig. 6).

The procedure is most easily performed with two operators: one to hold the penis and guide the insertion tube and one to manipulate the control knobs. Sterile lubricant should be applied to the tip of the insertion tube prior to entering the urethra (Fig. 7). Care should be taken to avoid lubricant contact with the endoscope lens, such that visualization is not hampered. The instrument should also be "white-balanced" to enhance color optics if that feature is offered with the endoscope (Fig. 8). Once inserted into the urethra, the insertion tube should be slowly advanced with intermittent air insufflation so that the field of view is easily discernible (Fig. 9). The lens may need to be rinsed occasionally if the image becomes blurred. As the endo-

Fig. 6. After thorough cleaning and rinsing of the penis, sterile gauze can be used to encircle the free portion of the penis to aid in holding the penis while performing an endoscopic procedure of the urethra.

Fig. 8. For endoscopes with this capacity, the endoscope should be subjected to "white balancing" prior to the examination to maximize coloration of tissues.

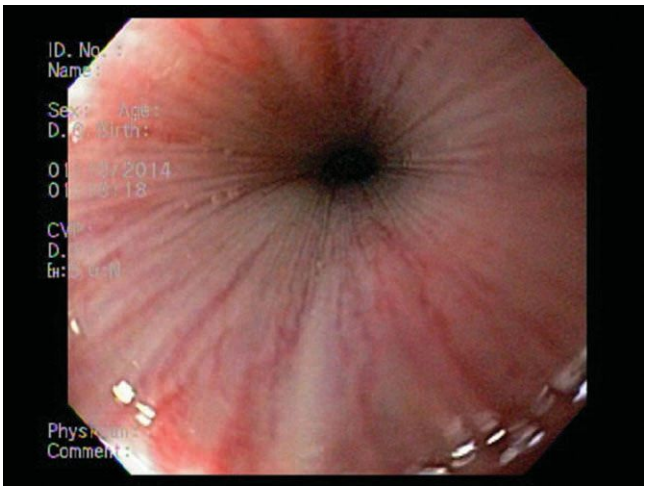

Fig. 9. Endoscopic view of the distal urethra following slight insufflation with air so that surfaces and lumen can be readily visualized.

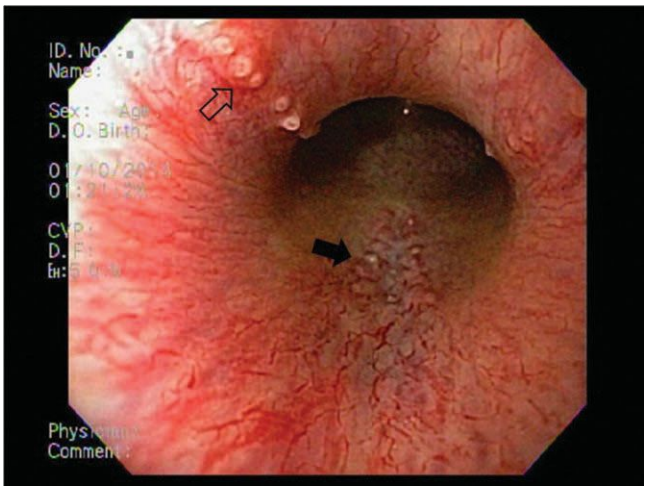

Fig. 11. Endoscopic view of the mid pelvic urethra, demonstrating the duct openings to the bulbourethral glands (closed arrow), and the duct openings to the urethral glands (open arrow).

scope is advanced toward the proximal penile urethra, the vascular pattern becomes quite prominent (Fig. 10). This is a normal finding and should not be confused with inflammatory changes. As one courses over the pelvic ischium, the openings of the bulbourethral glands can be found on the ventral midline of the pelvic urethra (when viewed endoscopically) whereas the openings of the scattered urethral glands are located more laterally (Fig. 11). More proximally, the seminal colliculus is a distinct protuberance located on the floor of the proximal urethra (recall that structures in this area are typically upside down when viewed endoscopically). It typically contains two prominent openings to the ejaculatory ducts (Fig. 12); however, the opening can be more slit-like and may require dilation with an atraumatic probe to become distinct (Fig. 13). Gentle forward pressure on the insertion tube while it is overlying the orifice of the ejaculatory ducts will generally allow admittance into the duct leading to the seminal vesicles. The procedure can be augmented by insufflation of air at the level of the opening or prior to insertion of an atraumatic probe (via the biopsy channel) into the opening to facilitate entry of the insertion tube. Numerous slit-openings to the prostatic ducts are detected on either side of the seminal colliculus (Fig. 12). The opening to the bladder, located at the most proximal point of the urethra, is oftentimes closed during the endoscopic procedure (Fig. 14), but access to the bladder lumen can be gained with slight forward pressure on

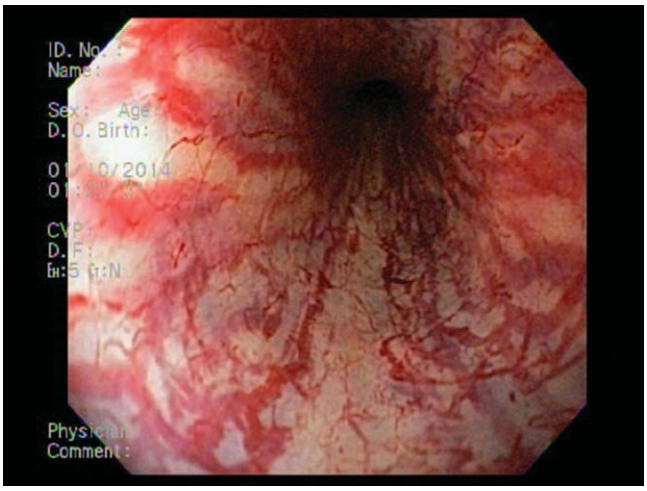

Fig. 10. Endoscopic view of the proximal penile urethra. Note the prominent vascular pattern in this segment of the urethra, a normal finding.

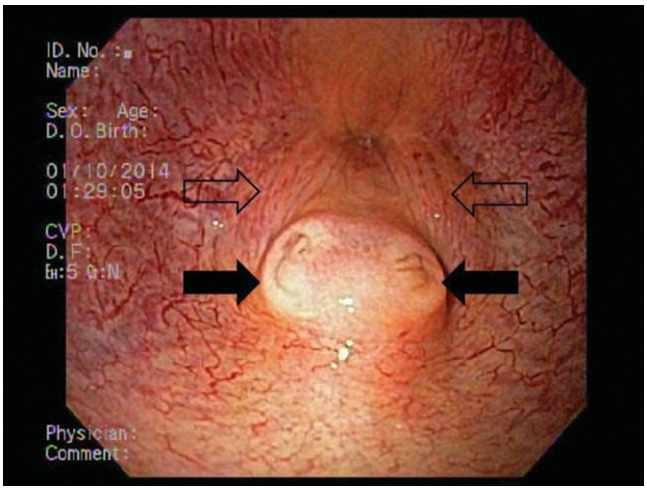

Fig. 12. Close-up endoscopic view of the seminal colliculus, demonstrating the ejaculatory ducts (closed arrow) and openings to the prostatic ducts, which partially encircle the seminal colliculus laterally (open arrow).

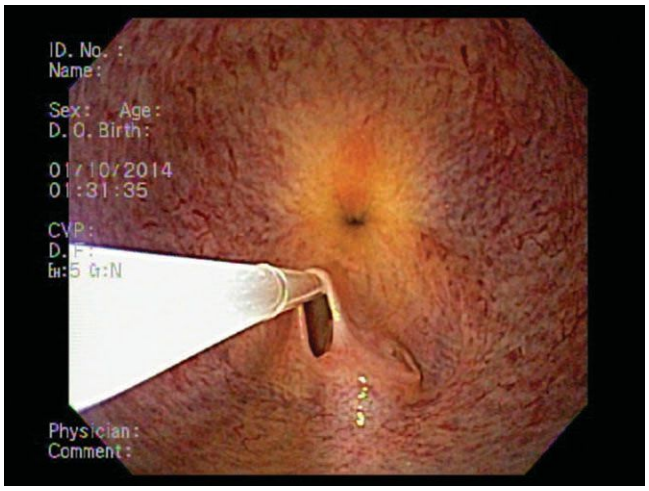

Fig. 13. Close-up endoscopic view of the seminal colliculus, demonstrating dilation of an ejaculatory duct opening with an atraumatic probe.

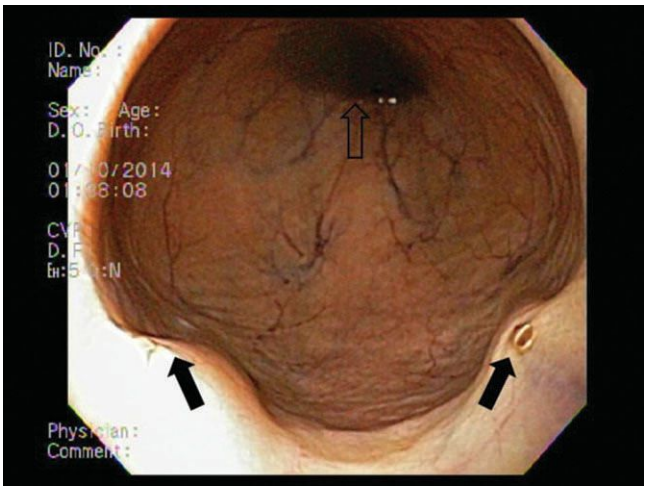

Fig. 15. Endoscopic view of the bladder after aspiration of most urine and partial insufflation with air. Note the ureteral opening near the bladder opening (solid arrows). Also note that the residual pool of urine is located dorsally (open arrow) because the insertion tube is typically "upside down" in this location.

the insertion tube. The urine in the bladder can be partially, or completely, evacuated with controlled aspiration so that the luminal contents and mucosal surface, and the ureteral openings can be identified (Fig. 15).

Disorders of the urethral and bulbourethral glands are essentially unreported, and prostatic lesions are rare. Although the most common disorder of the accessory genital glands is occluded ampullae, this condition cannot be confirmed via urethral endoscopy. Two disorders of the stallion that are most commonly confirmed by urethral endoscopy are seminal vesiculitis and rents in the pelvic urethra.

For confirmation of seminal vesiculitis, the insertion tube of the endoscope can be inserted directly into the seminal vesicles, whereby the interior of the gland(s) can be examined, samples obtained for cytology and culture, and gland lavage and infusion of medications can be conducted (Fig. 16). Alternatively, a sterile aspiration catheter or culture instrument can be advanced into the lumen of a seminal vesicle via an ejaculatory duct for procurement of samples (Fig. 17). This is also a good method for lavage of the seminal vesicles and for instillation of medications.

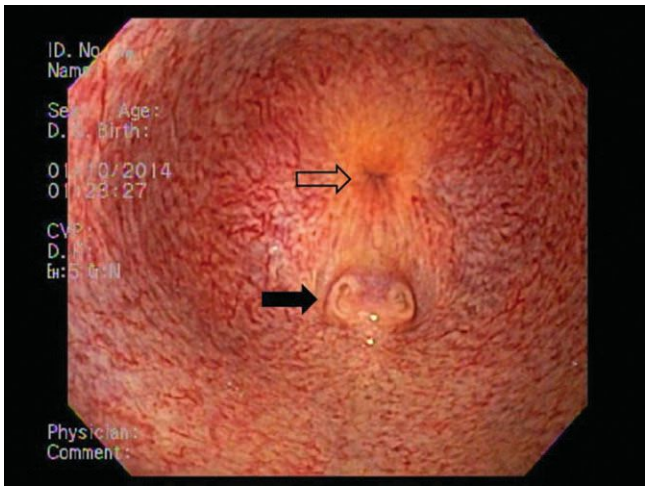

Fig. 14. Endoscopic view of the proximal pelvic urethra, demonstrating the seminal colliculus (solid arrow) and opening to the bladder (open arrow).

Fig. 16. Endoscopic view of the lumen of a seminal vesicle by direct entry of an insertion tube into the site. Procurement of samples or lavage/medication of the site can be performed in this manner.

Fig. 17. Advancement of a catheter into an ejaculatory duct for lavage of the lumen of a seminal vesicle.

Rents in the urethra are the most common disorder associated with hematuria/hemospermia in stallions where external lesions of the penis are absent. Profuse arterial hemorrhage in ejaculates usually results from an idiopathic defect in the urethra that communicates with the surrounding corpus spongiosum penis. Diagnosis of the urethral defect requires urethral endoscopy (Fig. 18). The defect can be small and difficult to identify. Endoscopy immediately following ejaculation may aid the examiner in locating the defect because blood can often be detected within the defect at this time. The rent occurs largely in a similar location in all affected patients, being located on the ventrum of the urethra (as viewed endoscopically) distal to the duct openings of the bulbourethral glands.

6. Precautionary Measures

Care must be used when performing a urethral endoscopy, as untended injury to the patient can occur. A primary precaution is to ensure that the endoscope and entry fluids are sterile prior to use, so as to prevent development of cystitis or seminal vesiculitis. Undue force on tissues can also induce injury as can aspiration of air from compartments when the endoscope tip is close to a wall, leading to traumatization of mucosa and underlying structures.

Fig. 18. A rent that communicates with the surrounding corpus spongiosum penis is identified in the urethra. A 1.5-inch 20-g needle has been passed into the urethral lumen from the subischial region to demonstrate the location of rent on the caudal aspect of the urethra.

As a general rule, aspiration should not be attempted when a visible lumen is not detectable. One should also guard against over-inflation of the urogenital tract with air when performing an endoscopic examination, as rupture of the bladder has occurred in such instances. It is wise to deflate the bladder and urethra when the examination is completed.

Acknowledgments

Conflict of Interest

The Author declares no conflicts of interest.

Footnotes

[a]Dormosedan®, Orion Corporation, Espoo, Finland.
[b]Betadine® Surgical Scrub, Purdue Pharma, Stamford, CT 06901.
[c]Nolvasan® Surgical Scrub, Zoetis Inc., Florham Park, NJ 07932.

Incidence of Complications Associated With Use of the Henderson Equine Castrating Instrument

Olivia E. Schroeder, VMD, DABVP (Equine); Helen W. Aceto, PhD, VMD; Steven J. Berkowitz, VMD; and David G. Levine, DVM, DACVS*

Use of the Henderson equine castrating instrument is becoming more widely accepted in equine ambulatory practice. Its use is associated with a low rate of complication in young horses (≤2 years of age) and a rate of serious complications in all ages of horses similar to other published techniques. Authors' address: University of Pennsylvania, 382 West Street Road, Kennett Square, PA 19348 (Levine, Aceto); 20612 276th Avenue SE, Maple Valley, WA 93038 (Schroeder); and 25 Webster Lane, Oxford, PA 19363 (Berkowitz); e-mail: dglevine@vet.upenn.edu. *Corresponding and presenting author. © 2014 AAEP.

1. Introduction

Castration is one of the most common surgical procedures performed in equine practice and complications of these surgical procedures are the most common cause for malpractice claims against equine practitioners. The Henderson equine castrating instrument is reported to result in a reduction of intraoperative and postoperative complications due to its technique of spinning and "tying" the vessels of the spermatic cord together with the vaginal tunic.

2. Materials and Methods

One hundred and eighty horses were included in this study and their medical records evaluated for the occurrence of complications. Castrations were performed in dorsal recumbency using injectable anesthesia. Two incisions were made and the testicles were removed using the Henderson equine castrating instrument. The incisions were left open to heal by second intention.

3. Results

The total complication rate was 10% (18/180). Of these complications, 16 were non-life-threatening (swelling, seroma). One horse bilaterally eviscerated upon recovery and was euthanized and another horse developed wound botulism postcastration. Horses three years of age and older were five times more likely to develop a postcastration complication than horses two years of age and younger.

4. Discussion

Use of the Henderson equine castrating instrument resulted in a low complication rate, especially among younger horses. This rate is similar to castrations performed with other published techniques and validates this technique's use in horses.

Acknowledgments

Conflict of Interest

The Authors declare no conflict of interest.

Research Abstract

NOTES

Complications Related to the Use of Intrauterine Glass Marbles in Mares

**Mariana Diel de Amorim, DVM, DVSc, DACT*;
Tracey Chenier, DVM, DVSc, DACT; Dawne Nairn, DVM; Jodyne Green, DVM;
Stephen Manning, DVM, MSc, DACT; and Claire Card, DVM, PhD, DACT**

Use of toy intrauterine glass marbles may lead to chronic endometritis, pyometra, and fragments of glass becoming embedded in the endometrium. Authors' addresses: Western College of Veterinary Medicine, Clinical Sciences, Department of Large Animal, Saskatoon, SK, S7N 5B4, Canada (Diel de Amorim, Nairn, Manning, Card); Ontario Veterinary College, Department of Population Medicine, Guelph, ON, N1G 2W1, Canada (Chenier); and 3661 15th Avenue, Prince George, BC, V2N 1A3, Canada (Green); e-mail: amorimm@uoguelph.ca. *Corresponding and presenting author. © 2014 AAEP.

1. Introduction

The effectiveness of intrauterine glass marbles for estrus suppression in mares varies in studies from 11-41.3%, but their use is still common practice. The purpose of this study was to describe a case series of five mares that developed complications related to intrauterine marble(s).

2. Materials and Methods

The history, reproductive examinations, cytologic, and microbial information from 6 mares with intrauterine glass marbles were reviewed. Intrauterine marbles were retrieved manually or under hysteroscopic guidance.

3. Results and Discussion

Three mares had 1 and 2 mares had 2 intrauterine glass marble(s), 5/5 mares had marbles for > 1 year, 2/5 had chronic endometritis, and 3/5 had pyometra. A marble or glass shards were adhered to the endometrium in 3/5 mares. This report demonstrates that the long term use of toy intrauterine glass marbles may have severe deleterious effects on reproductive health.

Acknowledgments

The Authors would like to thank the rDVMs.

Conflict of Interest

The Authors declare no conflicts of interest.

Research Abstract

NOTES

Pharmacokinetics of Metronidazole in Foals

Elsbeth Swain, DVM*; K. Gary Magdesian, DVM; Judy E. Edman, BS; and Heather K. Knych, DVM, PhD, DACVCP

Neonatal foals have reduced clearance of metronidazole compared to adults, with a prolonged elimination half-life. Based on the results of this study, dosage recommendation for foals includes 10–15 mg/kg IV or orally, q 12 h. Authors' addresses: William R. Pritchard Veterinary Medical Teaching Hospital (Swain); Department of Internal Medicine and Epidemiology (Magdesian, Edman); and Kenneth L. Maddy Equine Analytical Chemistry Laboratory (Knych), School of Veterinary Medicine, University of California, Davis, CA 95616; e-mail: elsbethswaindvm@gmail.com. *Corresponding and presenting author. © 2014 AAEP.

1. Introduction

Neonatal foals have different pharmacokinetics compared to adults, which may lead to accumulation of drugs and adverse effects when adult dosage regimens are used. The purpose of this study was to determine the pharmacokinetics of metronidazole in healthy foals when administered as a single intravenous (IV) and intragastric (IG) dose.

2. Materials and Methods

Twelve healthy foals at 1 to 2.5 days of age were randomized into two groups and received metronidazole (15 mg/kg) IV or IG. Foals in the IV group were studied again at 10 to 12 days of age to evaluate for maturational differences. Plasma metronidazole concentrations were measured using liquid chromatography mass-spectrometry. Pharmacokinetics were described using a noncompartmental model and were compared between age groups.

3. Results

For IV administration in foals 1 to 2.5 days of age, the maximum plasma concentration (C_0 after infusion) was 18.79 ± 1.46 μg/ml, elimination half-life was 11.79 ± 1.77 h, clearance was 0.84 ± 0.13 ml/min/kg, and the volume of distribution was 0.87 ± 0.07 L/kg. Oral bioavailability was 100%. Elimination half-life was significantly longer, and clearance was significantly lower, in 1–2.5 day-old foals compared to 10–12 day-old foals. Elimination half-life at 10 to 12 days of age was 9.07 ± 2.84 h.

4. Discussion

The reduced clearance and longer elimination half-life of metronidazole in foals compared to adults warrants altered dosing recommendations in foals.

Acknowledgments

Conflict of Interest

This study was supported by the Center for Equine Health, with funds from the Oak Tree Racing Association, the State of California pari-mutuel wagering fund, and contributions from private donors as well as the Henry Endowed Chair in Emergency Medicine and Critical Care.

Research Abstract

NOTES

Pulmonary Pharmacokinetics of Desfuroylceftiofur Acetamide After Nebulization or Intramuscular Administration of Ceftiofur Sodium to Weanling Foals

Lisa Fultz DVM*; Steeve Giguère DVM, PhD; Londa J. Berghaus MS; G. Scott Grover MS; and Dawn A. Merritt, PhD

Nebulization of ceftiofur sodium is well tolerated and results in significantly higher drug concentrations in pulmonary epithelial lining fluid (PELF) compared to administration of the same dose intramuscular (IM). Authors' addresses: Department of Large Animal Medicine, College of Veterinary Medicine, University of Georgia, Athens, GA 30602 (Fultz, Giguère, Berghaus); and Zoetis, Metabolism & Safety, Kalamazoo, MI 49001 (Grover, Merritt); e-mail: lfultz@uga.edu. *Corresponding and presenting author. © 2014 AAEP.

1. Introduction

Administration of ceftiofur sodium via nebulization has been recommended for the treatment of bronchopneumonia in horses despite the lack of pharmacokinetic and safety data. The objectives were: (1) to compare concentrations of desfuroylceftiofur acetamide (DCA) in plasma and pulmonary epithelial lining fluid (PELF) of foals after nebulization or intramuscular (IM) administration of ceftiofur sodium, and (2) to determine if nebulization of ceftiofur sodium induces airway inflammation.

2. Materials and Methods

Six weanling foals received ceftiofur sodium[a] (2.2 mg/kg daily for 5 doses) by the IM route and 6 foals received the same dose by nebulization.[b] Concentrations of DCA in the plasma and PELF were measured and bronchoalveolar lavage cytology was performed after dose 5.

3. Results

Foals receiving ceftiofur sodium via nebulization had significantly higher C_{max} (4.52 ± 2.91 vs 0.73 ± 0.73 μg/mL) and area under the curve (24.14 ± 14.09 vs 5.91 ± 3.28 μg h/mL) in PELF compared to the IM group. Nebulization resulted in PELF DCA concentrations above the minimum inhibitory concentration of bacterial pathogens commonly associated with bronchopneumonia for most of the dosing interval. Differential cell counts in bronchoalveolar lavage fluid of foals nebulized with ceftiofur sodium were not significantly different from those of foals nebulized with saline.

4. Discussion

Nebulized ceftiofur sodium warrants further investigation for the treatment of lower respiratory tract bacterial infections in horses.

Research Abstract

NOTES

Acknowledgments

Conflict of Interest

This study was funded by a grant from the Veterinary Comparative Respiratory Society and by Zoetis.

Footnotes

[a]Naxcel® Sterile Suspension, Zoetis, Madison, NJ 07932.
[b]Flexineb, Nortev Ltd., Galway, Ireland.

Use of Daily Diclazuril Pelleted Top Dress for the Prevention of *Sarcocystis neurona* Infection in Foals

Nicola Pusterla, DVM, PhD, DACVIM*; Andrea Packham, MS; Patricia Conrad, DVM, PhD; Philip Kass, DVM, MPVM, MS, PhD; and Sarah Mackie, DVM

The daily supplementation of diclazuril pelleted top dress at 0.5 mg/kg body weight has shown a significant reduction in seroconversion against *Sarcocystis neurona* in foals treated up to 12 months of age when compared to untreated control foals. Authors' addresses: Department of Medicine and Epidemiology (Pusterla, Mackie), Department of Pathology, Microbiology and Immunology (Packham, Conrad), and Department of Population Health and Reproduction (Kass), School of Veterinary Medicine, University of California, Davis, CA 95616; e-mail: npusterla@ ucdavis.edu. *Corresponding and presenting author. © 2014 AAEP.

1. Introduction

Therapeutic treatment strategies for the prevention of *S. neurona* infection in horses have been empirical. A pelleted top dress 1.56% diclazuril antiprotozoal drug recently introduced to the equine market and labeled for the treatment of EPM has the potential to be used for the prevention of EPM due to its convenient formulation. A low dose of diclazuril given at 0.5 mg/kg body weight has shown to reach plasma and cerebrospinal fluid (CSF) concentrations at steady-state in excess of the minimal concentration known to be inhibitory to *S. neurona* merozoite production in cell culture. The purpose of this study was to evaluate the temporal serological response against *S. neurona* in foals receiving daily diclazuril and in untreated herdmates.

2. Materials and Methods

Thirty-three foals from a farm with a high exposure rate to *S. neurona* were randomly assigned to either an untreated control group or a diclazuril-treated group. Treatment consisted of the administration of 0.5 mg/kg body weight of diclazuril pelleted top dress starting at 4 weeks of age until the foals were 12 months of age. Whole blood was collected from every dam and foal 24 hours post-foaling and monthly thereafter from every study foal for the duration of the study. The blood was tested for IgG against *S. neurona* using the indirect fluorescent antibody test.

3. Results and Discussion

Following ingestion of colostral antibodies to *S. neurona*, there was a steady and continuous decline in seroprevalence and antibody titers to *S. neurona* until the foals from both groups reached weaning age. Thereafter, untreated foals showed an increase in monthly seroprevalence ranging from 53 to 82%. Diclazuril treated foals showed significant

Research Abstract

NOTES

lower monthly seroprevalences ranging from 6 to 37%. The difference in temporal seroprevalence can be explained by the successful reduction of *S. neurona* infection in foals receiving daily diclazuril at a low dose.

Acknowledgments

Conflict of Interest

This study was supported by a grant from The Center for Equine Health, University of California, School of Veterinary Medicine at Davis and Merck Animal Health.

Pharmacokinetics of a Low Dose of Diclazuril Administered Orally as a Pelleted Top Dressing in Adult Horses

Laszlo Hunyadi, DVM, PhD*; Mark G. Papich, DVM, MS; and Nicola Pusterla, DVM, PhD, DACVIM

Diclazuril pellets given at a low dose reach plasma and cerebrospinal fluid (CSF) levels known to inhibit *Sarcocystis neurona* in cell culture. Authors' addresses: William R. Pritchard Veterinary Medical Teaching Hospital (Hunyadi); Department of Medicine and Epidemiology (Pusterla), University of California, Davis School of Veterinary Medicine, Davis, CA 95616; and College of Veterinary Medicine, North Carolina State University, Raleigh, NC 27607 (Papich); e-mail: lmhunyadi@ucdavis.edu. *Corresponding and presenting author. © 2014 AAEP.

1. Introduction

The economic impact of EPM in endemic areas on both performance horses and horse breeding industries is substantial. Although various approaches to the prevention of EPM have been investigated, replication of the disease in clinical settings has been elusive. The objective of this study was to determine if the low dose would attain plasma and cerebrospinal fluid (CSF) concentrations that are known to inhibit *Sarcocystis neurona* in cell culture.

2. Procedures

Six healthy adult horses received 0.5 mg/kg diclazuril pellets as a low dose orally once and plasma samples were collected at regular intervals for 168 h. Daily oral doses were then administered for 10 days. Plasma and CSF were collected once after the 9th dose. After the 10th oral dose, plasma samples were collected at regular intervals for 168 h. The study was repeated with the labeled dose at 1 mg/kg after a 4 week washout period. Plasma and CSF samples were analyzed by high-pressure liquid chromatography. A one-compartment pharmacokinetic model with first-order oral absorption was fitted to the single administration data. Steady-state pharmacokinetics was performed using noncompartmental analysis for steady-state analysis.

3. Results

The mean concentration of diclazuril in the CSF following the low dose was 26 ng/mL whereas the CSF in the labeled dose was 25 ng/mL. The CSF concentrations were only 5 to 6% of the corresponding plasma concentrations and therapeutic plasma concentrations occurred at steady-state after the 10th dose for both doses.

4. Discussion

The results of this study show that diclazuril pellets given at both a low and labeled dose reach plasma and CSF levels known to inhibit *Sarcocystis neurona* in cell culture after steady-state levels have been reached.

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Conflict of Interest

The Protazil[a] and pharmacokinetic analysis were sponsored by Merck Animal Health. The horses and CSF analysis were sponsored by University of California Davis. The Authors have no investments or consulting with the sponsors. Two authors are employees of University of California Davis; one is employed by North Carolina State University.

Footnote

[a]Protozil™ Merck Animal Health, Summit, NJ 07901-1330.

Safety and Efficacy of a New Formulation of Trimethoprim Sulfadiazine in Horses

Scott McClure, DVM, PhD, DACVS*; Craig Reinemeyer, DVM, PhD; Robbin Koenig, PhD; and Peggy Anne Hawkins, DVM, MS

A novel proprietary oral suspension of trimethoprim/sulfadiazine underwent safety and efficacy studies for an FDA new animal drug approval. The antimicrobial had no serious adverse effects on clinical, laboratory, or pathologic parameters of mature horses when administered at up to five times the intended combined dosage of 24 mg/kg twice daily for 30 consecutive days. Horses with lower respiratory tract infections caused by *Streptocccus equi* subsp. *zoopidemicus* were treated with combined trimethoprim/sulfadiazine (TMP/SDZ) oral suspension at a dosage of 24 mg/kg q 12 h for 10 days. The TMP/SDZ suspension effectively treated the clinical signs of respiratory infection and elimination of the organism from the respiratory tract. Authors' addresses: Iowa State University, College of Veterinary Medicine, 1600 S. 16th Street, Ames, IA 50011-1250 (McClure); East Tennessee Clinical Research, Inc., 80 Copper Ridge Road, Rockwood, TN 37854 (Reinemeyer); Biotechnical Services Inc., 4610 West Commercial Drive, North Little Rock, AK 72116 (Koenig); and Aurora Pharmaceuticals, 1196 Hwy. 3 South, Northfield, MI 55057 (Hawkins); e-mail: mcclures@iastate.edu. *Corresponding and presenting author. © 2014 AAEP.

1. Introduction

Potentiated sulfonamides combine sulfa with a diaminopyrimidine, utilizing the synergistic mechanisms of action of these drugs. Trimethoprim and sulfadiazine both function as bacteriostatic antimicrobials by interfering with folate metabolism in bacteria. The two antimicrobials function synergistically to inhibit bacterial DNA synthesis, reducing the potential for the development of antimicrobial resistance compared to either drug used alone. Potentiated sulfonamides are routinely used in equine practice for broad spectrum antibiotic therapy, particularly in the treatment of lower respiratory tract infections. This drug combination is popular because it can be administered orally.

Although paste formulations approved for horses are labeled for once daily administration at 30 mg/kg body weight, pharmacokinetic data for the potentiated sulfonamides suggest that twice daily dosing is more appropriate to maintain therapeutic plasma levels above the minimum inhibitory concentration (MIC). A combined study of field effectiveness and pharmacokinetics conducted in 26 horses with lower respiratory disease determined that the time-concentration profile of sulfadiazine in horses did not demonstrate linearity with increasing dosage. Increased dosages of the potentiated sulfa did not result in a significantly greater $t >$ MIC for either active moiety, particularly for sulfadiazine, where the plasma C_{max} was actually lower for the higher dose regimen. The 30 mg/kg q 12 h dosing regimen had no clinical advantage over 24 mg/kg q 12 h and the latter was associated with fewer incidents of antibiotic-associated loose feces.

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Based on this information, a proprietary oral suspension formulation of trimethoprim plus sulfadiazine (TMP/SDZ) has been developed, with label recommendations for dosing at 24 mg/kg body weight twice daily for 10 consecutive days. Because the formulation was different than those previously approved, the FDA requested a New Animal Drug Application. Two separate studies as part of this application are presented here. A controlled clinical study to determine the margin of safety of the suspension at dosages of 1×, 3×, and 5× the label recommendation, administered for 30 days (three times the recommended duration), and a broad field trial on the clinical efficacy of the antimicrobial on *Streptocccus equi* subsp. *Zoopidemicus* bronchopneumonia.

2. Safety Study

Objective

To evaluate the safety of a novel trimethoprim/sulfadiazine oral suspension in horses up to 5X the normal dose for 30 days.

Design

Blinded, randomized, controlled margin of safety study.

Animals

Thirty-two healthy horses.

Procedures

Dose multiples of 0×, 1×, 3×, or 5× the recommended dosage of 24 mg/kg bwt were administered at 12-hour intervals for a total of 30 days; three times the recommended treatment duration of 10 days. Daily health observations and fecal consistency was scored. Clinicopathologic data were obtained pretreatment and on days 6, 13, 20, and 29. At the completion of the 30-day period, complete necropsies were performed.

Results

Loose feces was the most common observation that was likely related to TMP/SDZ treatment. The incidence of loose feces was greater in groups treated with higher dosages of TMP/SDZ. All episodes were self-limiting and no horses were treated for diarrhea. Significant differences between the TMP/SDZ-treated animals and the control group were observed for albumin, creatinine, and gamma-glutamyl transferase (GGT) concentrations. These changes did not exhibit a correlation to dosage and none were considered clinically significant. All gross pathologic observations noted during necropsy were considered to represent normal biological variation, or were related to conditions existing prior to treatment, primarily to parasitic activity.

Conclusions and Clinical Relevance

No serious adverse effects on the clinical, laboratory, or pathologic parameters of mature horses were found when administered at up to five times the intended combined dosage of 24 mg/kg twice daily for 30 consecutive days.

2. Efficacy Study

Objective

To evaluate the effectiveness of a novel trimethoprim and sulfadiazine oral suspension in the treatment of naturally acquired *Streptococcus equi* subsp. *zooepidemicus* (*Strep. zoo*.) related pneumonia in horses under field use conditions.

Design

A randomized blinded placebo controlled field efficacy study.

Animals

Two hundred seventy horses that met inclusion criteria from 5 different sites.

Procedures

Horses with lower respiratory tract infections caused by *Streptococcus equi* subsp. *zooepidemicus* were treated with a novel formulation of combined trimethoprim/sulfadiazine oral suspension at a dosage of 24 mg/kg twice a day for 10 days.

Results

Of the 119 horses receiving treatment, 58% (69/119) were considered successes, compared to only 15% (9/61) of the saline control horses. Many of the failures were apparent by day 5 with 41% (25/61) of the control horses failing at day 5 compared to only 7.6% (9/119) of treated horses. By day 10, 46% (28/61) of the control horses had met the failure criteria compared to only 9.2% (11/119) of the treated horses. There were no adverse events associated with the TMP/SMZ administration. The fecal consistency scores assessed twice daily throughout the study period showed no significant differences between treatment groups.

Conclusions and Clinical Relevance

Improved bioavailability allows a 20% lower dose than previously published. The novel TMP/SDZ suspension effectively treated the clinical signs of respiratory infection and eliminated the organism from the respiratory tract.

Acknowledgments

Conflict of Interest

These studies were funded by Aurora Pharmaceuticals LLC, Northfield, MN. Dr. Scott McClure served as a paid investigator in the broad field trial. Dr. Craig Reinemeyer served as a paid investigator in the safety studies. Dr. Robbin Koenig served as a paid employee of Biotechnical Services Incorporated to facilitate FDA submission. Dr. Peggy Anne Hawkins was employed by Aurora Pharmaceutical as a

representative of the company during the FDA studies. The paper was reviewed by the sponsor; however, the sponsor did not exert editorial influence. All of the data presented was available to the author of these abstracts and all of the data is/will be publicly available through the FOI. The FDA does exert influence by limiting statements to those proven by the data.

Effect of CYP2D Genetic Polymorphisms on Drug Metabolism in the Horse

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Results of this study support the presence of polymorphisms in the CYP2D50 gene that appear to have functional implications with respect to metabolism of drugs that are substrates for this enzyme. Authors' address: K.L. Maddy Equine Analytical Chemistry Laboratory, School of Veterinary Medicine, University of California, Davis 95616; e-mail: hkknych@ucdavis.edu. *Corresponding and presenting author. © 2014 AAEP.

1. Introduction

Metabolism is an essential factor in the clearance of many drugs and, as such, plays a major role in the establishment of dosing regimens and withdrawal times. CYP2D6, the human ortholog to equine CYP2D50, is a drug-metabolizing enzyme that is highly polymorphic in humans, leading to widely differing levels of metabolic activity. In horses, it has been proposed that the CYP2D50 gene may be prone to polymorphism.

2. Materials and Methods

Blood samples were obtained from 150 horses, the CYP2D50 gene cloned, sequenced, and full-length sequences analyzed for single nucleotide polymorphisms (SNPs), deletions, or insertions. Pharmacokinetic data was collected from a subset of horses following administration of a single oral dose of tramadol or dextromethorphan and probit analysis was used to calculate metabolic ratios and correlate results of the genotyping studies with those of the phenotyping studies.

3. Results and Discussion

Alignment of the full-length sequences from over one hundred horses showed a distribution into three distinct haplotypes, which may correspond to phenotypes previously described in the human. A number of SNPs were discovered which may interfere with protein function, as determined by SIFT analysis of the protein sequence. Furthermore, probit analysis of the metabolic ratio of 2 CYP2D probe drugs administered to a subset of the horses revealed what appears to be poor, extensive, and ultrarapid metabolizers.

Acknowledgments

Conflict of Interest

This study was funded by the Grayson-Jockey Club.

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Long-Term Response of Equids With Pituitary Pars Intermedia Dysfunction to Treatment With Pergolide

Harold C. Schott II, DVM, PhD, DACVIM*; Julie R. Rapson, DVM; Judy V. Marteniuk, DVM, MS; and Sue Wismer, LVT

Long-term (>5 years) treatment of pituitary pars intermedia dysfunction (PPID)-affected equids with pergolide produces clinical improvement in nearly all affected animals, normalization of endocrine test results in >60% of cases, and high owner satisfaction. Authors' address: Department of Large Animal Clinical Sciences, College of Veterinary Medicine, Michigan State University, East Lansing, MI 48824; e-mail schott@cvm.msu.edu. *Corresponding and presenting author. © 2014 AAEP.

1. Introduction

There is growing interest in treatment of PPID-affected equids; however, limited data exist to document long-term treatment responses.

2. Material and Methods

Thirty equids with signs of PPID and supportive overnight dexamethasone suppression test (ODST) results or elevated (>50 pg/mL) plasma adrenocorticotropin (ACTH) concentrations were started on pergolide[a] (2 μg/kg, PO, q24h) in 2008 to 2009. Equids were reexamined after 0.5, 2.5, 3, 3.5, 4.5, and 5.5 years of treatment. Pergolide dose was increased to 4 μg/kg, PO, q24h when endocrine test results remained abnormal.

3. Results

Fourteen horses were euthanized (3 for chronic laminitis) and 4 died during the study. After >5 years of treatment, owners of the 12 remaining equids remained satisfied with clinical improvement and ⅓ are still on a dose of 2 μg/kg. At the most recent evaluation, ACTH concentration was <50 pg/mL in 71% and ODST results remained normal in 61%.

4. Discussion

Long-term treatment of PPID-affected equids with pergolide results in clinical improvement, normalization of endocrine test results, and owner satisfaction in a high percentage of cases. Further, this case series provides evidence that horses may continue to respond favorably to a 2 μg/kg pergolide dosage, rather than require a progressive increase in dose over time.

Acknowledgments

Conflict of Interest

This study was funded in part by Boehringer-Ingelheim Vetmedica, Inc.

Footnote

[a]Prascend, Boehringer Ingelheim Vetmedica, Inc., St. Joseph, MO 64506.

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Does Equine Pituitary Pars Intermedia Dysfunction Affect Immune Responses to Vaccination?

Amanda A. Adams, PhD*; Melissa H. Siard; Stephanie E. Reedy; Steve Grubbs, DVM, PhD; Marian G. Little, DVM; and Jim P. Little, DVM

Pituitary pars intermedia dysfunction (PPID) horses compared to non-PPID responded immunologically the same to the equine influenza virus component of the vaccine; however, there were some differences in immune response measured to the equine herpes virus and West Nile virus component of the vaccine. Authors' addresses: 108 Gluck Equine Research Center, University of Kentucky, Lexington, KY 40546 (Adams, Siard, Reedy); Boehringer Ingelheim Vetmedica, Inc., 3902 Gene Field Road, Saint Joseph, MO 64506 (Grubbs); and 2320 Fords Mill Road, Paris, KY 40361 (M. Little, J. Little); e-mail: amanda.adams@uky.edu. *Corresponding and presenting author. © 2014 AAEP.

1. Introduction

Over the past decade the aged horse population has significantly expanded with 20 to 30% of the equine population comprised of geriatric horses (≥20 years) and, of these horses, approximately 30% are affected by pituitary pars intermedia dysfunction (PPID), a progressive and debilitating endocrine disease. Along with advancing age, there is a decline in immune function, but currently there are no specific recommendations regarding vaccination of older horses, nor those affected by PPID. Furthermore, it remains to be determined if PPID affects immune responses to vaccination. Thus, the objective of this study was to determine if horses with PPID compared to non-PPID aged match controls would respond immunologically differently to vaccination.

2. Materials and Methods

A total of 33 aged horses of mixed sex and breeds were used in this study. All horses were pre-screened to determine equine influenza virus (EIV) antibody titers and PPID status based on responses to a dexamethasone suppression test (DST) and a thyrotropin releasing hormone (TRH) stimulation test. Furthermore, all horses were assigned a numeric value for severity of clinical signs (hair coat, muscle condition, etc.) of PPID by two blinded DVMs, via an established scoring system. Non-PPID horses were characterized by cortisol levels (≤0.2 ug/dL) at 19 hr post-DST and adrenocorticotropic hormone (ACTH) levels (≤35 pg/mL) pre-TRH stim and ACTH levels (≤110 pg/mL) at 10 min post-TRH stim test. The PPID horses were further characterized by either double positive (DP) [DST no suppression and pre-TRH ACTH (≥35 pg/mL) and 10 min post-TRH (≥110 pg/mL)] or single positive (SP) [DST suppression but pre-TRH ACTH (≥35 pg/mL) and 10 min post-TRH (≥110 pg/mL)] responders. Treatment groups were randomly blocked according to these measures, along with age,

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to the following vaccinate groups: (group 1) non-PPID horses, vaccinated IM[a] ($n = 12$), (group 2) non-PPID controls, receiving a sterile saline vaccination ($n = 3$), (group 3) PPID horses (DP, $n = 7$; SP, $n = 7$) vaccinated,[a] and (group 4) ($n = 6$) PPID horses receiving sterile saline vaccine (DP, $n = 2$; SP, $n = 2$). Peripheral blood for antibody titer measures to EIV, equine herpesvirus (EHV)-1, and West Nile virus (WNV) were collected prior to the first vaccination (week 0) and again at week 2 and 4 post-vaccination. A second vaccine[a] or saline control was given at week 4 and blood collected at week 6 and week 8 post-vaccination.

3. Results

The results indicated that all horses receiving vaccination responded significantly with an increase in EIV and EHV-1 antibody titers post-vaccination. Furthermore, the results indicated that there was no significant difference in EIV antibody titers vaccinations between non-PPID and PPID horses; however, there were significant ($p < 0.05$) differences in EHV-1 and WNV humoral immune responses among the PPID horses.

4. Discussion

This is the first study to determine the effects of PPID on immune responses to routine vaccination.

Acknowledgments

Conflict of Interest

This project was funded by Boehringer Ingelheim Vetmedica.

Footnote

[a]Vetera® Gold, Boehringer Ingelheim Vetmedica, St. Joseph, MO 64506.

How to Utilize an Open Source DICOM Viewer and Properly Transmit Diagnostic Images in Your Practice

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1. Introduction

The adoption of digital imaging has been swift and recent. The greatest advantage of digital imaging is the ability to manipulate contrast, zoom in on areas of interest, make measurements, and transfer them via the Internet for consultation. However, for optimal benefit, this adoption requires more technical "know how." As such, the need for image visualization and analysis has extended beyond the light box. Software for digital imaging and communications in medicine (DICOM) image viewing is often expensive, challenging to navigate, and limited in accessibility. Because of this, practitioners often send and receive radiograph, ultrasound, computed tomography, or magnetic resonance as lossy compressed images with .jpg or .jpeg (Joint Photographic Experts Group (JPEG)) file extensions for consultation on cases. However, when a diagnostic image is exported as a JPEG image important metadata and information in the DICOM header that tells the detector system, the radiographic technique, institution obtaining the radiographs, compression levels, and a host of other information is stripped. Most importantly, the information of scale is also stripped, so measuring lesions is not reliable. Furthermore, JPEG images are difficult to manipulate in a way to ensure that the reader is able to properly evaluate the entire image. Therefore, it is important that practitioners realize the advantage of reading and sharing DICOM images.

In order to share DICOM images, practitioners must have access to simple, user-friendly, and free/affordable DICOM imaging software and easy methods to transfer images to colleagues and specialists. Osirix[a] is an open source Mac OSX-based image processing application for DICOM image viewing. It is free and downloadable software from the Internet. This user-friendly software will aid practitioners in not only evaluating their own images such as radiographs and ultrasound, but it will allow them to manipulate, illustrate, and prepare images and even videos for presentations to clients and colleagues. Osirix is specifically designed to navigate and visualize 2D and 3D images while at the same time acting as a DICOM picture archiving and communication system (PACS) workstation (it can also

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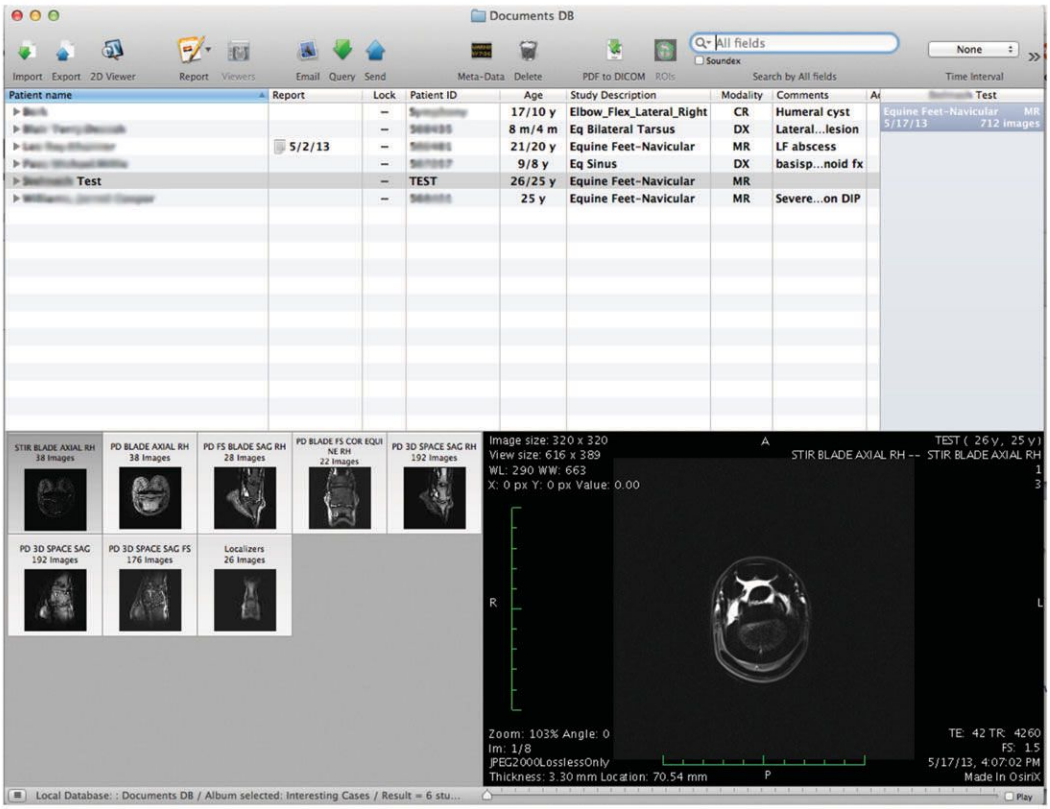

Fig. 1. The database window of Osirix with a list of the patients. The bottom half of the window displays the thumbnail previews. Along the right, there is a list of current and prior studies displayed.

handle 4D and 5D, but that is outside the scope of this discussion). Additionally, this program will easily transmit images via DICOM transfer or export the DICOM images for transfer via free file sharing programs. Learning to use software such as Osirix will improve practitioners' ability to evaluate medical images, consult with other professionals on cases, and even provide increased understanding of cases to clients. This article aims to provide practitioners with an overview of the many capabilities of Osirix, an open source DICOM imaging software and file-sharing options.[1]

2. Materials and Methods

Software

An open source stand-alone application has been developed that serves as a DICOM viewer and PACS. Osirix can be downloaded at www.Osirix-viewer.com to run on the latest version of OsX.. There are alternatives for the Windows users such as Ginkgo CADx,[b] however this "how to" will be confined to Osirix and free file-sharing software. There are numerous download options to choose from, including the free 32-bit version, a paid 64-bit version, a paid FDA approved 64-bit version for medical usage, and a paid version for iPad and iPhone users. Unless you are a radiologist or performing advanced imaging studies with very large volume sets, the free 32-bit version should suffice in most scenarios.

The Osirix program offers all the basic image manipulation functions of zoom, pan, contrast adjustment, image orientation, and measure, as well as allowing annotation and database organization by client or diagnosis. It also has the ability to hang multiple relevant prior exams. Additional advanced functions that can be performed include multiplanar reconstructions, convolution filters, variable slice thickness adjustments, volume rendering, minimum and maximum intensity projections, and surface rendering.

There are several free file-sharing programs commercially available. Dropbox, Google Drive, and Microsoft Onedrive are the most notable. Only Dropbox will be explored in depth in this "how to."[2] A Dropbox account can be created and the software can be downloaded at www.dropbox.com. This is available for Windows, OsX, as well as mobile versions. Dropbox is an installable program or web-based program that syncs images on a personal computer as well as a remote server, also known as the cloud. This program allows folders and files to

Fig. 2. This is the diagnostic window with a sagittal plane magnetic resonance image of the foot. The tool bar is located at the top of the screen with common tools such as pan, zoom, and window on the left side. Osirix automatically detects the number of displays and uses all available real estate to display the study. While the cursor is over the image, a right mouse click will also give options to change the mouse function. By default, left click is window/level, right click is zoom, and scroll is performed by dragging a finger forward and back.

be shared using links to the file storage site rather than e-mailing the entire files, which circumvents e-mail server limits on attachment file size.

3. Results

Both authors use Osirix almost exclusively to view 10 to 30 studies daily comprised of both clinical as well as research cases in an academic setting. One author uses Osirix as a PACS and web server as well as a DICOM viewer for the purposes of teleradiology. The study modalities are radiographs, ultrasound, nuclear medicine, magnetic resonance imaging, and computed tomography. Additionally, photographs or videos of patients included in prepurchase exams may be imported and reviewed.

The database window shows the list of patients (Fig. 1). From this window, there are a variety of options for the selected patient. Double clicking on the patient will open the image viewer (Fig. 2).

Table 1. List of Hot Key Actions in Osirix

Action	Key
Default window width and window level	~
Flip vertical	V
Flip horizontal	H
Zoom	Z
Pan/move	M
Rotate	I
Measure length	L
Measure angle	A
Text	T
Arrow	Q

Once the viewer is open, there is a customizable tool bar with icons and descriptive words for the icons along the top of the screen. The icons represent tools to manipulate the images. The most common tools used during review are the pan, zoom, and contrast. However, there are a variety of tools that are underutilized by practitioners, including arrow and text tools to annotate images. The default tool for the left click on the mouse is contrast. By default, right click (if you are using a single button mouse or touch pad, clicking and simultaneously holding down the control key is the same as a right click) and hold will allow the user to zoom centered on where the pointer is. The user can change the left click tool by clicking on the icons in the toolbar or right clicking and choosing. You can use the keyboard to reduce the number of mouse clicks. For example, holding down the option key with the left mouse click causes the curser/pointer to become the pan tool. When released, it returns to contrast. Using hot keys also may increase productivity (Table 1).

Osirix also has built in capabilities to display the current and relevant prior studies. If connected to your home or remote back up server, it will automatically check to see if there are additional studies for the current patient.

Osirix has robust capabilities to reconstruct surface renderings and cut away specific bones and soft tissue in cross sectional imaging. This capability is used by the authors weekly, if not daily, for surgical planning and teaching.

Right clicking on a study in the database window will give you several options, including export. A variety of options for exporting images are available. Exporting as DICOM file(s) is the appropriate choice for transmitting radiographs through file-sharing applications. Once this is selected, a window appears asking where to save the resulting file (Fig. 3). If you have already set up Dropbox, this folder will appear in the favorites bar in the finder window. There are also several options to check in the export window. The defaults settings are correct, which are checked boxes next to add DICOMDIR, hierarchical folder tree, don't modify the files for compression, and save as ZIP. The

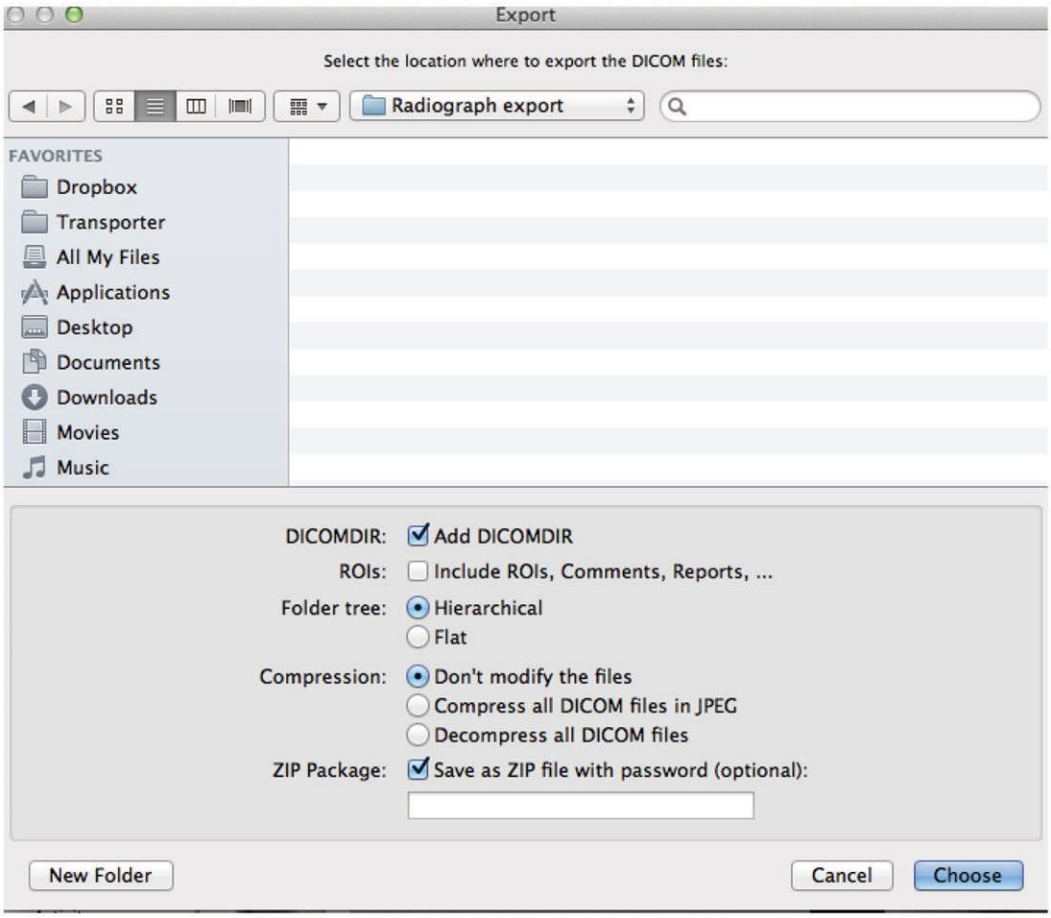

Fig. 3. Export window. By right clicking on the patient study in the database, an option for export is displayed. The window gives options for saving the study. The displayed settings are the most helpful.

authors recommend compressing (zipping) the diagnostic images (Fig. 3). This can be password protected if needed. This may be done by adding the desired password under the zip option. This also acts as a single file rather than multiple and is often easier to manage. Once in the Dropbox folder, the integration of the program allows a link to be generated (Fig. 4) for the file by right clicking on the file and selecting "share link." This link is then pasted into an electronic mail message and sent to a selected recipient.

4. Discussion

Osirix image viewing software and Dropbox file sharing software have revolutionized consultation capabilities within the medical field. Positive collaboration and consultations between practitioners and specialists is vital to the overall success of the patient. With the increased utilization and improved access to advanced imaging in equine veterinary medicine, this collaboration becomes even more important. Advanced imaging modalities such as computed tomography and magnetic resonance imaging are growing rapidly due to high sensitivity and specificity for pathologic change. However, the success cannot be achieved without accurate evaluation of the images produced by these modalities. Equally important is the ability of the image evaluator to describe and illustrate image findings in order to ensure an appropriate clinical therapeutic plan.

As previously mentioned, similar products as an alternative to Osirix for Windows-based users are available. One of the easier programs to navigate is Ginkgo CADx (http://ginkgo-cadx.com/en/downloads/). This also comes in a variety of download options for free with limited support to a paid version that offers professional support. The program is feature-rich with basic abilities similar to Osirix such as pan, zoom, magnify, measure, and DICOM send features.

The minimum system requirements for Osirix are summarized (Appendix 1). Similar recommendations should be employed on a Windows-based computer. The advantage of the 64-bit system is its use of system memory for performing tasks. As previously outlined, in large data sets such as cross sectional imaging, more memory must be allocated to

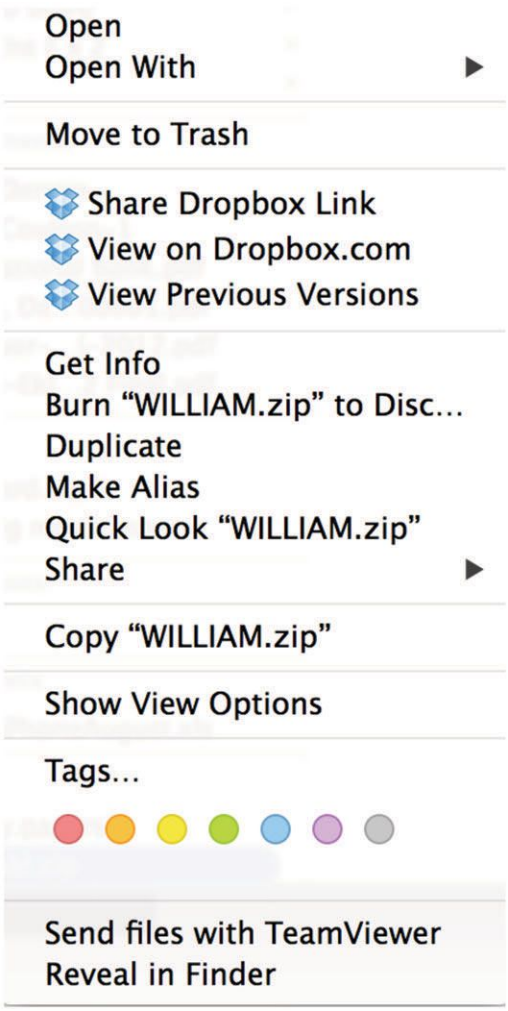

Fig. 4. This is the right click option of the exported study saved in the Dropbox folder. By selecting the "share Dropbox link" a link is saved to the computer's clipboard and is able to be pasted to an electronic message.

the program. In cases of radiographic and ultrasound review, the 32-bit Osirix program will perform well.

Recommendations for monitors to review cases on have been set by the American College of Radiology. Veterinary Radiology also uses the guideline that a 3 MP, medical grade, 350 cd/m^2 monitor be employed. Newer liquid crystal display monitors often exceed this recommendation. The authors currently use Apple 27 in. displays with specifications of 3.7 MP, 375 cd/m^2, and a contrast ratio of 1000:1. It should also be noted that viewing images is best in a dimly lit room. This increases the perceived contrast of the image and reduces glare on the screen.

The veterinarian often is encountered with clients wanting the images of the patient. Similar to other DICOM viewers, Osirix allows for the studies to be packaged with a "lite" version of the Osirix viewer and a Java-based DICOM viewer. In this regard, the packaged viewer is capable of displaying the images on both Apple and Microsoft operating systems. The Java based viewer comes with pan, zoom, window width, and level capabilities.

In summary, it is important that practitioners have an understanding of the advantages to DICOM image viewing and file-sharing. Osirix is easy to learn and can quickly become indispensible software to any practitioner utilizing digital imaging equipment in their practice. As such, Osirix can help bridge the gap between practitioners and specialists, which can lead to advancements in diagnostic capabilities, increased disease understanding, successful therapeutic planning, and ultimately improved clinical outcomes and client satisfaction.

Acknowledgments

Conflict of Interest

The Authors declare no conflicts of interest.

Appendix 1

The hardware recommendations for Osirix are outlined:

- MacOS X 10.8 or higher
- Intel processor

For best performance:

- 2–4 GB of RAM for Radiographic image review (DX and CR)
- 6 GB of RAM if you plan to open more than 800 images (CT and MRI, PET-CT)
- 8 GB of RAM for more than 1500 images (multi-slice CT and PET-CT) with OsiriX-64 bit
- 12 GB of RAM for more than 3000 images (cardiac or functional imaging) with OsiriX-64 bit

References and Footnotes

1. http://www.osirix-viewer.com/AboutOsiriX.html
2. https://www.dropbox.com/

[a]Osirix, Pixmeo SARL, Bernex, Switzerland,
[b]Ginkgo CADx, MetaEmotion S.L, Valladolid, Spain.

Radiographic Study of Throat Spray Distribution in the Horse

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Nasopharyngeal administration of barium showed consistent distribution within the nasopharynx, larynx, and dorsal soft palate. Oral administration of barium highlighted the ventral soft palate in the majority of horses, and this route may be appropriate for treatment of some palatal conditions. Authors' address: Colorado State University, College of Veterinary Medicine, 300 West Drake Road, Fort Collins, CO 80523; e-mail: aimee.colbath@colostate.edu. *Corresponding and presenting author. © 2014 AAEP.

1. Introduction

Although topical laryngeal and pharyngeal medications are routinely administered in upper airway disease, no studies have documented the distribution of these medications. The study objective was to determine the distribution of contrast medium administered orally or into the nasopharynx using a nasopharyngeal catheter in standing, unsedated horses using radiographic assessment.

2. Materials and Methods

Five adult horses without signs of respiratory disease were studied. Baseline radiographs were performed. Twelve milliliters of contrast medium was administered orally or using a nasopharyngeal catheter. Distribution of contrast material was evaluated using radiographic assessment. Contrast distribution was graded. Upper airway endoscopy was used to complement radiographic analysis.

3. Results and Discussion

Radiographs performed after nasopharyngeal administration revealed contrast in the nasopharynx in 5/5 (100%) horses, dorsal soft palate in 5/5 (100%) horses, larynx in 5/5 (100%) horses, and esophagus in 5/5 (100%) horses. Radiographs performed after oral administration identified highlighting of the ventral soft palate in 4/5 (80%) horses and the esophagus in 3/5 (60%). The study suggests throat spray administered using a nasopharyngeal catheter will result in topical distribution of the larynx, dorsal soft palate, and nasopharynx. Oral administration may be utilized for selected soft palate conditions but did not highlight laryngeal structures. Endoscopy confirmed radiographic findings.

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Research Abstract

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Conflict of Interest

The Authors declare no conflicts of interest.

Detection of Fractures of the Palmar Processes of the Distal Phalanx in Young Foals

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Without oblique radiographs 35% of palmar process fractures in foals will be missed. Authors' address: Western University of Health Sciences, College of Veterinary Medicine, 309 E. 2nd St., Pomona, CA 91766; e-mail: bfaramarzi@westernu.edu. *Corresponding and presenting author. © 2014 AAEP.

1. Introduction

Cross-sectional imaging and histopathology has confirmed a high incidence of palmar process (PP) fractures in foals. Obtaining good quality radiographs of foal hooves under field conditions is challenging. The availability of digital radiography has improved radiographic quality.

2. Materials and Methods

The forefeet of nineteen 6- to 8-month-old foals were radiographed under field condition using a standard portable x-ray machine and a digital image acquisition system. Lateral, horizontal beam dorso-planar, and three oblique projections were made of each foot.

3. Results

Ten of nineteen foals were diagnosed with at least one fracture. Three foals had one or more fractures in each foot. Four foals had fractures of both the medial and lateral PP. If only the lateral and dorso-planar projections were evaluated, only 65% of the fractures were identified. The remaining 35% were identified only on one or more of the dorso60°proximal-palmarodistal oblique (Dr60Pr-PaDiO) and the two complex oblique projections.

4. Discussion

Fractures were identified in 53% of the subjects. Digital radiography has significantly improved radiographic quality in equine ambulatory practice, allowing immediate review of the images so that positioning abnormalities can be corrected and suspicious areas can be re-evaluated as necessary. Using the proper diagnostic technique and good quality radiographs are critical in diagnosing PP fractures in foals since clinical signs can be easily missed. It has been reported that PP fractures may be associated with conformational abnormalities such as a club-footed conformation; however, the assessment of the long-term effects of PP fractures warrant further research.

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Conflict of Interest

The Authors declare no conflicts of interest.

Research Abstract

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Review of the Complete Ultrasound Examination of the Hind Suspensory Ligament

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A thorough knowledge of anatomy, ultrasound principles, and ultrasound machine settings are required to be proficient at ultrasound examination of the hind suspensory ligament. Multiple approaches (medial, plantar, and abaxial) with the limb in weight bearing and nonweight bearing positions are necessary to perform a complete examination. Author's address: Equine Diagnostic Imaging, Inc., 9418 SW 67th Drive, University of Florida, Radiology Department, Large Animal Clinical Sciences, Gainesville, FL 32608; e-mail: equinedxim@yahoo.com. © 2014 AAEP.

1. Introduction

Suspensory ligament (SL) injury is an important source of lameness in performance horses.[1,2] Injury to the SL can include both soft tissue and osseous components that can occur together, or in isolation.[3,4] Ultrasound and radiographs are frequently used to aid in the diagnosis of SL injury. Ultrasound provides valuable information about soft tissue injury and superficial bone abnormalities, while radiographs can provide information about bone injury that is both superficial and deep to the bone surface. The anatomy of the SL is complicated. It contains regions of fat and muscle (Figs. 1 and 2), and changes in shape multiple times from the origin to the distal extent of the body (Fig. 3). In addition, the proximal SL (PSL) is deep to a number of soft tissue structures and positioned between the second and fourth metatarsal bones, further obscuring its visibility with traditional ultrasound methods. However, new ultrasound techniques and approaches provide a complement to the traditional techniques. Using multiple techniques together allows a method for a comprehensive ultrasound examination of the hind SL. The purpose of this review paper is to describe a comprehensive ultrasound examination of the hind SL.

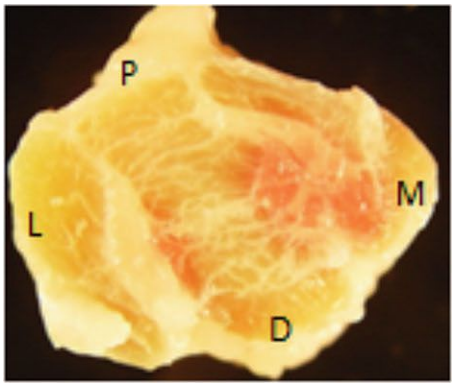

Fig. 1. Image showing cut surface of the hind SL when it is oval in shape, with regions of fat (white) and muscle (pink) surrounded by yellow ligament fibers with interspersed connective tissue. P, plantar, L, lateral, D, dorsal, and M, medial.

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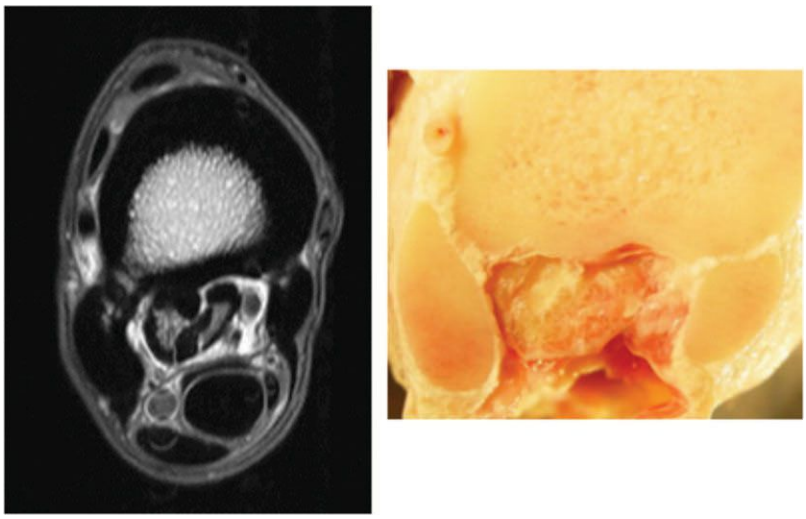

Fig. 2. Corresponding MR and US images of the hind SL showing the anatomic correlation. On the MR image, the ligament fibers are black and the regions of fat and muscle are light and medium gray.

2. Materials and Methods

In order to perform an ultrasound examination of the hind SL and properly evaluate the images, it is imperative to know the anatomic features of the ligament, to understand the techniques required for a thorough examination, including the effects of ultrasound beam angle on different tissue types, and to know how to properly adjust the ultrasound machine settings such as depth, frequency, gain, and focal zones.

Anatomic Characteristics

The hind SL has a proximal branch that is oval in shape and extends from the level of the tarsometatarsal joint to the fourth tarsal bone. Beginning at the level of the tarsometatarsal joint, the hind SL begins as a rectangular to triangular shaped soft tissue structure at the proximal aspect of the third metatarsal bone. The triangle is thinner medially than it is laterally and is located lateral of midline, placing it closer to the fourth metatarsal bone than the second metatarsal bone. The ligament is separated from the second and fourth metatarsal bones, as well as the third metatarsal bone distal to the attachment by connective tissue that is most prominent along the medial margin of the ligament. As the ligament continues distally it can become heart-shaped in some cases. It then becomes round and then oval, until it reaches the level of the bifurcation. Throughout the transitions in the shape of the SL, the apex of the triangle and the heart shape, which is the plantar margin of the ligament, is directed plantar lateral. Therefore, the majority of the SL is lateral of midline and adjacent to the axial margin of the fourth metatarsal bone. The fourth metatarsal bone is larger than the second metatarsal bone and the axial margin of the fourth metatarsal bone is curved and wraps around the lateral margin of the SL. The anatomic relationship between the hind SL and the metatarsal bones is important when considering ultrasound (US) examination of this structure. This relationship becomes less important distally as the size of the fourth metatarsal bone decreases relative to the SL.

The hind SL typically has a prominent area of central fibers as well as peripheral fibers, and is not bilobed like the forelimb. Random areas of fat and muscle can dissect through the ligament to the peripheral margin and asymmetry between limbs exists. Similar to the SL in the forelimb, regions of fat and muscle converge into a zigzag pattern as the ligament takes on an oval shape.

Ultrasound Examination Methods

Three different approaches should be used when performing an ultrasound examination of the hind SL. The medial, the plantar, and the abaxial approaches should be used in order to complete a thorough examination of the hind SL. For all approaches, the depth is set such that the echogenic line or lines representing the osseous landmarks are visible, without excessive space deep to their margins. In certain instances only the third metatarsal bone will be visible in the images and in other circumstances the second, third, and fourth metatarsal bones will be visible. This will be further described where applicable. Following selection of the proper depth, the frequency and focal zones are set to maximize visibility of the SL and the osseous landmarks. Performing the ultrasound examination in a consistent manner and using the same measurement and marking systems will improve the recognition of abnormalities. Comparison to the opposite limb is beneficial in all initial ultrasound examinations of the hind SL.

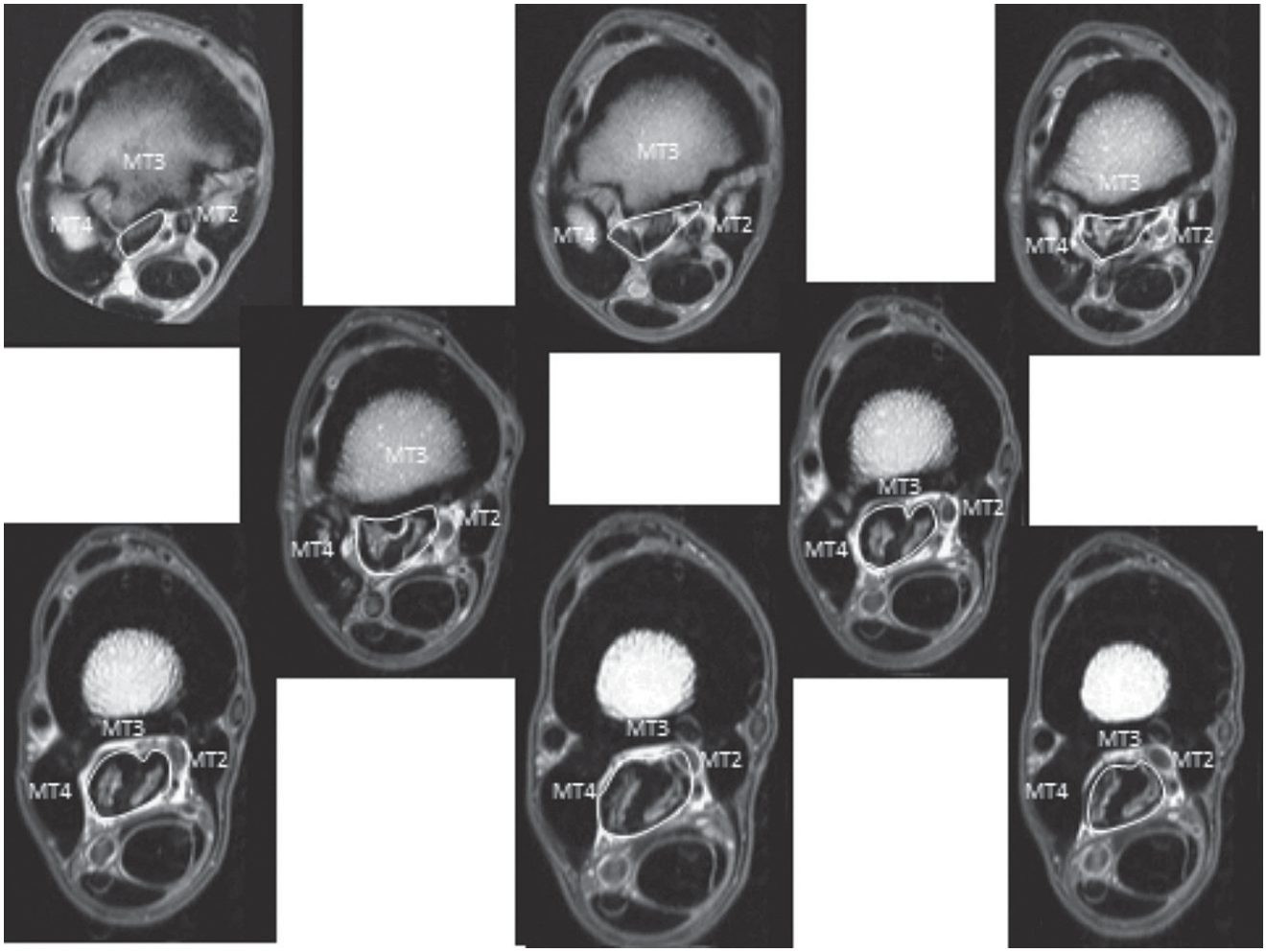

Fig. 3. Series of MR images demonstrating the changes that occur over the length of the SL (outlined in white). In addition, we can evaluate the relationship between the metatarsal bones and the SL, as well as the changes in the shape and size of the fourth metatarsal bone.

3. The Medial Approach

The standard technique for evaluation of the SL in the hind limb involves using a medial approach, as has been described for the proximal aspect of the SL.[5] Using this approach the probe is placed on the deep digital flexor tendon (DDFT) medial aspect of the limb immediately distal to the chestnut. Both longitudinal and transverse images are obtained (Figs. 4 and 5). The probe is directed dorsolaterally towards the SL.

Longitudinal Images

When imaging the SL longitudinally, the plantar margin of the third metatarsal bone should be a smooth line with a mild slope. Bundles of fibers can be identified extending to the bone. However, regions of nonlinear tissue can also be identified extending to the bone, representing the regions of fat and muscle. The bone-ligament interface should be closely evaluated for any indication of resorption or proliferation. It is important to note that the SL is wider than the ultrasound beam. Therefore, fanning the probe until the axial margins of the second and fourth metatarsal bones are identified is required to evaluate the entire ligament when scanning longitudinally. As the entire length of the ligament is imaged, continue to fan the probe medially and laterally between the second and fourth metatarsal bones as the probe slides distally.

Transverse Images

Transverse images are obtained using the second, third, and fourth metatarsal bones as osseous landmarks. The ultrasound probe is typically centered over the DDFT and directed dorsolaterally. However, in contrast to the longitudinal image, the majority of the ligament is visible on one image bordered by the metatarsal bones. The axial margins of the second and fourth metatarsal bone must be apparent on the image to ensure that the maximum visibility of the SL is obtained. Once the os-

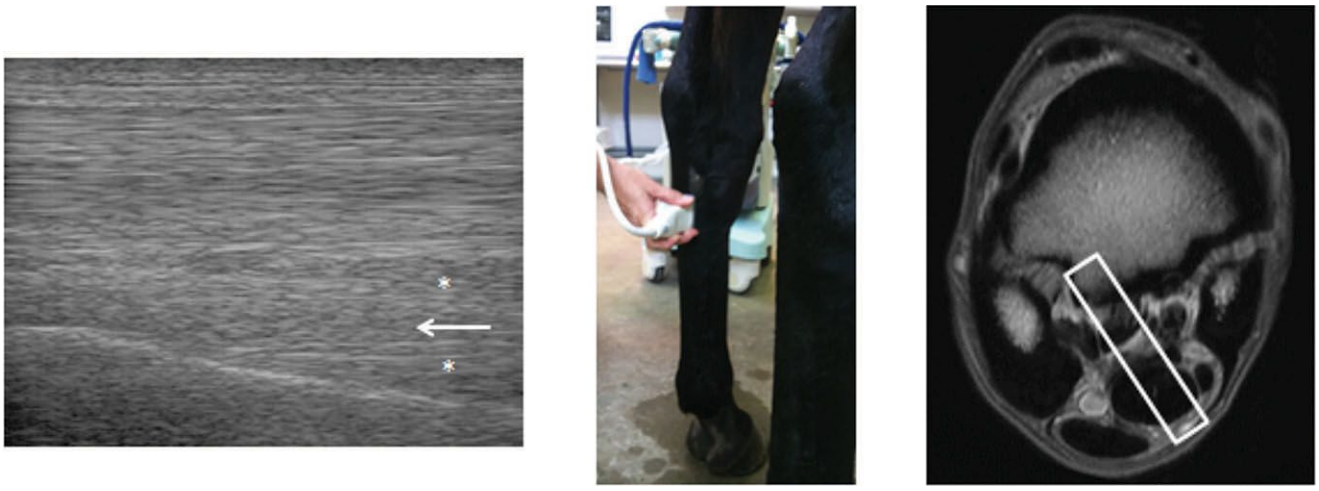

Fig. 4. Ultrasound reference image and probe position used to create a longitudinal image of the SL origin using the medial approach. Note the region of nonlinear tissue (arrow) within the ligament that is consistent with the bundles of fat and muscle. The region of fat and muscle is bordered dorsally and plantarly by ligament fibers (asterisks). The MR image shows the ultrasound beam width relative to the SL width, making fanning the beam necessary to image the entire ligament. Machine settings should then be adjusted before continuing with the examination.

seous landmarks are identified on the image, the SL is then identified based on its position, shape, and fiber pattern. The SL is scanned proximally to distally keeping the osseous landmarks in view as the probe slides distally. The changes in shape of the SL should be identified as the length of the ligament

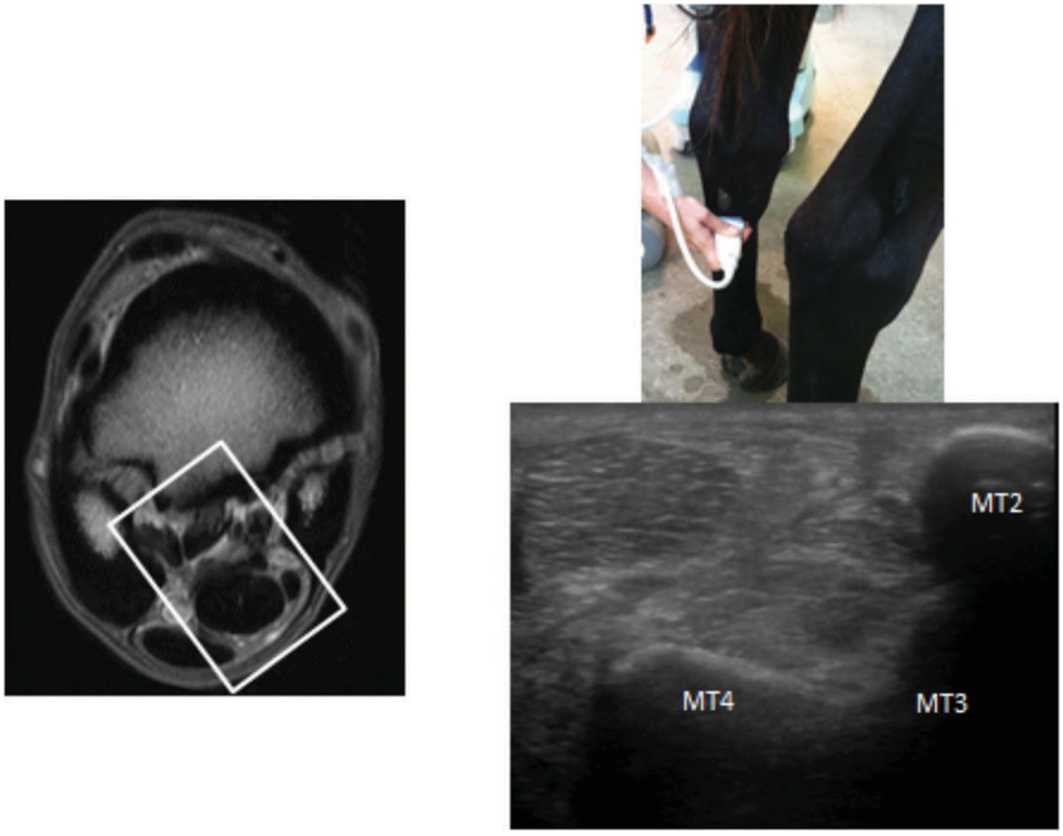

Fig. 5. Ultrasound reference image and probe position used to create a transverse image of the SL origin using the medial approach. Osseous landmarks (metatarsal bones) should first be identified, followed by the SL. The MR image shows the ultrasound beam direction and position relative to the SL. The ultrasound beam is perpendicular to the bone-ligament interface.

is imaged. The non-uniform echo pattern is expected, based on the regions of fat and muscle.

General Comments About This Technique

This approach can be performed with the limb in weight bearing and nonweight bearing positions. If there is minimal enlargement of the SL or other soft tissue structures of the limb at this level, then a contact artifact will begin to occur due to the normal anatomic depression between the SL and the DDFT. A stand off pad or flexor tendon laxity achieved with the limb in a nonweight bearing position can be used to improve the images if contact artifact occurs. However, even with the use of a stand off pad when the limb is in a weight bearing position, the ligament may be partially obscured. In addition, this technique places the ultrasound beam at an oblique angle relative to the SL bone interface. This may obscure certain types of abnormalities. The weakness of this approach is offset by the addition of the plantar abaxial approaches.

4. The Plantar Approach

The plantar approach is performed with the limb in a nonweight bearing position. In most cases, having the horse stand with limb unweighted, resting on the toe, will provide enough flexor tendon laxity to perform this technique (Fig. 6). The laxity in the flexor tendons creates a surface for the ultrasound probe that is wider than the SL and decreases the depth to the ligament, allowing greater visualization of the ligament at less depth. Therefore, the ligament can be imaged at a higher frequency than when the limb is weight bearing, increasing image detail.

Fig. 6. Reference images for the plantar approach. The ultrasound beam is positioned perpendicular to the longitudinal axis of the ligament and such that the plantar margin of the third metatarsal bone is a horizontal echogenic line bordered by the second and fourth metatarsal bones. The SL associated connective tissue will be present within the osseous landmarks.

Transverse Images

The ultrasound probe is placed on the plantar aspect of the limb immediately distal to the chestnut and positioned such that the plantar margin of the third metatarsal bone is a horizontal echogenic line on the screen. The axial margins of the second and fourth metatarsal bones should be visible as curved echogenic vertical lines that are perpendicular to the plantar margin of the third metatarsal bone. From this probe position the proximal branch of the SL can be imaged extending to the fourth tarsal bone (Fig. 7). Sliding the probe distally will allow imaging of the proximal SL and body. As the probe is moved distally, the metatarsal bones must remain visible and the plantar margin of the third metatarsal bone should remain horizontal. This technique and probe position places the ultrasound beam perpendicular to the bone-ligament interface, improving visualization of this region, which is commonly affected with SL injury.

Longitudinal Images

Longitudinal images can be obtained using this technique and should be used to document abnormalities and evaluate the bone at the attachment. However, transverse images will provide the most information using this technique.

5. The Abaxial Approach

The medial and lateral margins of the ligament are evaluated by placing the ultrasound probe on the medial or lateral aspect of the limb, similar to the probe positioning used to image the SL branches. The ultrasound probe is placed as proximally as is possible to allow visualization of the peripheral surface of the SL margins that are not obscured by the second or fourth metatarsal bones. This approach will place the ultrasound beam perpendicular to the medial or lateral surface, allowing visualization of the curved peripheral ligament margin, which is not as well visualized using the plantar and medial approaches. This approach also allows visualization of periligamentous tissue proliferation and evaluation of the relationship between the SL and the plantar metatarsal fascia, extending from the plantar margins of the second and fourth metacarpal bones. A stand off pad can be useful in this approach, similar to what is described in the medial approach when a contact artifact occurs.

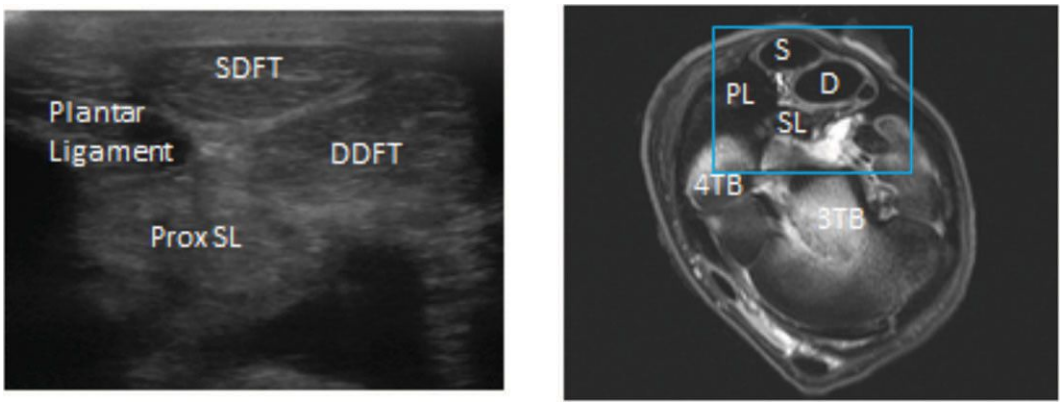

Fig. 7. Transverse ultrasound image and corresponding MR image of the proximal branch of the SL at the level of the fourth tarsal bone. The MRI is oriented to match the US image with the dorsal at the bottom of the image.

6. The Effect of Beam Angle

The complicated anatomy of the SL requires additional US techniques to be employed for complete visualization of the ligament and to correctly identify its anatomy (Fig. 8). One of the challenges of SL US is that the regions of fat and muscle create variations in the ligament echogenicity. This echo pattern makes it difficult to determine if these variations in echogenicity are the result of injury or the normal SL anatomy. A technique that can be used to correctly identify regions of SL fibers versus areas of fat and muscle is called off-angle or oblique incidence imaging. The beam angle affects the echogenicity of soft tissue structures with linear fibers, which is apparent every time we adjust the angle of the US probe proximally or distally in order to create

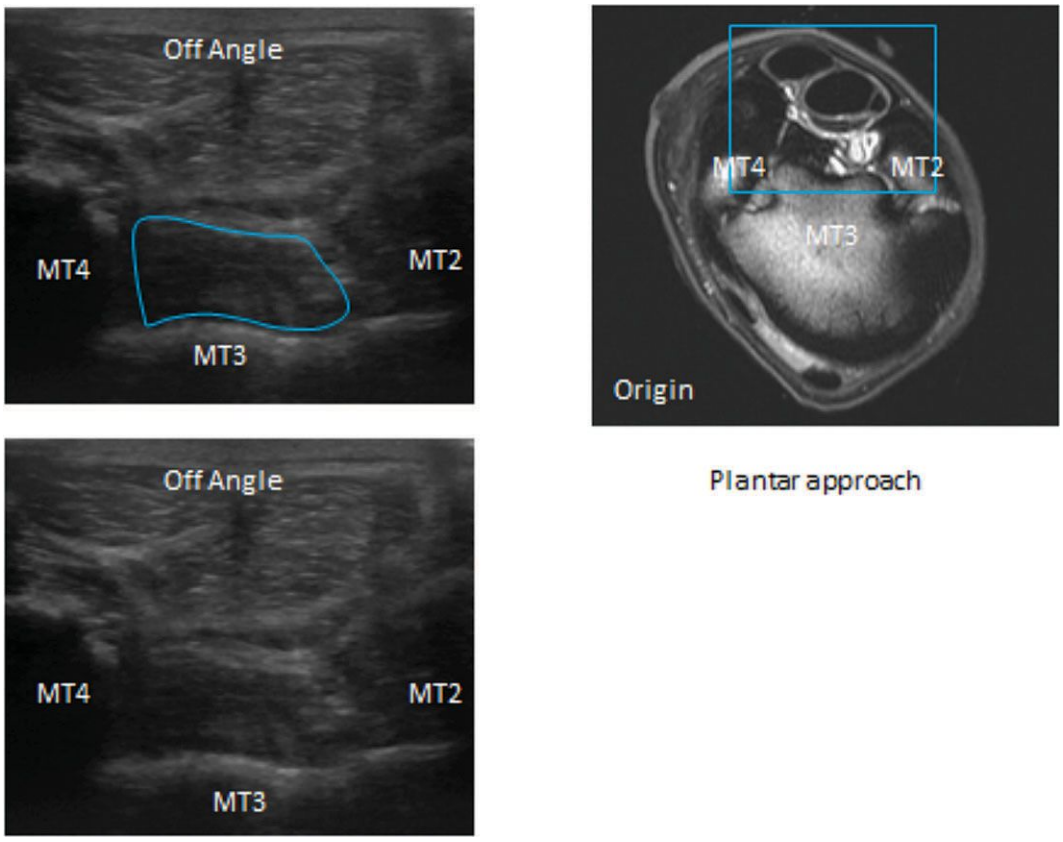

Fig. 8. Series of reference images showing the SL origin to the body with on- and off-beam angle imaging using the medial and plantar approaches. The SL is only outlined in certain images to allow better assessment on the nonmarked images. The MRI and gross images are oriented to match the ultrasound images.

maximum echogenicity when examining the palmar metacarpal flexor tendons in the transverse plane. When the US beam is truly perpendicular to the longitudinal axis of linear fibers within a normal tendon, maximum echogenicity is created. When the US beam is not perpendicular to a normal tendon we can create decreased echogenicity in that structure. In contrast to normal tendon or ligament fibers, the echogenicity of fat and, to a lesser degree, muscle is not dependent on the angle of the US beam. The difference between the echogenicity of SL fibers versus the regions of fat and muscle becomes very apparent when the US beam is not perpendicular to the longitudinal axis of the SL fibers. This change in the position of the probe can be used to identify regions of fibers versus regions of fat and muscle in the SL.

Normal SL fibers will be echogenic when the US beam is perpendicular to the longitudinal axis of the fibers and will become hypoechogenic when the US beam is no longer perpendicular to the fibers. In contrast, regions of fat and, to a lesser degree, muscle will remain echogenic regardless of beam angle. Therefore, comparing the appearance of the SL with the beam both perpendicular and not perpendicular to the ligament allows identification of fibers versus regions of fat and muscle. Therefore, regions of mottling or decreased echogenicity identified in the SL with US can then be further investigated using changes in beam angle to determine if the source of the decreased echogenicity is ligament fibers or regions of fat and muscle. This technique provides a method for determining the actual tissue source, which allows us to determine if this is part of a normal pattern of the SL or a region of injury.

Similar to the areas of fat and muscle, the echogenicity of the connective tissue surrounding the SL is not beam angle dependent (remains echogenic, regardless of beam angle). Placing the US beam slightly oblique to the SL allows differentiation of the ligament margins from the surrounding echogenic connective tissue.

7. Discussion

Ultrasound is an extremely useful tool for the diagnosis of SL injury. Due to the complicated anatomy and location deep within the limb, ultrasound examination of the SL often requires the clinician to go above and beyond the techniques that can be used to provide a diagnosis for the flexor tendons. In certain cases, the mid to distal aspects of the SL body must be imaged from the plantar aspect of the limb, because at this location the medial aspect of the limb does not provide an adequate contact surface to allow visualization of the ligament, even with a stand off pad. This is especially true when the ligament is focally affected with areas of enlargement and/or fiber abnormalities. As discussed above, the PSL is located adjacent to the fourth metatarsal bone. By using the DDFT as a window and directing the US beam dorsal and lateral, the entire SL can be visualized as opposed to the planter technique with the limb in a weight bearing position where the ligament is partially obscured. However, the beam angle required for this technique is not ideal for the ligament-bone interface and for imaging the dorsal aspect of the ligament. The addition of the plantar approach with the limb in a nonweight bearing position complements the medial approach with increased detail and places the ultrasound beam perpendicular to the dorsal aspect of the ligament as well as the bone ligament interface. These are important advantages when using ultrasound to diagnose SL injury.

The hind SL changes shape more significantly than the front SL. In addition, subtle changes in shape as a result of ligament injury are often visible before fiber abnormalities are detected. Therefore, comparison to the opposite limb is imperative. In addition, the size and shape of the fourth metatarsal bone changes dramatically at the level of the PSL. The size and shape of the fourth metatarsal bone and its relationship to the third metatarsal bone can be used to ensure that the comparisons between the right and left hind limbs are being made at the same level.

Abnormalities in the size, shape, margin, and echogenicity can all indicate abnormalities in the SL. Focal variations in the ligament echogenicity identified using the standard technique require further investigation with the limb in a nonweight bearing position. Re-examining these regions with the limb in a nonweight bearing position and varying the US beam angles will establish if they are associated with SL fibers or regions of fat and muscle. Regions of marked injury in the SL fibers will have decreased echogenicity, regardless of beam angle. They will appear as decreased echogenicity with the beam angle not perpendicular to the fibers. Their appearance will remain unchanged as the beam is moved perpendicular to the longitudinal axis of the SL and the surrounding normal fibers become echogenic. In contrast, scarring will be echogenic, regardless of beam angle and can have associated ligament enlargement. The echogenicity of mature fibrous tissue is independent of the beam angle, creating its echogenic appearance despite changes in the beam angle.

Often, injury to the SL alters the fat and muscle distribution and creates indistinct margins between regions of fibers and areas of fat and muscle. The regions of fat and muscle can become less evident with diffuse enlargement of the ligament and with focal regions of fiber injury and enlargement. Loss of the normal fat-muscle distribution in combination with abnormal fibers is often seen in conjunction with an SL injury.

The margins of the SL should be closely examined. This is best done with the limb in a nonweight bearing position and the US beam not perpendicular to the SL. Dorsal margin fraying or tears and focal areas of enlargement along the dorsal border of the

SL are best identified using an off-angle or oblique angle technique. Abnormalities in the SL margin will be evident adjacent to echogenic connective tissue. It is important to recognize that some amount of edge artifact is present on the medial and lateral margins of the SL. Therefore, small margin tears on the medial and lateral margins of the SL may be best visualized using an abaxial approach, placing the US beam perpendicular to the affected margin. These abaxial and medial approaches will also best identify periligamentous tissue proliferation and allow evaluation of the palmar metatarsal fascia on the medial and lateral aspects of the ligament, while the plantar approach will best define periligamentous tissue proliferation on the plantar aspect of the ligament.

In addition, abnormalities on the plantar margin of the third metatarsal bone will be identified with the plantar approach and recognized with the beam angle, both perpendicular and not perpendicular to the osseous surface, as well as during evaluation in the longitudinal plane. However, it is important to note that the echogenicity of bone is mildly beam angle dependent. The beam angle that best characterizes the appearance will depend on the lesion margins and configuration. The plantar margin of the third metatarsal bone as well as the axial margins of the second and fourth metatarsal bones and their relationship to the SL should be assessed. The beam angle should be adjusted as much as is possible to be perpendicular to the surface or region of interest.

Ultrasound will have limitations in the diagnosis of soft tissue injury as compared to magnetic resonance imaging. In addition, MRI is the only imaging modality that can definitively identify fluid in bone. However, ultrasound remains as the primary method for imaging of the SL and these techniques provide a comprehensive method for imaging the ligament.

Acknowledgments

Conflict of Interest

The Author declares no conflicts of interest.

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How to Perform Ultrasound-Guided Injection of Corticosteroids Into Subchondral Bone Cysts of the Medial Femoral Condyle in the Standing Horse

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1. Introduction

The most common location for subchondral bone cysts (SBCs) in the horse is the medial femoral condyle (MFC).[1] SBCs are often bilateral[2] and are thought to arise either as a manifestation of osteochondrosis or as a consequence of local trauma.[3] Horses are generally presented for evaluation and treatment of MFC SBCs in one of two scenarios:

1. The horse is lame and the lameness has been localized to the medial femorotibial joint by intra-articular analgesia, with subsequent radiologic identification of a SBC
2. A young horse is presented following prepurchase or survey radiographs, which identified a SBC in a horse, which may not be lame

In either situation, radiographic confirmation typically remains the mainstay for diagnosis of MFC SBCs.[4] The use of ultrasound for the diagnosis of medial condyle SBCs has, however, been described in the standing horse.[5] This modality has the advantage of allowing rapid assessment of joint, ligament, meniscus, and other soft tissues, which, in addition to evaluation of the SBC, are prognostically important.[5–7] Ultrasound of the equine stifle can be easily performed using widely available portable ultrasound equipment and a linear probe.[6,8] Concurrent radiographic and ultrasonographic examination in cases of MFC SBCs is often recommended.[5]

Several conservative treatment options have been proposed for treating MFC SBCs including restricted exercise and intra-articular steroid injection. Other techniques, which are more invasive and require general anesthesia and surgical facilities include: arthroscopic enucleation and forage[9,10], and cancellous bone grafting.[11] Such procedures can, however, lead to medial meniscal injuries,[12] possible postoperative enlargement of the cyst, and disruption to the articulator surface.[13] They may also require a long convalescence.[9] Additionally, debridement of lesions greater than 15 mm wide is associated with a poor prognosis.[10]

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Other studies have focused on reconstruction of the SBC using platelet-rich-plasma (PRP) and/or cultured stem cells[14] or the use of transcondylar lag screws.[15] The aforementioned techniques require general anesthesia in a surgical facility.

Arthroscopic-guided injection of corticosteroids directly into the fibrous cyst lining has been recommended[13] to attenuate the inflammatory process associated with cytokines within the cystic lining.[16] The cytokine production is thought to contribute to lameness and bone resorption. It is hypothesized that reduction in the production of such inflammatory mediators may aid resolution of the SBC. Treatment via direct infiltration with corticosteroids yielded a success rate of 77%.[1] The procedure resulted in faster recovery times and similar or improved results over surgical intervention. However, arthroscopic-guided injection of SBCs requires a surgical facility, general anesthesia, expensive arthroscopic equipment, and may represent considerable expense to the client.

A less invasive ultrasound-guided injection of corticosteroids directly into the fibrous cyst lining under general anesthesia has been used with success to alleviate lameness arising from SBCs.[1,14] However, the materials and methods of the technique have not been reported. Additionally, application of this technique in standing horses has not been described in the literature. Ultrasound-guided injection of corticosteroid into MFC SBCs in the standing patient may represent advantages over existing treatment methods including: rapid treatment time, avoidance of general anesthesia, reduced cost, and ability to treat patients on the farm. The widespread availability of ultrasound equipment and the relatively simple ultrasonographic anatomy make this technique a useful alternative for treatment of SBCs in the MFC in appropriately selected cases.

The purpose of this paper is to describe the technique used to identify and treat femoral SBCs in the standing horse using ultrasound guidance.

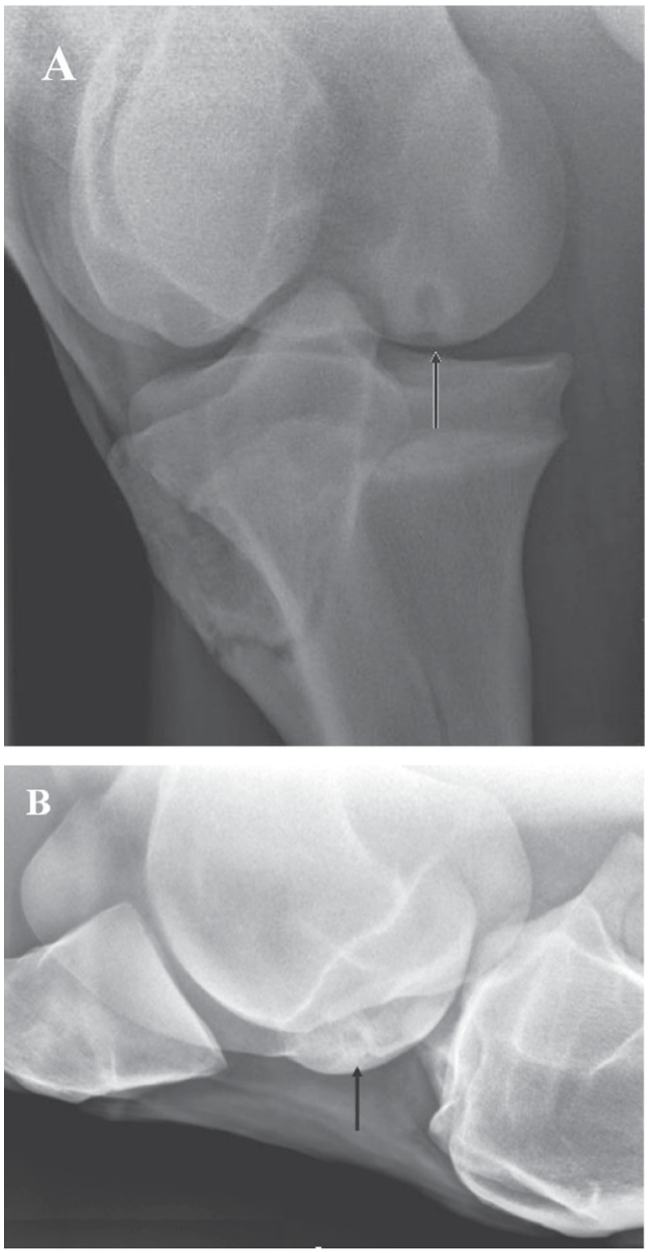

Fig. 1. The 20° caudolateral-craniomedial oblique radiographic projection (A) and the flexed lateral (B) demonstrate a medial femoral condyle SBC (arrows). Radiographs are the primary diagnostic modality for identification of these lesions; however, radiography may not be a sensitive method of evaluating the healing response.

2. Materials and Methods

Evaluation of the MFC and SBC

A standard series of stifle radiographs should be obtained[17] prior to ultrasonographic evaluation and treatment, with the caudo 20 lateral-craniomedial oblique, flexed lateromedial and caudocranial views being of particular importance (Fig. 1). These lesions are often bilateral, so images of both stifles should be obtained.

Following sedation, a wide area around the medial femorotibial joint should be clipped with a #40 blade as the overlying skin will move considerably when the limb is flexed. A routine ultrasonographic examination of the stifle should be performed to identify associated soft tissue injuries.[6] Most SBCs are present on the weight-bearing surface of the cranial aspect of the MFC.[18] Ultrasonographic evaluation and treatment of the MFC lesion must be performed with the stifle flexed. Care should be taken when imaging the MFC with the limb flexed in fractious horses; appropriate sedation is recommended as is the help of a capable assistant to maintain the limb in the flexed position. To evaluate the MFC SBC, the hindlimb is lifted to maintain stifle flexion at 90°. The foot can either be placed on a wedge block or placed on a hoof jack (Fig. 2). In this position,

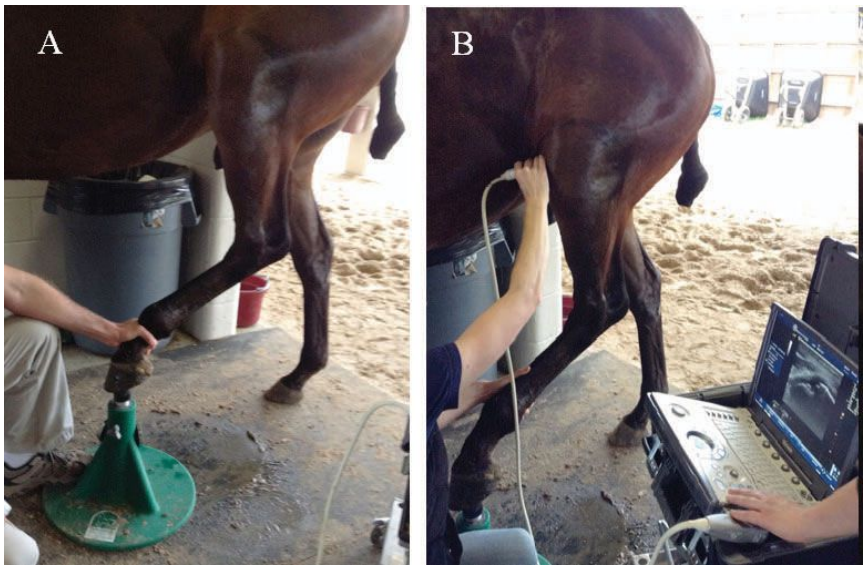

Fig. 2. A, the affected hindlimb is placed on a hoof jack or block to flex the stifle to 90°. B, in this position, the operator can easily image the femoral condyle and assess the best trajectory for needle entry to the SBC. Note the SBC is visible on the ultrasound screen.

the MFC becomes more superficial and can be easily evaluated using standard linear transducers (Fig. 3). To obtain a parasagittal image, the transducer is placed in long axis, proximal to the tibial crest and axial to the middle patellar ligament. The ultrasound transducer is directed approximately 45° in a craniodistal to caudoproximal direction to image the medial condyle and SBC. The probe is then rotated 90° to obtain transverse images of the condyle and the SBC. The femoral condyle is more rounded in the transverse plane than the parasagittal plane. In a normal MFC, the articular margin is a smooth, convex bone surface with a thin layer of overlying anechoic cartilage. The SBC is often a concave disruption of this smooth contour and may be associated with thickening of the cartilage layer around the cloaca (Fig. 4). By obtaining images in 2 planes, it is possible to judge the contour and size of the cloaca and articular surface. This allows the formation of a plan for needle trajectory, saving valuable time in the surgically prepared patient.

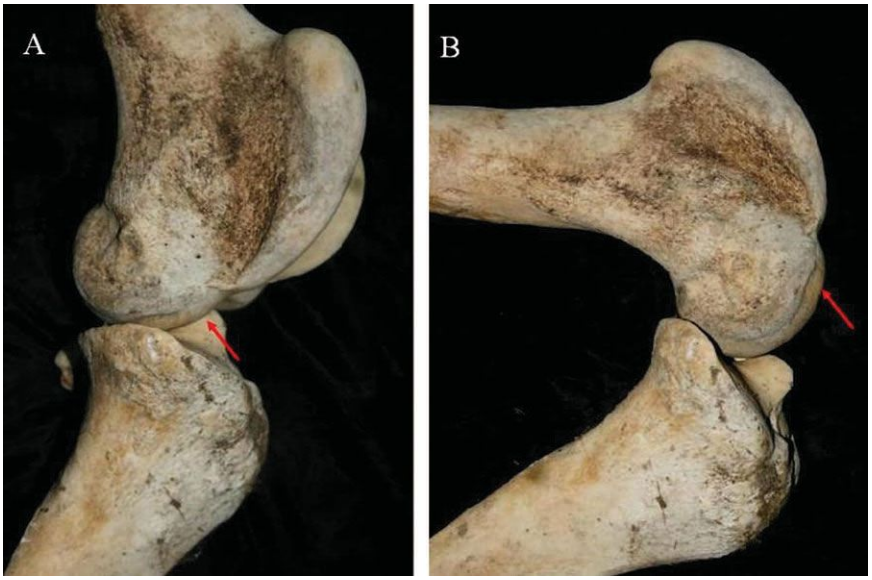

Fig. 3. A, in the standing position, the weight-bearing medial condyle is not accessible to ultrasound imaging (arrow). B, by flexing the stifle joint 90°, the medial condyle becomes superficial and easily identifiable on ultrasound examination (arrow).

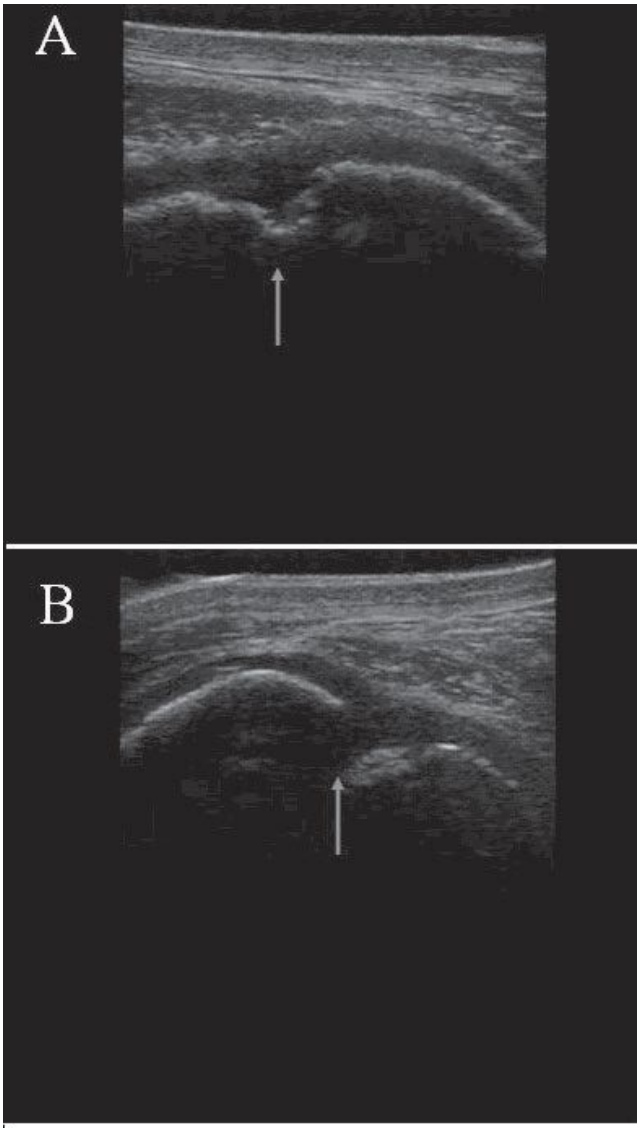

Fig. 4. Ultrasound images of a MFC SBC obtained in parasagittal (A) and transverse (B) orientations in the flexed stifle. The normal smooth convex hyperechoic subchondral bone is disrupted by the SBC defect (arrows). (A: distal is to the left. B: medial is to the left).

Patient Preparation

Following a thorough ultrasonographic examination of the stifle, the area from the base of the patella to the tibial crest is surgically prepared with the horse fully weight-bearing on all 4 limbs. The patient should be resedated if necessary to ensure complete compliance.

With the horse weight-bearing, a subcutaneous line block using mepivicaine should be placed at the level of the base of the proximal patella. This should be sufficiently proximal to avoid gas artifact appearing in the ultrasound image.

The limb is then lifted and the foot placed on the hoof jack and stabilized throughout the procedure by an assistant. In this position, the final surgical scrub is applied, and the area copiously doused in alcohol as an ultrasound coupling agent.

A 7.5 to 10 Mhz linear or curvilinear probe is placed inside a sterile sleeve with sterile lube between the transducer and the glove.

The probe is placed over the medial condyle in both parasagittal and transverse planes to determine the best needle trajectory.

A 3.5 inch 18 G spinal needle is placed through the skin adjacent to the transducer and advanced, in the ultrasound beam, towards the SBC. It is preferable to perform this freehand, rather than with a biopsy guide. The needle will appear as a hyperechoic line, which may cast an acoustic shadow (Fig. 5). The needle should be advanced into the far wall of the cyst, which may not be visible on ultrasound if the cyst is wider than the cloacal opening.

The needle should contact bone and be fairly firmly placed prior to removal of the stylette and injection of 10 to 15 mg of sterile triamcinolone acetonide. It has been recommended that injection in multiple sites within the cyst lining may be beneficial,[7] and this can be attempted with ultrasound depending on patient compliance. The total dose of triamcinolone should not exceed 18 mg.

In less cooperative horses, the technique can be performed under general anesthesia. Following premedication with xylazine (0.8mg/kg bwt i.v.) and butorphanol (0.04 mg/kg bwt i.v.), anesthesia is induced with diazepam (0.05 mg/kg bwt i.v.) and ketamine (2.2 mg/kg bwt i.v.). The horse is then placed in dorsal recumbency, a sterile rectal sleeve placed over the foot on the affected limb, and the limb flexed to allow evaluation of the medial condyle. The surgical technique is similar to that described in the standing patient, with the stifle held in a 90° flexed position by the assistant (Fig. 6). An adhesive bandage strip should be placed to prevent contamination during recovery from anesthesia. This procedure can be performed under short acting intravenous general anesthesia of 20-minutes duration.

Following the procedure, the patient should be stall rested for several days, provided with systemic anti-inflammatory medications, and observed for any negative sequalae to joint injection.

3. Results

The authors have successfully performed this technique in over 45 horses (2011–2013). For the purposes of this manuscript, however, case selection was limited to juvenile Thoroughbred horses in race training presenting with sudden-onset unilateral hindlimb lameness within the first 6 months of commencement of training. The source of lameness being localized to the medial femorotibial joint by a positive response to intra-articular anesthesia of the medial femorotibial joint with radiologic confirmation of a MFC SBC with no additional ultrasonographic abnormalities. Twelve cases met

Fig. 5. A, demonstration of needle placement into the medial femoral condyle in the path of the ultrasound beam (left femur, lateral to right of image). B, corresponding ultrasound image from a treated case. The hyperechoic line (arrows) represents the needle as it enters the SBC under ultrasound guidance.

Fig. 6. In cases that cannot be treated standing, the horse is placed in dorsal recumbency following intravenous anesthesia. The stifle is flexed through 90° and the needle placed as for the standing patient.

the selection criteria for inclusion in this study. Twenty horses were identified from our medical records to comprise a retrospective control group. Controls were Thoroughbred horses of the same age and stage of training as case horses with a known history of lameness resulting from a unilateral MFC SBC. All horses had been subjected to ultrasonographic evaluation of the stifle with the SBC confirmed as the only significant pathological finding. Controls had been treated with intra-articular triamcinolone acetonide injection and were, thus, considered to have been treated conservatively. For both groups, treatment was considered a success if the horse returned to training and remained free from stifle lameness for at least 1 year. This was assessed by direct observation by the authors over the first 6 months and then by detailed trainer/owner follow-up conversations. Long-term follow-up was assessed by the ability to start a race. Comparison of outcome variables between groups was performed using Fishers Exact Test ($P < 0.05$). The number of days from treatment to resumption of training was compared using analysis of variance (ANOVA). The number of repeat injections and changes in radiographic SBC appearance were also compared between groups.

Eleven (91.7%) cases required only a single ultrasound-guided injection of the fibrous cyst lining to achieve soundness. Ten (83.3%) resumed training without lameness, and 9 (75%) successfully started a race. Only 11 (55%) conservatively treated control horses became sound following a single intra-articular injection with 14 (70%) returning to training. Eleven (55%) subsequently started a race. Although there was no statistically significant difference in likelihood of a positive outcome between the methods, cases treated with ultrasound-guided SBC injection were significantly more likely to require

Table 1. Summary of Results

	U/S guided cases	Intra-articular controls	
Number of horses	12	20	
1 treatment required	11 (91.7%)	11 (55%)	$P = 0.05$
>1 treatment required	1 (8.3%)	9 (45%)	
Returned to training	10 (83.3%)	14 (70%)	
Started a race	9 (75%)	11 (55%)	
Average days from initial treatment to return to training	50.8	65.1	$P = 0.04$

only one treatment than controls (P = 0.05 two sided Fishers Exact Test) with an average time from diagnosis to return to training of 50.8 (95% confidence interval [CI] 8.1) days in case horses compared to 65 (95% CI 15.2) days in control horses. The radiographic appearance of the SBCs did not differ between groups and was not a useful predictor of a successful outcome. The results are summarized in Table 1.

4. Discussion

This is the first time that ultrasound-guided injection of SBCs has been compared to conservatively treated controls from a similar population. Ultrasound-guided injection of corticosteroids into the fibrous lining of MFC SBCs is a viable method to alleviate lameness caused by the subchondral bone cysts and appears to have similar success rates to several surgical treatment options. With practice, it is technically easy to perform using readily available equipment and obviates many of the shortcomings of existing surgical methods such as the requirement for general anesthesia and transport of the patient to a surgical facility. It also offers cost benefits to the client while achieving similar results to previously documented treatment methods. If the treatment is unsuccessful, arthroscopic investigation can be subsequently performed.

Proponents of arthroscopic-guided injection as an alternative to ultrasound-guided injection contend that arthroscopy is preferable, as it allows debridement of cartilage defects. However, less than 10% of cases may actually require debridement.[7] One potential benefit of arthroscopic injection is the ability to observe the "bulging" of the cyst lining during injection. This direct visualization is not possible using ultrasound guidance. The reported outcomes appear to be comparable between the techniques.

It should be noted that the procedure is not without risk to the operator and the horse, especially if the patient were to move or become uncooperative during the procedure. However, with appropriate case selection, competent assistants, adequate restraint, and skin anesthesia, the authors have encountered no negative outcomes using this method. The authors have not found it necessary to place anesthetic solution into the medial femorotibial joint when undertaking the procedure as has been reported in standing arthroscopic evaluation of the stifle.[19] Additionally, many potential risks can be reduced by performing the technique under intravenous general anesthesia if the above precautionary prerequisites cannot be achieved.

This study included only juvenile Thoroughbred racehorses with lameness originating from a single SBC in a single limb. Moreover, ultrasonographic examination was used to ensure no significant soft tissue pathology was visible in any of the horses used in this study. The usefulness of this technique on older horses, different breeds, or in the presence of coexisting soft tissue pathology is, therefore, unknown and should be considered prior to undertaking this procedure in such cases. The authors have performed the technique in horses of different breeds and ages but have not objectively evaluated the outcome. It is possible that the successful outcome in the cases reported here was due to lack of concurrent pathology associated with an immature cyst in a young horse.

As has been reported in previous studies, radiologic resolution of the SBC was not a useful predictor of treatment success. Using the ability to start a race as an objective outcome variable is a widely accepted method for assessing treatment success in racehorse studies, but care should be taken when interpreting results of the current study for several reasons: the study population was small, which has the potential to over or underestimate significance of results. Also, the class or ability of race was not investigated. It is possible that horses that raced may have done so at a lower level than if they had not suffered from the disease. It is not appropriate to comment on the significance of this in small groups such as in the current manuscript. Future studies with larger populations should further investigate this novel technique.

No negative sequalae were observed in this study, but care should be taken throughout this procedure to ensure maintenance of a sterile environment during needle placement and injection, as the needle has to enter the medial femorotibial joint during this procedure.

In summary, ultrasound-guided injection of corticosteroid into the lining of subchondral bone cysts of the equine MFC is easy to perform and offers similar success rates to previously reported surgical treat-

ments in this group of young Thoroughbreds and may offer advantages to both owner and patient in appropriately selected cases.

Acknowledgments

Conflict of Interest

The Authors declare no conflicts of interest.

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How To Perform Ultrasound-Guided Arthrocentesis and Injection of the Equine Scapulohumeral (Shoulder) Joint

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The equine shoulder joint can be a significant and severe source of lameness in horses. The proximal location and complex anatomy of the surrounding area often make arthrocentesis of this joint a challenge to the practitioner. Use of an ultrasound-guided injection technique can greatly facilitate the ease and accuracy of shoulder joint injection in the horse. Assuring the correct placement of anesthetic or therapeutic medications in the joint will allow for a confident and accurate assessment of the response to diagnostic analgesia and intra-articular medications. Authors' addresses: University of Georgia, Veterinary Biosciences and Diagnostic Imaging, 501 DW Brooks Drive, Athens, GA 30602 (Selberg); and 2716 Landmark School Road, The Plains, VA 20198 (Allen, Norvall, Johns); e-mail: ktselberg@gmail.com. *Corresponding and presenting author. © 2014 AAEP.

1. Introduction

The shoulder or scapulohumeral joint is a ball and socket joint with the chief movement of extension and flexion. It is mainly stabilized by four muscles and their tendinous attachments: the supraspinatus, infraspinatus, subscapularis, and teres minor. Unlike joints with similar movements, there are no collateral ligaments. Instead, there are relatively small glenohumeral ligaments. Although lameness related to the shoulder joint in horses is less common than that associated with the distal limb, when present it often causes significant lameness and decreased performance.[1,2] A thorough understanding of the pertinent clinical anatomy and the available techniques for arthrocentesis of the shoulder joint is necessary when performing diagnostic analgesia or administering intra-articular medications. In addition to radiography, ultrasound examination of the shoulder joint provides additional important diagnostic information. It also can help facilitate safe and accurate arthrocentesis or injection of the joint.

Both traditional (blind) and ultrasound-guided techniques for shoulder joint injection have been described.[3,4] A recent comparison of ultrasound-guided versus non-guided attempts at intra-articular shoulder injection revealed that blind techniques resulted in a 50% extra-articular injection rate and more attempts at needle repositioning for successful injection.[5] Practitioners frequently use aspiration of joint fluid as criteria for confirmation of correct needle placement. However, in diseased joints synovial proliferation and joint capsule fibrosis may obstruct the bore of the needle, thereby precluding aspiration of synovial fluid. Redirecting the needle multiple times to aspirate fluid may lead to fenes-

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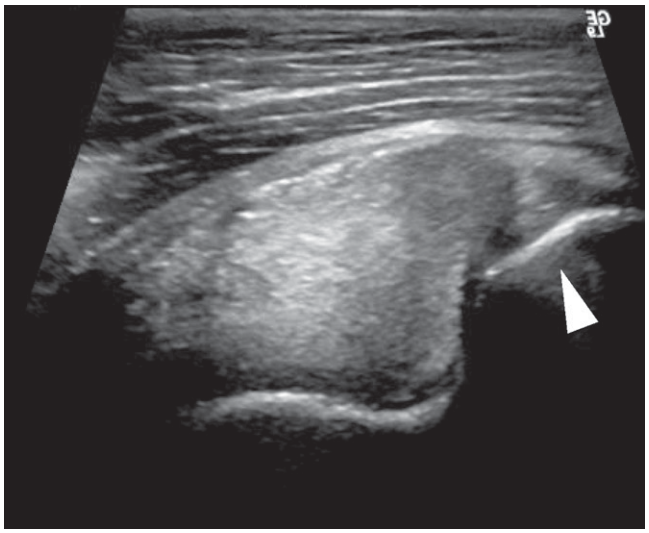

Fig. 1. Transverse image of the biceps tendon at the level of the bicipital groove. Medial is to the left of the image. The arrowhead is highlighting the cranial part of the greater tubercle, comprising the lateral border of the biceps tendon.

Fig. 2. Lateral aspect of the scapulohumeral joint. Cranial is to the right of the image. The great tubercle consists of the cranial and caudal portions (arrowheads). Along the cranial-lateral aspect of the shoulder joint there is a synovial out-pocketing (arrow).

,tration of the joint capsule and extravasation of the local anesthesia or medication. Current techniques for ultrasound-guided injection of the shoulder describe needle placement in the joint in a transverse plane.[4] However, the articular margins of joints are also effectively visualized in the sagittal (para) plane (longitudinal axis), perpendicular to the joint. Ultrasound-guided needle placement in the equine shoulder is accurate, safe, and easily performed. Assuring correct placement of anesthetic or therapeutic medications in the joint will allow for confident and accurate assessment of the response to diagnostic analgesia and administration of intra-articular medications. This paper and presentation will demonstrate how to use ultrasound guidance to accurately and safely perform intra-articular injection of the shoulder joint in horses.

2. Materials and Methods

Ultrasonographic evaluation of the shoulder should be performed before sterile preparation of the injection site to accurately identify joint margins and suitable access sites to the joint. Horses may be lightly sedated with xylazine HCl (75–100 mg, IV) for the procedure with minimal impact on subsequent lameness evaluation.[6] Optimal visualization of the joint is achieved by clipping the hair over the entire shoulder region with a no. 40 clipper blade. The skin should be washed and ultrasound gel applied. The joint should be examined at the highest frequency possible (7–12 MHz) at a scanning depth of 4 to 6 cm. A linear or curvilinear ultrasound transducer will provide optimal images, allowing ultrasound guidance of needle penetration into the joint.

The biceps brachii tendon is often the most easily identifiable structure upon initial ultrasound examination. To visualize the tendon, the ultrasound transducer is placed on the point of the shoulder in the transverse plane. As the biceps tendon passes over the humerus, through the bicipital groove, it is bilobed. The lateral aspect of the biceps tendon is bordered by the greater tubercle of the humerus (Fig. 1). The greater tubercle consists of the cranial and caudal parts (Fig. 2). The cranial part forms the lateral border of the bicipital groove and serves as an attachment for the lateral branch of the supraspinatus muscle. The caudal part serves as an attachment for the infraspinatus muscle. The lateral aspect of the caudal portion of the tubercle is covered in cartilage, over which the tendon of the infraspinatus passes. Subjacent to the infraspinatus tendon lies a small bursa, which is often not seen during examination when pressure is applied to the ultrasound transducer. From the cranial greater tubercle, the joint is visualized by rotating the transducer 90° and moving slightly caudally and proximally, between the cranial and caudal portions of the greater tubercle. The joint margin is most easily visualized in the (para) sagittal plane or long axis of the limb. Along the cranial aspect of the scapulohumeral joint, there is a synovial recess to which a needle can be easily directed. This is the synovial recess, which is preferred by the author; however, the joint can be accessed from the lateral and caudal aspects as well.

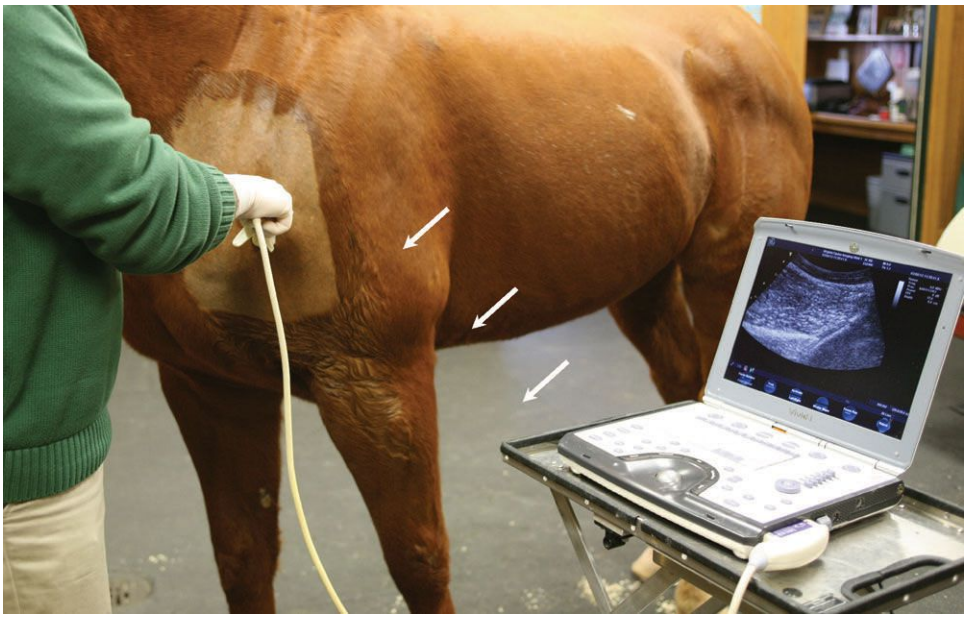

Fig. 3. The ultrasound is placed caudal to the shoulder with the screen facing cranially. The ultrasonographer is standing just cranial and lateral to the shoulder. This minimizes awkward body positioning during the procedure.

The ultrasound machine is prepared by placing a sterile glove filled with coupling gel over the transducer, adjusting the ultrasound image to 6 cm of depth, and, if a variable mHz probe is used, setting the frequency of ultrasound transducer to approximately 5 to 10 mHz. Patient preparation consists of a sterile surgical preparation of the shoulder region. Local anesthetic (subdermal injection at the injection site) may be used to desensitize the skin; however, needle placement is generally well-tolerated. Ideally, the ultrasound machine is positioned behind the plane of the shoulder, with the screen

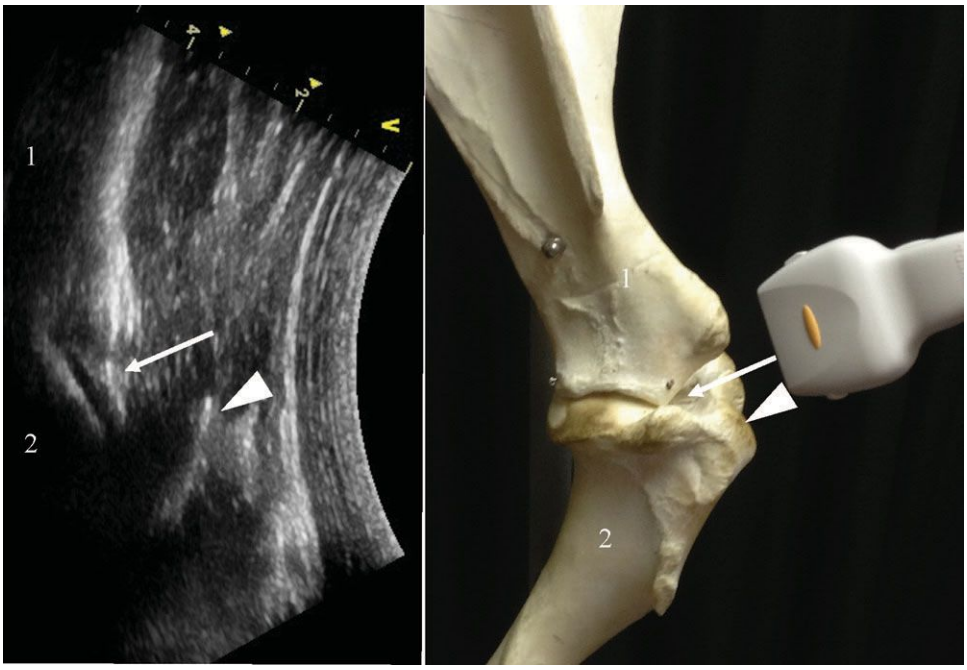

Fig. 4. The image on the left is a parasagittal ultrasound image of the shoulder (proximal is to the top of the image) with the corresponding lateral aspect of a skeletal specimen (right image). 1, Scapula, 2, humerus. The arrows are highlighting the joint space of the scapulohumeral joint. The arrowheads are highlighting the cranial part of the greater tubercle.

Fig. 5. Parasagittal (lateral aspect) ultrasound image of the shoulder joint (left image). Proximal is to the left. The vertical yellow line represents the handle of the transducer. The white angled line represents the proposed path of the needle. The angle between these two lines (20–30°) indicates the angle required between the needle and the transducer handle in order to achieve the proposed needle path. The right image is demonstrating needle placement in relation to the transducer. Cranial is to the left.

facing cranial (Fig. 3). The joint margin and joint space are visualized in the (para) sagittal plane (Fig. 4). While examining an area it is important to keep the object of interest in the middle of the screen. The needle (3.5 in. 21–18 g spinal needle) should be placed at the proximal aspect of the ultrasound probe, generally at an angle of 20° to 30° proximal to the transducer handle (Fig. 5). The initial angle of the needle can be judged by imaging the joint space and visualizing an angled line extending from the proximal aspect of the ultrasound image into the recess of the joint. An additional vertical line, representing the ultrasound beam or transducer handle, can be visualized extending from the center of the ultrasound image to intersect the angled line in the joint space. Redirection of the needle is easily performed in the plane of the ultrasound beam by pulling the hub of the needle toward the transducer to direct the tip of the needle deeper or pushing the hub towards the skin to direct the tip of the needle more superficially. The needle should be visualized in the joint space or synovial recess of the shoulder before injecting (Fig. 6). While injecting, joint distention and/or fluid turbulence in the joint may be observed as evidence of correct needle placement and confirming intra-articular placement of the solution. Ultrasound visualization precludes the need to aspirate joint fluid to confirm intra-articular placement of the needle. A synopsis of the techniques for ultrasound-guided injection of the shoulder joint in the horse is listed in Table 1.

3. Results

Medical records of horses presenting for lameness evaluation at a busy sports medicine practice in which either diagnostic analgesia or intra-articular medication of the shoulder joint was performed were identified. From 2007 to 2013, 69 horses received either diagnostic (n = 50) or therapeutic (n = 19) intra-articular injections in the shoulder joint. This represented 2% of all horses evaluated for lameness during the same period. In all horses,

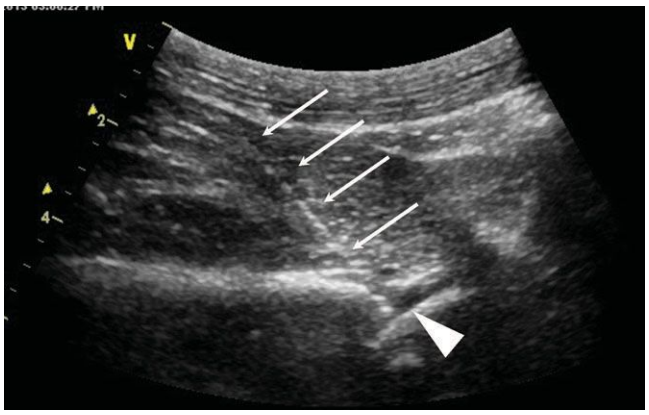

Fig. 6. Proximal is to the left. A 3.5 in. spinal needle (arrows) is placed through the infraspinatus muscle into the shoulder joint space.

Table 1. Step-by-Step Procedures for Ultrasound-Guided Injection of the Shoulder Joint

1.	Image the biceps tendon in the transverse plane.
2.	Locate the cranial portion of the greater tubercle; the lateral border of the biceps brachii tendon.
3.	Move the ultrasound probe laterally and caudally approximately 4 cm, between the cranial and caudal portions of the greater tubercle.
4.	Rotate the transducer 90° and move proximally approximately 2–3 cm to visualize the joint space.
5.	Prep the patient and ultrasound.
6.	Insert the needle just proximal to the transducer handle at an angle 20–30° proximal to it.
7.	Adjust the tip of the needle as needed to achieve the appropriate needle path to the joint space.
8.	Visualize the needle entering the joint space and distention of the joint capsule while injecting.

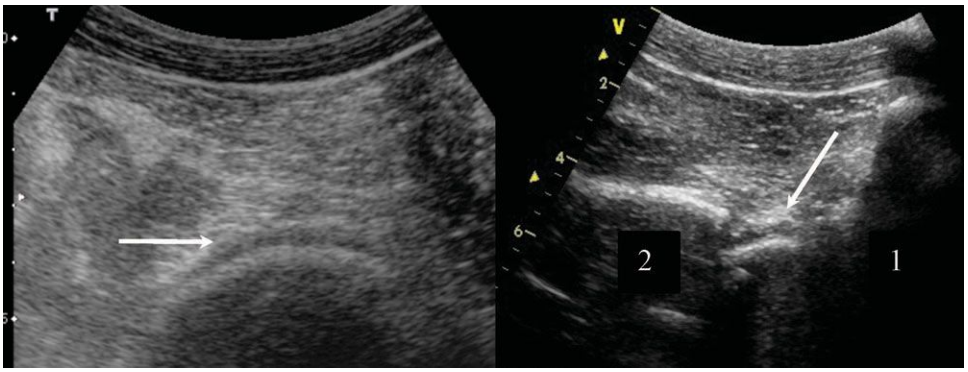

Fig. 7. Comparison ultrasound images of the shoulder joint space in the transverse (left) and parasagittal (right) ultrasound images. In the transverse image, cranial is to the left. 1, Humerus, 2, scapula. The arrows are highlighting the joint capsule/synovial lining (thin hyperechoic line).

ultrasound guidance was used to facilitate injection. Of the 50 horses that received ultrasound-guided diagnostic anesthesia, only 19 (38%) exhibited a positive response (improved lameness grade). Ultrasound visualization confirmed successful intra-articular injection in 69/69 (100%) of horses. No immediate or long term (>3 month) complications were associated with the procedure.

4. Discussion

Common diseases and injuries to the shoulder region include osteochrondrosis lesions, osteoarthrosis, fracture, luxation, septic arthritis, and soft tissue injuries to the regional myotendinous units and bursae.[1,7] Clinical examination (lameness examination, palpation, and flexion) and stepwise diagnostic anesthesia lead to isolation of lameness to the shoulder region, which is then confirmed with intra-articular anesthesia.

Ultrasound-guided injection of the shoulder is readily and easily performed by the equine practitioner in the hospital or a field situation.[4] While best performed with a linear or curvilinear probe at 7 to 12 mHz, a linear rectal probe may be used with success. The added visualization of the needle placement increases the accuracy of the injection[5] and assurance that the medication is being placed where the practitioner intended. More traditional blind techniques which require aspiration of synovial fluid to confirm needle placement may result in iatrogenic trauma to the surrounding soft tissues if the needle is redirected multiple times. Additionally, multiple punctures of the joint capsule during diagnostic anesthesia of the shoulder joint may result in extravasation of the anesthetic solution and inadvertent anesthesia of the suprascapular nerve with subsequent paralysis of the associated shoulder musculature.[8]

A technique for ultrasound-guided shoulder injection currently exists in the literature.[4] The previously described technique is performed with the ultrasound probe positioned in the transverse plane, parallel with the joint space. Reportedly, this allows for more surface area of the joint to be visualized for examination or needle placement (Fig. 7). Conversely, in the report presented here, the ultrasound probe is positioned in the sagittal (para) plane. The sagittal orientation displays the joint contour and margins in a plane that are perpendicular to the joint space, more closely resembling the anatomy of a standard lateral radiographic projection of the shoulder joint. This anatomical relationship is potentially more intuitive and familiar to the practitioner, making it easier for a beginner to perform ultrasound-guided procedures. The authors routinely utilize the sagittal ultrasound-guided technique to ensure correct placement of the needle into the synovial cavity and minimize the risks associated with the shoulder joint.

The use of ultrasound guidance to facilitate shoulder joint injection provides the practitioner with a more dependable method of assuring the correct placement of anesthetic or therapeutic medications into the joint as compared to more traditional (blind) techniques. Knowing the joint was entered successfully allows the practitioner to make confident and accurate assessments of responses to diagnostic analgesia, administration of intra-articular medications, and further treatment recommendations. In conclusion, the authors recommend the sagittal technique as an additional approach for ultrasound-guided injection of the shoulder joint to ensure accurate and dependable placement of the needle into the synovial cavity and minimize the risks associated with shoulder joint injection.

Acknowledgments

Conflict of Interest

The Authors declare no conflicts of interest.

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Comparison of Arthroscopy to Ultrasonography for Identification of Pathologic Changes in the Equine Stifle

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Ultrasound and arthroscopy should be combined to increase the likelihood of detecting stifle pathology since each modality has its own limitations. When combined, these modalities can provide a more global assessment of pathological changes within the equine stifle. Authors' addresses: Department of Environmental and Radiological Health Sciences (Adrian and Barrett); Department of Clinical Sciences and Orthopaedic Research Center, College of Veterinary Medicine and Biomedical Sciences (Goodrich, Kawcak); and Department of Statistics, College of Natural Sciences (Chapman), Colorado State University, Fort Collins, CO 80523; and Department of Small Animal Clinical Sciences, College of Veterinary Medicine, University of Florida, Gainesville, FL 32610 (Werpy); e-mail: laurie.goodrich@colostate.edu. *Corresponding and presenting author. © 2014 AAEP.

1. Introduction

Although descriptions of the arthroscopic and ultrasonographic boundaries of the normal femorotibial joints exist, there are few examples in the literature comparing the pathologic changes observed with each imaging modality. Our objective was to compare the diagnostic capability of arthroscopy to ultrasound in detecting pathologic changes in the equine stifle and to describe how arthroscopic and ultrasound examinations may differ in the grade/severity of the lesion.

2. Methods

Retrospectively, the structures of the stifle joint were evaluated and graded by scoring arthroscopic and ultrasonographic exams and levels of pathologic changes were graded and compared between the modalities.

3. Results

Ultrasound detected significantly more lesions within the medial meniscus than arthroscopy. The appearance of insertional desmopathies of the medial cranial meniscotibial ligament could be better assessed with ultrasound compared to arthroscopy; however, arthroscopy had an improved diagnostic capability in the detection of tearing of the medial cranial meniscotibial ligament. Arthroscopy detected more cartilage defects within the medial femoral condyle, but ultrasound identified more osteophytes along the medial femoral condyle. Four patients had defects within one of the

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patellar ligaments, with this being the major clinical finding in one patient.

4. Conclusion

When combined, arthroscopy and ultrasound can provide a more global assessment of pathological changes within the equine stifle.

Acknowledgments

Conflict of Interest

Drs. Goodrich and Kawcak are shareholders in Advanced Regenerative Therapies, Inc., and Dr. Goodrich is a speaker for Bayer Pharmaceuticals, Inc.

Outcome of Palmar/Plantar Digital Neurectomy in 50 Horses With Foot Pain Evaluated With Magnetic Resonance Imaging

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Palmar or plantar digital neurectomy (PDN) can resolve lameness in horses with foot pain unresponsive to medical therapy. However, horses with core or linear lesions of the deep digital flexor tendon subjected to PDN may have residual lameness or early recurrent lameness postoperatively. Authors' addresses: Department of Veterinary Clinical Medicine, College of Veterinary Medicine, University of Illinois, Urbana, IL 61802 (Gutierrez-Nibeyro, M.A. Mitchell); Department of Small Animal Clinical Sciences, College of Veterinary Medicine, University of Florida, Gainesville, FL 32610 (Werpy); Marion duPont Scott Equine Medical Center, Leesburg, VA 20177 (White); Fairfield Equine Associates, 32 Barnabas Road, Newtown, CT 06470 (R.D. Mitchell, Edwards); BW Furlong & Associates, Oldwick, NJ 08858 (Gold); and Virginia Equine Imaging, 2716 Landmark School Road, The Plains, VA 20198 (Allen); e-mail: sgn@illinois.edu. *Corresponding and presenting author. © 2014 AAEP.

1. Introduction

Palmar or plantar digital neurectomy (PDN) can be performed in horses with chronic foot pain, allowing horses to return to athletic activity. However, there is limited knowledge of the foot lesions that influence the outcome of PDN.

2. Materials and Methods

Medical records of 50 horses subjected to PDN due to chronic foot pain were reviewed. Age, breed, sex, athletic activity, duration of lameness, affected limb(s), response to anesthesia of the palmar/plantar digital nerves, MRI findings, and surgical technique were analyzed together with follow-up data to identify factors that influenced outcomes.

3. Results

Forty-six of 50 horses (92%) responded to surgery; forty (80%) were able to return to their previous athletic use for a median time of 20 months (range: 12–72 months). Horses with pre-existing core or linear lesions of the deep digital flexor tendon (DDFT) had a significantly shorter period of lameness resolution after surgery than horses with dorsal border lesions of the DDFT or other foot lesions.

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4. Discussion

The results show that horses with core or linear lesions of the DDFT can experience residual lameness or early recurrent lameness after surgery. The use of MRI to rule out core or linear lesions of the DDFT before surgery may be an adjunctive diagnostic tool for patient selection.

Acknowledgments

Conflict of Interest

The Authors declare no conflicts of interest.

Association of Catastrophic Fracture of the Condyle With Bone Changes of the Thoroughbred Racehorse Fetlock Identified by Standing Magnetic Resonance Imaging

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Standing MRI (sMRI) identified a greater degree of bone changes in horses with catastrophic failure of the front fetlock from condylar fracture when compared with control horses. These changes may be useful as a screening test to identify horses at increased risk of fetlock fracture during racing. Authors' addresses: Equine Medical Center of Ocala, 7107 W. Hwy 326, Ocala, FL 34482 (Peloso); Texas A&M University, College of Veterinary Medicine, College Station, TX 77843 (Cohen); Orthopedic Institute, 4500 Newberry Road, Gainesville, FL 32607 (Vogler); and Gulfstream Park Racing and Casino, 901 S. Federal Hwy, Hallandale Beach, FL 33009 (Marquis); e-mail: jpeloso@emcocala.com. *Corresponding and presenting author. © 2014 AAEP.

1. Introduction

The fetlock joint is the most common site of musculoskeletal disease leading to euthanasia in Thoroughbred racehorses in the United States, Canada, United Kingdom, and Hong Kong. Identifying alterable risk factors to reduce condylar fractures could have a significant impact on reducing the occurrence of catastrophic failure of the fetlock (CFF).

2. Materials and Methods

Bone change scores from Florida Thoroughbred racehorses with CFF due to condylar fracture (n = 18) were compared to racehorses (n = 53) euthanized for reasons other than CFF (e.g., pneumonitis). The association between the dichotomous outcomes of condylar fracture with independent variables was assessed using bivariable and multivariable logistic regression analysis with significance set at $P < 0.05$.

3. Results

The proportion of cases with >50% sclerosis of the lateral condyle (67%; 12/18) was significantly ($P < 0.0001$) greater than that among controls (13%; 7/53). The proportion of cases with lateral condyle short tau inversion recovery (STIR) signal increase (50%, 9/18) was significantly ($P < 0.0001$) greater than that among the controls (0%, 0/53).

4. Discussion

Performing a sMRI on Thoroughbred racehorses with lameness localized to the fetlock joint may help reduce the occurrence of CFF. The identified bone changes may be useful as a screening tool to identify horses at increased risk of fetlock fracture during racing.

Acknowledgments

Conflict of Interest

The Authors declare no conflicts of interest.

Research Abstract

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Financial Management and Planning Foundation: The Chart of Accounts

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1. Introduction

Garbage in, garbage out. More than an acronym for computer data-input error, GIGO describes a plethora of practice management challenges every administrator faces. Your best practice management decisions pivot on methodically-gathered data from your own practice.

Solid data collection systems result in reliable financial information. Over time, trends in the unique financial and transactional information of your practice become the basis of intelligent decisions and strategies.

With a scientific approach to financial data management, you can change the GIGO acronym to one with much more appeal: Good In, Good Out.

Envision what "Good Out" might mean for your practice:

- Good (ethical) **standards** (business, tax, client, patient, employee, operational)
- Good (reliable) **benchmarks** of practice and practitioner progress
- Good (healthy) **financial decisions** from equipment purchases to compensation and benefit arrangements
- Good (unbiased) **feedback** to doctors and support staff and partners
- Good (accurate) **recognition** of effort and contribution
- Good (appropriate) **fee structure** for which guilt and apologies are not necessary
- Good (consistently applied, state-of-the-art) **patient care**
- Good (timely, appropriate, accurate) **client communications**
- Good (timely, appropriate, accurate) **employee performance reviews** and recognition
- Good (replicable, consistent, strong) **internal control** and operational systems
- Good (attractive, transferable, documented) business methods supporting **succession plans**
- Good (higher, sustainable, supportable, transferable) **practice/goodwill value**
- Good (commensurate with risk, effort, time) **return on your investment** in practice ownership

Good In, Good Out philosophy resides in a time continuum. Like good patient care and like good animal husbandry, practice management is a lifelong activity. One time vaccinations and one time management conference drenchings do not result in

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a sustainable practice wellness plan or healthy business.

2. The Accountant's Five Point Plan

Business texts and well-versed advisors recognize five essential managerial elements. The five point financial and operational strategy is based on:

Accounting
Reporting
Comparing
Analyzing
Planning

These five activities evolve into practice management systems, controlling daily, weekly, quarterly, and annual administration and the strategic positioning of the practice as the years proceed.

The **accounting** element dictates whether the financial and managerial information is accurate and complete. Practice accounting systems are the basis for controlling assets and for structured record keeping.

The second management element, **reporting**, requires qualified personnel both within and outside the practice to ensure correct summarization of data. Practice leadership decides which reports will be generated in-house and which will occur through the practice's engaged accountant. The reporting activity requires management to have basic understanding of financial statement interpretation. Standardized practice financial data reporting is important to making informed decisions.

Practice administrators use the **comparing** and **analyzing** elements concurrently. Statistics are compared in the practice as well as to outside. Ratio **analysis** is one of the tools that occurs in a review that preludes the last element of management, **planning**. Underlying financial and transactional data are compared and analyzed to reassess practice growth, client and patient activity, fee schedules, to establish budgets, and to monitor staff development success, employee relations, and client satisfaction.

3. Accounting Element

"Accounting" refers to the accumulation of data in a meaningful way. Accumulated data is translated into reports that can be used in comparison and analyzing, and finally, in planning for the future. Practice accounting systems must be integrated to efficiently provide a variety of functions:

- Bookkeeping
- Tax reporting
- Financial accounting
- Managerial accounting
- Internal control

In the veterinary industry, data collection from a revenue production standpoint has been greatly expedited and enhanced through the use of computerized systems. Managing and accounting for the expense side of the practice has been a slower evolving segment. With the advent of relatively inexpensive software programs, the ability to accumulate data from both an income and expense standpoint within the practice has significantly improved to serve the variety of functions spanning from client invoicing to tax preparation and internal control procedures.

The foundation for any practice's financial data management requires a standardized **chart of accounts** enabling systematic categorization of capital outlays and inflows. A chart of accounts uses numerically organized categories based on the following chronologically listed broad transactional types:

- Practice Assets (what the practice ***owns***)
- Practice Liabilities (what the practice ***owes*** outside parties)
- Owner's Equity or Capital (book value of the residual, i.e., assets minus liabilities, owing to owners)
- Revenues (from veterinary practice operations)
- Expenses (assets consumed to generate revenues)
- Other Revenues and Expenses (not from the conduct of veterinary activities)

The practice's chart of accounts lists all possible areas of expense and revenue transaction categorization, as well as assets, liabilities, and owner equity accounts. The practice bookkeepers, receptionists, and bill payers, effectively log each transaction using the chart of accounts, whether they are aware of it or not. Bookkeepers usually use account numbers to code each transaction, dependent upon its nature. Timely coding and logged, each dollar-measured transaction builds a story. A definitive reporting date and defined span of reporting time results in data summarization, compiled into financial statements and information transferred to tax returns.

4. How Does a Chart of Accounts Work?

Every time a bookkeeper records a transaction, s/he decides which account title best describes it in a way that creates useful management reports in a cost-efficient manner. For an unusual or unfamiliar transaction, the bookkeeper refers to the chart of accounts as necessary to decide which account title best fits the particular transaction.

Generally, the bookkeeper dates the transaction in the records on the day it occurred. Over a specific period, say a month, all of the transactions accumulate, resulting in monetary amounts in each account.

For example, during the month:

- All veterinary fees collected and deposited are added to the business checking account.
- All invoiced veterinary services are recorded as revenues.
- All employee payments made through paychecks are recorded as payroll expense and accurately posted as gross wages to appropriate accounts reflective of job descriptions (receptionist wages, technician wages, employee veterinarian wages, etc.).

At the end of the month, the bookkeeper's computer tallies all transactions; there could be hundreds if not thousands of them over the course of 30 days. The bookkeeper produces a variety of financial reports. Computerized bookkeeping programs give the flexibility of quick turnaround of just about any information a manager would want to evaluate.

The most common financial report produced would be the month's profit and loss report (otherwise called a P & L, or a statement of revenues and expenses, or an **income statement**). All transactions the bookkeeper classed as a revenue item or as an expense are included in this report.

Another common financial report is the **balance sheet**, also called the statement of assets, liabilities, and equity. This report shows the month end balances of all the practice's asset, liability, and equity accounts.

Each transaction's correct coding builds a financial story, a history. The story can be fact or it can be fiction, depending on the care and importance given to consistent and accurate use of the practice's chart of accounts.

If the bookkeeper correctly codes transactions in accord with the practice's chart of accounts, management can rely on the reports to make sound financial decisions.

5. Why Should a Practice Use a Standard Chart of Accounts?

Many practice managers cannot make an accurate analytic assessment of the sum total of all the activity in the course of the year. They don't know if the financial reports accurately represent practice activity results. They may not be able to make sound decisions. The practice might be on track of financial targets or perhaps the reports give false information.

Have you ever felt that cash flow was tight? Were you quickly able to pinpoint the reasons why? Were you able to decide if the practice was making good use of **capital** resources to generate more money, or if underutilized assets of talent, equipment, and supplies had overextended the practice, or whether the problem was simply out of control credit policies?

Do you wonder if the practice can afford to add additional employee benefits? Can you predict the overall cost of investing in a loyal labor force?

Many significant financial decisions become nothing more than "best guesses" unless accounting reports accurately measure the practice's progress. The best way of measuring the practice's progress is to compare its current period results against those of past periods but only when they are reliable and replicable. The records should match the depletion of assets with income and profit generated over the same period.

Planned asset depletion should yield more than enough revenues and profits to replenish them, but do you know if this is really the case? Without accurate records created through a systematic process of coding transactions, a practice manager doesn't always know the answers to these questions.

Assure you have accurate information about your practice to know if it is on track to accomplish goals, objectives, ideals, vision, and satisfy obligations. Accounting for practice transactions using uniform standards gives assurance that decisions are based in fact and lead to profit over time.

6. What Else will a Chart of Accounts do for your Practice?

- Result in consistent, replicable reports that present trends.
- Provide information for accurate tax reporting and other governmental requirements.
- Satisfy lenders as to the practice's financial strength.

When practices properly implement accounting systems using an industry-defined chart of accounts, almost everything else falls into place. You gain both a consistent measurement of your practice's economic progress over time and a meaningful comparison of its results with other practices that use the same systems and organization. The Equine Veterinary Practice Chart of Accounts (EVPCOA) will result in reliable, relevant data for financial decision-making.

If your practice does not properly maintain a uniform chart of accounts or has not redesigned one within the last 5 to 8 years, then you most likely are experiencing 2 or more of the following scenarios:

- Your functional managers depend on their own month end reports to explain operating results and make little use of the month-end financial reporting package available on most accounting software.
- Your financial analysts are handicapped by a lack of reliable, relevant detail when performing their studies.
- The key-punch entry of ledger data into spreadsheets at month-end is a time-consuming and error prone activity.
- There is a 2 to 20 day delay between the final ledger closing and the issuance of your complete month-end reports.

We recommend using the Equine Veterinary Practice Chart of Accounts for the following reasons:

- It promotes consistency and accuracy for your practice's financial comparison to itself as the years go by.
- It promotes consistency for comparative measurement purposes within the equine veterinary profession as a whole.
- It encourages practice administrators to become familiar with the underlying structure of practice **financial statements** based on correct bookkeeper coding and classification.
- It forms the framework for organizing practice financial data of all sources.
- Its use results in reports that give administrators a better grasp of the practice's current financial status and the ramification of business decisions made.
- It enhances practice internal control systems through support of **double-entry accounting** methods with affordable off-the-shelf software.
- It reflects the practice's economic health by organizing, measuring, and quantifying information that can then be readily compared with other practices.
- It results in historically reliable reports that enhance quantification of practice value during appraisal for succession purposes, buy-in, practice sale, and purchase.
- It is an essential practice system component that reassures outside third parties about management's attention to sound business methods, financial record reliability, and long-term economic stability.
- It helps managers make better use of practice budgets and forecasts by building estimates of future activity based on reliable reports of past results.
- It supports data accumulation leading to efficient creation of a large number of essential reports, including summarization for tax reporting purposes.
- It provides for detailed analysis of financial activity or very broad assessments, depending on intended user need. For example, improved tax return disclosure through increased detail appears to decrease compliance audits by government agencies.
- It is highly usable by every practice, no matter the required level of detail. The Equine Veterinary Practice Chart of Accounts design is flexible to meet the needs of all equine veterinary practices of any size or required level of financial detail.

Understand that capturing data accurately is an investment. Besides equipment and software setup, you must engage qualified personnel who can summarize the data based on the knowledge of the practice and overall management interest in the time permitted.

We believe, with the variety of excellent software programs available today, that the most economical way is to source the majority of data collection and accumulation within your practice. However, do not rely exclusively on a single individual to maintain bookkeeping systems and input data.

Be careful. With all internal data collection and transaction management, division of asset control from recordkeeping responsibility must occur. Establish checks and balances through segregation of duties among personnel. Division of responsibilities is the basic premise of strong internal control within a practice. Weak controls exist when the person collecting and depositing receipts also prepares the records of what transpired. In all cases, practice owner oversight is crucial to thwarting defalcation.

The practice's Certified Public Accountant (CPA) is an excellent resource for examining existing systems and identifying weaknesses. Such a consultation helps you decide the assignment of record keeping responsibilities as compared to who has control of the asset so the practice is protected to the extent possible with the given personnel resources.

In summary, the success and profitability of your practice rests with you. Act as a leader with vision. Adopt the new version of GIGO activity and philosophy: Good In, Good Out. Establish systems that allow you to make proactive and meaningful conclusions. Search out those individuals who have knowledge of the veterinary profession and give sound advice. Be wary of quick fixes and pat answers. You know your practice. You must take responsibility for what occurs in it.

Do not abdicate responsibility for systems once they are in place. Oversight maintenance is the responsibility of management. *You are management*—not your associate, not your practice manager, not your accountant, not your management consultant. Use your head. Discern and choose the data, systems, reports, and people important to your vision of practice. Garbage In, Garbage Out will steadily and surely evolve to Good In, ***Gold*** Out**.**

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Conflict of Interest

The Author declares no conflicts of interest.

Collaboration By Choice—Membership in a Veterinary Management Group

Stephen Curvey

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1. Introduction

The first veterinary management group (VMG) was started in 1984 by a past president of the American Animal Hospital Association (Bob Featherston, DVM) and a radiologist from the University of Georgia (Bill Lewis, DVM). Borrowing a concept from the Automobile Dealers Association, these two veterinary leaders formed a "20s group," comprised of several prominent independent noncompeting small animal practices from across the United States. The group formed to address opportunities in practice and focused on topics ranging from profit and cost center analysis to standards of care. The primary objective: practice success through collaboration.

The initial group recognized that didactic seminars were useful for specific issues but provided limited understanding of the complexities of veterinary practice management for the attendees. They were determined to focus on meaningful debate among competent peers of similar experience to produce more useful ideas for implementation within their respective practices.

Veterinary management group (VMG 1) continuously refined its focus and procedure through trial and error over the years. In 2000 the executive committee of the VMG was approached by Dr. Bill Rood of Rood and Riddle Equine Hospital and asked to provide structure to the formation of an equine group. Recognizing the potential application of the concept to other groups, the leaders of the VMG decided to support the request and subsequently formed an umbrella corporation, VSG, Inc. (Veterinary Study Groups, Inc.), to oversee group development activities, which included the creation of policies and protocols which would support the successful operation of additional groups.

Thirty years later, the same policies and protocols apply to the operation of 25 VMGs. Today's groups are comprised of more than 450 members who own and operate 670 practices, with nearly 2900 full-time equivalent veterinarians providing care for more than 6 million pets and horses.

While the groups are physically similar and operate according to the same VSG Policies and Procedures and Protocols, there are numerous variations related to medical focus. There are three Emergency/Referral/Specialty groups, five private practice equine groups, one equine academic study group, one feline group, one mixed animal group, and fourteen small animal primary care practice groups.

The group formed by Dr. Rood became known as VMG III and remains a remarkable affiliation of some of the most iconic leaders and hospital-based

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Table 1. Length of Study Group Participation

0–2 years	2–5 years	6–10 years	10+ years
• Satisfaction of belonging to a larger group	• Collaboration	• Positive financial results	• Relationships
• Initial financial benefits	• Accountability	• Sharing	• Motivation
	• Fostering relationships	• Self-development	• Cost savings and services

equine practices from across the veterinary profession. Each of the equine groups, be they hospital-based, ambulatory, or academic, represent equine practice excellence.

Today's equine practice owner faces significant challenges in successful business management. Market fundamentals and trends related to changing demographics, equine populations, regulation, and client participation require careful consideration when developing a durable business strategy.

While membership in a VMG will not alter some of the more troubling trends, year-round interaction with inspired equine colleagues can lead to goal attainment through strategic planning, more innovative medical and business practices, and peace of mind acquired through shared experiences and ideas with a committed peer group.

In late 2012, the VSG asked members about their experience and value of being a part of a study group. Members placed the financial benefits and results since joining a study group as being the most important. The poll also pinpoints that access to intellectual capital, camaraderie, personal, and professional growth immediately follow. The analysis corroborates that veterinary study group participation promotes a momentum of member enrichment and growth as time progresses (Table 1).

It would appear that "cost savings" are attributed to participation in a group purchasing program, whereas "financial benefits and results" tend to describe the benefit of developing self-efficacy as applies to business acumen. The VSG places cost savings related to purchasing programs as tertiary to the broader and more lasting benefits associated with "financial benefits and results" derived from idea exchange and access to intellectual capital.

While the specific financial effects of participation in a VMG vary, it is not unreasonable for VMG members to report a slight reduction in COGS and improvement to an average practice's earnings before interest, taxes, depreciation, and amortization. Quantifiable improvement is normally incremental across multiple expense, revenue, and efficiency categories; slight improvements in these categories contribute to business results that lead the profession.

In summary, participation in a VMG provides an opportunity for "collaboration by choice." Dr. Dick Goebel, an original member of VMG 1 once wrote, "Do you find it lonely at the top? With whom does a practice owner discuss his/her challenges, identify appropriate goals and share success stories? Why not with professional colleagues who face similar challenges and who struggle to attain similar goals? Why not share your success with a trusted group of professional colleagues as well? A VMG can be an advisory board for your practice."

Acknowledgments

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The Author declares no conflicts of interest.

How to Offer Veterinary Services as an Equine Relief Veterinarian

Tricia Arnold, DVM

This presentation addresses how to establish a reputation for competence and commitment in providing relief veterinary services, while honoring an individual equine practitioner's practice style and established client base. Author's address: Cedar Springs Equine Veterinary Services, 15200 NE 81st Way, Unit K104, Redmond, WA 98052; e-mail: tmarnold49@gmail.com. © 2014 AAEP.

1. Introduction

One of the most common problems, especially facing solo practitioners, is the struggle to provide 24/7 veterinary care. Some solo practitioners do not have the client base, financial means, or desire to hire a full time associate. While waiting for practices to grow enough to warrant a second or third veterinarian, a balance in lifestyle may be overlooked. As the demographics and desired life styles of equine veterinarians continue to evolve, there is a growing need for competent equine relief veterinarians. Incorporating relief services is a responsible and dedicated option to ensure a high standard of continual veterinary care.

Many *small animal* veterinary practices, especially in larger urban areas, have established pools of relief practitioners. For example, the State of Washington offers a list of relief veterinarians through the Washington State Veterinary Medical Association. Rarely is an equine practitioner listed among the large pool of small animal veterinarians. There is an opportunity to use equine relief veterinarians to enhance both the personal and professional lives of equine practitioners.

Since beginning an equine relief practice in October 2012, a number of different circumstances have warranted the need for service. Some examples include the following:

1. Veterinarian A: scheduled to play polo, out of state, every other weekend. Relief services provided every other Thursday through Sunday during the polo season.
2. Veterinarian B: requested coverage to attend the AAEP convention with additional time for an extended vacation.
3. Veterinarian C: an equine practitioner opting to additionally offer small animal veterinary services, thus requiring intermittent equine relief services.
4. Veterinarian D: requested relief for an extended vacation of 2 weeks.
5. Veterinarian E: the sole equine practitioner on an island, requesting relief services for prepurchase exams.
6. Veterinarian F: requiring relief for personal surgery and recovery for 3 weeks.
7. Veterinarian G: opting for relief coverage one week a month during March through September.

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8. Veterinarian H: coverage of Mondays and Tuesdays for several weeks for increased personal time.

These are several examples of relief services that have been required and provided. *The need for relief veterinarians is endless, including both life celebrations and vacations or during unfortunate times of illness or family emergencies*.

2. Implementation

Equine practitioners have often provided years of commitment and dedication to establishing their practices. Although dedicated to providing excellent and available care, personal commitments may require planned or emergency relief coverage. Steps to establishing oneself as an equine relief veterinarian include, but are not limited to:

1. Mail letters to potential veterinarians who may require relief work.
2. Invite colleagues to meet for coffee to discuss relief options.
3. Drop by visits of potential practice locations.
4. Volunteer time for a daily ride-along.
5. Attend horse events and introduce yourself to the veterinarian on call.
6. Provide potential references, if requested.

In establishing a relief practice, I have both incorporated and licensed my business, thereby working as a subcontractor. Another scenario is to act as an employee, if a practice requires ongoing scheduled relief work.

Transportation has been with either using a practice vehicle with the practice's gas card or using my own vehicle and keeping a mileage log. When using a practice vehicle, all drugs and equipment are provided. When using my own vehicle, a rate, as established by the Internal Revenue Service (currently 56 cents/mile), is billed to the practice and all drugs are reimbursed at cost. Equipment such as radiograph and ultrasound machines are provided by practices for which I am providing relief services.

A variety of forms of compensation have been accepted:

1. A guaranteed daily base pay plus emergency (ER) fees.
2. A guaranteed daily base or 50% of professional service fees/per day, whichever is greater, plus ER fees.
3. A daily service fee (2× base pay) with *no* emergency call fee.
4. A guaranteed base pay or 3/8 of gross revenue earned, whichever is greater, plus ER fees.

The base pay varies, depending on a variety of factors, including the time to commute, cost of commute (fuel, bridge/ferry fees), and availability of work. If the practice is outside of the relief veterinarian's area to allow for an effective response to emergencies, accommodation is either provided or compensation for a motel is billed to the practice. Follow-up calls concerning care, lab work, referrals, etc. are transferred back to the practice owner once they have returned.

3. Discussion

This career opportunity can benefit veterinarians, providing relief services in the following ways:

1. Setting your own schedule to work.
2. Allow part time work.
3. Experience in a variety of practice styles.
4. Experience in running your own business on a smaller scale.
5. Flexibility for time off (e.g., parent of children).

Admittedly, there have been some issues that have arisen requiring open and honest communication between both parties.

Failure to Pay for Compensation at the Time of Service

As a relief veterinarian, I expect payment from clients at the time of service. In doing so, I expect to be paid immediately following my scheduled days worked. Unfortunately I have experienced tardy payment of up to 6 weeks by a veterinary practice. I will work only for practices providing timely compensation.

Different Practice Styles

With open and respectful minds, both parties can gain valuable knowledge on various drug protocols and veterinary procedures. I utilize their practice protocols and techniques as long as I am providing a high standard of care. Most veterinarians are open to conversation and welcome alternatives. At the end of the day, as a veterinary contractor I am legally responsible for the cases in which I provide care.

Record Keeping

In my experience, there is a wide variety in type and completeness of medical records. The documents I write may be requested for legal reasons and, thus, I try to write complete records. Also, the practice requires detailed medical records for appropriate follow-up care.

Concern Over Case Poaching

I continue to establish myself as an equine relief veterinarian who can be trusted. My reputation as an equine relief veterinarian is dependent on establishing confidence in both my integrity and practice style. My intent is not to poach clients but rather be an alternative to veterinarians who desire time off for whatever reason. This continues to be an issue for some veterinarians and they must decide if it is worth the inherent risk to contract a veterinarian for relief services.

There is a need and opportunity for individuals to become equine relief veterinarians. As the demographics of equine veterinary medicine continue to change, there likely will be growth in this field.

Acknowledgments

Conflict of Interest

The Author declares no conflicts of interest.

An Accounts Receivable System for Equine Veterinarians

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1. Introduction

We can all agree that equine veterinary practice is very hard work. What makes it even tougher after the long hours, fractious horses, challenging cases, and miles on the road is when we do not get paid for our services. Despite this, many equine vets resist implementing an accounts receivable (AR) strategy. Whether it is because we are afraid of losing clients or a lack of appreciation of the value of our services, we hate asking for money. Our excuse is, "that's just the way equine practice has always been and no horse-owning clients will ever pay at time of service." At the same time, we are expected to pay our bills within 30 days and, yet, most of our clients take 60 days or more to pay us. What happens when that gap gets too wide? We have to borrow money from the bank so we can pay our bills. Something is wrong with this picture.

Our practice and others are proof that clients can learn to pay at the time of service. We instituted a simple to follow, but very effective, system that has resulted in the majority of clients paying at the time of service, while a few remaining excellent clients retain the privilege of paying within 30 days. Here is how we did it.

Average Days AR

The first thing we needed to do was use a marker that we could measure our efforts against. The best metric is called average days AR. It tells us, on average, how long it takes to collect payment on the services we sell. The formula we use is:

accounts receivable total/(sales for previous year/365)

Ideally, we would like this number to be less that 30 since we have to pay our supplier bills in 30 days. We want to get paid before our bills are due. The closer that number is to zero, the better.

Client Categories

Once we had developed this metric, we split our clients into three groups: new clients, excellent clients who always paid within 30 days, and chronically late payers. We realized that the best way to avoid AR problems was to not extend credit to our customers. With that in mind, we developed a policy that all new clients were to pay at time of service, with no exception. Our customer service receptionists would email or fax a new client form to every new client that detailed our credit policy. It asked the new client to give us a credit card to keep on file and to sign their agreement to the policy. Ninety-nine percent of all new clients did this without bat-

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ting an eye. Those who refused always had an excuse such as "I don't have a credit card." Our attitude was: if Visa or MasterCard won't offer you credit, why should we? We wouldn't say this of course, but we told these people that our vets would expect to be paid with a check or cash at the appointment. Right away, we were on the right track to reduce our average days AR by getting paid at the time of service.

The slow payers were sent a letter informing them of the new policy and that, because they were historically late on their accounts, we now expected them to pay at the time of service. We enclosed our credit policy and asked for a credit card on file and a signed acceptance of our new policy. I made sure to be at the office for a few days after sending these letters, expecting a huge backlash from certain clients. I am happy to say that there was not one call from a client.

The last group were the great clients. We also sent them a letter explaining our new policy, but let them know that since they have always been reliable we weren't going to insult them by expecting them to pay at the time of service. However, if they strayed even once past 45 days, they would automatically become a "cash on delivery" client. Again, not a whimper from anyone about our new terms.

The end result was that we did not receive one client complaint or threat to move to another practice and, at the end of the year, we actually had an increase in both sales and new clients. We may have quietly lost a few who did not agree with our new policy, but it appears that they were the ones we could stand to lose and they were quickly replaced by those who were willing to pay for veterinary services.

2. Conclusion

It has been 4 years since we instituted our new credit terms and there have been a couple of things we learned along the way:

- We must be diligent with the policy. Once we slack off even a little bit, we see our average days AR creep up a little bit.
- If associates are paid on production, commission should be earned only on the client invoices that are paid. This way, associates(?) won't work for just anyone to increase their sales, while at the same time the office must be vigilant about not sending them to work on bad paying clients. Not all practice management software programs can run a report that gives you this information, thus you may want to investigate your own software system to see if you can benefit from this feature.
- Detail what the method of payment will be for every appointment on a day sheet for each vet. This lets the vet know whether the clients will have a credit card on file, will be paying by check, or if they are a client that is allowed credit terms.
- Have a very safe system to store credit card information. Most practice software systems can do this. Some veterinarians have had good luck using smartphone apps such as Square, which allows you to swipe a credit card using your smartphone. In this manner, a veterinarian can take a payment at the appointment even when on the road.

Of course, with every system, there are exceptions to the rule. For example, this system can be challenging to implement in a racetrack or breeding practice. The business model of these practice types depend on numerous part-owners and trainers that attempt to control the process. At the same time, our clientele has many absent owners in show horse barns and boarding stables, but we have not had an issue with acceptance of our system.

Before we instituted our new AR system, our average days AR was in the 60-day range. It was taking us twice as long to collect our money as it was to pay our bills. Now we are at less than 30 days and have been for the 4 years since we instituted this policy. Our cash flow is much better because money owed to us is actually in our hands and not in the pockets of our clients. Our vets are much happier knowing they are working for clients that are paying when they are supposed to. The customer service representatives spend most of their time making appointments instead of chasing after money. The main thing is that we haven't lost a client because of our credit policy, except those few who likely wouldn't want to pay anyhow.

Acknowledgments

Conflict of Interest

The Author declares no conflicts of interest.

How to Implement a Lean Inventory Management System for a Solo Equine Practitioner

Jorge L. Colón, DVM

Inventory costs are the number one component of a solo practitioner's expenses against income. In essence, any inventory held longer than its purchase credit period must be financed through debt or through the tying up of capital that could be invested elsewhere in the practice. For most solo practitioners, decreasing their cash-to-cash cycle time is a sounder financial investment than holding cash in inventory. Implementation of a lean inventory management system will allow the solo practitioner to minimize total inventory costs and eliminate waste. Author's address: Jorge L. Colón, DVM, PLLC, PO Box 11631, Lexington, KY 40576; e-mail: jorgecolondvm@ me.com. © 2014 AAEP.

1. Introduction

The equine ambulatory solo practitioner has two types of inventory riding in his/her vehicle: that which will be used in the process of providing service and that which will be dispensed as a whole unit as part of the process of providing service. Because economies of scale cannot be applied by solo practitioners, the manageable aspect of the total cost of in-stock inventory will mostly rely on the quantity of stock desired to be had on hand to allow for the provision of service.

In my reproductive ambulatory practice, inventory costs for drugs and medical supplies have increased from 22.2% of expenses against income to 30.8% over the last 6 years. During the same time period, inventory expenses as a percent of total expenses have increased from 52.5% to 61.9%, making them the most significant expense against profits in my practice. Assuming that inventory is not the most sought after place for cash investments for most solo practitioners, we look for ways to improve cash flow that do not tie cash assets to inventory that is not actively helping to generate revenue. The following is a brief description on how to manage these significant costs by loosely following some of the teachings of the Toyota Production System[1] in order to 'go lean' and reduce waste.

2. Total Cost of Inventory

There are three separate costs associated with the total annual inventory cost for the practice: purchase costs, ordering costs, and holding costs. Because of the inability to apply economies of scale, solo practitioners are left to deal with ever-increasing prices through their collaborative relationships with distributors and through the proper utilization of specials and promotions, while taking into account necessary cash flows and investing opportunities. Today, most distributors provide free-of-charge next-day or two-day delivery that, combined with the simplicity of ordering and receiving, make ordering costs a negligible part of the equation. Holding costs, on the other contrary, make up the part of the equation that most significantly impact the cash flow of the solo practitioner and yet are the

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hardest to grasp and understand. Suffice it to say that the items purchased with an 8.3% savings through a "buy 11, get one free offer" cost more than just the dollar amount of items times price; there is a cost associated with having those items on the shelf. In addition of that, those that are not consumed during the vendor's billing cycle and, therefore, generate no revenue for the practice while creating a cash outflow, are a drain on cash resources that significantly impact the practice's potential profitability. The practitioner's veterinarian mentality has not been trained to recognize the significance of the holding costs of inventory and is therefore content in not realizing the excess inventory's impact on income.

Collaboration, in terms of loyalty to distributors and pharmaceutical representatives, is critical for the management of purchasing costs when one does not have the power of economies of scale. Loyalty to two or three vendors creates a beneficial relationship for all parties. First, favorable billing terms and superior service can be obtained because of loyalty. There's no more efficient way to make your money work for you than by generating and collecting revenue from your supplies before payment for them is due. Second, having more than one supplier allows for maintenance of supply streams that avoid stock-out risks. It also promotes healthy competition for your business between a controlled group of vendors, which ultimately leads to improved service and reduced prices.

Ordering costs can be deemed negligible based on current delivery terms offered by distributors. Meaning that, other than the time spent in ordering and receiving, increased number of orders do not translate into increased ordering costs because we benefit from free shipping services. Ordering and receiving do not, in fact, consume any more time than what is already allotted to vehicle restocking at the end of the day. Having said this, if a significant expense is incurred every time an order is placed or received, this ordering cost will make an impact on the total annual inventory cost and must therefore be calculated. The person in charge of inventory can apply the scientific method to ideas on how to improve the system in a continuous attempt to reduce waste. Implementation of proven ideas generated by front-line workers serve to streamline the inventory management process and increase the efficiency of the system.

The inventory holding cost is the dollar value of holding one unit of inventory; its formula is defined by the cost of the item and its holding rate. It is this holding rate that is almost impossible to define or quantify and is notoriously constantly underestimated. For the drugs and medications that we use, this holding rate could easily reach 25%[2] (pharmaceuticals firms use 20–30%[3]). In simplistic terms, the holding rate entails, amongst others, the expenses associated with physical storage, insurance, shrinkage, expiration, taxes, etc., together with the forgone opportunity of the capital strapped to an item that could provide for an investment elsewhere. As important as the former are, it is the latter, the cost of capital, that can be easily measured and managed by the practitioner. By definition, if the inventory holding period is longer than the purchase credit period, then the holding period in excess of the credit period must be financed from debt or from the stockholder's equity sources of capital. The practice's cost of capital will dictate what their actual holding rate should be, based on experience. Based on the practitioner's historical knowledge of annual demand for inventory, together with the common quantity per order, the practitioner can measure the metrics of the annual inventory turnover, days of supply, and inventory holding period.

There is a mathematical formula called the economic order quantity that provides the order quantity that minimizes the total annual inventory cost by balancing ordering costs against holding costs while taking into account the total purchase costs. This economic order quantity does not usually apply for the solo practitioner since the formula assumes a constant demand rate for inventory, with no variability, and a constant price per unit, amongst others. If the particular situation dictates that the ordering costs are negligible, the total inventory cost will truly be dependent on the negotiated prices with vendors and on the holding costs of those items. If a significant cost is associated with an order, then the number of orders will make an impact on total cost and must be factored in. Because holding costs mathematically decrease when the quantity ordered is reduced (which would imply more frequent ordering), the goal is to find an order quantity that satisfies the service provision requirements (cannot lose profit opportunities by not having inventory on hand needed to provide service) but that does not leave unused items on the shelf whose holding period exceed the purchase credit period. This has to be balanced against the potential benefit of specials and promotions, which could make more financial sense if cash is readily available for investment and investing in inventory is a sounder financial opportunity. A modified total inventory cost formula that takes into account the holding rate and the reduced prices (and order cost, if significant), based on higher purchase quantities, can be utilized to make a sound financial decision when cash is available or when extended credit terms can be secured.

3. Lean Inventory Management System

I have generated a modified fixed-time-interval system that incorporates a reorder point from a fixed-order system. I have termed it my "whatever I need plus one" system. The solo practitioner has a good sense, based on experience and actual usage/consumption, of how much inventory is being utilized, depending on the season. Assuming a lead time of two days for delivery, a practitioner could have on hand whatever they need of an item to cover

the order interval period, plus the amount deemed as necessary safety stock. I operate under a one day lead time and, therefore, hold whatever will cover the next day, plus my safety stock. When that "plus one" gets transferred to the "in-use" supply, an order of a specific quantity is triggered with the intent of bringing back the inventory level to the target inventory established. In reality, almost everything that I utilize in my reproductive practice covers me for at least several days and I end up having an order trigger event twice a week at most. This type of system provides an inventory turnover that purposely entails the fewest days of supply on hand possible in order to assure a short inventory holding period. The end result being a reduction in inventory costs associated with holding rate and capital financing; the least amount of cash possible ends up attached to stocked inventory. Manipulation of my credit card payment cycle around my vendors' billing cycle allows me to reduce my cash-to-cash cycle time,which leads to receipt of cash before paying for the inventory used.

In essence, the forgone opportunities and assumed risks associated with holding inventory act in a contra fashion to the desired profitability of the practice by tying up cash that could better be utilized elsewhere. A solo practitioner should never order more inventory than what will be consumed during the vendor's billing cycle unless a favorable financial condition exists with bulk ordering. If the individual's cost of capital is lower than the break-even point cost of capital between the bulk and regular-priced orders, then it would actually be cheaper to buy in bulk and hold the inventory (if the individual's return on invested capital is lower than the holding rate, then the cash is better invested in the inventory). Regardless, the goal is to make your inventory work for you by generating profits not only from the inventory, but also from the cash received from it before having to pay the supplier. The frequency of ordering will be dependent on the practitioner's level of desire for control of actual inventory on hand and on whether a cost is accrued with each order. Interestingly, experience has shown me that active management of inventory usage has led to the unintended consequence of more accurate procedures performed/billing records before leaving a farm call. An attempt at improving my profits by not wasting tied up cash on inventory has led to increasing my profits by avoiding the failure of complete billing; a major problem in ambulatory practice.

4. Conclusion

Improperly managed inventory leads to an increase in the cost of inventory against income and, with continuously rising pharmaceutical prices, will continue to represent an even bigger proportion of a practice's total expenses. Additional revenue must be generated, billed, and collected to pay for expenses that are not being utilized themselves to produce revenue during the billing cycle. Improperly managed inventory costs generate the need for more work and reduce the amount of cash available to the practice. The inventory system must allow for the availability of inventory necessary to provide services, while avoiding the common practice of holding on to excess inventory that is not being used to generate revenue (see the Appendix for the formulas used in this article). Equine ambulatory solo practitioners must learn how to implement an inventory management system that reduces costs by eliminating waste and allows the enactment of lean processes by utilizing collaborative relationships with vendors and suppliers, by reducing costs created by waste, and by eliminating activities that consume resources but provide no added value to the customer.

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Conflict of Interest

The Author declares no conflicts of interest.

Appendix: Formulas

Total annual inventory cost	TAIC = (annual demand × unit cost) + [(annual demand/order quantity) × (order cost)] + [(order quantity/2) × (holding rate × unit cost)]
Economic order quantity	EOQ = square root of [(2 × annual demand × order cost)/(holding cost)]
Reorder point	ROP = (demand rate) × (lead time)
Demand rate	d = (annual demand)/(days open)
Order quantity	Q = (target inventory) − (inventory position)
Inventory turnover	IT = (annual demand)/(order quantity/2)
Days of supply	DS = [(order quantity/2)/(annual demand)] × (days open)

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How to Mathematically Decide if That Distributor Special is Good for Me

Jorge L. Colón, DVM

Knowing the total actual cost of an inventory purchase will empower the practitioner to make sounder financial business decisions in the face of a distributor special. Author's address: Jorge L. Colón, DVM, PLLC, PO Box 11631, Lexington, KY 40576; e-mail: jorgecolondvm@me.com. © 2014 AAEP.

1. Introduction

Specials and promotions by distributors and pharmaceutical companies are frequently placed in front of practitioners, especially at the AAEP trade show during our annual convention. Practitioners wanting to financially maximize the business side of their practice are usually left trying to figure out what specials to take advantage of, without having an exact idea of the actual financial cost of their decision. Whereas the common practice is to look at the per item promotion price multiplied by the potential quantity of the purchase order and compare the obtained number to the number without the special, the true cost of the order is dependent on other factors that will affect the total actual cost of the inventory purchased.

2. Total Actual Inventory Cost

Inventory calculations are usually based on annual figures and the annual cost of inventory is termed the total annual inventory cost; it is calculated utilizing the annual purchase cost, annual holding cost, and annual order cost.

Because we are interested in the numbers associated with purchases that will most likely only cover a few months, we will term the equation "total actual inventory cost" and subtract from the original formula the financial benefit obtained from delayed billing for the special order. The new formula being:

Total actual inventory cost	TAIC = PC + HC + OC − DB
Annual purchase cost	PC = (demand) × (cost + tax)
Annual holding cost	HC = (quantity/2) × (holding rate) × (cost + tax)
Annual order cost	OC = (demand/order quantity) × (ordering cost)
Delayed billing	DB = (delay billing months/12) × (PC) × (line of credit rate)

This general formula was used to generate a downloadable Excel spreadsheet that allows the practitioner to determine the total actual inventory cost when assessing a special offer in order to compare it to the actual total cost of inventory if the offer is not taken. The break even holding rate for the special offer is calculated based on the input information. The spreadsheet is divided into four parts

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and addresses the four most common types of offers: (1) buy X, get Y free, (2) limited time price reduction, (3) future cash back rebate, and (4) extra purchase to get a future rebate.

3. Break Even Holding Rate

The mathematical computations in the spreadsheet provide the more business knowledgeable practitioner with the break even holding rate, or break even cost of capital point, so they can compare it to their known practice's potential return on investment. This rate is the rate at which the total actual inventory cost when taking the offer is the same as the total actual inventory cost when not taking the offer. If the known opportunity cost of capital is less than the break even holding rate, the special offer will be the better investment, meaning that it would be cheaper to buy in bulk through the offer and hold the inventory.

4. Demand and Order Quantity

Financial inventory calculations are usually based on annual numbers for demand and quantity; however, we are more interested in the numbers associated with purchases that cover a few months. To do this, the spreadsheet needs an estimation of the product quantity normally consumed per month during the specific time period so that it can calculate how many months it would take to consume the total quantity of the special offer order. In addition, it allows the spreadsheet to calculate the monthly order quantity if the offer is not taken and, hence, the number of orders to be placed to fulfill an equal total demand.

5. Delayed Billing, Future Rebate, and Line of Credit

Delayed billing and future cash back rebate provide a reduction to the total actual inventory cost. Because of the time value of money, any inventory that does not need to be paid until the future will reduce the actual cost of the inventory. Because of the present value of future money, the value of a rebate to be received in the future is less than the face value of the rebate at the time of the offer. Both of these computations reduce inventory cost based on the time difference between the purchase and future transaction. A vendor or distributor should provide an adequate estimation of how many months in the future before the bill is payable or before the rebate is to be received. The line of credit rate provides a framework to estimate the cost of borrowing the money from an institution for this specified time.

6. Special Offers

Buy X, Get Y Free

Distributors prefer to move merchandise by the case and will create specials that promote the sale of full cases (i.e. buy 11, get 1 free).

Limited Time Price Reduction

The vendor is offering a special price that is either lower than the regular price, or it's a base price before a known price increase.

Cash Back Rebate

The vendor is offering a percentage cash back rebate based on purchase level.

Need Extra Purchase for Cash Back Rebate to Kick in

The vendor is offering a percentage cash back rebate if you reach a certain amount by a certain date. You have made some purchases and need to make an extra purchase to reach the rebate kick-in level.

7. Conclusion

A sound financial inventory purchase decision can only be made if the total actual inventory cost is known. This cost is more than just price times quantity. Calculations generated on the downloadable spreadsheet at https://db.tt/cWnKLLfK will provide for a sound estimate of the actual total cost of inventory from a distributor special based on the information provided. The ability to carry out these calculations on-site through a smart phone or tablet should provide the practitioner with a powerful tool that will empower him/her to make sound financial purchasing decisions.

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The Author declares no conflicts of interest.

How to Use Vehicle Global Positioning System Tracking to Improve Ambulatory Efficiency

Rachel Bourne, DVM

Collaborating with colleagues on new technology before implementation is a great way to improve success and reduce the stress of change for veterinarians and staff. Field service scheduling is challenging but with GPS vehicle tracking, cost and time savings are attained. Author's address: Wisconsin Equine Clinic and Hospital, 39151 Delafield Road, Oconomowoc, WI 53066; e-mail: rbourne@wiequine.com. © 2014 AAEP.

1. Introduction

A global positioning system (GPS) is a space-based satellite navigation system that, when installed into veterinary vehicles, can provide location, speed, and time information. In an ambulatory veterinary practice this information can provide reliable information as to a vet's location to aid in a more efficient response to emergencies, more efficient scheduling, and improved customer service. The technology can also be utilized to aid in vehicle maintenance management, to decrease fuel costs by allowing appointments to be scheduled by location more efficiently, and to assist doctors and office staff by plotting the quickest route between locations.

2. Materials and Methods

Installation of GPS technology was first discussed within the practice in 2011. Early conversations raised concerns with privacy issues for the veterinarians and, at the time, higher costs for individual GPS units prohibited their installation. For eight months we partnered with a veterinary practice in the United Kingdom and experienced their ups and downs with installing GPS tracking devices before we implemented this technology. Together, we learned about the technology, shared ideas, and addressed issues before our practice installed the system. During this time we researched the various companies offering the technology to find the most practical and cost effective for an ambulatory veterinary practice. In 2013 the costs for individual units and their installation had decreased as fuel costs were increasing. Our clinic was looking for ways to improve scheduling efficiency, cut costs, and improve customer service. Since vehicles were only being used for work purposes we elected to install the units in 9 clinic-owned vehicles instead of using cell phones or other devices to respect the individual veterinarian's privacy. The evaluation parameters included the following:

- Operational cost savings
- Ambulatory scheduling efficiency both reactive (emergencies) and proactive (routine scheduling)
- Vehicle maintenance management
- Cultural effects on staff

3. Results

During the first five months of operation, fuel costs decreased, on average, $101/month/vehicle. Im-

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proved scheduling efficiency has been demonstrated in the following ways: decreased time for emergency response since office staff know where the doctors are and can select the closest veterinarian to the emergency; when scheduling, multiple locations can be entered into the system and the most convenient route, including appropriate drive times, can be assessed; directions can be e-mailed to doctors from their current location to the next; when clients call wondering if the doctor is running on time our office staff has a better idea of their current location to assist the client. Our system can also track vehicle mileage to schedule vehicle maintenance. E-mails are sent to a central location and the doctors that remind them when the maintenance is due. Culturally, the doctors were initially concerned about their whereabouts being known ("Big Brother"). Management was also concerned that the technology would not be embraced and as a tool it would be underutilized and "forgotten." Over time these have proven to be of little concern. Our front office staff utilizes the technology all the time. Overall, implementing this system has saved the practice money, improved scheduling and emergency response time and thus customer service, and the cultural concerns with staff have been minimal.

4. Discussion

Implementing vehicle GPS units in your practice is not a difficult process and by following these steps, you will improve the outcome:

1. *Talk* with colleagues that use GPS tracking and learn from their experiences: the good, bad, and ugly.
2. *Research* several service providers to find the right fit and price point for your practice. Demo the programs to get the best feel for the capabilities of the system.
3. *Discuss* with key personnel: the veterinarians, office administrative assistants, and owners, etc. and address potential concerns and value.
4. *Monitor* the system and empower staff to use it as a tool. Use the tools contained within the system and other metrics, including fuel costs, to assess cost savings and efficiency improvements.

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The Author declares no conflicts of interest.

How to Use Excel to Improve Client and Colleague Communication

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1. Introduction

Our current world is inundated with technology. Some of it is useful, and some of it is not. Microsoft Excel has persevered over the past several decades as one of the most versatile, useful, and readily available software. This program is generally thought to be used for accounting and data analysis. While it excels at these functions, the versatility it affords makes it quite adaptable for daily use in equine practice. Excel is extremely useful for doing rapid calculations along the whole spectrum of simple to complex. Equine veterinary practice requires the daily, and often repetitious, use of calculations. This software has proven to be a useful tool in doing many of these calculations as well as providing a quick and accurate method of providing follow up and discharge instructions to clients. A high percentage of equine practitioners are also business owners, which provides an added opportunity for practitioners to use Excel for monitoring financial metrics within an equine practice. This paper speaks to several of the ways that it can be utilized to improve communication to clients and colleagues.

Excel has come to be the most widely used and accepted spreadsheet software on the market today. Because of its popularity, it is an easy way to share files with other users. At the time of writing this paper, Microsoft has developed the website www.onedrive.live.com which allows users to share, edit, and create Excel files. This arrangement gives the opportunity for multiple users to simultaneously view and edit a given document.

A comprehensive explanation and tutorial on Microsoft Excel is well beyond the scope of this discussion. There are innumerable opportunities and venues elsewhere to learn and understand this software. However, some basic understanding of the program is necessary to consider its use in equine practice. Excel is laid out as individual sheets within a file. When the program is first opened, 3 blank sheets are presented. The tabs labeled Sheet 1, Sheet 2, and Sheet 3 at the bottom of the screen allow the user to toggle between sheets by simply clicking on the tab (Fig. 1). Each sheet is made up of "cells" aligned in vertical columns and horizontal rows. The cells are where data, text, or formulas can be entered. The beauty of Excel is that it is not a program to simply arrange and analyze data. Rather, it is a software program that allows the user to write formulas to tell it what the user would like the program to do. This makes Excel a very versatile program with the ability to adapt and conform to many different uses. Formulas can be entered into

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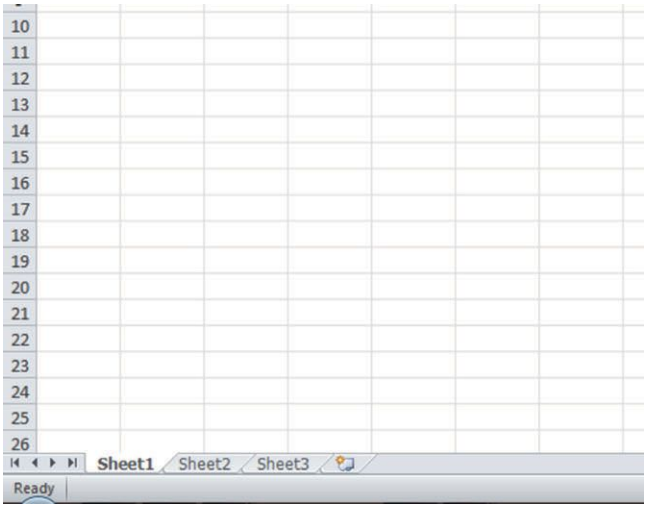


Fig. 1. Multiple sheets default.

any cell by first typing the equals sign "=". Immediately after the equal sign the user then enters the formula to be applied. For example, if the user would like the cell to fill in today's date, begin by typing "=TODAY()" (Fig. 2). The cell will then always return the current day's date. Often when writing a formula the user would desire for the formula to be applied to an entire range of data. For example, if the SUM of a series of numbers is desired, write a formula in the cell in which the user desires for the SUM value to be expressed. In that cell the user will begin by typing "=" followed by "SUM." The range of values to sum together would then be entered in parenthesis immediately thereafter. Therefore, as shown in Fig. 3, if the user wishes to determine the sum of the range of values in cell B2 through cell B6, the formula would be written "=SUM(B2:B6)." The data to include in the formula can be delimited by a comma, rather than selecting an entire range of data. For example, if the user wishes to determine the average of the values in cells E1, E3, and E6, the formula would

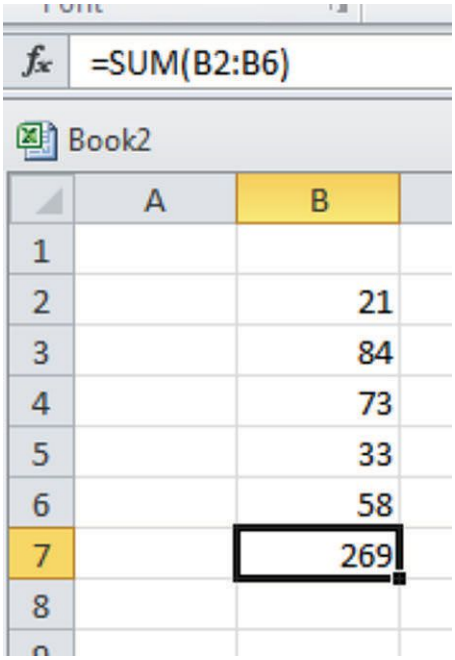


Fig. 3. Example of "SUM" function.

be written "=AVERAGE(E1,E3,E6)," as demonstrated in the Fig. 8.

Many of the simple math functions can be written without the use of a function. Thus, if the formula "=2+2" is entered into a cell, it will return the value of "4." Likewise, if the formula "=D2*E2" is put into a cell, it will return a value of the product of multiplying the 2 values in these cells (Fig. 4). This arrangement then allows the user to later change the values in these cells without having to rewrite the formula to see the resulting product. This ability to change values in input cells is key to applying Excel to equine practice. This feature allows the user to write functions and formulas once, and the reported values will change as the input values are changed.

Another key function to consider in the application of Excel is the use of formulas that are logical

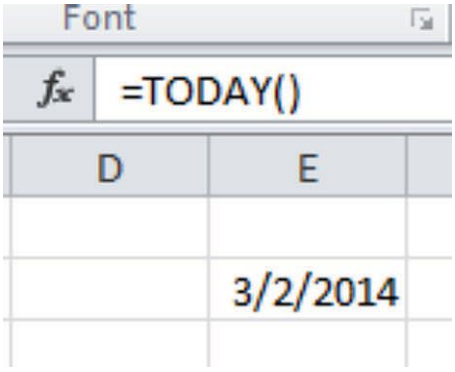


Fig. 2. Example of "TODAY" functions.

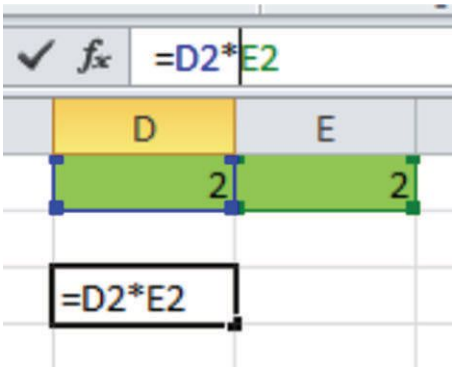


Fig. 4. Example of simple math functions.

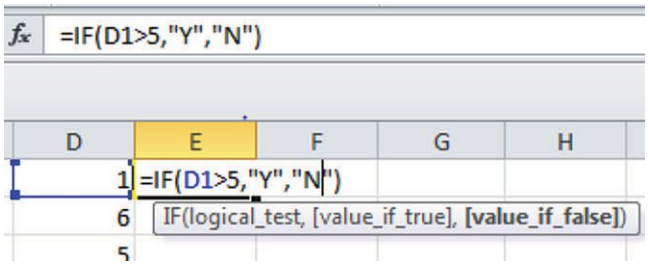


Fig. 5. Example of "IF" function.

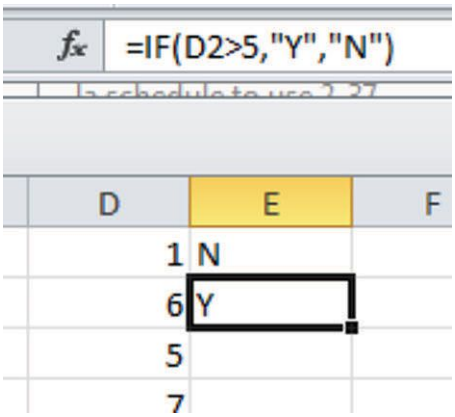


Fig. 6. Example of value returned with "IF" function.

arguments (IF, OR, AND, etc.). These formulas can be found in the toolbar under the "Formulas" heading. This is a way to tell Excel to only return a value when the specified argument is met. One particular logic formula that is helpful to grasp is the "IF" function. When this function is applied and the criteria that the user supplies is met, the cell will return the specified value. When it is not met, it will return a different specified value. For example, if we have a value in cell D1 and we want Excel to tell us if it is greater than 5, we can write a formula "=IF(D1>5,"Y","N")." As seen in Fig. 5, Excel will tell the user which criteria to enter in the formula. Thus, the first value is the logical test (D1>5). The second value is what to return if true. Here the text is included in quotations to tell Excel that this is the text it needs to return. The third value is what value to return if false (i.e., not greater than 5). In this case "N" was used (Fig. 6). The "IF" function can be used on departing instructions to tell Excel to only include the instruction if certain specified criteria is met. This application of the "IF" function will be illustrated in the examples that follow.

Values to be included in any given formula can also be pulled in from another sheet within the same file or from another Excel file altogether. This utility allows the user to have one sheet for which data is entered and a second sheet for which the formula is applied or reported out. It is important to understand some of these basic concepts in the application of Excel to equine practice.

2. Client Communications

One of the most useful applications of Excel in equine practice is in the construct of departing instructions when a series of dates and times must be given for the application of future actions or treatments. In the author's practice, a client letter has been developed that gives the owner of a pregnant mare the dates for which vaccination and subsequent reproductive exams will be required. This sheet has only 2 cells for which data needs to be entered, one is for the mare's name and the other is for the ovulation date of the mare. From these 2 fields, the dates for vaccination and subsequent reproductive exams will automatically be calculated (see Fig. 9). The subsequent dates are incorporated within a cell that aligns within the text of the letter. Each calculated date then has a formula that is written to add "x" number of days to the ovulation date. Thus, if the user desires to return a date of 25 days after ovulation, where the ovulation date has been entered into cell C14, the formula would be written "=C14+25" (Fig. 10). A good practice is to have a separate sheet for which to enter the input data (Fig. 7). This prevents the end user from being tempted to make adjustments or changes to the master template. It also gives the ability to hide

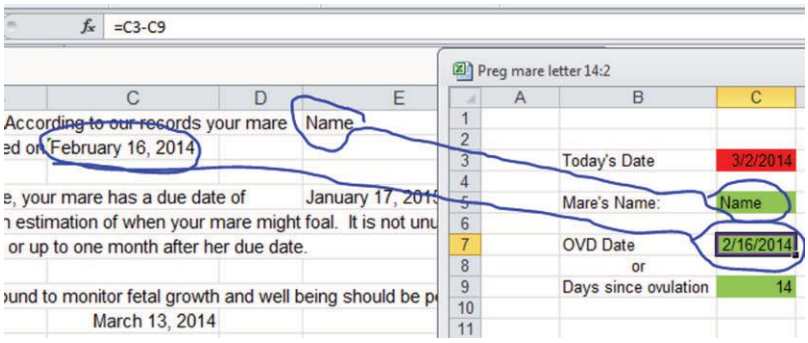


Fig. 7. Illustration of separate data entry and reporting sheets.

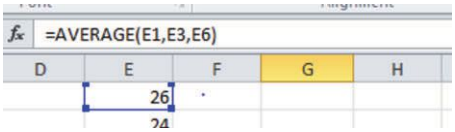


Fig. 8. Example of "AVERAGE" function.

variables that the user does not wish to print out on the client information sheet.

A similar process can be employed for other departing instructions that require set times and dates for future events. One additional example is for providing a schedule for ophthalmic treatments. Subpalpebral lavage systems have become increasingly popular in private practice. These cases often rely on the owner delivering the medication at prescribed times. It can be challenging for owners to keep track of the medication schedule because of the number of medications and the high frequency for which they need to be administered. By using these same principles of building off a separate data entry sheet and output sheet and the formulas that Excel affords, it is possible to build an ophthalmic treatment schedule that is easy for owners to understand. Note, that the "IF" function discussed previously is used here so that the doses are filled in only when the specified criteria is met. If that criteria is not met, then "-" is returned. Figure 11 shows the separate input and reporting sheets.

A useful tip when building and using these sheets is to paint input cells green. Correspondingly, cells that have formulas in them that are not to be overwritten are painted red.

**Please note that the dosing schedules on these examples are for illustration purposes only and do not constitute any recommendation for actual treatment.

3. Dosage Calculating

The use of Microsoft Excel for client communication is only one of the many ways this program can be utilized in equine practice. It is also useful for dosage calculating or calculating fluid administration rates. Equine practitioners are familiar with doing these types of calculations on a daily basis. Excel

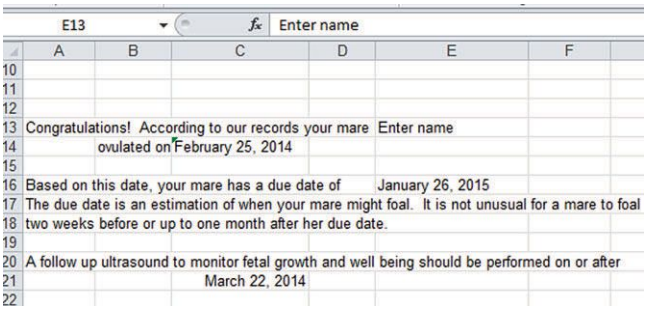


Fig. 9. Sample pregnant mare entry fields.

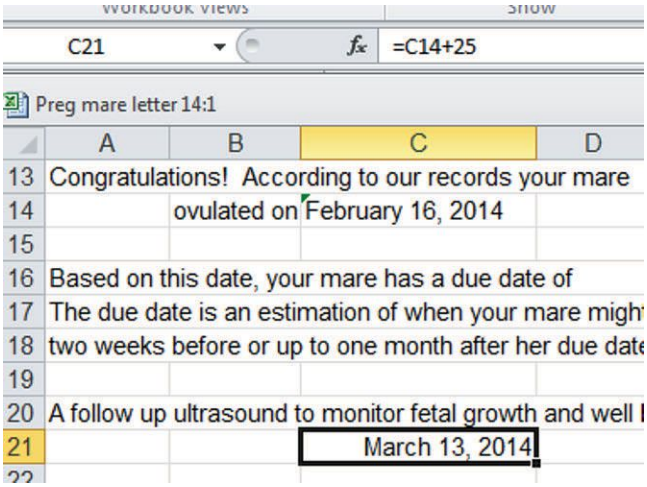


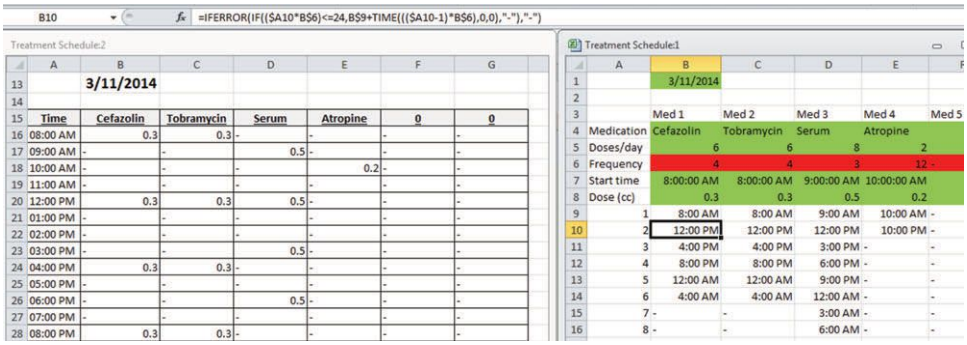
Fig. 10. Example formula for computation of future date.

makes it possible to build a customized calculator to do these calculations more rapidly and the ability to transmit these calculations to other clinicians or staff involved in the case. Having these basic calculators standardized for a given practice helps to ensure continuity of care. The accompanying image (Fig. 15) demonstrates a relatively simple calculator of anesthesia induction doses. The incorporation of the IF function returns a specified value only if the specified conditions are met. In this case, if the text in the Y/N column is marked "Y" in the corresponding row for that medication, then the administration volume will be calculated. If it is marked anything but "Y," an administration dosage will not be calculated. Similar sheets can be developed and customized for any number of protocols or fluid administration plans.

4. Scheduling

Because of the largely mobile nature of most equine practitioners, it can be a challenge to have a schedule that multiple users can simultaneously interact with and access from any location at any given time. Excel has many functions that allow it to be readily adaptable for calendars and scheduling (Fig. 14). The ability for the practitioner to build their own schedule template gives limitless possibilities as to how the schedule is configured. The author practices in a multi-doctor ambulatory and in-clinic practice. The coordination of schedules of multiple doctors is crucial to smooth operation of the practice. One method of accomplishing this goal is to develop a template in Excel and then upload it to Microsoft's Onedrive site (https://onedrive.live.com). Calendar templates can also be downloaded from: https://store.office.live.com/templates. A scheduling calendar is fairly easy to create in Excel by mimicking the layout of most any appointment scheduler book.

Utilization of the Onedrive website requires a Microsoft ID, which is provided free of charge. Simply



Treatment Schedule:2

	A	B	C	D	E	F	G
13		3/11/2014					
14							
15	Time	Cefazolin	Tobramycin	Serum	Atropine	0	0
16	08:00 AM	0.3	0.3	-	-	-	-
17	09:00 AM	-	-	0.5	-	-	-
18	10:00 AM	-	-	-	0.2	-	-
19	11:00 AM	-	-	-	-	-	-
20	12:00 PM	0.3	0.3	0.5	-	-	-
21	01:00 PM	-	-	-	-	-	-
22	02:00 PM	-	-	-	-	-	-
23	03:00 PM	-	-	0.5	-	-	-
24	04:00 PM	0.3	0.3	-	-	-	-
25	05:00 PM	-	-	-	-	-	-
26	06:00 PM	-	-	0.5	-	-	-
27	07:00 PM	-	-	-	-	-	-
28	08:00 PM	0.3	0.3	-	-	-	-

Treatment Schedule:1

	A	B	C	D	E
1		3/11/2014			
2					
3		Med 1	Med 2	Med 3	Med 4
4	Medication	Cefazolin	Tobramycin	Serum	Atropine
5	Doses/day	6	6	8	2
6	Frequency	4	4	3	12
7	Start time	8:00:00 AM	8:00:00 AM	9:00:00 AM	10:00:00 AM
8	Dose (cc)	0.3	0.3	0.5	0.2
9	1	8:00 AM	8:00 AM	9:00 AM	10:00 AM
10	2	12:00 PM	12:00 PM	12:00 PM	10:00 PM
11	3	4:00 PM	4:00 PM	3:00 PM	-
12	4	8:00 PM	8:00 PM	6:00 PM	-
13	5	12:00 AM	12:00 AM	9:00 PM	-
14	6	4:00 AM	4:00 AM	12:00 AM	-
15	7	-	-	3:00 AM	-
16	8	-	-	6:00 AM	-

Fig. 11. Separate input and reporting sheets.

upload the schedule template that the user desires to use to this site. The file can be opened and edited through this site alone, and does not require separate Excel software to edit or view the file. The online version of Excel lacks some of the more advanced features of the full version. This format allows multiple users to view and edit the file simultaneously. By this means, multiple reception staff are able to schedule appointments for multiple doctors. The doctors in the field are likewise able to view and access the site real time on a laptop, tablet, or smartphone (an internet connection is required). With this format, appointments can be highlighted or coded by color for appointments that require special circumstances, such as radiograph or ultrasound equipment, new client, routine care, breeding work, etc. The flexibility in application is limited only by the creativity of the user.

5. Data and Financial Analysis

A primary utility of Excel is its ability to analyze and process large amounts of data with very little effort on the part of the user. The analyzed data can then be configured to automatically represent the results in a graphical format which, in the author's experience, is the best way to analyze, share and discuss the key performance metrics of the business. This works best if one sheet is designated for data entry only. Most veterinary software has the ability to export raw data in a CSV or TXT file. These files can then be opened by Excel and the data imported into Excel format. Alternatively, the key data that one wants to track can be manually entered into the data entry sheet. Once the raw data is brought into Excel, a second sheet can be built from which calculations can be made referencing the imported data. Once the required calculations are

Congratulations! According to our records your mare Name ovulated on February 16, 2014

Based on this date, your mare has a due date of January 17, 2015
The due date is an estimation of when your mare might foal. It is not unusual for a mare to foal two weeks before or up to one month after her due date.

A follow up ultrasound to monitor fetal growth and well being should be performed on or after March 13, 2014

The gender (filly or colt) of the fetus can be determined with ultrasound between April 17, 2014 and April 27, 2014

Reconfirmation of pregnancy with routine rectal palpation is recommended on or after May 17, 2014

In order to protect your new investment, vaccination of your mare as described below is strongly recommended. It is best if these vaccines are administered within two weeks of the dates listed.

Rhino	May 17, 2014
Rhino	July 16, 2014
Rhino	September 14, 2014
Rhino	November 13, 2014
WNV+EWT	December 13, 2014
Influenza & Rhino	December 13, 2014

Please continue to deworm your mare per your normal deworming schedule. We do not recommend the use of "Quest" in pregnant mares. All of the other oral dewormers are safe for use in a pregnant mare, when dosed appropriately.

Please feel free to call if you have any further questions or concerns.

WMVS

Fig. 12. Sample pregnant mare letter.

2/24/2014 All doses are in units of cc

Time	Cefazolin	Atropine	Serum	Tobramycin	0	0
08:00 AM	0.3	-	-	0.3	-	-
09:00 AM	-	-	0.4	-	-	-
10:00 AM	-	0.2	-	-	-	-
11:00 AM	-	-	0.4	-	-	-
12:00 PM	0.3	-	-	0.3	-	-
01:00 PM	-	-	0.4	-	-	-
02:00 PM	-	-	-	-	-	-
03:00 PM	-	-	0.4	-	-	-
04:00 PM	0.3	-	-	0.3	-	-
05:00 PM	-	-	0.4	-	-	-
06:00 PM	-	-	-	-	-	-
07:00 PM	-	-	0.4	-	-	-
08:00 PM	0.3	-	-	0.3	-	-
09:00 PM	-	-	0.4	-	-	-
10:00 PM	-	0.2	-	-	-	-
11:00 PM	-	-	0.4	-	-	-
12:00 AM	0.3	-	-	0.3	-	-
01:00 AM	-	-	0.4	-	-	-
02:00 AM	-	-	-	-	-	-
03:00 AM	-	-	0.4	-	-	-
04:00 AM	0.3	-	-	0.3	-	-
05:00 AM	-	-	0.4	-	-	-
06:00 AM	-	-	-	-	-	-
07:00 AM	-	-	0.4	-	-	-

Fig. 13. Sample dosing schedule.

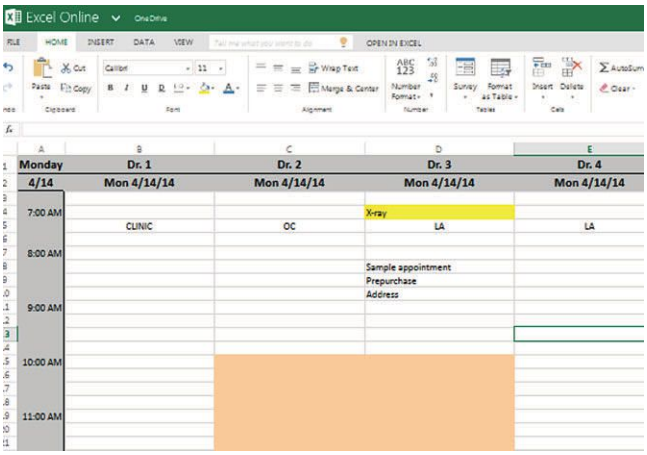


Fig. 14. Example schedule.

made, a third sheet can be used to draw graphs of the calculations. The ability to easily chart data is crucial to rapidly identifying and understanding the trends that are happening within the business. (Please note illustrated examples contain completely random data.) Below is an example of what charts can be generated from the input data (Fig. 17). Representation of practice performance into a graphical format, as demonstrated in the accompanying figure (Fig. 16), is an outstanding way to quickly and easily share, analyze, and discuss practice performance with multiple partners.

Developing these charts within Excel requires several steps, which are beyond the scope of this discussion. A future talk could be dedicated to the topic of building business metric charts in Excel. However, the same principles and calculations apply here as they do to the other examples discussed previously.

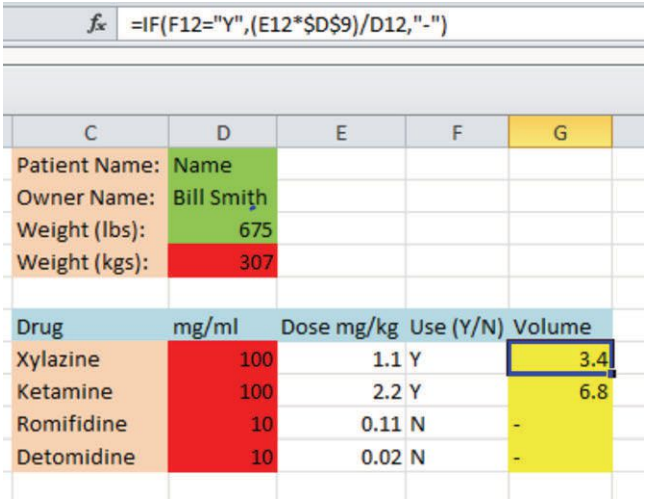


Fig. 15. Example dosage calculator.

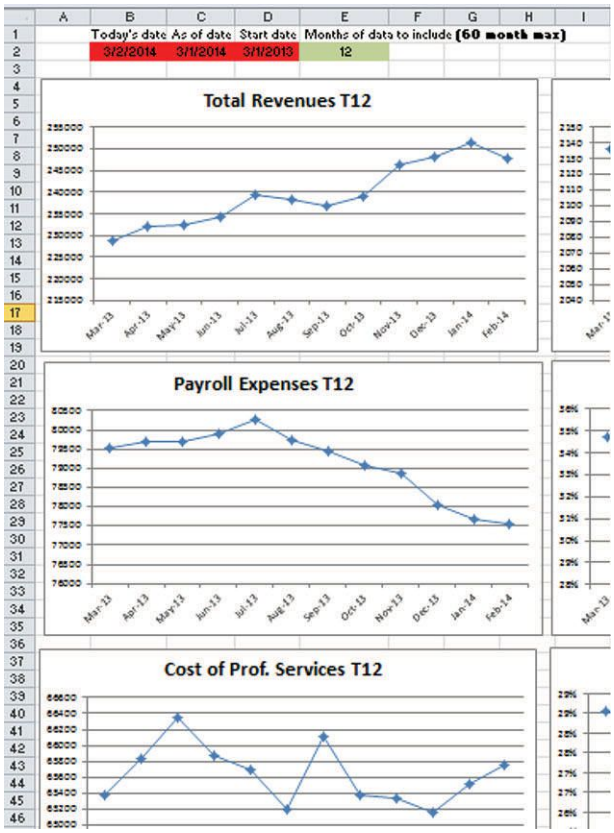


Fig. 16. Example financial metrics charts.

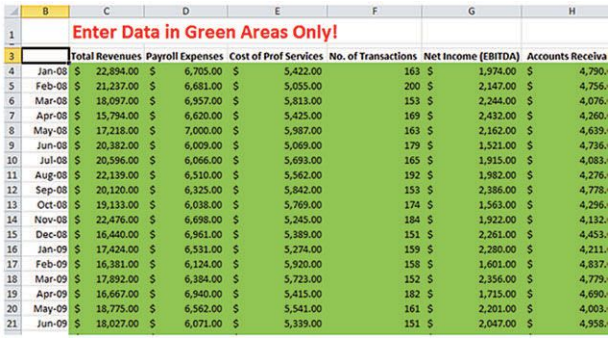


Fig. 17. Data entry sheet for financial metrics.

Excel is an extremely customizable and adaptable program. Its widespread use and acceptance makes it a great vehicle to share information with clients and colleagues. It is one of many tools equine practitioners can utilize to increase their efficiency and accuracy of practice, while simultaneously improving the client experience and ultimately patient care. The creativity of its uses in equine practice is limited only by the end user.

Acknowledgments

Conflict of Interest

The Author declares no conflicts of interest.

Equine Immunology and Vaccine Strategies

Elizabeth Davis, DVM, PhD, DACVIM

Equine immunology is an expansive field of study. Vaccines are designed to induce antigen specific immunity that provides host protection at the time of pathogen challenge. Although a great deal of knowledge exists in this field, there are areas that require further investigation. Many vaccines exist that provide strong protection while some vaccines are unsuccessful for provision of protection in all individuals or provide only short term immunity. Additional work is required to better understand vaccinology for specific diseases such as equine herpes myelitis that will eventually lead to protection from this serious infectious disease. Author's address: Kansas State University, 1800 Denison Ave., Manhattan, KS 66506; e-mail: edavis@vet.k-state.edu. © 2014 AAEP.

1. Introduction

The primary goal of vaccination is the induction of long-lasting and protective immunity. During the initial stages of pathogen challenge, innate immune responses are induced that may provide nonspecific clearance of the challenge. However, in addition to providing immediate protection, innate immune mechanisms also initiate adaptive immunity in the event that memory responses are required for long-term immune protection. The fundamental goals of a successful vaccine program are to safely induce immunity that provides antigen specific protection that is efficacious and long lasting. Although vaccination provides an immunologic advantage at the time of challenge, vaccine protocols should be appropriately designed in combination with effective environmental control strategies that include mosquito control programs.

2. Innate Immunity

Effective immunity requires interplay between host immune mechanisms and pathogen challenge. The initial element of host protection from such a challenge involves innate immune mechanisms. Disruption or penetration of superficial epithelium and/or mucosal barriers will result in pathogen exposure to host immunity. If immune clearance is ineffective, the potential for disease development exists. Beneath the superficial epithelium lies components of the innate immune system, which provides the host with a well-orchestrated collection of immune mechanisms that will target non-self constituents that are typically encountered during early and acute stages of pathogen challenge. If the challenge is low-grade, innate mechanisms may completely eliminate the challenge. Among the constituents of innate immunity are leukocytes that express surface proteins capable of directly signaling additional cells or secreting protein factors that will arm the host to be protected during the course of pathogen interaction.

The presence of invading pathogen is initially detected by sentinel cells that include macrophages, dendritic cells (DCs), and mast cells. These sentinel antigen presenting cells are highly capable of recognizing invading pathogen due to the presence of surface protein receptors. These receptors bind with pathogen via their pathogen associated molec-

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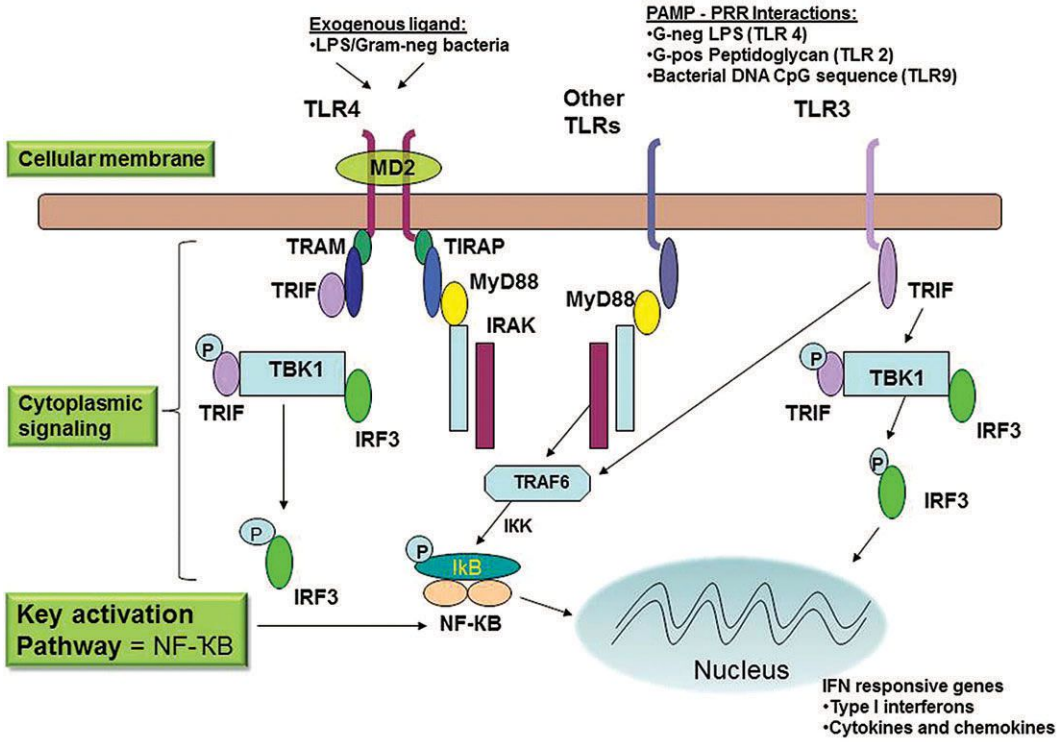

Fig. 1. Pathogen associated molecular patterns such as LPS interact with host-expressed pattern recognition receptors, which can initiate cellular signaling that results in inflammatory mediator expression.

ular patterns (PAMPs). Pathogen associated molecular patterns are expressed by a variety of pathogens such as bacteria, fungi, and viruses. Since pathogens mutate at a rapid rate, the receptors that can identify invading pathogens do not specifically identify individual microbes but rather can identify classes of pathogens that result in a global host response. Receptors on sentinel cells are referred to as pattern recognition receptors (PRRs), which may be classified into four separate categories. Pattern recognition receptors may be secreted, free receptors located in the extracellular space and in general circulation; PRRs may be membrane bound and phagocytic in function; PRRs may also be membrane bound and responsible for cellular signaling; or they may be cytoplasmic in their location. A primary example of host PRR includes the toll like receptors. Examples of PAMPs include Gram negative lipopolysaccharide (LPS), Gram positive peptidoglycan, and acid fast bacterial glycolipids. These molecules are unique to classes of microbial pathogens and are not expressed by mammalian species, which therefore provides a mechanism for host identification of pathogen challenge. Binding of PAMP with PRR results in cellular activation to trigger inflammation and other innate immune pathways (Fig. 1).

3. Adaptive Immunity

Immunologic members of the adaptive (memory) immune response include antigen presenting cells, lymphocytes, and cytokine mediators. Collectively, these constituents provide effective clearance of pathogen challenge conferring immunologic specificity and memory. Antigen presenting cells include dendritic cells, macrophages, and B lymphocytes. Dendritic cells are the most efficient antigen presenting cells, as they are the only cells that can present antigen and effectively activate naïve T lymphocytes (Fig. 2). Immature DCs are located throughout the host, and they are specifically designed to capture foreign antigen. Once stimulated by foreign antigen, DCs mature and become highly effective with regard to antigen presentation to host T cells. In order to effectively express antigen, DCs must express surface major histocompatibility molecules (MHC) class II molecules, which are essential for propagation of this process.

Dendritic cells ingest foreign antigens, process antigen in an efficient manner, and present peptides within MHC II to T cells, via the T cell receptor. Macrophages are an additional antigen presenting cell, but because they have the capacity to destroy ingested material, they are less efficient with regard to antigen presentation when compared with DCs. B lymphocytes also have antigen presenting capabilities and are highly effective during a secondary immune response.

Dendritic cells are divided into two categories, classified as myeloid and plasmacytoid. Plasmacytoid dendritic cells are particularly important for immunity and are major producers of type I inter-

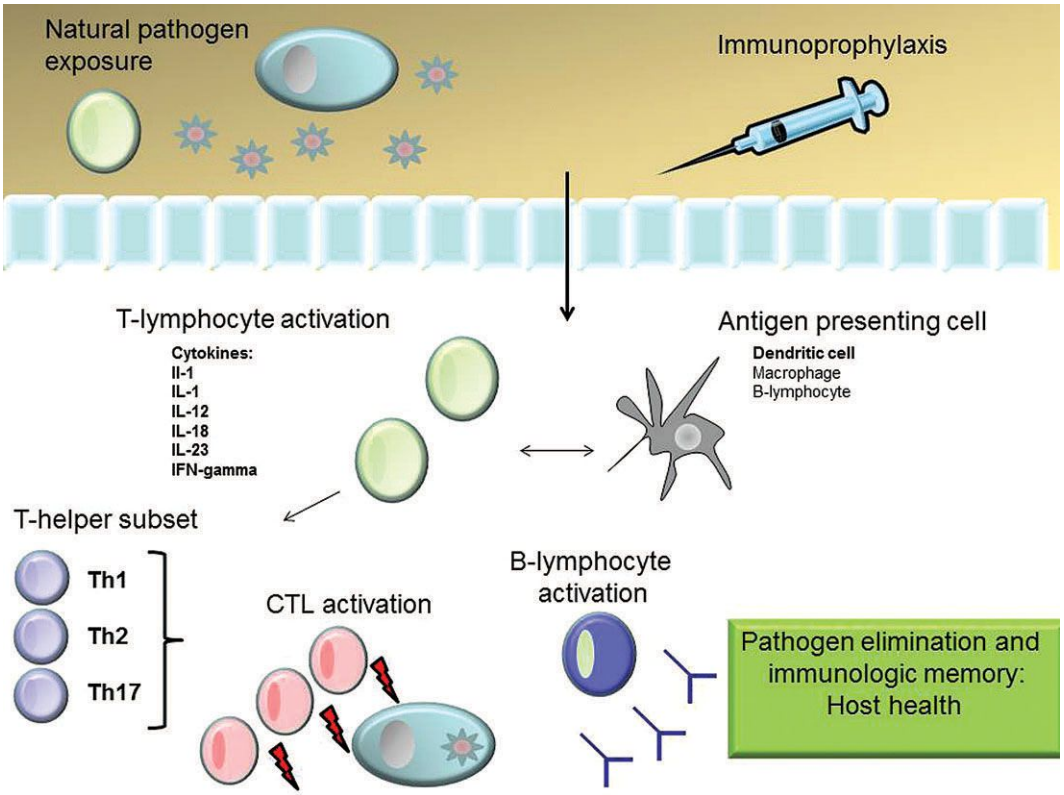

Fig. 2. Adaptive immunity involves the interaction of antigen presenting cells with T-lymphocytes in an antigen specific manner. This interaction results in clonal expansion of antigen specific lymphocyte populations capable of orchestrating pathogen clearance mechanisms.

ferons, such as interferon alpha and beta, which demonstrate pronounced antiviral properties.

Lymphocytes are found within lymphoid organs, in circulation, and scattered under mucosal surfaces. Although they morphologically appear similar, they are characterized by their cell surface molecules and their behavior. The pattern of cell surface molecules defines their specific immunophenotype. Through the process of immunophenotyping, it is possible to identify many lymphocyte subpopulations.

Helper T cells possess antigen receptors (TCRs) that consist of two peptide chains. These receptors have an antigen binding groove that can bind to antigenic peptides linked to major histocompatibility complex molecules on antigen presenting cells. The antigen binding chains of the TCR are linked to a complex signal transducing component called CD3. Each TCR is associated with either CD4 or CD8. CD4 binds to MHC class II molecules on the antigen presenting cell. CD8 binds to MHC class I molecules expressed on all nucleated cells. In order to respond to antigens, T cells must bind to antigenic peptides within the MHC protein cleft. In addition, they must also receive co-stimulation from cytokines and other co-stimulatory molecules. The signals from an APC to a T cell are communicated through an immunological synapse. There are three major types of helper T cell subpopulations (Fig. 2). Th1 cells are stimulated by interleukin 12 and secrete interleukin (IL)-2 and interferon gamma. This response classically leads to a cell-mediated immune response. Th2 cells are stimulated by IL-1 and secrete IL-1, IL-13, and IL-10. This signaling pathway generally leads to antibody production. Th17 cell development is stimulated by IL-6, transforming growth factor beta and IL-23. This cell population secretes IL-17 and promotes neutrophilic inflammation. Cytotoxic T lymphocytes are activated CD8+ expressing T lymphocytes that are essential for effective clearance of virally infected or altered self cells. Cytotoxic T cells are an important component of cell-mediated immunity.

The requirements for generating protective immunity vary with the nature of the infecting organism. Many effective vaccines work by inducing antibodies targeted toward specific pathogens. For many pathogens, including extracellular organisms, antibodies can provide protective immunity. This is not the case for all pathogens, however, such as intracellular viruses, which may require additional cell-mediated immunity that is provided by activated CD8+ T lymphocytes.

Effective protective immunity against microorganisms requires the presence of preexisting antibody at the time of infection. Antibody proteins

will aid in preventing damage from the presence of the organism or prevent infection altogether. An example of protection involves tetanus vaccination, which provides antibodies that bind even low levels of exotoxin liberated at that time of challenge. An added mechanism by which antibody proteins provide protection at the time of challenge includes the production of neutralizing antibodies. Neutralizing antibodies are capable of preventing infection by viral pathogens.

4. Host Specific Factors That Impact Immunity

Additional factors for consideration include host immunity at the time of vaccination. Horses that have undergone a stressful event such as long distance transport, exertion,[1] or have received immunosuppressive therapy[2] should not be vaccinated during times of immune suppression. It should be noted that vaccine formulation should be considered when characterizing the impact of physiologic stress on the equine patient. For instance, although exertional exercise may impede an optimal immune response, mucosal immunity appears effective in response to intranasal influenza vaccination in ponies despite high intensity exercise.[3] Specific therapeutic medications that should be taken into consideration at the time of vaccination include both corticosteroids and non-steroidal anti-inflammatory (NSAID) therapy. Previous work has demonstrated that routine doses of immunosuppressive corticosteroid therapy results in immunosuppression when used at the time of vaccine administration resulting in suppressed IgG expression.[4] Even though immunosuppressed horses are capable of producing antibody in response to vaccination, the levels of protective IgG antibody are markedly reduced when compared to IgG production in healthy horses that had not received immunosuppressive corticosteroid therapy.[4] Therefore, corticosteroid therapy is not recommended at the time of vaccination in horses. More recent data has demonstrated that the administration of an NSAID at the time of vaccination and continued for a total of 3 days can attenuate cellular and antibody responses.[5] Collectively, administration of medications that have the intended purpose of attenuation of inflammation should be used with caution if used at the time of vaccination.

Attention to health programs for young foals should be specifically designed for at risk individuals. Foals that ingest adequate colostrum will be protected from a variety of pathogen challenges during the first four to six months of life. From an immunologic standpoint, the optimal time to initiate a preventative health vaccine program is when maternal antibodies have waned, likely as the foal approaches 1 year of age.[6] From a practical standpoint, however, this is not possible, so the goal of management is to wait until maternal antibodies have fallen to a sufficient level that endogenous immunity is capable of responding with a pronounced memory response that will provide specific and long lasting protection.

We recently evaluated healthy foals that received adequate colostrum for their ability to respond to vaccination beginning at 3 months of age.[7] Measures of immunity were characterized by IgG antibody expression and CD4+ and CD8+ cellular cytokine production. When compared with foals that were initially vaccinated at 6 months of age, the foals vaccinated at 3 months of age demonstrated a similar immune profile for cellular cytokine expression including IL-4 and interferon gamma. When immune responses were examined at 11.5 months of age, similar cellular expression of interferon gamma was observed in CD4+ and CD8+ lymphocytes in response to Eastern equine encephalomyelitis (EEE), Western equine encephalomyelitis (WEE), West Nile virus (WNV), influenza, and equine herpesvirus (EHV-1/4) indicating that the foals vaccinated at 3 months of age responded in a similar manner to foals vaccinated at 6 months of age ($P < 0.05$). In addition, the foals vaccinated at 3 months of age demonstrated a greater than fourfold increase in IgG expression when receiving a booster vaccine against influenza, EHV-1/4, and tetanus administered at 11 months of age. These data provide evidence that when healthy foals are initially vaccinated at 3 months of age, they are capable of demonstrating an antigen specific immune response.

Age-associated changes in immunity have been investigated in a variety of mammalian species.[8–10] Collectively, these investigations have demonstrated that there is an overall age-associated reduction in circulating T lymphocytes. Similarly, the concentration of circulating lymphocytes is reduced in aged horses.[11–13] Additional age-related changes involve lymphocyte subset concentrations. Consistent with a reduction in total lymphocyte numbers, there is a reduction in the total number of CD4+, CD8+, and B lymphocytes in circulation. However, older horses display an increased percentage of CD4+ lymphocytes.[14] Subsequently, the CD4:CD8 ratio is altered in older horses resulting in an apparent proinflammatory immune bias similar to what has been reported to occur in older people.[10]

An important characterization of lymphocyte function is the assessment of cellular proliferation induced by cytokine, mitogen, or antigen specific response in vitro. Similar to elderly humans, an impairment of lymphocyte proliferation has been observed in horses.[14,15] Regardless of the methods used to enhance this response, such as with IL-2 supplementation or changes in CD25 (IL-2 receptor) expression, cellular proliferation is not restored to adult-age responses. The conclusion from this finding is that similar to geriatric humans, horses have reduced T lymphocyte proliferation that may contribute to impaired immune function, in particular, with regard to antigen specific responses at the time of vaccination.

Since the number of geriatric horses is increasing, there is an increased need for veterinary care of this population. Many older horses continue with competitive activities into their late 20s, which may increase their risk for exposure to infectious disease. An important management program for elderly horses is to maintain appropriate and specific vaccine protocols for this at-risk population. Although there are no current recommendations that suggest older horses require more frequent vaccination than their younger stable mates, there is evidence that they may be at greater risk for disease even in the face of an appropriate preventative health program. Specifically, Adams and colleagues have demonstrates that despite having preexisting immunity to influenza, geriatric horses may still be susceptible to viral infection.[16] Therefore, the goal for equine veterinary clinicians should be to identify the specific individual or group of individuals that require vaccination. Core vaccination for potential lethal disease and those with significant zoonotic potential should be included in the protocol to include: EEE, WEE, WNV, tetanus, and rabies. Risk based decisions should also include the age and use of the horse. Respiratory pathogens such as influenza and EHV-1/4 should be included in most vaccine programs. Horses that are at low risk of other respiratory pathogens are best managed with effective environmental and biosecurity programs that minimize the potential for disease exposure. Horses at high risk for exposure should have a program that also includes additional risk-based vaccines in an effort to minimize the risk of serious infectious disease.

AAEP Vaccine Guidelines
http://www.aaep.org/info/guidelines-48

Core vaccines

EEE/WEE/WNV
Tetanus
Rabies

Risk based vaccines

Anthrax
Botulism
Equine herpesvirus (EHV-1 and 4)
Equine viral arteritis
Equine influenza
Potomac horse fever (PHF)
Rotavirus/rotaviral diarrhea
Streptococcus equi subsp. *equi* (Strangles)

Young Horse Vaccination

- Use the AAEP core vaccine protocol beginning at 4 to 6 months of age.
- Use an initial three-dose series to ensure an effective memory (secondary) immune response has been induced. This is particularly true for inactivated vaccines.
  - Administer the first two doses approximately 30 days apart.
  - Administer the third dose approximately 60 to 90 days later.
  - If the series was initiated early in the year, administer a booster vaccine at approximately 12 months of age followed by product appropriate booster vaccines.
- If the risk for disease is high, such as EEE or WNV, consider starting the series at 3 months of age to complete a three-dose series by the time infectious disease challenge is present.
  - Administer booster vaccine at 11 to 12 months of age to substantially enhance and maintain the memory immune responsiveness.

Mature Horse Vaccination

- Use AAEP core vaccine protocols for EEE, WEE, WNV, tetanus and rabies vaccination.
- If not previously vaccinated administer a second booster vaccine approximately 30 days later.
- Administer risk-based vaccines as indicated based on use and potential for pathogen exposure; consider geography and horse population for such decisions.

Elderly Horse Vaccination

- Use appropriate biosecurity protocols during infectious disease outbreaks.
- Use AAEP core vaccine protocols for EEE, WEE, WNV, tetanus, and rabies vaccination.
- Administer risk-based vaccines as indicated based on use and potential for pathogen exposure; consider geography and horse population for such decisions.
- Maintain effective booster protocols in the elderly horse population to maintain a sufficient memory immune response.
- Recognize that lymphocyte populations are reduced and altered in their expression in elderly horses; biosecurity protocols should be carefully implemented to aid in protection of this population from infectious disease.
- Identify and manage geriatric horses in an ideal preventative and health maintenance program.
  - Pituitary pars intermedia dysfunction (PPID) affected horses may require pergolide therapy to maximize overall health that includes immune function.

5. Pathogens that Provide Additional Preventative Health Challenges

Streptococcus equi subspecies *equi* (Strangles)

Vaccination for the protection of strangles is recommended in high risk individuals, such as those in-

troduced to a farm with endemic disease or a recent outbreak. Two types of *Streptococcus equi* subsp. *equi* vaccines are currently commercially available in the United States: an inactivated subunit vaccine administered intramuscularly and a modified live vaccine (MLV) administered by the intranasal route. Specific information regarding disease pathogenesis and control of an outbreak were reported in the 2005 American College of Veterinary Internal Medicine (ACVIM) consensus statement: http://onlinelibrary.wiley.com/doi/10.1111/j.1939–1676.2005.tb02671.x/pdf.

Although vaccination appears to reduce morbidity, vaccination is not without risk for adverse event. For this reason, when determining the indications to vaccinate against strangles, consideration should be made to vaccinate horses specifically at high risk for exposure to this bacterium. A potential adverse event with use of the MLV product is for inadvertent localized abscess formation. If administering concurrently with other vaccines, care should be implemented to complete administration of all other vaccines and once complete then prepare and handle the modified live product. Using such precautions will aid in avoiding inadvertent intramuscular injection of live bacterial organisms. An additional potential complication associated with use of any strangles vaccine is the development of purpura hemorrhagica, a generalized immune-mediated vasculitis that can occur following recovery from strangles infection or following vaccination, particularly if vaccination is employed during a course of disease outbreak. For these reasons, it is important for the equine clinician to carefully consider vaccine risks and consult the ACVIM 2005 consensus statement to ensure the risk for adverse event is minimized.

Horses not previously vaccinated against strangles that are receiving the MLV should receive an initial intranasal series of two vaccines approximately 3 to 4 weeks apart. Booster vaccination is recommended at 6 to 12 month intervals based on risk assessment and manufacturer instructions. Adults receiving the inactivated vaccine should be vaccinated with an initial intramuscular series of two vaccines with an approximate 4 week interval and a subsequent booster vaccine at 6 to 12 month intervals based on risk assessment and manufacturer instructions. Foals in high-risk situations should be vaccinated with the inactivated vaccine beginning at 4 to 6 months of age with an initial series of three vaccines administered at 4 to 6 week intervals. Booster vaccines should be administered at 6 to 12 month intervals. The modified live intranasal vaccine has been utilized in foals and may be administered with an initial two-dose series, starting at 6 to 9 months of age with a 3 week interval between doses. Recent evidence has suggested that the risk of adverse events associated with vaccine administration may be increased when the MLV is administered to foals less than a year of life.[17] In this reported investigation, six-month-old pony foals were vaccinated and developed clinical signs of strangles (4/4); in one foal, disease progression occurred, which resulted in mesenteric abscess formation that was diagnosed at post mortem examination, while the other three recovered without clinical intervention. When administering this vaccine in horses <1 year of age, monitoring for adverse event should be implemented.

Strangles Vaccination Considerations

- Subunit M protein vaccines.
- Modified live intranasal vaccine.
- Vaccinate in high-risk situations according to AAEP risk-based vaccine recommendations.
- Previously infected and recovered horses should be carefully considered for vaccination based on the ACVIM *Strep equi* consensus statement.
- Risk for vaccine complication and development of immune-mediated disease is increased if vaccine is administered in a disease outbreak situation.
- The risk of vaccine-associated adverse events is increased when the MLV product is administered to young foals.

West Nile Virus

Protection against WNV-induced encephalomyelitis, similar to other vector-mediated encephalitides requires strict attention to control of insects as well as an effective vaccination program. Four vaccines are currently USDA licensed for use in horses to aid in the protection against WNV. Two inactivated vaccines, a nonreplicating canary pox-vectored vaccine and an inactivated flavivirus chimeric vaccine.

Administration of inactivated WNV vaccines, consistent with label instructions, involves administration of an intramuscular dose at 3 to 6 weeks apart followed by a 12-month revaccination interval. Previous evidence has demonstrated that the clinical benefit from this class of vaccine is directly associated with protection from disease.[18] Immunologic efficacy has also been demonstrated through induction of cell-mediated immunity and antigen specific Ig subclass antibody expression.[19]

The inactivated flavivirus chimera vaccine provides host protection by expressing WNV antigens that induce protective immunity. The vaccine antigens are expressed by a yellow fever vector that safely and effectively provides antigen exposure to the host. Label instructions include a primary series of two doses administered approximately 3 to 4 weeks apart followed by an annual booster. This vaccine is approved for the aid in reduction of disease, encephalitis, and viremia.

The recombinant canary pox-vectored vaccine protective antigens are expressed in a canary pox vector, which does not replicate in the horse and contains an adjuvant. Label recommendations include intramuscular injection with a primary series

including a 4- to 6-week interval followed by a 12-month revaccination period.

An adult horse that has previously been vaccinated for WNV should receive an annual vaccine in the spring, prior to the onset of the mosquito vector season. An adult horse that is unvaccinated for WNV (or unknown vaccine status) should receive a primary series of two vaccines administered with a 3- to 6-week interval followed by annual revaccination. It should be noted that although there is limited data evaluating the safety[20] and efficacy of vaccinating pregnant broodmares for WNV, due to the inherent risk of disease it is commonplace for veterinarians to vaccinate pregnant mares. In order to induce an optimal and effective immune response, a primary vaccine series should be completed before breeding whenever possible. A booster vaccine is recommended approximately 4 to 6 weeks before expected foaling to provide WNV-specific IgG among colostral constituents.

A previous investigation demonstrated that foals, which received colostrum from WNV vaccinated mares, were capable of producing WNV specific antibodies when vaccinated at 180 days of age.[21] Current recommendations include a three-dose series for WNV vaccination to begin at 4 to 6 months of age. Booster vaccination should be administered 4 to 6 weeks after the initial vaccine with a third vaccine administered at approximately 10 to 12 months of age, prior to the onset of vector insect season the following spring. Foals that are delivered by unvaccinated mares (or those with an unknown vaccine status) should receive WNV vaccines at 3 to 4 months of age with the goal to complete the three vaccine series prior to the peak mosquito season. The duration between the first and second dose should be approximately 30 days with approximately 60 days between the second and third dose. If vaccination is taking place during the mosquito vector season, the entire protocol should be completed closer to an 8 week interval to provide optimal immunity at the time of peak mosquito season.

A recent investigation evaluated the humoral immune response to WNV vaccination with regard to serum neutralizing (SN) antibody titers generated when WNV vaccines administered as a monovalent injection or as a component of a multivalent WNV containing vaccine.[22] Vaccines that were evaluated included three commercially available WNV containing multivalent vaccines compared with their counterpart multivalent vaccines without WNV. Horses receiving a non-WNV containing multivalent vaccine were given a second injection containing the manufacturer's monovalent WNV vaccine at the same time as the multivalent product so that all groups received the same vaccine factions (EEE, WEE, tetanus, influenza, EHV, and WNV). This investigation revealed that when WNV was a component of a multivalent vaccine, WNV SN, titers were significantly diminished compared to titers generated when a monovalent WNV vaccine was administered ($P < 0.05$). These results were observed following both initial and booster vaccinations and were consistent across all three vaccine manufacturers. Many questions remain regarding all factors that contribute to a diminished antibody responses from the multivalent products, such as total antigen concentration for each vaccine and what influence other antigens such as EEE and tetanus have on immune responsiveness following vaccination. Overall, if an ideal and maximal antibody response is the goal, such as with an elderly horse or one that will encounter high risk conditions, results from this study clearly reveal that monovalent WNV vaccine will induce a stronger serologic response compared with a multivalent vaccine that contains WNV.

West Nile Virus Vaccination

- Four USDA licensed vaccines available
  - Inactivated (two manufacturers)
    - — Two- or three-dose initial series, depending on age at initial vaccination; Annual booster
  - Canarypox vectored vaccine
    - — Two- or three-dose initial series, depending on age at initial vaccination
    - — Annual booster
  - Inactivated flavivirus chimera vaccine, yellow fever vectored
    - — Two- or three-dose initial series, depending on age at initial vaccination
    - — Annual booster

Equine Herpesvirus

Equine herpesvirus-1 is associated with respiratory disease, abortion, and neurologic disease. EHV-4 is most commonly associated with respiratory disease. All horses on breeding, boarding, and training farms should be routinely vaccinated for rhinopneumonitis (respiratory form of EHV-1/4). Current commercial products include several inactivated vaccines and one modified live vaccine. Vaccination is recommended for use in pregnant mares to aid in the prevention of abortigenic EHV-1 and prevention of EHV-1/4 induced respiratory disease (rhinopneumonitis) in foals, weanlings, yearlings, young performance, and show horses that are included in the high risk group for viral exposure. There are two high antigen load inactivated vaccines labeled for protection from abortion in pregnant mares. None of the currently available vaccines has a label claim for prevention against equine herpes myelitis.

There are no vaccines that have proven efficacy for protection against the neurologic form of EHV-1 (aka, equine herpes myelitis, EHM). A comprehensive approach to manage a potential outbreak situation should be implemented when risk for outbreak exists. Outlines and recommendations have recently been reported in the 2009 ACVIM consen-

sus statement on the topic of EHV-1.[23] Current recommendations involve using appropriate biosecurity measures to minimize the potential for viral exposure. Several inactivated vaccines are commercially available, including those licensed for protection against respiratory disease alone, characterized by vaccines that contain a low antigen load and two that are licensed to provide protection against both respiratory disease and abortion, which contain a high-antigen load. Efficacy of the inactivated low-antigen load respiratory vaccine is variable, with some vaccines outperforming others. Performance of the inactivated high antigen load respiratory abortion vaccines is superior resulting in higher antibody responses and some evidence of cellular responses to vaccination. This factor may provide good reason to select the high antigen load respiratory/abortion vaccines when the slightly higher cost is not a decision factor. The most recent evidence to support the use of high antigen load vaccination for the prevention of equine herpes myelitis was reported by Maxwell and colleagues in an challenge study that aimed to determine whether an individual three-dose series of a high-antigen load vaccine[a] was effective for protecting against EHM in an established model setting.[24,25] Parameters of interest included ataxia scores, rectal temperature, and clinical scores. Control horses demonstrated more severe neurologic signs with 83% (5/6) horses affected compared to 16% (1/6) in the vaccinated group that had a minimum of two grade change in ataxia. Vaccinates had lower temperatures at times of biphasic fever spikes, significantly lower viremia and an overall clinical score 20% lower than control horses. Overall, vaccination with a high antigen load EHV-1 vaccine decreased clinical signs of infection following challenge with the neuropathic strain of EHV-1. While this investigation was a pilot study and the differences between treatment and control horses were not statistically significant, the results support subsequent investigation to establish the protective effects of vaccination in the prevention of EHM using a more robust study design.

Effective vaccination against EHV-1 should include an initial three-dose series, using an approximate 4 to 6 week interval between doses. When administering the initial series in foals, vaccination should be initiated at 4 to 6 months of age with a 4 to 6 week interval between the first two doses and the third dose being given at 10 to 12 months of age. Booster vaccines are recommended to maintain adequate immunity at approximately 6 month intervals. Pregnant broodmares should be vaccinated at 5, 7, and 9 months of gestation with a high antigen product labeled to protect against abortion. Many equine clinicians in high-risk breeding settings initiate the vaccination series at the third month of gestation. Effective colostral antibody protection is optimally induced with an inactivated EHV-1/4 vaccine booster administered 4 to 6 weeks before expected foaling. Horses maintained on breeding farms such as barren mares, stallions, and teaser stallions should be vaccinated at the beginning of the breeding season with 6 month booster vaccines administered. Adult horses should be vaccinated every 6 to 12 months, young horses (high risk, travel, commingling) will be optimally protected with booster vaccines every 6 months.

Equine Herpesvirus Vaccination Considerations

- Rhinopneumonitis (EHV-1/4) among young horses, performance horses, non-pregnant mares, initial series of three vaccines with boosters administered at 6-month intervals or according to risk of exposure.
  - Young horses <5 years of age.
  - Horses traveling to competitions and in contact with groups of horses.
  - Horses on breeding farms in contact with broodmares.
- Vaccine programs should be aimed at reducing the frequency of EHV associated disease despite recognizing that the majority of the equine population is latently infected with equine herpesviruses.
- Specific EHV-1 vaccine considerations for broodmares.
  - EHV-1 high antigen load vaccine labeled to prevent abortion administered at 3, 5, 7, 9 months of gestation.
  - EHV-1/4 rhinopneumonitis vaccine 4 to 6 weeks prior to expected foaling to enhance colostral antibody production
- No USDA licensed vaccine has a label for the prevention of equine herpes myelitis.
  - High antigen load vaccines and the MLV vaccine may induce a stronger serologic and cell mediated immunity (CMI) response that may provide the equine host with an immunologic advantage if the highly pathogenic EHV-1 is encountered.

Additional risk based vaccines:

Anthrax

Anthrax is a serious and rapidly fatal bacterial disease that has specific geographic regions associated with disease outbreak. Infections develop secondary to ingestion, inhalation, or wound contamination where soil contamination has occurred. It is important to recognize that infection is restricted to areas where alkaline soils exist, which favors bacterial survival. One USDA licensed vaccine is available for use in a variety of livestock species that includes horses. The vaccine is prepared from an attenuated, encapsulated variant of *Bacillus anthracis* strain B. Initial vaccination should include a series of two vaccines administered by the subcutaneous route followed by an annual booster while the horse is maintained in at risk areas. Vaccination is not recommended in broodmares; there is no infor-

mation regarding vaccination in horses less than a year of age. Adverse reactions have been reported in young and miniature horses, and caution should be used when use is considered in such instances.

Botulism

Botulism is a serious and frequently fatal infectious disease that develops secondary to liberation of toxin from *Clostridium botulinum*, most commonly *Cl. botulinum* type B and C. Four forms of disease occur in horses: wound botulism, shaker foal syndrome, forage poisoning, and equine grass sickness. An inactivated toxoid *Cl. botulinum* type B vaccine is licensed for use in horses in the United States. The indication for vaccination is for the prevention of shaker foal syndrome in horses less than 1 year of age. Pregnant broodmares should be vaccinated in at-risk geographic regions such as central Kentucky and the mid-Atlantic region. There is no evidence for cross protection against other *Cl. botulinum* toxins; therefore, vaccination against type B will not protect against other forms of disease.

The vaccine protocol involves an initial three-dose series in pregnant broodmares with a final vaccine booster administered approximately 4 weeks before the expected foaling date. This protocol will provide the mare the opportunity to maximize colostral antibody concentration at the time of parturition. Annual boosters are recommended at time of prefoaling vaccination. Foals in high risk regions should begin a vaccine series at 2 to 3 months of age. Foals born to unvaccinated mares or foals moving to high risk geographic locations should begin the three-dose vaccine series at 2–4 weeks of age with boosters at 4 week intervals. Other horses located in high risk regions should receive an initial three-dose series with annual booster vaccination. Horses that have suffered from *Cl. botulinum* type B infection that have recovered from disease should begin a three-dose vaccine series following recovery.

Equine Influenza

Equine influenza is a common upper respiratory infection resulting from viral colonization of the upper respiratory tract. Equine influenza is classified among the orthomyxoviruses, which are single stranded RNA viruses. Influenza viruses are classified among three types depending on surface and internal protein antigens, A, B, and C, yet only type A influenza has been reported to infect horses. Major viral antigens include neuraminidase and hemagglutinin. Two type A subtypes that are known to cause disease in horses include H7N7 and H3N8. Among viruses H3N8 is considered to be of greater pathogenicity than H7N7.

Influenza is most common in horses commingled under stressful conditions such as race or show training. Infection occurs via inhalation of viral particles, which are abundant in an environment containing infected horses, resulting from severe paroxysmal coughing that occurs in affected individuals. Once inhaled, the virus infects the respiratory ciliated epithelium, leading to loss of the mucociliary escalator, which substantially diminishes pathogen and particle clearance. Therefore, viral infection predisposes affected individuals to secondary bacterial colonization.

Clinical manifestation of disease includes a short incubation period of 1 to 3 days, high fever, depression, and paroxysmal coughing, which can be severe. Nasal discharge typically begins as a serous fluid, yet with disease progression and bacterial contamination mucopurulent discharge may be observed.

There are currently three influenza vaccines available:

1) *Inactivated (intramuscular administration)*: This vaccine contains many of the A2 stains of influenza that are currently in circulation. These vaccines are recommended to include a three-dose priming series with the initial dose administered with a 4 to 6 week interval; the third vaccine should be administered 8 to 12 weeks after the second dose. These vaccines are well suited for prefoaling series, which will provide high levels of colostral immunoglobulin.
2) *Modified live cold-adapted equine influenza A/2 (intranasal administration)*: This vaccine is administered intranasally and has been shown to effectively provide rapid protection to naïve individuals.[3,26,27] The product is labeled for protection of horses 11 months of age or greater. Vaccination in horses 6 months of age and older is recognized to safely provide protection from disease. Label claims report 6 months of protection; however, it is recognized to confer protection for 12 months. Circulating immunoglobulin levels are not markedly elevated following vaccination; local mucosal immune protection is recognized to confer immunity to individuals following vaccination.
3) *Canary pox-vectored vaccine (intramuscular administration)*: This product is administered as an intramuscular injection and is safe to administer as early as 4 months of age. A strong humoral response is observed following vaccination, suggesting that this is also an appropriate product to administer during late term pregnancy to enhance colostral antibody levels.

Adult horses that have previously been immunized and are at risk for exposure can be effectively vaccinated twice annually. If risk for disease in relatively low, annual booster vaccination should be sufficient to provide protection from disease. Previously unvaccinated adult horses can effectively be vaccinated with an individual dose of the MLV intranasal vaccine and boostered at 6 month intervals. Alternatively, an initial two-dose series of the canary pox vectored vaccine or a three-dose series of

an inactivated vaccine should provide protection. When administering a three-dose series of the inactivated vaccine a period of 3 to 4 weeks between the initial two vaccines followed by a period of 3 to 6 months before the third vaccine will provide optimal protection. Subsequent revaccination should be performed at 6 to 12 month intervals, depending on the use and risk of exposure of the horse.

Horses in the first year of life and less than 11 months of age should begin receiving vaccination at approximately 6 months of age. If the MLV intranasal vaccine is selected, the initial dose should be administered at 6 to 7 months of age with a booster vaccine administered at 11 to 12 months of age. If the canary pox-vectored vaccine is administered, it should be initiated at approximately 6 to 7 months of age with a booster vaccine administered in 4 to 6 weeks. If the inactivated vaccine is selected, it should be administered in a three-dose series with the initial dose at approximately 6 months of age, the second dose approximately 4 to 6 weeks later, and a third dose 3 to 6 months later. If the foal is born to a mare that is suspected to be unvaccinated, it is still likely that some maternal antibody transfer has occurred; due to the effect of maternal antibody interference, the vaccine series should be initiated at approximately 6 months of age. Broodmares previously vaccinated should receive a booster vaccine approximately 4 to 6 weeks before expected foaling with either an inactivated product or the canary pox-vectored vaccine. If a broodmare is previously unvaccinated, a three-dose series of inactivated product should be administered with an interval of 4 to 6 weeks between the first two vaccines followed by a third dose at approximately 4 to 6 weeks before expected foaling. Alternatively, a two dose series of the canary pox-vectored vaccine can be administered so that the second dose of vaccines is administered approximately 4 to 6 weeks before the expected foaling date.

Potomac Horse Fever

Equine monocytic ehrlichiosis is caused by *Neorickettsia risticii* (formerly *Ehrlichia risticii*) and causes a seasonal course of disease in late spring through early fall. Clinical signs may include fever, diarrhea, laminitis, colic, and reduced gastrointestinal motility. In endemic regions, one component of reducing disease severity includes vaccination.

Two commercial inactivated vaccines are available for use in horses considered high risk for disease development. Pregnant mares that develop disease are at risk for abortion; neither of the commercially available vaccines claims to protect against abortion. Adult horses that have previously been vaccinated should be vaccinated prior to the peak season associated with disease, typically spring months. Booster vaccine administration is recommended at 3 to 4 month intervals in high risk situations, as the duration of immunity induced by vaccination appears to be short lived. Horses that have not been previously vaccinated should receive an initial series of two vaccines at 3 to 4 week intervals with the second dose being completed approximately 2 to 3 weeks before the anticipated peak incidence of disease. Broodmares should receive a booster vaccine approximately 4 to 6 weeks before expected foaling. If the mare has not been previously vaccinated for Potomac horse fever (PHF), she should receive a two dose series with the second dose being administered approximately 4 to 6 weeks before expected foaling. In foals, the initial dose should be given at 5 to 6 months of age with a second dose to follow in 3 to 4 weeks; a third dose should then be administered at 10 to 12 months of age. Boosters are then recommended at 3 to 6 month intervals depending on the risk of disease. Boosters should be given in May to June in endemic regions.

Rotavirus

Rotavirus is a nonenveloped RNA virus that is an important infectious cause of foal diarrhea. Although many factors play important roles for the development of diarrhea in foals, such as environmental conditions and exposure to infected individuals on high density farms, mare vaccination against rotavirus results in high antibody levels in colostrum and subsequently increased foal serum antibody concentrations. Evidence to support mare vaccination is based on field trials, which have demonstrated attenuation of rotaviral diarrhea cases.

One inactivated vaccine is commercially available, which contains group A rotavirus and is indicated for use in mares to enhance colostral immunoglobulin concentration against equine rotavirus (group A). Mares should receive a series of three doses of intramuscular vaccines at 8, 9, and 10 months of gestation, regardless of previous vaccine status. This vaccine is used frequently in regions where large populations of mares and foals are housed; in addition, mares that are being prepared to be shipped to high density areas should be vaccinated in preparation for exposure to rotaviral challenge. It is important to ensure that foals receive an adequate quantity of colostrum from mares that have been vaccinated against rotavirus. There is no evidence to suggest that newborn foals require vaccination against rotavirus. Colostral antibodies will decline at approximately 60 days of age; although there is some risk for development of rotaviral diarrhea at this age, the risk is considered low and if disease develops it is generally self-limiting.

Rattlesnake Bite Envenomation

Venomous snake bites occur in certain areas of North America. Based on risk for exposure there may be an indication for vaccination of horses in high risk areas with the *Crotalus atrox* (Western Diamondback Rattlesnake) toxoid vaccine. Currently there is one toxoid vaccine with a conditional license indicated for use in horses that are at risk for rattlesnake envenomation. The label suggests that

there may be cross protection against the Western Rattlesnake (including Prairie, Great Basin, Northern, Southern, and Pacific varieties) Sidewinder, Timber Rattlesnake, Massasauga, and Copperhead. Partial protection may be provided against the Eastern Diamondback but not the Water Moccasin (cottonmouth), Mojave Rattlesnake, or Coral Snake.

The toxoid vaccine is labeled for use in horses older than 6 months of age. The primary series includes three vaccines to be administered at monthly intervals with subsequent booster vaccination at 6 month intervals. Use of this vaccine in pregnant broodmares has not been specifically investigated, and if needed, direct consultation with the manufacturer is recommended.

6. Summary

Effective vaccine programs are best achieved by identifying AAEP established core and risk based needs. Recognition of all factors that may influence equine immunity should be carefully evaluated. An understanding of equine immunology provides the practitioner with a strong foundation for selecting specific vaccine protocols. Identification of factors that may influence immune function such as age, stress, and pharmacologic agents are important considerations when establishing the ideal vaccine protocol. Specific infectious diseases may provide unique challenges based on disease manifestation and available commercial vaccine products. Due to the expansive nature of the equine industry and the diversity of the equine population, it is imperative that equine veterinarians continue to serve as the primary source of information as horse owners and equine professionals design and implement vaccine programs.

Acknowledgments

Conflict of Interest

The Author declares no conflicts of interest.

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[a]Pneumaboart K®, Zoetis, Kalamazoo, MI 49007.

Integrated Parasite Control—How to Strike That Balance

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Parasites have evolved and methods of controlling them must also evolve. A "silver bullet" anthelmintic no longer exists, but all formulations still have a place in equine parasite control. Parasite surveillance information, generated via quantitative fecal egg count, is essential for monitoring the efficacy of parasite control programs. Fecal egg counts should be used in guiding drug selection, treatment frequency recommendations, and classifying the pasture contamination potential of individual horses.

Baseline treatment recommendations for adult horse coincide with other preventive health measures, such as spring vaccinations and fall dental examinations. Veterinarians who provide direct parasite control services along with other preventative health measures are more vertically integrated into the total health care needs of their patients. Ongoing work is underway to investigate the relationship between inflammatory reactions to parasite burden and anthelmintic treatment as well as the possible interaction with host response to vaccination. One study in horses and one study in cattle suggest superior vaccine response in horses and cattle with low parasite burdens. Author's address: M.H. Gluck Equine Research Center, University of Kentucky, Lexington, KY 40546-0099; e-mail: martin.nielsen@uky.edu. © 2014 AAEP.

1. Introduction

Parasite control is an integrated part of general equine management and is generally viewed as routine annual care along with vaccinations. Traditional approaches for deworming have largely resembled vaccination schedules; medication is applied to all horses with regular intervals year-round and often with a degree of rotation between products.[1–4] However, mounting evidence of ever-increasing anthelmintic resistance is forcing the equine industry away from this approach, and surveillance-based strategies are recommended instead. Equine parasite control has become the art of treating just enough and choosing the right drug for each treatment. This is a challenge that requires veterinary involvement to design a structured approach to strike the balance between over- and under-treatment.

It is often stated by parasitologists that no one-size-fits-all program exists for equine parasite control, and each strategy has to be based on conditions on the given farm. While this is true, it does not provide any guidance to the end-user. Instead of pointing to the complexity of parasite control, it is more helpful to outline the general features that characterize equine parasites and identify the elements that should be considered essential in a successful parasite control program. This paper will attempt to outline some of the important elements in an integrated parasite control program.

NOTES

Parasite Species Resistance Patterns

Equine parasite control is complicated by the magnitude of different parasite species always infecting horses in coinfection. These differ in biology, pathogenicity, and in levels of anthelmintic resistance, so many control recommendations may be appropriate for one parasite category but not others. Despite this, it is striking how uniform the equine parasite fauna appears to be across the world. Despite vast climatic and geographic differences, equine operations all appear to face the same classical intestinal parasite categories: the group of cyathostomins (small strongyles), tapeworms (*Anoplocephala perfoliata*), and ascarids (*Parascaris equorum*). These can be considered truly ubiquitous and present in any establishment. The only exception to this pattern is the group of large strongyles that have declined dramatically in prevalence during past decades, and occurrence now appears to be dependent on the parasite control strategy used.[5]

In addition to the mere presence of parasites, the occurrence of anthelmintic resistance in these appears to be strikingly uniform. Of the four parasite categories mentioned above, two of them have developed widespread resistance to commonly used anthelmintic drug classes. Cyathostomins are now widely resistant to benzimidazole-type drugs, and pyrantel resistance is commonly reported both in Europe and North America.[6,7] Furthermore, it is of great concern that recent studies have documented reduced egg reappearance periods after ivermectin and moxidectin treatment[8,9]; this has been associated with resistance in immature stages of these parasites.[10–12] A few studies have documented reduced fecal egg count reductions at two weeks post ivermectin treatment.[6,13,14] Resistance in *Parascaris equorum* is also widespread, with a situation complementary to what is described with the cyathostomins: Resistance to ivermectin and moxidectin is reported across the world[15] while only a few studies have reported resistance to pyrantel salts.[16,17] Although anecdotal evidence suggests resistance to benzimidazoles in *P. equorum*, no confirmed reports exist in the peer-reviewed literature.

The possible existence of anthelmintic resistance in large strongyles has been discussed,[18] but the general consensus is that this group of parasites has maintained susceptibility to all anthelmintic drug classes. This explains the rare occurrence of these parasites in managed horse populations. As long as treatment intervals are kept frequent enough to break the life cycle, transmission appears to be effectively interrupted. Recent work performed in Denmark has illustrated that if treatment intensities are lowered below two treatments per horse per year, *Strongylus vulgaris* can occur at up to 80% of farms.[5] It remains unknown if this can be associated with a higher risk of parasitic disease.

The equine tapeworms have not been documented resistant to any of the anthelmintics labeled for tapeworm treatment, but this probably reflects the lack of established methods and protocols for determining anthelmintic resistance in this parasite category. Certainly, resistance is biologically plausible with any parasite category.

Educated Anthelmintic Use

Despite the widespread occurrence of anthelmintic resistance, none of the currently marketed formulations can be considered completely useless. As outlined above, they all have issues of resistance in some equine parasites, but all also maintain good efficacy against others. Essentially, the equine anthelmintics have all gone from being broad-spectrum to being more narrow-spectrum. Thus, the large majority of cases should offer at least one good and efficacious treatment option for each of the important equine parasites mentioned above. However, the real challenge is to make the right treatment choice. This can only be carried out with some degree of parasite surveillance. This is best exemplified with the question of whether a foal or weanling is primarily infected by ascarids, strongyles, or both. Fenbendazole and possibly pyrantel are likely to represent good treatment options for ascarid treatment, but cyathostomins may very well have developed resistance to one or even both of these drugs. On the other hand, macrocyclic lactones would probably be drugs of choice for cyathostomin treatment in most cases but is unlikely to be efficacious against ascarids on many farms across the world. So in order to choose the right anthelmintic, it is necessary to perform fecal egg counts that will provide the needed information. If these samples are followed up with post-treatment egg counts, the veterinarian will gain useful knowledge about treatment efficacy and possible resistance on the given farm.

2. Fecal Egg Counts

Quantitative fecal egg counts are the cornerstone of equine parasitological diagnostics. However, it is important to understand the nature of egg counts in order to make the appropriate interpretation of the results. There are numerous egg counting techniques available and even more different modifications of these. Classic egg counting techniques that are widely used include Stoll, Wisconsin, and McMaster. In essence, all methods are based on the same principle, which is flotation of eggs to allow them to separate from the fecal matter making them available for microscopy and counting. The two most important features characterizing any given egg counting technique are the detection limit and the level of variability between repeated counts. The detection limit is synonymous with the multiplication factor for the technique, i.e., the smallest egg count detectable with the method. The level of variability is considerable with most egg counting methods, and it is important to take this into account when results are interpreted. One recent

study illustrated that the large majority of variance between egg counts exist between repeated counts performed on the same subsample and between different subsamples. Additional variance between different samples collected at different time points on five consecutive days was negligible.[19] Thus, variance can be largely reduced by performing a few repeated counts on the same sample, yet no additional gain is obtained in collecting samples for consecutive days. In practice, reducing the variability will mostly be important when egg counts are near the cutoff value for treatment or when screening for parasite resistance.

Egg counts can be performed for several different purposes, and it is important to realize that they are not necessarily equally useful for all these possible purposes. The four most important purposes are:

1. Fecal egg count reduction test (FECRT) for screening of anthelmintic efficacy.
2. Detection of patent ascarid infection in foals.
3. Identification of high, medium, and low egg shedders in herds of horses for selecting which horses to deworm and with which anthelmintic drug.
4. Clinical diagnosis as part of the clinical work-up for a horse with suspected clinical disease.

In the following, we will outline the usefulness of fecal egg counts for each of these purposes.

Screening for Resistance

The fecal egg count reduction test remains the gold standard for detection of anthelmintic resistance in equines. There are several factors that should be considered in order to generate meaningful results with the FECRT. First of all, it is important to emphasize that anthelmintic resistance is diagnosed in the parasite population, which is shared by the herd of horses. Thus, each horse is a biological sample of the parasite population and resistance can never be diagnosed in individual horses. With the levels of variability mentioned above, there will always be differences in FECRT results observed in a herd of horses. Thus, the FECRT results should always be generated as mean reductions observed on the herd level.

The detection limit of the egg counting technique is very important for the FECRT. A small reduction of anthelmintic efficacy will be expressed in smaller numbers of eggs in the post-treatment samples. If the egg counting method does not have a low detection limit, these eggs may go undetected. If, for example, the pretreatment egg count level in a horse is 300 eggs per gram (EPG), and it is treated with a drug with a 90% efficacy, the post-treatment egg count should theoretically be 30 EPG. However, if the egg counting method has a detection limit of 50 EPG, the post-treatment result would falsely be 0 EPG, leading to an observed FECRT of 100%. In essence, a high detection limit can falsely overestimate the efficacy of the drug. There are two possible ways to avoid this bias; one is to ensure that horses selected for the FECRT have very high egg counts pretreatment. If egg count levels are more than 1000 EPG, then an egg counting method with a detection limit of 50 EPG would be able to detect eggs in the post-treatment samples even when the efficacy is 90%. However, such high egg counts are rare in adult horses, and it is unlikely to find more than a few on any given farm. The other option would be to use an egg counting method with a lower detection limit. McMaster techniques are widely used in veterinary practice because of their simplicity, which makes them easy to perform in a practice setting. The detection limits are typically in the range of 25 to 50 EPG, making them quite unsuitable for the FECRT. The FLOTAC technique has a detection limit of 1 EPG,[20] making it highly suitable. However, improved detection limits come with a cost, as centrifugation of specially manufactured counting chambers is required, which makes the method more time consuming and requires adequate centrifuge equipment. A simpler mini-FLOTAC system has been developed, which does not require centrifugation but operates with a detection limit of 5 EPG. The choice of egg counting technique always represents a trade-off between the costs and ease of use compared to the detection limit and variability.

Identification of Ascarid Infection in Foals

As outlined above, it has become imperative to determine whether a given foal is primarily infected with ascarids, strongyles, or both. Efficacious drugs exist for both parasite categories but not necessarily in the same anthelmintic formulation. The young foal is highly likely to harbor *Parascaris equorum* infection during the first months of life while their immune system most often will have eliminated these parasites by yearling age. The age around or shortly after weaning is the transitional period, where the ascarids gradually disappear and the strongyles take over. The only method to determine when this occurs in a given foal is a fecal egg count. Routine egg counts are rarely performed in foals, but useful information can be generated if they are incorporated in the management program.

Identification of Consistent Egg Shedders

It is well established that adult horses have a strong tendency to return to the same strongyle egg count levels regardless of the anthelmintic treatment.[21] This is particularly pronounced for low egg shedders shedding less than 200 EPG. Thus, horses can be defined to have a certain level of strongyle egg shedding, which they are highly likely to maintain over time. This has become the foundation for the selective therapy principle, where egg counts are used to identify horses exceeding a predetermined threshold

value for treatment. By treating adult horses exceeding egg counts of 200 EPG with an anthelmintic with 99% efficacy, it has been shown that about 50% of horses will be left untreated, while maintaining an overall egg reduction above 95%.[22] Thus, by performing systematic surveillance over time, fecal egg counts can reliably identify consistent low, medium, or high strongyle egg shedders within a herd of adult horses. Typical cut-off values used for categorizing horses into these three groups are as follows: Low shedders = <200 EPG, medium shedders = 200 to 500 EPG, and high shedders = >500 EPG. However, the choice of cut-off may vary depending on conditions on the given farm. As opposed to with the FECRT, the McMaster technique is generally very suitable for identifying high, medium, and low shedders. Also, it should be emphasized that the consistent egg shedding patterns are much less pronounced with horses younger than 4 years-of-age, as egg count levels are generally higher and more variable. Therefore, selective therapy is not recommended in foals and young horses.

Correlation of Fecal Egg Counts to Clinical Parasitism

The previous sections have illustrated how useful information can be generated with fecal egg counts. Despite this, many veterinarians and horse owners remain skeptical about their reliability. The main reason for this is probably that fecal egg counts are not useful tools for diagnosing clinical parasitic disease. There are several reasons for this:

1. The mere presence of parasite eggs and/or larvae in the feces does not equate parasitic disease. The majority of equine parasites are more or less omnipresent, and parasitic disease represents the exception rather than the rule. In addition, false negative egg counts are not uncommon.[23]
2. Migrating or encysted strongyle larvae do not produce eggs but remain the most pathogenic stages. Egg or larval counts strictly represent progeny produced by less pathogenic adult parasites present in the intestinal lumen. Currently, there are no commercially available diagnostic assays capable of detecting migrating or encysted parasite larvae in horses, but several are under development.[24]
3. There is no direct linear relationship between the actual egg counts and the size of the worm burden,[23] so a higher egg count simply does not mean more worms. However, cutoff values in the range of 100 to 500 EPG all corresponded to lower worm burdens in horses below the cutoff compared to those above.[23] Thus, there is data supporting a cutoff in this EPG range.

Taken together, a fecal egg count does not provide useful information when dealing with cases of suspect parasitic disease, and there is no reason to consider it part of a clinical work-up.

3. Baseline Treatments

There is general consensus that a foundation of baseline treatments, which should be considered for all horses, can be identified. Parasite surveillance performed on the farm will help identify needs for additional treatments.

In adult horses, the general foundation is two yearly treatments, preferably with a macrocyclic lactone, typically applied in the spring and early fall. These are generally applied to break the life cycle of the more pathogenic large strongyles, but a tapeworm drug can be included if there is evidence of high tapeworm transmission. Egg counts will identify consistent high strongyle shedders that could receive additional treatment during the grazing season. A large majority of adult horses are likely to not need more than the two baseline treatments. It should be emphasized that these baseline treatments could be integrated with other preventive health measure programs such as yearly vaccinations and dental work.

In foals and young horses, parasite control should be approached in a different manner. Egg counts can provide useful information in foals, but they should be used differently than in adult horses. Given the ubiquity of *Parascaris equorum*, it is justified to focus the program on this parasite during the first few months of the foal's life. The main concern is the risk of small intestinal impaction, which typically occurs at the age of 4 to 8 months.[25] Ironically, treatment with an efficacious anthelmintic has been identified as a significant risk factor for this condition,[25] so treatments should be planned with great consideration. It should also be noted that in case of macrocyclic lactone resistance, no drug formulation will provide efficacy against migrating larvae when given in a single dose. Larvae migrate in the liver and lungs for about four weeks before returning to the intestinal lumen, so there is little or no value to treating foals at less than 30 days-of-age.

The first treatment for foals should be considered around 2 to 3 months-of-age. This allows the first ascarid parasites to complete their migration, reach the intestine, and become available for treatment. Benzimidazole-type drugs appear to be the best choice for treatment at this time because of the apparent lack of anthelmintic resistance in *P. equorum*, but other drug classes can be considered if they have been found efficacious. Furthermore, other drug classes with a paralytic mode of action may be more associated with the risk of verminous small intestinal impactions than the benzimidazoles, which interact with parasite metabolism.[25] The second treatment should be considered about three months later, which could coincide with weaning in many foaling operations. It is recommended to consider anthelmintic treatment prior to weaning.

Fecal egg counts are useful to determine the relative presence of strongyles versus ascarids at this time. If both categories are found, priority should be given to treat the ascarids first and then possibly follow up with ivermectin if needed. A 5-day regimen of fenbendazole given at 10 mg/kg can be considered to also remove migrating ascarid larvae, but no studies have been performed to evaluate any possible benefits of such an intervention. Third and fourth treatments should be considered at about 9 and 12 months-of-age, respectively, and treatment should primarily be targeting strongyles. Tapeworm treatment should be included on one of these latter treatment occasions. Taken together, a baseline treatment for foals consists of four treatments within the first year. Additional treatments can be added if evidence suggests circumstances with high infection pressure or cases of parasitic disease. However, it should be borne in mind that the selection pressure for anthelmintic resistance has traditionally been very high in this age group, and maintaining treatment regimens with very frequent treatments is strongly associated with a risk of selecting for multi-drug resistant ascarids.

Young horses between 1 and about 3 to 4 years-of-age are slowly transitioning from the foal to adult programs outlined above. As a rule of thumb, it is recommended to treat young horses about three times a year, with the third treatment occurring in the middle of the grazing season. Treatments should mainly target strongyle parasites and tapeworms. Disease due to encysted cyathostomin parasites is rare, but horses in this age group appear to be at higher risk compared to foals and adult horses.[26] Thus, larvicidal treatment can be considered in this age group. Anthelmintics with labeled larvicidal efficacy against encysted cyathostomins include moxidectin (single dose), and a 5-day regimen of double-dose fenbendazole.

4. Parasites and Inflammation

Given the ubiquity of equine gastrointestinal parasites, one relevant question to ask is whether the host-parasite relationship has a degree of symbiosis to it. Does the horse actually, to some extent, benefit from its parasite burdens? Research with parasites in other animals as well as in humans has illustrated that gastrointestinal nematodes possess anti-inflammatory properties and that inflammatory reactions can be largely modulated by these parasites.[27,28] This is the basis for treating human inflammatory bowel syndrome (Crohn's Disease) with viable eggs of the porcine whipworm, *Trichuris suis*[28].

Little is known about the relationship between parasites and inflammatory reactions in horses, but some information has been generated in recent studies. One study evaluated acute phase inflammatory markers in horses experimentally infected with mixed species cyathostomins as well as *S. vulgaris*. A subtle acute phase reaction could be associated with strongyle infection, but the most potent inflammatory marker, serum amyloid A (SAA), remained unaffected throughout the study.[29] Another study evaluated systemic inflammatory reactions to treatment with pyrantel pamoate, oxibendazole, and moxidectin in naturally infected Shetland ponies. Again, only subtle inflammatory reactions were found in any of the three treatment groups, with SAA remaining unaffected.[30] This is contrasted by other studies reporting substantial local inflammatory reactions in the mucosal walls associated with numbers of encysted larvae or larvicidal treatment with fenbendazole.[31,32] Thus, it appears that inflammatory reactions to cyathostomin infection are primarily local with very little spill-over to the systemic circulation in otherwise healthy horses.

One study illustrated an interaction between gastrointestinal worm burdens and vaccination response.[33] Horses with high parasite burdens had significantly lower immunoglobulin levels as well as lower mRNA levels of the pro-inflammatory cytokines interleukin (IL)-4 and interferon gamma (IFNγ) when compared to horses with medium or low parasite burdens. In addition, vaccine-specific antibodies were found to be generally higher in the low parasite burden group. Altogether, this suggests a possible interaction between gastrointestinal worm burdens and the immune response to antigen stimulation. A recent study performed in cattle documented a beneficial effect of deworming calves two weeks prior or at the day of vaccination with a combination vaccine against respiratory viruses.[34] A current study is evaluating the possible interaction between vaccination and anthelmintic treatment in three cohorts of ponies. Preliminary results indicate a decreased inflammatory reaction in ponies treated with ivermectin compared to those treated with pyrantel pamoate and untreated controls. Further, there are indications of interactions with antibody titers to the vaccines tested, but a thorough data analysis is needed before any conclusions can be drawn.

5. Conclusion

Equine parasite control has become more complex in recent years, which is likely to be perceived as a negative by many. However, this represents an opportunity for veterinarians to help guide horse owners in formulating the best plan for parasite control programs. If veterinarians are well-educated on all issues relevant to parasite control, they will be able to provide a meaningful service to their clients. Because of the evolution of parasite susceptibility to anthelmintics, all present control programs need veterinary involvement to remain effective.

Acknowledgments

Conflict of Interest

The Author declares no conficts of interest.

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Translating the Science of Equine Wellness Into a Business Model/Wellness Plan and What This Can Do for Your Practice From an Economical Standpoint

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A wellness program is not a bundle of discounted services. That is a discount plan and many people (including the author) have tried unsuccessfully to sell a discount plan as a wellness program. A wellness plan is a service that requires the commitment of the lay and professional staff to operate successfully. When done properly, a wellness plan is a tool to set your practice apart, increase client visits, and increase opportunities to improve client loyalty. Author's address: Brazos Valley Equine Hospital, 6999 Hwy 6, Navasota, TX 77868-7465; e-mail: bbuchanan@bveh.com. © 2014 AAEP.

1. Introduction

As defined by Merriam-Webster (www.merriam-webster.com), "wellness" is the quality or state of being in good health especially as an actively sought goal. This is an appropriate definition for a wellness plan in veterinary medicine as well. When a practitioner is developing a wellness program there are several aspects that should be considered. What is the goal of the wellness plan? Is it the right time to develop a wellness plan? What is in the wellness plan? How to execute the plan?

A wellness plan can encompass many aspects of the care of the horse. Depending on the practice's population, it can be vaccines and general exams, it could be serial radiographs and lameness exams, it could be dentistry, or it could be acupuncture and chiropractics. To begin to build a wellness plan, the practice must first decide on the ultimate goal. Is the goal to introduce a new service, increase use of a current service, or to continue building client loyalty? As with every service, one goal is to be profitable. How a wellness plan can be profitable depends on how it is designed. The author discourages discounting and bundling services. While successful in small animal practices, this approach has been a failure for us and many equine veterinarians. In this approach, the belief is that the client will either not utilize the services that are prepaid or that by giving free exams, the client will spend money on diagnostics leading to higher average transactions per client. Our philosophy has been to encourage clients to utilize the wellness plan to both benefit the horse and also to benefit the owner by relieving them of the worry about "what does my horse need and when?" Because you are taking care of it for them, it helps build loyalty and trust of the veterinarian as an essential partner in taking care of the horse and establishing the vet as the go-to person for the healthcare needs.

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2. When to Invest in a Wellness Plan

A wellness plan may not be appropriate for every practice. If the doctors are booked out 2 weeks, implementing a wellness plan does not make sense, as they will not be able to provide additional services. If there are consistently 1 to 2 appointments not filled on the schedule or a seasonally slow period, investing in a wellness plan is worth considering. In a general practice where vaccine sales or dentistry are small parts of the total visits or income, a preventative wellness plan may be worth implementing. The more involved you are in the "little things" (dentals, vaccination, and deworming), the more vertically integrated you will be in the total healthcare of that individual horse making yourself more essential to that individual client.

One other scenario to consider for implementing a wellness plan is when there are similar style practices competing for the same clients. Offering a wellness plan is one way to build client loyalty and to elevate your practice above the competition. The decision to implement a wellness plan should involve the professional staff, the lay staff, and office staff. Encouraging their input will help to build ownership in the plan and improve staff buy-in. If the office staff feels the plan is too complicated or the professional and lay staff do not understand the plan or make recommendations counter to the plan, it will not be successful.

3. Designing the Plan

Once the decision has been made to design a wellness plan, several questions should be addressed.

- Who is the target client?
- What is included in the plan?
- Is a farm call included?
- What is not included in the plan?
- Are there multiple levels or customization of the plan?
- Why is that beneficial to the horse?
- How will this be beneficial to the horse owner?
- Why is that beneficial to us?

There is a tendency to add up all the services you want to offer and mark them down 10 to 20% and sell the plan as a great deal. While this is a great deal, it is the author's impression that the horse owners in search of a great deal buy their vaccines and wormers at the feed store. Building a plan for clients who don't seek routine veterinary services is not logical. Including some free services can be helpful as long as those services are likely to lead to other diagnostics. A detailed oral exam may lead to more dentistry. A semi-annual lameness exam may lead to more joint therapy and orthopedic diagnostics. Including customization will increase the attractiveness to clients but also increases the work load on the office staff. If the plan is too complicated for the staff to keep straight, clients will not understand it and will not buy into the plan.

After deciding what is in the plan, you must put a price on it. Will you charge clients monthly? Charge them once a year? When billed out monthly, client retention is highest. When billed out once a year, the chances of losing money on the plan are reduced as the client has prepaid for all the services. When pricing the plan, add up everything included in the plan at full value. Divide this number by 12. That is the monthly cost to the client for you to administer the plan and treat the horse. There are multiple companies that you can outsource the monthly debit or bank withdraw reducing the workload on your office staff. Autopay from a bank account is preferred to a credit card, as bank accounts do not expire and are less frequently canceled. Consider asking for a bank account as a backup for clients who want to set up the plan on a credit card.

Consider your clientele: Will they pay this monthly fee? If not, consider removing some things from the plan. Will there be a onetime setup fee? You need to decide what to do with clients who want to cancel the plan and to be very clear on how to handle scenarios involving sale or death of the horse. We will allow clients to cancel a plan, but they must pay the full retail price for everything they have used and not yet paid for.

4. Internal Marketing and Training

Once you have decided what is in your plan and the cost, you still have to "sell" it to clients. Just like any new service, some marketing and client education is a must. Having the staff buy-in is critical in recommending or offering the plan to clients when they call to make an appointment, when they arrive and check in, during the examination, and on any follow-up contacts. It is most useful to "sell" the plan to clients during well horse visits that are related to components of your plan. It is not helpful to try and "sell" clients a preventative wellness plan when the horse presents for an illness. The staff needs to understand all aspects of the wellness plan and be able to articulate this to the clients. The staff should be able to answer most questions the client asks. Most importantly, the staff must believe that the wellness plan is in the best interest of the horse, client, and practice. Having some written material on the website and as handouts is important to allowing clients to review the details. Where we have failed in "selling" our wellness plan has been poorly training our staff and having doctors make recommendations counter to the plan. The AAEP has multiple resources available to clients and staff in relation to core vaccines and deworming strategies. Utilizing these tools is one method to get all the veterinarians and staff on the same page when discussing the wellness plan with clients. No client is going to invest in a wellness plan that recommends twice a year influenza if the veterinarian advises them that once a year is all they need.

Another reason some doctors are not enthusiastic about a wellness plan is when they are paid on production. When there are multiple doctors in a practice, the client payment for the wellness plan is attributed to the practice and not a specific doctor. The client pays upfront and is not charged when the wellness work is done. Frequently, doctors do not understand how they can generate a commission when the charge to the client is $0 for that visit. In our practice management software, a horse marked as wellness will not create a cost to the client that day for vaccines. The client is billed monthly for that horse and the income does not go to any doctor's production. Rather we run a wellness report to identify what services were performed by the doctor and hand calculate a commission. An alternative is to create a payment category of wellness that is used to pay for all of a client's wellness charges. In this scenario the doctor sees the production that is generated, but the office staff has to recognize what is a wellness charge and what is a non-wellness charge that must be paid separately. If the professional staff is confused or not enthusiastic, the effectiveness of the wellness plan will suffer.

5. Implementing the Plan

Once everyone is on board with the plan and trained, start small. Introduce the plan to the few key clients you were likely thinking about when the plan was developed. Make sure the office policies are working and that the clients don't have any unexpected questions. After everyone is comfortable with the plan, a larger marketing campaign is appropriate. Use of mailers, social media, posting in the feed stores and tack shops, and local advertisements will attract current and new clients. Such materials are available through not only the AAEP but are also provided by pharmaceutical companies. The staff must be able to handle the increased numbers for the return on the marketing to be worthwhile. Once the clients have bought in, a reminder system to alert them to unused services will increase appointments and the potential for additional services.

6. Evaluation

Finally after the wellness plan has been developed, the staff has been trained, and the plan marketed, evaluation of the reenrollment rate will give you an idea of how your clients perceive the plan. Our target for client reenrollment is 80%. A low reenrollment suggests that the plan is not meeting the needs of your clients. A periodic analysis of supply costs, technician costs, administrative costs, and doctor compensation compared to revenue generated directly is valuable. As long as the plan is profitable, the value to the client can be indirectly evaluated by looking at the reenrollment percentage. While very little data is available in equine practices on reenrollment, tracking this data will help your wellness plan mature into a profit center and a tool that builds client loyalty.

7. Conclusion

A wellness plan is a service that needs to be approached like any new service added to a practice. It should include significant planning, staff training, and marketing. It requires complete buy-in from the staff and doctors in the practice and reminders to be sent to the clients. The success of the plan can be evaluated by calculating the reenrollment rate. When done properly, a wellness plan is a tool to set your practice apart, increase client visits, and increase opportunities to improve client loyalty.

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Conflict of Interest

The Author declares no conflicts of interest.

Managing and Training Mustangs in the Bureau of Land Management Wild Horse and Burro Program

Joan F. Guilfoyle; Albert J. Kane, DVM, MPVM, PhD; Gus Warr; and Fran Ackley

The Bureau of Land Management (BLM) is responsible for managing approximately 49,000 wild horses and burros on 32 million acres in the western United States (U.S.). Approximately 48,000 are also held in off-range holding locations such as pastures, corrals, or training and adoption centers. The BLM provides care and humane treatment throughout the Program and offers excess trained and untrained animals for sale and adoption through the Adopt a Horse or Burro Program and partnerships with private and public organizations. Authors' addresses: U.S. Department of Interior, Bureau of Land Management, Division of WH&B, 20 M Street SE, Washington, DC 20003 (Guilfoyle, Warr, Ackley); and U.S. Department of Agriculture, Animal and Plant Health Inspection Service, Veterinary Services, 2150 Centre Ave., Bldg B MS2E6, Fort Collins, CO 80610 (Kane). © 2014 AAEP.

1. Introduction

Few images capture the spirit, pride, emotion, and nostalgia of the American West as quickly and easily as that of the wild mustang. Their appearance and *type* are as varied as their colors: from little brown or black ponies; to sorrel, bay, or grey saddle horses; to flashy palominos, duns, grullas, and pintos; drafty sorrel or gray work horses; bay or chestnut Thoroughbred, or stock type horses and beyond. Their histories are as varied as are their types. Most trace their origins to modern domestic stock released or escaped within the last 100 to 200 years. At one time broodmare bands were often managed as free-roaming herds on western rangelands and horses have been released or escaped from ranchers, sheep herders, miners, or cavalry remount stations countless times in the past 100 years. Other horses, particularly those with stronger genetic links to the Spanish or New World Iberian lineages, could have a more romantic history. These may be descendants of horses reintroduced to North America by Spanish explorers or those used by early American Indians.[1]

Burros roaming the public lands of the western United States have a similar, although perhaps slightly more straightforward, history. After Spanish Conquistadores brought donkeys to the Americas, a Jesuit Priest, Padre Eusebio Kino, is credited with bringing the first burros to a Spanish mission at San Xavier de Bac in the Arizona Territory in 1679.[2] This was part of his agricultural development and exploration work to aid in the expansion of the American Southwest where he used and promoted them as pack animals to carry supplies and

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ore to and from mining operations. Later in the mid-1800s when gold was discovered in the lower Colorado River Valley, the popularity of burros soared and the gold rush became a burro rush with many mines and prospectors counting on their burros as a critical partner in their operations. When the gold rush declined in the late 1800s many of these animals were abandoned along with the mining claims; the hardy animals, already well-adapted to desert living, thrived in areas where water was available.

The varied appearance and history of mustangs and burros contributes to their broad appeal in the U.S. and even in many European countries. It also contributes to the controversies, confusion, and disagreement over how they should be managed and even what they should be called.

Biologists technically consider today's free-roaming horses and burros *feral*, that is, animals living in the wild following their escape or release from domestic stock. However, in the 1971 Wild Free-Roaming Horses and Burros Act (the Act), Congress legally declared that the "*wild* free-roaming horses and burros" roaming public lands managed by the U.S. Department of Interior Bureau of Land Management (BLM) and the U.S. Department of Agriculture (USDA) Forest Service (FS) were "living symbols of the historic and pioneer spirit of the West" that "contribute to the diversity of life forms within the Nation and enrich the lives of the American People."[3]

This same legislation protected the animals from commercial exploitation and ordered the BLM to manage the animals where they existed on BLM and FS lands in 1971. Key elements of this legislation include that it only applies to animals living on lands managed by the BLM and the FS, but not to free-roaming feral horses living on lands managed by the National Park Service, U.S. Fish and Wildlife Service, Department of Defense, or state or tribal governments. The Act gives the direction that all management activities shall be at the "minimum feasible level," that will "preserve and maintain a thriving natural ecological balance and multiple-use relationship," in keeping with the Bureau's multiple use mandate to manage public lands for many uses such as fish and wildlife development and utilization, mineral and energy development, livestock grazing, timber production, rights of way, and outdoor recreation. The intention of the Act was not to create herds of animals intensively managed similar to domestic animals, but instead to preserve the wild free-roaming nature of the animals while also protecting the "natural ecological balance of all wildlife species which inhabit such lands, particularly endangered wildlife species." The Act also includes provisions for removing excess animals where the BLM determines overpopulations exist and directs the agency to achieve "appropriate management levels" by removal, destruction, or sterilization of animals. While the law provides for the destruction of "old, sick and lame" animals as well as excess animals "for which an adoption demand by qualified individuals does not exist in the most humane and cost efficient manner possible," these are provisions which the BLM has never fully implemented. Initially, this was because of public opinion and later because subsequent Congressional appropriations language prohibited the destruction of healthy animals.[4] It was not until 1976 and 1978 that additional legislation authorized the BLM to use helicopters and motorized vehicles to gather and remove animals from the range and provided appropriated *funding* for the management of wild horses and burros.[5] The BLM and the FS are land and resource management agencies, and wild horses and burros are the only animals for which these agencies have direct management responsibilities.

From the 1950s through 1971 it was estimated there were about 25,000 wild horses and burros on BLM lands; by 1978 this estimate had grown to 60,000.[6] Today the BLM estimates they manage about 40,815 wild horses and 8,394 wild burros roaming approximately 32 million acres in the western U.S. (Fig. 1).[7] The majority of the animals (about 25,000) are in 85 herd management areas (HMAs) in Nevada, with smaller numbers of animals in Wyoming, California, Utah, Oregon, Colorado, Arizona, Idaho, Montana, and New Mexico (Table 1). Nationally, the free-roaming population exceeds the number (about 26,700) that the BLM has determined can exist in balance with other uses and rangeland resources by about 22,500 animals.

As early as 1982, the number of excess animals removed from public lands exceeded the number that could be adopted to the public. The Act required the BLM to humanely destroy unadoptable animals, but limited initiation of this action drew a public response that caused the BLM in 1984 to put a moratorium in place on the destruction of healthy animals. Large scale adoptions in the early 1980s also met with disapproval since many of these animals were resold for slaughter. As a result, regulations implemented in 1986 prevented further large scale adoptions. In 1998 a clause was added to the adoption agreement that required adopters to promise that they had no intention of selling adopted animals for slaughter. Adoptions to good homes peaked in 2002 with 7746 animals adopted that year. Since that time adoptions have faced a steady decline, with only 2311 animals adopted in 2013. During this time, removals of excess animals ranged from about 6000 to 12,000 animals per year. About 40% of these animals were mares, and most mares are pregnant when removed from the range. Although no breeding is allowed following removal from the range, a significant number of foals are born in captivity, which also adds to the numbers in holding. The result of this imbalance between supply (of excess horses that must be removed from the range) and demand (interest in adoption to good homes) is that although the BLM has adopted over

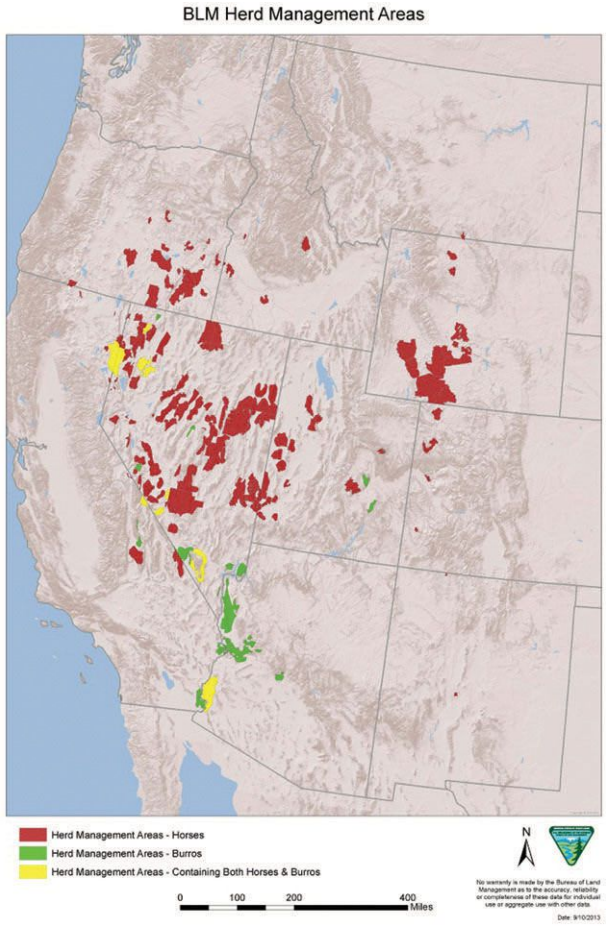

Fig. 1. The BLM herd management areas (HMAs) contain both wild horses and burros in 10 western states. Red = Herd Management Areas—Horses; Green = Herd Management Areas—Burros; Yellow = Herd Management Areas—Both Horses and Burros.

230,000 animals since 1971, there are still approximately 16,000 animals in the Bureau's short-term corrals and adoption centers and another 32,000 animals being held on long-term holding pastures in the Midwest.

In 2004 another amendment to the Act required animals that are older than 10 years old or have been offered for adoption unsuccessfully three times to be offered for sale without limitation.[3] The Bureau's sales policies and contracts have been created in response to the overwhelming public sentiment that animals should not be sold unless safeguards are in place to prevent wild horses and burros from going to slaughter. In addition, Congressional appropriations language prohibits the euthanasia of healthy animals and sale without limitation of certain animals that remain unadopted. For these reasons, it seems likely that the majority of animals currently on long-term holding pastures will remain there or in some sort of government funded sanctuary for the remainder of their natural lives. The cost of feeding animals currently consumes about two-thirds of the BLM's total operating budget for the Wild Horse and Burro (WH&B) Program, which was $72 M in fiscal year 2013. About 6% of the budget is spent on gathering and removing excess animals from the range and another 10% is spent on adoptions. This leaves about 20% of the budget for all other activities in the Program such as monitoring and managing horses on the range, caring for animals in short- and long-term holding, and other operating costs.

Strategies for putting the WH&B Program on a more sustainable track have been reviewed and developed in the last 5 years. Several expert reviews of the Program, some of which are described here, have been solicited by the BLM; and over 40,000 public comments have been received and considered during these processes. There are four general elements of these strategies:

1. Reduce the number of horses removed from the range for a period of three years while reviewing the science used to support the Program and continuing to monitor habitat conditions. A National Academy of Sciences review was contracted by the BLM in June 2011[8] and the Bureau has responded by incorporating

Table 1. Populations of Wild Horses and Burros in BLM Herd Management Areas (HMAs) Across the Western U.S. in Fiscal Year 2014 Compared to the Established Maximum Appropriate Management Level (Maximum AML).[7]

State	HMAs	Horses	Burros	Total	Maximum AML
AZ	7	333	4411	4744	1676
CA	21	4086	1922	6008	2184
CO	4	1205	0	1205	812
ID	6	668	0	668	617
MT	1	160	0	160	120
NV	85	23,347	1688	25,035	12,789
NM	2	146	0	146	83
OR	18	3120	60	3180	2715
UT	19	3979	313	4292	1956
WY	16	3771	0	3771	3725
Total	179	40,815	8394	49,209	26,677

recommendations from that review into new management approaches where possible.
2. Continue to protect the health and well-being of animals in the Program with a new Comprehensive Animal Welfare Program initiated in 2012 and expected to be fully implemented in 2015. The BLM will use this animal welfare auditing approach to improve animal care and handling, wherever possible, with a new emphasis on consistency, monitoring, accountability, and reporting and demonstrating the BLM's success in this area within the Agency as well as to the public and political interests.
3. Increase the use of contraceptives, fertility control, and other techniques as methods of population growth suppression and increase research in this area through a new aggressive call for innovative and problem-solving research in the field.
4. Increase the use of public/private partnerships to expand adoption opportunities for WH&B and move more excess animals that have been removed from the range into lower cost Eco-Sanctuaries.

There may never be a perfect solution to the sustainable management of wild horses and burros that protects their habitat and provides for the productivity and other uses of the public lands under BLM and FS management. However, the BLM and FS are committed to the protection, management, and control of wild free-roaming horses and burros and continue to strive for that thriving natural ecological balance.

2. On the Range Management

Under the 1971 Act, WH&Bs are considered an integral part of the national system of public lands in the areas where they were found in 1971. Under this law, the Bureau is not permitted to introduce WH&Bs into new areas where they did not exist in 1971 and determines through land use planning decisions where animals will and will not be managed. The BLM's goal is to manage healthy WH&B populations on healthy rangelands. To achieve this goal, the BLM designates herd management areas (HMAs) for the long-term maintenance of WH&B herds and collects data about the animals and their habitat. Wild horses and burros are considered comparably with other resource values in planning for their management. This means WH&Bs are the same as other resource values (e.g., cultural, historic, scenic, rangelands, timber, and minerals). Wild horses and burros are a resource value, as opposed to a land use (e.g., livestock grazing or timber harvest). The BLM is chartered to sustain the health and productivity of the public lands for the use and enjoyment of present and future generations, within a *multiple-use environment*. In addition to managing the land, the BLM is responsible for managing the resource values such as WH&Bs as well as resource uses such as fish and wildlife habitat, mineral and energy development, livestock grazing, timber production, cultural resource protection, rights of way, and outdoor recreation.

Habitat

The habitat for WH&Bs is composed of four essential components: forage, water, cover, and space. These components must be present within the HMA in sufficient amounts to sustain healthy WH&B populations and healthy rangelands over the long term. If one or more of the key habitat components are missing, the HMA may be considered unsuitable for year-round use. In these situations, the BLM may consider not designating the area for the management of WH&Bs and remove all of the horses and burros from the area. Along with inadequate habitat, other factors contribute to a decision not to manage some areas for WH&Bs. The analysis and rationale for these decisions are presented in land use plans. The following are reasons that portions of the original 53.8 M acres of lands originally identified as having WH&Bs present in 1971 are not being managed for them: land was never under BLM management (6.7 M); intermingled with private lands or where water was privately owned (7.5 M acres); transferred to other agencies (2 M); withdrawn due to urban development, court decisions, highway construction (1.5 M); populated by privately owned and claimed horses (1.5 M); substantially in conflict with other resource values such as habitat for threatened or endangered species (1.6 M), and missing a critical habitat component such as winter forage and, therefore, unsuitable for WH&B use (1.2 M). This means that today the BLM manages WH&Bs on approximately 32 M acres of which 27 M acres are lands under BLM management and 5 M are lands managed by other agencies or under private ownership.[7]

Many WH&B populations live in very arid low productive rangeland sites. Available water is often the most limiting factor in an HMA. Due to this low production and other uses of public lands, large acreage of lands may be needed to sustain relatively small populations of WH&Bs. Typically BLM HMAs do not have 100 horses on 50,000 acres in a contained area but hundreds of horses on hundreds of thousands or millions of acres of open rangelands. These are hot and cold or high altitude desert ecosystems often characterized by the types of brush and scrub trees present more than the edible plants or grasses present. Grass typically does not cover the ground as one would expect to see in the East, Midwest, or even in the short or tall grass prairie areas of the West. The majority of wild horse habitats are located in the cold desert areas of the Great Basin, where altitudes are typically >5000 feet above sea level and temperatures vary from below 0°F in the winter to above 100°F in the summer. In the winter WH&B, typically forage on residual grasses that are available on windswept ridges or

under shallow snow depths and on palatable shrub species such as winterfat, bitter brush, and some varieties of sagebrush. Water is often not available, forcing animals to rely on snow to meet their needs. This works adequately when forage is limited along with water, but snow as a source of water can be a fatal combination with hay or abundant amounts of dry forage. When dry forage is fed to horses that must rely on snow as their only source of water, impactions usually develop because the horses simply cannot consume enough snow to meet their need for water.

Improvement projects such as seeding, prescribed fire treatments, emergency fire rehabilitation, or shrub and tree removal have potential to increase the forage available for WH&B use and to improve habitat conditions. However, these improvements may also cause grazing animals to concentrate their use within project areas. Structural improvement projects (e.g., fences, cattle guards, or water developments) are often constructed or removed to protect the wild free-roaming nature of WH&Bs and provides for more normal herd distribution and movement as well as genetic interchange over larger groups of animals than may be possible otherwise.

Population Management

Wild horses and burros are managed in a manner designed to achieve and maintain a thriving natural ecological balance (TNEB) and protect the range from the deterioration associated with WH&B overpopulation. They are managed as self-sustaining populations of healthy animals in balance with other uses and the productive capacity of their habitat. Some selected HMAs may be managed for nonreproducing wild horses to aid in controlling on-the-range population numbers. To achieve a TNEB on the public lands, WH&Bs should be managed in a manner that assures significant progress is made toward achieving the Land Health Standards for upland vegetation and riparian plant communities, watershed function, and habitat quality for animal populations, as well as other site-specific or landscape-level objectives, including those necessary to protect and manage Threatened, Endangered, and Sensitive Species.[9] Wild horse and burro herd health and welfare are promoted by achieving and maintaining a TNEB. The appropriate management level (AML) is the number of WH&Bs (expressed as a number range) that has been determined to be the appropriate population size for a specific HMA over time. The AML upper limit is established as the maximum number of WH&Bs, which results in a TNEB and avoids a deterioration of the range. This figure should be below the number that would cause rangeland damage due to excessive grazing. The BLM must monitor the rangelands and WH&Bs located on public lands to determine if there are an excess number of animals present. In making this determination, the field specialists analyze grazing utilization and distribution, trends in the ecological condition of the range, actual grazing use of the plants, weather data, current population inventories, wild horse and burro locations (whether they are inside the HMA or have strayed outside their HMA), and other factors. The term "excess animals" is defined as those animals which must be removed from an area in order to preserve and maintain a TNEB and multiple-use relationship in that area. Annual monitoring objectives will include determining whether or not forage and water are adequate to support the animals in a healthy condition throughout the year. Additionally, a visual assessment and evaluation of animal condition will be done as part of the monitoring. Population surveys are done using helicopters or fixed-wing airplanes to estimate the population size, growth rate, and distribution of animals within a specific area. At a minimum, population surveys should be conducted every 3 years, whenever possible, and within 6 to 12 months prior to establishing the need to gather and remove excess WH&Bs.

Management of WH&Bs also requires specific emphasis given to the animals themselves. The age structure and sex ratio in the population can influence herd health, social interactions, and population growth rates of reproducing WH&B herds. Typically, males and females are born in equal proportions over time, but a herd's sex ratio may have been altered by previous BLM removal policies or natural mortality. Similarly, age ratios will vary depending on previous BLM removals as well as the herd's survival characteristics and the typical environment in a given HMA, particularly regarding droughts or severe winters. Age structures are estimated from the best available data, which may include annual foal counts and the age distribution of captured animals during a gather. Population growth rates represent the net difference between births and deaths and the numbers of animals that enter and leave the area (ingress and egress) over time. Population growth rates can be highly variable and influenced by habitat quality, forage production, water availability, and other factors. Typically, 60 to 75% of breeding-age mares will foal each year and the average population growth rate for populations managed by the BLM is 15 to 20% a year. Survival rates are lowest for animals less than one year of age and over 20 years of age.[10] The foaling season for wild horses on the range is generally March 1 to June 30 for most herds. This has a great deal of variability due to the location of individual HMAs and past management practices and, in some areas, newborn foals may be seen in any month. Wild burros typically foal year-round.

Genetic Monitoring

The BLM uses hair follicle testing to monitor genetic diversity in each herd to characterize the genetic makeup of the herds and protect against inbreeding depression. The breeds of horses most represented in each herd's genetic makeup varies, with about 6

herds showing stronger ties to old Spanish blood than most others.[11] This connection is indirect in some herds, that is, through the introduction of domestic horses in modern times, but this Spanish heritage may also be direct in other more isolated herds. While there are no completely unique genetics among BLM herds that cannot also be found among domestic horses, there are herds with distinctive phenotypes and a higher proportion of Old World alleles. However, conserving unique alleles comes at the price of losing genetic diversity and, at some point, maintaining a desirable level of genetic diversity (avoiding inbreeding depression) may be important to maintaining herd health and fitness. The BLM establishes baseline genetic information on each of the individual HMAs. Once the baseline is established, additional sampling is done to reassess genetic diversity, on average, every other gather or removal period (e.g., every 6–10 years). If testing indicates genetic diversity is low or in question, the herd is reassessed more frequently (e.g., every gather). The probability of natural gene flow in free-ranging horses and burros varies among individual herds. When concerns are noted and the likelihood of natural ingress is low, a few animals (usually young mares) from nearby but genetically different herds can be translocated to boost genetic diversity in the receiving herd.

Controlling Population Growth Rates

The management of population numbers that exceed what the range can sustain without causing ecological damage is the biggest challenge within the WH&B Program. During herd management area planning, the BLM considers a range of alternatives to reduce population growth rates and avoid or at least extend the period of time between gathers. Alternatives may include but are not limited to: use of fertility control, adjustments in the sex ratio in favor of males, a combination of fertility control and sex ratio adjustment, and management of selected HMAs for nonreproducing wild horses. The BLM has supported the development of an effective contraceptive agent for wild horses since 1978. Over the years, several approaches—such as hormone implants and vasectomy or castration of harem stallions—were tried but abandoned as ineffective or impractical as tools for suppressing population growth rates over time. Currently, the most promising fertility control agents are porcine zona pellucida (PZP) and gonadotropin-releasing hormone (GnRH) vaccines developed starting in the 1990s.[11] The BLM currently uses PZP in two formulations. The most effective is a one-year liquid vaccine that must be re-administered annually.[12] This vaccine[c], was registered with the Environmental Protection Agency in February 2012 for preventing pregnancy in wild horse mares. When it can be delivered annually (and preferably in the 2–3 months prior to the breeding season), this is a highly effective contraceptive for horses. This approach is used and encouraged by the BLM when it is practical. However, it is not feasible to treat most wild horse herds every year. In a few herds, horses can be treated by darting each year, but darting is not practical for the majority of BLM herds because it is difficult to approach most wild horses closely enough on Western rangelands. Gathering wild horse herds every year to administer this form of the vaccine is also not practical as the horses in almost all herds become harder and harder to capture when gathers are repeated with just a few years in between. For these reasons, the BLM mostly uses a longer-lasting 22-month pelleted PZP agent (commonly known as PZP-22).[13] The pelleted vaccine has been successfully administered by darting into a few mares, but typically it is hand-injected after the mares have been captured during gather and removal operations. Treatments that can only be administered after an animal is captured mean that during gathers, extra animals need to be captured (for treatment and release); more than would actually be removed from the range if removal was the only goal. While this is usually possible, it can be difficult to capture a large enough proportion of the population so that significant numbers of mares can be treated and released and at the same time remove enough animals to achieve an AML. Maximum effectiveness of PZP-22 is achieved when the mares are treated during a three- to four-month window prior to foaling. Recent research evidence from free-ranging and captive trials has also revealed that PZP-22, another form of PZP[d], and the GnRH vaccine[e] are not working as well as expected, based on early trials. The PZP vaccines generally seem to work well the first year, but recent evidence suggests that none of these products reliably reduces foaling by more than 50% after the first year.[a] Additional management alternatives, such as surgical sterilization of mares, may be considered in the future, pending further research.

Capture and Removal Methods

At the present time, the capture and removal of excess horses from the range is the only proven and reliable method available for the control of population numbers on most HMAs. Wild horse and burro gathers are done on a regular basis to protect and conserve rangeland resources and remove excess animals. The gather cycle, the interval between gathers, is every 3 to 5 years on average. Gather frequency is a function of the AML range, population growth rates, and environmental factors. When the removal of excess WH&Bs from the range is possible (depending on the limitations of budget and holding space), gathers typically have been prioritized in the following order: emergencies to prevent the starvation of animals, court orders, nuisance animals on highways or in urban or developed agricultural areas, impacts to threatened and endangered species, WH&Bs located outside the HMA in areas not designated for their long-term

maintenance, landowner requests to remove animals from private land outside an HMA, and finally, the goal of achieving and maintaining population size within the AML on an HMA.

Capture and removal from the wild can be a stressful process regardless of the techniques used. More passive capture methods using bait or water to lure animals into a capture pen can be effective in some areas, but it is not always possible. Animals that are too suspicious of man-made structures will usually move to alternative water and feed sources if they are available in the area. Water trapping is thought by some to be easier on the animals, but this is not always the case. In most instances, water trapping involves some degree of natural or forced water deprivation before animals can be lured into a capture pen. This, in itself, can create problems, particularly for the very young animals. The first 25% of the animals may be relatively easy to capture, the next 25% may be moderately difficult, but the last 50% may be very difficult or impossible to capture. Depending on the temperament of the animals, power of attraction of the water or bait (which has to be a limited resource in the area), and the percent of animals that need to be captured, bait or water trapping may or may not be feasible.

Helicopter drive trapping where animals are herded into a capture pen much like a border collie herds sheep is usually the most effective and humane way to capture large numbers of animals. Unlike riders on horseback or motorcycles or ATVs, a helicopter will very rarely lose sight of the animals and only needs to apply pressure when the animals need to be turned or are brought into the funnel section of a trap. A rider pursuing on horseback must maintain a fast pace or risk losing sight and control of the animals. A skilled helicopter pilot can allow the animals to move at their own pace along their normal trails and routes of travel, applying pressure only when needed and releasing that pressure when targeted animals are traveling in the general direction of the capture pens. There is no "stampede" involved. Not only would that be inhumane, but it would be counterproductive, as that type of uncontrolled herding of the animals would make it difficult, if not impossible, to guide them into a corral.

Protecting animal welfare during the gather process starts by managing rangeland resources and population numbers to prevent the animals from getting into poor condition prior to the operation and then doing everything possible to limit the exertion associated with the pursuit by considering the condition of the animals, terrain, weather, snow depth, etc. Selecting the right location for a trap, orientation of the trap to the terrain and natural trails in the area, and construction of the trap itself all influence how readily animals can be brought near the trap and put into it. On arrival at a trap, animals should be only slightly to moderately winded; that is, not blowing really hard or showing signs of labored breathing or exhaustion. They should be in a condition similar to that of trail horses entering an endurance ride checkpoint, not Thoroughbreds finishing a race. They may be sweaty, as is typical following exercise, but they should not be lathered up and soaked with sweat. Recovery from the exercise associated with the capture itself should typically be within about 10 min.

Following capture, horses show more signs of being nervous or agitated compared with burros, but most settle down and start showing typical species-specific exploratory and social behaviors in their new environments within 30 min to an hour once they are left undisturbed. If animals show signs of exhaustion or distress during the capture process this should be a concern, and those supervising the activity should try to determine the underlying reason(s). These may be related to the initial condition of the animals, environmental conditions, or the manner in which the animals were captured. Modifications to the capture process such as shortening pursuit distances, breaking trails through deep snow, or limiting capture activities to a cooler part of the day can all be used to address concerns revealed by the condition of the animals following capture.

In one author's experience (Kane) with the capture of 12,297 feral horses and burros, 57 animals or about 0.5% of captured animals died due to an injury or accident during gather operations. When the rate of serious accidents and injuries exceeds this typical injury fatality rate, that is, less than 1% of the animals captured, then the environment and gather operation should be reexamined to try to identify factors that may be contributing to the higher than expected rate of serious injury. Some groups of animals may simply be more excitable than others, but increasing the use of visual barriers on gates and panels as well as modifying trap location and design or animal handling methods may also be indicated to help address higher than expected injury rates during roundups. Capture methods each have advantages and disadvantages, but the most common cause of suffering of animals during and after capture is the poor condition of the animals due to drought or starvation prior to removal. These conditions affect animal health and well-being more than any other factor and is the principle reason that a proactive approach to controlling population numbers is needed.

3. Protecting the Health and Welfare of WH&B

Seasoned by the often-harsh environment in which they live, WH&Bs are known to be tough, hardy animals that are often able to survive under extreme environmental conditions. These are not simply poorly behaved or unhandled domestic animals, but feral animals that have survived in the wild for generations. Like all equids, however, they also have their vulnerabilities. Regardless of how they came to live in their present ranges and how they may be viewed by man—wild or feral, cultural re-

source, or pest—they were put on the range by mankind and the BLM and the FS have been assigned the responsibility of caring for them and providing for their well-being. This includes keeping them healthy for their own sake as well as that of the larger equine population of which they are a part. The BLM is committed to protecting the health and welfare of WH&Bs under their care and, through an interagency agreement, the BLM conducts most operational work associated with gathers, removals, off-range holding, and adoption for the FS.

Over the past 40 years, the BLM has developed an infrastructure of WH&B specialists and veterinarians who work within the program to provide for the health of WH&Bs under their jurisdiction. Wild horse and burro specialists are typically field employees of the BLM who have a background in range management and animal husbandry. These specialists monitor the condition of the animals much like an owner or caretaker of domestic horses would monitor the health and well-being of their animals. When problems are discovered the underlying cause(s) might be addressed using their expertise in range ecology and grazing management and a cadre of veterinarians is available to assist with medical and welfare issues. On the range, during gather and removal activities, and when a veterinary assessment is needed following adoption, the BLM most often relies on a partnership with the U.S. Department of Agriculture Animal and Plant Health Inspection Service (APHIS) to provide veterinary support for the Program in the form of consultation, assessment of animals in the field, triage of health problems, and, to the extent possible, emergency first aid or treatment. There are about 30 APHIS veterinarians and another 20 or so animal health technicians who have assisted the WH&B program on various occasions over the past 15 years. One coauthor (Kane) manages the partnership between the two agencies and serves as a full time advisor for the program in the field as well as for animal health and welfare concerns that come up in relation to Program policy, training, or research that may involve WH&Bs. Within the BLM's facilities the Bureau relies largely on local private practitioners to provide the primary veterinary care for the animals in each facility. The APHIS veterinarians and epidemiologists are available in the event that an outside assessment or second opinion is needed. Following adoption, it is the new owners who are responsible for ensuring their animals receive adequate healthcare. The WH&B specialists perform post-adoption compliance visits to adopters' facilities, with routine assistance from APHIS veterinarians in some areas. APHIS is also available to provide an outside expert veterinary opinion, if necessary, in cases of suspected or alleged animal neglect or abuse. Following adoption the BLM also relies on private practitioners who are often asked to certify that adequate care has been provided to adopted animals before an adopter can obtain a certificate of title for ownership of the animal. The BLM follows up on all documented and reported cases of inhumane treatment or animal cruelty to WH&Bs that are under their jurisdiction, which includes animals that have not yet been titled or transferred into private ownership.

Health problems of animals on the range are uncommon. Wild horses and burros are robust and hardy animals, but they are still susceptible to many of the same diseases and conditions as domestic horses. The ailments commonly associated with intensively managed and housed domestic horses (e.g., obesity, early onset osteoarthritis, hoof abscesses, laminitis, colic) are rare, but injuries and disease do occur. Poorly healed injuries and manifestations of developmental orthopedic disease tend to be the most common serious conditions seen on the range. Although seemingly no more or less common in WH&Bs compared to domestic animals, flexural deformities of the lower limb (primarily club feet) and angular limb deformities affecting the fetlocks, carpus, and tarsus seem to be the most common conditions noted on the range and during gather operations in the authors' experience. Horses with club feet, in particular, seem to be overrepresented in some herds, evidence that would support the idea that the condition is heritable. It also appears that without the luxury of rest or restricted exercise for affected animals, acquired and developmental orthopedic abnormalities of the lower limbs tend to become more severe than one normally encounters among domestic stock. Animals may still be ambulatory and "get around OK" but can, for example, develop grossly deformed hooves that "mushroom" out the sides and back of a club foot so it is two to three times the size of a normal hoof. Similarly, while poor conformation and mild limb deformities are seen, severe manifestations such as 45° angular deformities of the fetlock or carpus are also seen from time to time on the range. While the causes of many of these conditions are known to be multifactorial, it is clear that genetics sometimes plays a role. The best approach to prevention is not to release animals affected by any manifestation of developmental orthopedic disease back onto the range but rather to remove them from the population.

One of the myths about WH&Bs is that they are somehow inherently more resistant to infectious disease than domestic equids, or that they do not have parasite problems because of the desert environment. Internal parasites (ascarids, Oxyuris equi, and large strongyles) are common in horses fresh off the range. Parasite burdens can be quite heavy in adolescents and adults with large numbers of adult forms passed in manure following an animal's first several deworming treatments. Surprisingly, heavy ascarid burdens are often seen in recently removed feral horses of all ages, including adults in which one might expect to see immunity.

Without question the most common and challenging health problem facing WH&Bs once they are

removed from the range and held in corral facilities is infectious upper respiratory disease. Both bacterial (*Streptococcus equi* and *S. zooepidemicus*) and viral (equine herpes and influenza viruses) agents are problems, but upper respiratory infections caused by *S. equi* and *S. zooepidemicus*, along with the less common sequelae of internal abscesses and immune mediated reaction (purpura hemorrhagica) associated with these bacteria, are most common. Exposure to both viral and bacterial respiratory agents has been documented in free-roaming wild horses and burros,[14] but exposure levels and effective contact rates for disease spread seem low in the wild. The real problems occur when animals are removed from the wild and congregated in captivity. Most of the animals are immunologically naïve to infectious respiratory disease agents when they are captured. Combined with the stress of adapting to captivity, this makes them particularly susceptible to infection. Complete isolation is very difficult in large dry-lot facilities and exposure to the agents that typically cause infectious upper respiratory disease in the domestic world seems inevitable. While vaccines for these agents are commonly used, they generally are not highly efficacious in this situation.

The best approach to controlling disease outbreaks seems to be as follows: (1) isolate new arrivals as much as possible when first removed from the wild, (2) use a broad vaccination protocol (equine herpes and influenza as well as *Streptococcus equi* as soon as possible following capture depending on animal condition, and (3) allow many of the respiratory conditions to "run their course" once they start to occur. Vaccination protocols that include periodic boosters for respiratory disease agents seem to keep outbreaks that do occur in check but, to some extent, such outbreaks seem inevitable, given the ecology of the disease agents involved and the susceptibility of the large numbers of animals that must be handled during the transition from free-roaming to domestic life. Large-scale treatment of groups of animals with sulfa antibiotics added to drinking water is sometimes used to try to decrease the spread and severity of infection, but it is difficult to know how beneficial this is in practice. Fortunately, while morbidity may be high, these conditions are usually self-limiting without treatment, serious complications and mortality due to pneumonia or internal abscesses are rare. Treatment of individual animals housed in a group setting is not without added risk and stress associated with handling, so this type of treatment is usually reserved for those individuals who appear to be suffering from complications of the upper respiratory infections.

Preventive healthcare and biosecurity measures in place at BLM facilities include managing animals in groups according to their risk and exposure profiles, to the extent possible, given the demands of moving large numbers of animals. For example, the most active centers for receiving animals fresh off the range have traditionally managed animals in groups as they are gathered from different HMAs. To the extent possible, these facilities would usually be emptied and cleaned and handling facilities would be disinfected prior to the start of each gather season. Animals removed from the range are routinely tested for equine infectious anemia and vaccinated for eastern and western equine encephalomyelitis, tetanus, equine herpes and influenza viruses, West Nile virus, *Strep. equi*, and rabies. The diagnosis and treatment of animals is performed by or under the supervision of a veterinarian and all surgical procedures are performed by a veterinarian. Specific protocols for diagnostics, treatments, and anesthesia are the responsibility of the attending veterinarian; however, it is BLM policy that all castrations be performed by a veterinarian under general anesthesia. All animals moved interstate by the BLM do so with a Brand Inspection Certificate, where required, and an Interstate Certificate of Veterinary Inspection.

Animal Welfare

Protecting the health and well-being of feral horses and burros begins with a healthy environment on the range. When range conditions provide adequate forage and water, the animals are remarkably hardy and do well, even in the harsh high desert environments typical in the Western U.S. During periods of drought or when forage utilization levels are too high, the ecology of the area suffers with decreased plant health and diversity. Ultimately, the animals suffer because of this. Excess numbers on the range above what the habitat can support is the biggest welfare problem in the Program and it has no easy solution.[15]

The natural condition where stallions, often more than one different stallion, will breed a mare several times a day throughout estrus is a very effective breeding model. Foaling rates are high. Typically, 60 to 75% of breeding age mares will foal each year and mares often foal well into their late teens and twenties. Unlike animals that may not twin or have larger litters in response to harsh conditions, horses generally invest heavily in just one offspring. In addition, where conception and birth are separated by several months, the grazing season that affects conception may be very different from that of the foaling season. Reduced fecundity is not often seen and the mechanisms of so called "self-limitation" are too often mechanisms of starvation. Starvation usually means reduced survival of newborn and nursing foals in the summer and a higher than usual die-off of older adults over the winter.

It is not uncommon for young wet mares and older horses to show dramatic changes in body condition during the course of the year. Many horses will fluctuate from a body condition class of 5 (the Henneke 1–9 scale)[16] following the peak of forage production in the spring to a body condition of 4 or even 3 in the fall or winter months, depending on drought and winter snow cover. This can represent a sea-

sonal change of 100 to 200 pounds of body weight. It is not uncommon for WH&Bs to travel long distances each day or in the span of 2 or 3 days, often 10 and even 20 miles, to and from water and forage. Horses whose body condition falls to a class of 2 or less do not survive this environment. Horses with a body condition of 3 or better can generally recover if adequate water is available so they can "tank up" and travel to reach adequate forage, if harsh environmental conditions do not persist year after year and if the demands of foaling and early lactation do not coincide with a significant shortage of water and forage. Environmental conditions, such as snow depth and the crust on fallen snow, also affect the horses' ability to survive under harsh winter conditions. The percentage of animals in a herd that might be expected to be in thin (3) or very thin (2) body condition will vary from one environment or HMA to the next. The bigger problem is that when body condition has already declined due to drought and a lack of forage or water, there has already been catastrophic damage to wildlife habitat, plant communities, and soil, particularly in riparian areas. Environmental recovery from this kind of damage—if that is even possible—can take many years and even decades. This impacts the horses, wildlife, livestock, soils, and water quality. Managing rangelands to protect the natural resources and optimize the ecological balance are the best ways to provide for the welfare of WH&Bs on the range.

BLM's Comprehensive Animal Welfare Program

Charged with the protection of WH&Bs since the inception of the Program, the BLM has always taken this responsibility seriously and worked to improve animal capture, care, and handling techniques. In late 1999 the BLM hired Dr. Temple Grandin, a noted authority on the humane handling of livestock, to review several of the BLM's corral and handling facilities as well as the handling procedures in use at the time. Dr. Grandin made several recommendations to improve the equipment and techniques used in the Program and reported to the BLM in September of 2000 that "overall handling was excellent."[b]

Since the inception of the APHIS/BLM WH&B Partnership in the late 1990s, the Bureau had used APHIS veterinarians to observe and examine animals during capture operations, particularly during high-risk or high-profile gathers such as those involving compromised animals or a significant media presence. In 2010, in response to allegations raised by the public and concerns of BLM management about ensuring that they could provide and demonstrate the best possible handling and humane treatment of animals during gathers, the Bureau made it standard practice to have a veterinarian on site during WH&B capture operations. At about this same time, the Program began the development of a new Comprehensive Animal Welfare Program (CAWP). The first steps included reviews of animal care and handling throughout the Program by outside independent observers. These included animal scientists and veterinarians organized by the American Horse Protection Association (AHPA), the Humane Society of The United States, and, most notably, a review requested by the Bureau by an American Association of Equine Practitioners Task Force. While overwhelmingly positive in their characterizations of the Program and finding that animals are handled in a humane manner by knowledgeable employees and contractors who demonstrate real concern for the well-being of the animals, the results of these reviews helped inform the public and BLM management about both the strengths and weaknesses observed regarding animal care, equipment, facilities, and handling throughout the Program. Many of the serious allegations from the public such as "stampeding animals to exhaustion" during helicopter roundups or employees and contractors being cruel and showing callous disregard for the well-being of the animals were proven false by these independent reviews.

The ongoing phase of the CAWP is the development and implementation of an animal welfare auditing program. The CAWP is "comprehensive" in that it will be implemented throughout the WH&B Program from on the range management, through gather and removal operations, through preparation for adoption and holding of animals in facilities and long term pastures or eco-sanctuaries, and through adoption and post-adoption compliance. This approach, similar to that used in other livestock industries, starts by identifying and establishing best practices and standard operating procedures (SOPs) for all aspects of animal care and handling throughout the program. This is done by reviewing what currently works well and what doesn't within the Program and by using outside experts in animal handling and welfare to address areas that can be improved. Training of BLM employees and contractors is provided where needed and clear guidance and policy established throughout. The monitoring or auditing phase of the CAWP involves periodically applying a Welfare Assessment Tool to measure and record compliance with the established standard operating procedures and demonstrates the BLM's ongoing efforts and commitment to ensuring humane treatment in a transparent manner for the public. Throughout the process, areas that can be improved will be identified and addressed to ensure the best possible care and humane treatment of animals throughout the WH&B Program.

4. Mustangs as Saddle Horses and Adoption Partnerships

Once considered untrainable renegades, mustangs have received greater acclaim as intelligent, loyal, trainable saddle horses over the past decade. This is due to a number of factors, including better mar-

keting, popular high profile events featuring knowledgeable trainers, improved training techniques, and prison inmate training programs geared specifically towards mustangs. The BLM has been working with state prison programs since 1986 in an effort to make mustangs more adoptable by offering some level of training prior to adoption. Recently, the expansion of prison programs is being explored, particularly in the eastern U.S. In the earlier years, horses of any age were gentled, halter-trained, and then offered for adoption. While there was success in training the younger horses, the older horses were not as amenable to training and success for the older ones was not nearly as frequent. At the time the prison training programs began, the BLM had thousands of mustangs in corrals and there was just one long-term holding pasture available. The prison training programs, coupled with alternative adoption methods and some policy changes, were successful in reducing the backlog of unadopted horses to the point that the BLM withdrew most funding for these programs in 1992. Realizing the rehabilitative benefits to inmates, Colorado's program continued to operate on a limited basis by offering saddle training services for a fee to adopters. Funding to operate the program was no longer provided by the BLM but was primarily derived from training fees charged to adopters.

In the late 1990s, the BLM began a concerted effort to maintain wild horse and burro numbers within the AML. Many more mustangs were removed from the range than could be adopted and the BLM again had to consider alternatives for holding or adopting these horses. Short-term holding facilities were expanded, long-term pastures were leased, and state prisons once again were solicited to help with holding and adopting mustangs. The BLM now has agreements in place with prison facilities in five states: Canon City, Colorado; Hutchison, Kansas; Carson City, Nevada; Gunnison, Utah; and Riverton, Wyoming. Collectively, these facilities train and adopt approximately 265 mustangs each year. The programs are not funded with state taxpayer dollars but are operated through correctional industries, which is the profit-making arm of state prisons. Programs within correctional industries must at least break even financially in order to stay in operation. The inmate training programs in these states differ in how they are financially structured, but all of the programs pay some of the cost in caring for and training the mustangs. These costs are either recouped directly from the adopter or paid for by the BLM and recouped through higher BLM adoption fees for saddle-trained mustangs. Sharing the financial burden for care and training creates an incentive to produce a quality saddle trained mustang as efficiently as possible. In Colorado, correctional industries pay for the feed and training and then recoup that cost from the adopter. In this case, the majority of the fee paid by adopters (this usually starts at around $1000 but may be higher at adoptions with competitive bidding) is paid to the prison for training and the standard $125 adoption fee is paid to the BLM. In order to better match an adopter with the right horse, saddle-trained mustangs are only offered for adoption when their training is complete. Most finished saddle horses are adopted from the facility within two weeks; others may be offered through competitive bidding at special adoption events.

In addition to inmate training programs, there are a couple of other notable training programs that operate in partnership with the BLM. The Mustang Heritage Foundation, created by the BLM in 2007 and funded primarily through the BLM to market and adopt mustangs, has been responsible for promoting the trainability of mustangs through several different venues, including the Extreme Mustang Makeover Events and a Trainer Incentive Program. Mustang Makeover events are held in several states each year and have been a big hit with adopters and the general public. Prospective trainers are assigned a mustang from a BLM holding facility and have 90 days to train it. At the end of 90 days, a competition is held, prizes are awarded, and the mustangs are offered for adoption to the public in a live auction. Trainers are given a stipend to help with transportation and care and also receive a portion of the adoption fee, which usually averages over $1000. The Trainer Incentive Program recruits prospective trainers to find adoptive homes for mustangs after providing some level of training. Trainers are reimbursed for expenses after the horse is adopted. Working with the BLM, the Mustang Heritage Foundation has helped adopt nearly 5000 of the 23,000 total animals (21%) adopted from the BLM in the past 7 years. The BLM has also used private contracts to train and adopt mustangs to the public. The Mantle Ranch Wild Horse Adoption and Training Center, located in Wheatland, WY, has been in operation since 1998. The center, which is paid a fixed amount for each adoption, offers training services and trained horses to adopters, clinics on wild horse handling and gentling techniques, and operates a year-round facility open to the public for adoptions during special events and by appointment. On average, the Mantle Ranch adopts about 75 mustangs annually and has placed over 1,200 animals into good homes since it opened.

Since the inmate programs share some of the financial risk in training and adopting saddle-trained mustangs, the horses selected by the staff must be readily adoptable at a higher fee compared to an untrained mustang. Allowing prison staff to select the horses for training promotes buy-in among the staff and management for the training and adoption process. This encourages good customer service, which, in turn, creates a higher adoption demand for the saddle-trained mustangs. While each staff trainer may differ some in their selection criteria

depending on the local market, the basic selection criteria include the following:

- Geldings are preferred over mares because from a marketing perspective, most adopters want geldings.
- The best age for saddle training is 3 to 4 years old. Horses are physically able to handle the rigors of training at this age. Since many of the mustangs have been in holding facilities for a couple of years and are accustomed to human presence, sometimes 5- to 6-year-old mustangs are also selected for training.
- The horse should be around 15 hands or taller when mature. In this author's experience (Ackley), only about 15% of the mustangs in the BLM's program will attain this height. Again, this is a market-driven criterion. While there is some demand for shorter horses, most adopters look for a horse of this size. A three-year-old mustang will likely gain another 1 to 2 inches in height by the time it is fully mature.
- No blemishes or obvious conformation faults are evident.
- Rounded hips and well-developed withers that will hold a saddle are preferred.
- The temperament must be suitable for training. Neither high-headed flighty horses nor pushy disrespectful horses are desirable.

Colored horses such as pintos, palominos, buckskins, etc., are occasionally selected for training but color is not a good criterion for selection at the expense of the other previously mentioned characteristics. Disposition, conformation, and intelligence are the primary factors. In general, adopters that spend more money on a saddle-trained mustang are more concerned with these attributes than with color.

After the initial selection, horses are started in a round pen and worked for 2 to 3 weeks or less. If a horse's temperament is suitable the training will continue. If the temperament is not suitable the horse is given back to the BLM and will eventually be sent to a long-term pasture. By utilizing the previously identified selection criteria, the success rate for mustangs in training is about 95%. Regardless of how good the temperament might be, saddle-trained mustangs are not recommended for children or inexperienced riders working without experienced coaching. This is simply a function of age, not breed. The training process normally takes 3 to 4 months, depending on the weather, horse temperament, and other factors. If there is any lameness or physical problem not previously observed, it usually manifests at some time during training. Towards the end of training, wolf teeth are extracted and teeth are floated if necessary. The horse must meet certain training objectives before being considered finished and available for adoption. These include things like being caught, haltered, picking up feet for trimming and shoeing, standing during saddling and mounting, changing gaits on command, simple lead changes, side passing to open a gate, dragging a log, allowing a rope to be thrown, and loading into a stock or slant load trailer. When the horse and trainer have satisfied these objectives, the mustang is offered for adoption either at an event or through the local website. Finished horses are usually adopted within two weeks. Other than the U.S. Border Patrol, most horses that go through the Canon City program are adopted for use as trail horses.

The most obvious benefit to the BLM and taxpayers is that more mustangs are adopted through the training programs, which results in reduced holding costs and allows the BLM to better manage rangelands by removing animals when necessary. However, there are indirect benefits as well, such as the following:

- Adopters have more money invested in the saddle-trained mustang, which usually translates to a higher level of commitment to the animal. Additionally, there are fewer compliance problems and facilities are of better quality.
- Trained mustangs have a better chance of success and a productive quality life with the adopter.
- Saddle-trained mustangs fill an additional market niche. Most adopters of saddle-trained mustangs would not adopt an untrained mustang.
- The training programs provide a rehabilitative work experience for inmates. Successful inmates develop good work ethics, derive a sense of pride and accomplishment by training a mustang from start to finish, and perhaps learn to overcome fear in a nonviolent manner. The programs provide a useful, meaningful, and productive way for inmates to serve their sentence to the benefit of the BLM and adopters. The skills that are learned can be utilized by inmates once their sentence has ended and they are looking for employment.
- The training programs promote the mustangs as sound, intelligent, durable, riding animals that can be used in a variety of equine disciplines.
- The prisons offer around-the-clock free security for the horses and burros housed there.
- The prisons provide an adoption venue that is open to the public (with certain restrictions). This varies from state to state, but in Colorado, adopters are allowed to visit the facility two days each month.

Adoption Partnerships and Who Adopts Trained Mustangs

Most of the trained mustangs are adopted by the public for trail riding, packing, and other recre-

ational uses. A few go to guest ranches, outfitters, and guides. Several are adopted each year to organizations that utilize horses for their therapeutic value in treating emotional problems.

In Colorado, over the past twenty years approximately 80 trained mustangs have been adopted to the Westernaires. This is a mounted precision drill-team organization composed of roughly 1000 children from 9 to 19 years of age. Since 1949, Westernaires has been thrilling spectators around the state and the west with their high-speed precision riding. Many mustangs adopted by the Westernaires are used in these riding exhibitions and an equal number of trained mustangs have been adopted by families that are members of the Westernaires. Trained mustangs have also been adopted to other federal agencies, including the U.S. Army at Ft. Carson, CO, the U.S. Marine Corps in Camp Pendleton, CA for their color guard units, the U.S. Park Service, and the U.S. Forest Service.

The largest adopter of saddle-trained mustangs has been the U.S. Border Patrol. Project Noble Mustang was initiated by the Colorado Wild Horse Inmate Program, the BLM, and the northern Border Patrol in 2007. Initially, the mustangs were adopted to help with Border Patrol recruitment efforts through attendance at parades, rodeos, and other events. As their success with mustangs increased, they began adopting and utilizing mustangs for mountain patrol. They had been leasing horses but decided that adopting their own would cost less. Mustangs are now patrolling the border country of Washington, Idaho, and Montana. Their focus is on monitoring smuggling activities in the mountainous border areas that cannot be reached other than by horseback and where wilderness designations preclude the landing of helicopters. Project Noble Mustang's success culminated with a trip to the 2009 inauguration parade in Washington, D.C. Ten mustangs trained at the Colorado Wild Horse Inmate Program, representing six BLM herd management areas, as well as the U.S. Forest Service, rode through the streets of Washington, D.C. on a very cold blustery winter day. Several of the mustangs had been picked up from the Inmate Program less than six months prior and some were ridden by dignitaries that had little, if any, previous riding experience.

As the success of Project Noble Mustang spread throughout the Border Patrol community, agents from the southern states began to inquire and the focus shifted to providing horses to them. With approximately 350 horses in service, the Southern Border Patrol has a much larger horse patrol function. Border Patrol horses are worked hard and regularly, making the mustangs a good fit for their needs. With 350 horses in service, there is a constant need to replace retired stock. Mustangs on the southern border are used not only for routine patrol and apprehension of illegal immigrants but also to counter smuggling operations for drugs, humans, and other contraband. Since Project Noble Mustang began in 2007, the U.S. Border Patrol has adopted 220 saddle-trained mustangs from inmate training programs in Colorado, Kansas, and Nevada. These mustangs are now helping protect the nation's borders in the three northern states mentioned as well as California, Arizona, New Mexico, and Texas along the southern border.

Collectively, inmate training programs, the Mustang Heritage Foundation, and the Mantle Ranch Wild Horse Adoption and Training Center have been responsible for the adoption of over 1100 trained mustangs annually. All have worked to raise the public awareness and value of mustangs by demonstrating that mustangs are intelligent, trainable, versatile, sound and durable horses, and that adopters are willing to pay for quality training.

Acknowledgments

Conflict of Interest

The authors are employees of the U.S. Department of Interior, BLM (Guilfoyle, Warr, Ackley) and the U.S. Department of Agriculture, Animal and Plant Health Inspection Service (APHIS), Veterinary Services (Kane). Dr. Kane serves as an advisor to the WH&B Program and is supported by funding provided from the BLM to the USDA APHIS.

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Review of Fertility Control Research

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1. Introduction

A considerable amount of research and expertise has been directed toward fertility control in domestic and wildlife animals, including free-ranging horses, over the past 30 years. The purpose of this review is to assess current fertility control methods for free ranging feral horses. While methods for fertility control serve as an attractive alternative for reducing large-scale population growth rates for free-ranging or semi-captive feral horses throughout the United States, it also serves as an alternative means of maintaining limited equid population growth in smaller-scale scenarios. Because of the large number of published reports on contraception in free-ranging horses and its related issues, this review will attempt to focus on published reports salient to how we have arrived and where we are today with available fertility control agents. Any failure to cite other relevant reports is not intended. A more detailed and comprehensive review of the literature can be found in the National Research Council (NRC), 2013, "Using Science to Improve the BLM Wild Horse and Burro Program: A Way Forward."[1]

Despite accomplishments in the development of fertility control agents for horses, an optimum contraception strategy has yet to be achieved for wildlife suitable for management of large population considerations. A primary difficulty in the development of the optimal fertility control agent for large scale population reduction may be due to the failure of current methods to meet suitable management strategies on a large-scale basis and to meet the requirements set forth by regulatory agencies responsible for the horses and their stakeholders. Historically, the requirements set forth to limit populations of free-ranging horses suggest that agents used for fertility control must adhere to the following criteria: 1) be reversible and temporary, 2) the strategies used for contraception must be noninvasive procedures, 3) have no adverse effects (long-term or short-term) on targeted animals, 4) contraception agents capable of passing along the food-chain (predatory/prey) are not acceptable, 5) behavior must not be altered, 6) social structure and band integrity must not be interrupted, 7) a single injection achieving long-term contraception is essential for management considerations, and 8) it must be practical and economically suitable for ongoing management strategies of large-scale populations of free-ranging horses. These requirements justifiably were designed to ensure the overall management of reducing large populations of wildlife where necessary and ensure the humane treatment of the targeted animal while maintaining its natural state as much as possible. Recently, however, the overwhelming need to limit population growth rates has forced management agencies to rethink some of the limitations previously prescribed for fertility control agents. Longer lasting and permanent contraceptive agents are now of interest to managers of wild

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and feral animals. While protection of the food chain, the environment, and the welfare of the animals are still primary concerns, some agencies have come to accept that some side effects on social organization, behavior, and even the animals themselves (such as injection site reactions) may be unavoidable. As long as these effects do not have an adverse impact on animal welfare, they are now regarded by some as unfortunate but necessary for the application of effective fertility control on a large scale.

Several agents designed for use as fertility control agents in free-ranging horses are identical to those used for domestic horses to suppress estrus behavior. Therefore, it is worthy of clarifying that the formulations of the agents used to suppress estrous behavior and those used as fertility control agents are strikingly different, and its intended use and the desirable length of effectiveness varies considerably.

For horses, there are several chemical agents suitable and capable of inhibiting fertility. However, the use of these agents is dependent upon the reason(s) for their use and to which population of horses they are intended. While the majority of agents used in domestic horses are targeted primarily toward suppression of estrus behavior, the agents do disrupt cyclicity and, thus, could be considered a form of indirectly inhibiting fertility. For example, exogenous progesterone/progestins as well as gonadotropin-releasing hormone (GnRH) analogs are commonly used to suppress short-term estrus behavior in domestic mares. The agents have also been investigated for long-term contraception use, albeit in different formulations, in free-ranging horses and will be discussed later.

Agents used in domestic horses to suppress estrus behavior may also indirectly serve as agents for inhibiting fertility by prolonging the corpus luteum. Examples of agents used for extending the corpus luteum include administering 1500 to 3000 IU of hCG when a 30 mm follicle develops during the luteal phase of the estrous cycle, thus, inducing a luteal phase ovulation and extending the luteal phase of the estrous cycle.[2] The use of 60 units of oxytocin daily before day 10 after ovulation can also extend the luteal phase of the estrous cycle for at least 30 days.[3] Another method used to enhance prolonged endogenous secretion of progesterone is the insertion of a sterile 35 mm glass marble ball[4] or 20 mm water- filled propylene ball into the uterine lumen to mimic pregnancy in the mare.[5] In these two studies, the persistence of corpus luteum lasted for 60 to 90 days if the devices remained in the uterus of the mare (approximately 50% were spontaneously expelled) with no reported evidence of adverse effects on the uterus and mare.[4] Although these methods have primarily been investigated for use solely for estrus suppression in domestic horses and can also be considered an indirect form of inhibition of fertility, the present format would not be suitable for contraception use in large scale wild horse populations due to 1) the extensive monitoring required to ensure the correct timing of the estrous cycle when administering the agent, 2) the requirement for multiple injections or applications, and 3) the relatively short period of the effects.

For longer-term contraception targeted for large scale population use, several chemical agents have been investigated in stallions and mares.

2. Fertility Control in the Male

In earlier studies, testosterone propionate was investigated for use in free-ranging horses. Several studies using various doses of testosterone propionate (200 μg to 1.3 g) reported successful attainment of oligospermia and reduction of foal crops in treated harems.[6] However, multiple doses were required in the majority of the studies, and the treatment was recognized as impractical for large-scale management purposes. GnRH vaccines have also been tested and reported in the stallion.[7–10] These reports demonstrate the effectiveness of GnRH vaccines to significantly reduce sperm quality while changes in behavior varied. However, multiple doses are also required during treatment, and in some studies a complete removal of sperm production was not evident.[9] Use of GnRH agonists in stallions has also been reported in earlier studies.[11–14] It appears that only transient effects were found following its use, and it has been postulated that perhaps larger doses may be required for a desired effect.

Vasectomy

Surgical vasectomy has been performed and reported in free-ranging horses.[15,16] Dominant stallions were used in these studies, and the results were successful in reducing foal production, particularly in bands where subordinate stallions were not present. However, foal production was eminent in bands where subordinate and bachelor stallions were present. While surgical vasectomy of stallions may not be a practical or economically feasible method for reducing large-scale populations of free-ranging horses, clearly, vasectomy of stallions, if performed in feral horse herds, should be inclusive of dominant, subordinate, and bachelor stallions, and a very large number of stallions being treated are a must in order to have any impact on foal production. Scientific scrutiny must provide evidence of its long-term effectiveness prior to its acceptance for large-scale use. Potentially, vasectomy, whether used chemically or surgically in a large number of stallions within select herd management areas, may have a deleterious effect on the genetic diversity of the herds. Surgical and chemical vasectomy has been investigated on the Sheldon Wildlife Refuge in the Western states. While the surgical procedures were thought to be successful, the vas deferens of the animals injected with a chemical vasectomy agent remained patent when examined some time later (Kane, personal commu-

nication). Chemical vasectomy is a minimally invasive procedure, and although it would more than likely be considered a sterilant if performed successfully, it may be an attractive alternative to limiting foal production in select areas where free-ranging horses are present and stallions thought to be a limiting factor. Development of chemical vasectomy, if proven successful and applied, will inevitably raise issues of stallion-targeted fertility control such as which and how many stallions will be required to have an effect on large-scale population dynamics and whether long-term effect on behavior or genetics will occur.

3. Stallion Versus Mares as Targets for Contraception

Controversy exists as to whether the male should be targeted rather than the female for large-scale population reduction. It is the author's opinion and speculation that the development of a chemical agent of fertility control in stallions is more than likely to have an effect on sexual behavior over time and is dependent upon the temperament and experience of the stallion. Any effect on sexual behavior in free-ranging dominant stallions could/would be an invitation for bachelor and subordinate stallions to meet the challenge and/or for mares of the affected stallion of a herd to wander during estrus. Additionally, a single fertile stallion (dominant, subordinate, or bachelor) may be capable of impregnating approximately 40 mares per year, although this scenario of access to mares would be unlikely in free-ranging horse herds. Regardless, in view of the number of bachelors available in free-ranging horse herds, it would require treating the majority of stallions (dominant, subordinate, and bachelors) in a given territory to have any effect on reducing foal crops for a given year. Would this have an effect on genetic diversity? What percentage of the stallions would be required to be treated to have an effect on population growth? Is this strategy feasible from a management perspective? These are a few of the many questions that require further investigation. Modeling studies may help answer questions of whether inhibition of fertility to favor males or females, or a combination of both, would be the best measurements of limiting foal production on a yearly basis. However, accurate data on population demographics and dynamics is required to inform and validate these models before they are truly useful.

4. Fertility Control in the Female

A number of agents for use as contraceptives in female equids have been studied. The agents comprise of steroids, GnRH agonists, immunocontraceptives, and intrauterine devices.

Intrauterine Devices

Several forms of intrauterine devices have been investigated in the horse. Presumptively, the mechanism of action in horses is to incite a low grade endometritis, with the devices serving as a nidus, stimulating persistent inflammation within the uterine lumen as long as the device remains in the uterus. Persistent low grade endometritis represents a hostile environment within the uterus and is incompatible with survival of the embryo. Flexible, silastic O-ring devices were inserted into the uterus in six domestic mares.[17] The mares cycled normally when monitored by palpation and ultrasound. None of the mares conceived throughout one breeding season but all conceived after removal of the intrauterine devices. Another study examined the effectiveness of a copper T shaped intrauterine device in 15 feral mares over a three year period.[18] The mares were exposed to fertile stallions for 60 days and found 20 percent of the treated mares to have conceived compared with 75 percent of the controls in the first year of the study. However, 75% to 80% of the treated mares were found to be pregnant over the next two years with no evidence of the intrauterine device being present in the pregnant mares. The authors speculated that the intrauterine devices were spontaneously expelled from the uterus of treated mares after the first year. As mentioned previously, the insertion of glass marbles and water-filled propylene balls into the uterus of mares may also be considered as intrauterine devices, and they may also have an effect on fertility. However, the objective of these two investigations was aimed at estrous suppression and not long-term contraception. Subsequent and consistent losses over time of glass marbles inserted into mares when housed with stallions suggests that intrauterine devices may not serve effectively as a long-term contraceptive strategy for large-scale population growth reduction.

Estrogens and Progesterone

In several earlier studies, the use of native estrogen and progesterone as long-term contraception agents in horses proved not to be as successful as expected.[19,20] It is speculated that its failure to achieve a desired effectiveness was due to the rapid metabolism and enzymatic degradation of the native steroids and that significantly and impractical higher doses were required for a desired effect. These earlier studies led to the successful use of synthetic estrogens and progesterone or its combination thereof for contraception in mares. Formulations of the synthetic steroid ethinyl estradiol and natural progesterone into silastic implants for long acting effects have been reported.[21,22] The authors report that contraception effect from intraperitoneal-inserted implants in treated mares was 85% to 100% effective for 2 to 3 years. In another study by one of the investigators, ethinyl estradiol implants alone, when inserted into the peritoneal cavity of mares at concentrations of 1.5 g, 3.0 g, and 8.0 g, yielded a 75% to 100% contraception rate based on the rate of decline of plasma ethinyl estradiol.[23] Despite the successful outcome and relative inva-

siveness of the procedure, no data were collected on the behaviors of the treated mares, which may have potential adverse effects on band integrity and social organization. Thus, its acceptance for large-scale use in wild horse populations is questioned. Additionally, the use of steroids (synthetic or natural) as a contraceptive agent in feral horse populations may provide potential risks of passing along the food chain and may not be acceptable by regulatory agencies.

Progestagens

A significant number of studies have been reported on treatment of mares with progesterone or its synthetics. However, the majority of the studies were aimed at manipulation of the estrous cycle for short-term control and ovulation in domestic mares. Although synthetic progestagens formulated in implants are successfully used in zoo environments and in women, multiple injections and monitoring of the estrous cycle is required for beneficial effects and, thus, may not prove useful for fertility control in large-scale feral horse populations. One study using native progesterone (8 or 24 g) in silastic implants proved unsuccessful albeit estrous behavior was suppressed and ovulation and conception resulted from the treatment group.[22] At this time, altrenogest[a], a consistently effective progestagen for suppressing reproductive function, appears to be the only progestagen approved for use in domestic mares. The effect of altrenogest on reproductive function persists for approximately 30 days. Its potential as a contraceptive for fertility control in feral horse populations may warrant further investigation. However, side effects have been reported after long-term use in domestic mares such as clitoromegaly[24] and compromised foals at birth[25] and may require alternative formulations for use in large-scale feral horse population control.

Gonadotropin-Releasing Hormone Analogs

Gonadotropin-releasing hormone, produced by the hypothalamus, is the master control of reproductive function in the male and female. Upon production and secretion, GnRH stimulates the pituitary gland to produce follicle-stimulating hormone and luteinizing hormone, which in turn is responsible for follicular development and ovulation. Inhibition of GnRH with synthetic or native analogs prevents reproductive function through the absence of follicular growth, oocyte maturation, and subsequent ovulation. (Fig. 1B)

Several formulations of GnRH have been investigated for use as potential contraception agents in the horse. Many other formulations, however, have been tested for use in the manipulation of the estrous cycle and ovulation in domestic mares and may not be relevant in this review. In one of the earlier studies aimed at contraception, immunization of domestic mares against GnRH conjugated with human serum albumin resulting in high variability of response and antibody titers.[27] However, three of the five inoculated mares ceased ovulation for a period of 4 months. Another study using GnRH conjugated to keyhole limpet hemocyanin and triple adjuvant in 29 feral mares showed no difference in foaling rates compared with controls following treatment.[28] More recent work with GnRH[b] as contraception agents in feral horses demonstrated that 93%, 64%, 57%, and 43%, after the first, second, third, and fourth year respectively, were not pregnant when the mares were inoculated with GnRH conjugated to keyhole limpet hemocyanin.[29] Another formulation of GnRH[b], was investigated in 24 free-ranging feral horses. In this study, 61%, 58%, and 69% in years 1, 2, and 3, respectively, were found to be not pregnant over a three year period.[30] Both investigations used the GnRH vaccine[b], and a M. avium adjuvant[c].

Studies using other GnRH conjugated formulations[d,e] also inhibit cyclicity in mares, and there are considerable variations as to the length of estrus and follicular suppression among these studies.[31–34] Inhibition of estrus suppression, cyclicity, and follicular development varied from 4 to 100 weeks. Formulations of GnRH vaccine for use in contraception, although limited in published reports in horses, appear at this time to be a potential GnRH formulation for the application and management suitability of fertility control for the large-scale reduction of feral horse populations.

GnRH Agonists

Actions of GnRH agonists on reproduction function in the mare are similar to those of native GnRH. The most widely used GnRH agonist is deslorelin acetate and is formulated as implants for subcutaneous insertion. Although the agonist implant[f] is designed specifically to induce ovulation in the cycling mare, another injectable[g] was developed for contraception use in other animal species. The injectable form[g] is commonly used as a contraception agent in captive wildlife and is designed to be effective for 12 to 18 months. However, the implants have not been tested in horses specifically for contraception to the author's knowledge. Despite the requirement for multiple injections to achieve a long-term desired effect, the injectable form[g] may serve as an attractive potential for long-term contraception in horses if reformulated as a single injection agent.

Porcine Zona Pellucida-Based Vaccines

Porcine zona pellucida (pZP) is the acellular membrane that surrounds the oocyte. This membrane is responsible for sperm binding through receptors, penetration and prevention of polyspermy, and is a prerequisite for fertilization in the horse and many other mammalian species. Inoculation of porcine zona pellucida, a similar but heterogenic set of proteins, into mares elicits an antibody response, which prevents the binding of sperm to sperm receptor

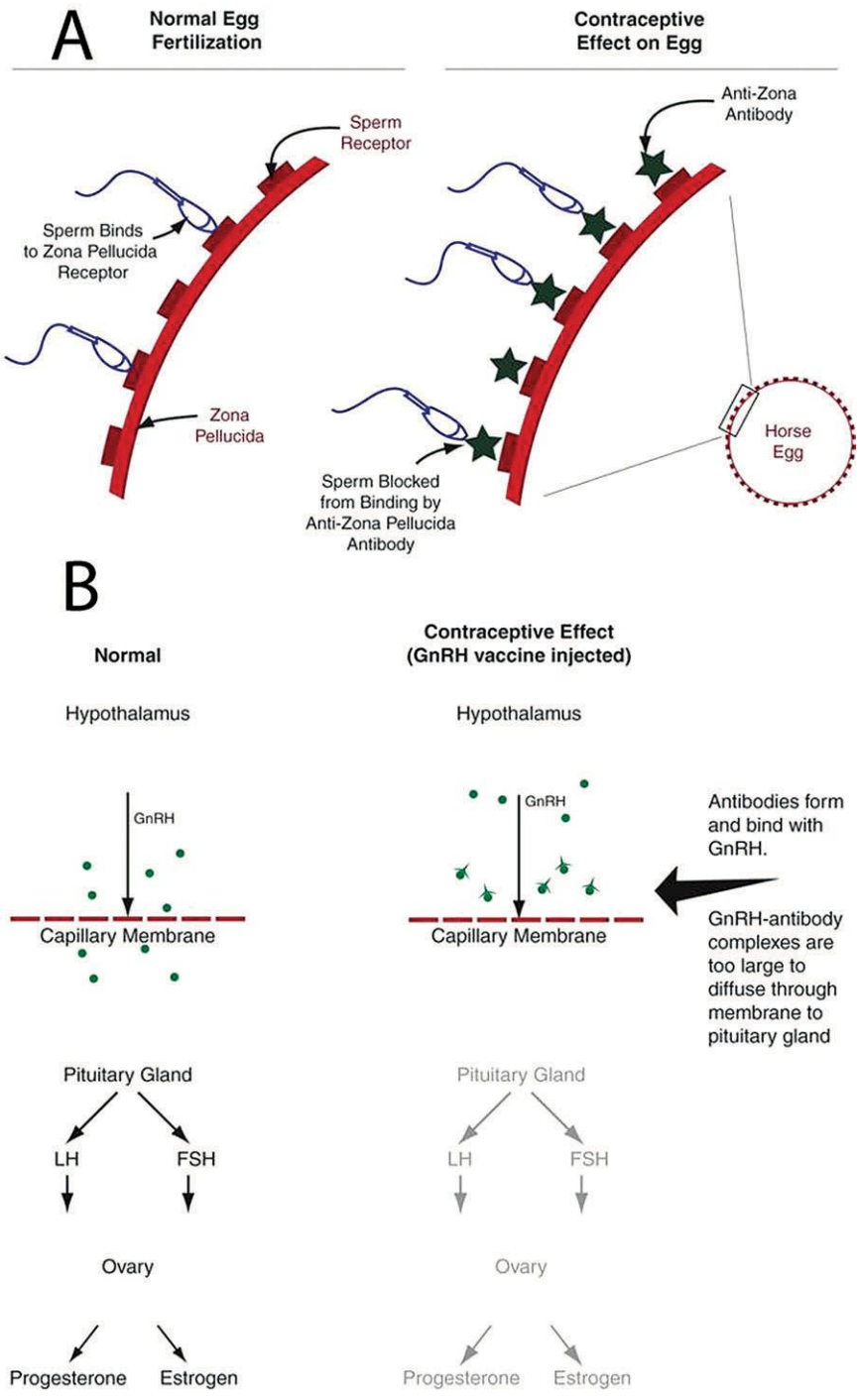

Fig. 1. A, Mechanism of the action of porcine zona pellucida in mares. Normal and inhibition of sperm binding by anti-zona pellucida antibody.[1] B, Mechanism of action of gonadotropin-releasing hormone. Normal and inhibition of GnRH function by anti-GnRH antibody.[1] Adapted from Asa et al. (1996).[26]

sites on the zona pellucida of horse oocytes. When binding of sperm is inhibited, fertilization is prevented (Fig. 1A).[35]

Porcine zona pellucida-based vaccines have been the most extensively studied contraception agent in free-ranging horse populations. Initial studies on the successful use of pZP in wild horses was reported in the late 1980s, and progress in its development for optimal use in the management of free-ranging horses has been slow. There are currently three formulations of pZP-based vaccines used under experimental conditions in an effort to find a suitable

formulation to meet the needs of large scale reduction of free-ranging horse populations. Although all three formulations are pZP-based, two formulations, a liquid pZP emulsion[h] and pZP-22 (a pelleted form of pZP) use pZP harvested from pig ovaries similarly whereas a third pZP-based vaccine[i], is produced slightly different. Additionally, pZP incorporated in liposomes[i] incorporates a diffusion-release mechanism (lipid bilayer liposomes). PZP-22 uses liquid pZP[h] as a primary inoculation and pellets containing pZP incorporated in d-lactide-glycolide copolymers as a controlled, time-release mechanism and QA-21 as the adjuvant for long-acting, booster effects when administered as a paired injection. Liquid pZP[h] is not formulated with controlled release mechanisms and must be administered on a yearly basis to maintain long-term contraceptive effect. The differences in methods of production of pZP from pig ovaries as well as the formulations for long-term effects may be related to the differences in results obtained when used as contraception agents. In particular, the differences between long-term and adverse effects are evident when pZP incorporated in liposomes[i] is administered in comparison with liquid pZP and pZP-22. In general, the major differences are related to effects on follicular development, uterine pathology, and reversibility post-inoculation. Reductions in the number and sizes of follicular development and persistent uterine edema have been reported after the administration of alternate formulations of pZP incorporated in liposomes[i].[29,36] In addition, evidence of reversibility of the contraceptive effect after the administration of pZP incorporated in liposomes[i] have not been consistently observed or reported. Although oocyte evaluation was not performed, the use of liquid pZP[h] also reported a decline in ovarian estrogen production and ovulation rates in treated mares after 3 to 7 years of consecutive yearly injections of the agent. The rate of decline for ovulations reached 10% after seven consecutive years of treatment. In this study, the absence of ovulation was determined by an assay for nonspecific progesterone metabolite iPdG assay from fecal samples.[37] No effects on cyclicity in the mare have been reported as yet after the use of pZP-22. However, further investigations are required to evaluate its effect on cyclicity in the mare post-injection. It is speculated that disruption of cyclicity of mares post pZP treatment may be 1) due to the presence of other ovarian proteins present in the inoculate, in view of the manner in which pZP is currently being processed and used and 2) additional mechanisms of infertility induced by pZP other than blocking of attachment of the zona pellucida by steric hindrance.

Injection site abscesses have also been reported following the use of liquid pZP[h] and pZP-22.[38] In this study, significantly large numbers of abscesses, nodules, and transient swellings were found in mares when pZP was remotely delivered with a rifle. Hand injection of the agent, in contrast, produced minimal numbers of presumptively granulomatous nodules at the injection site.

The contraceptive effectiveness of pZP-based vaccines vary considerably throughout the literature and may lead to much confusion among the reading audience. In general, however, pZP-based vaccines are considered to be 85% to 100% effective for the first year. With long-acting, single injection formulations, the effectiveness begins to decrease after the second and third years at a range of 83% to 85% and 68% to 85%, respectively.[29,39] Unfortunately, recent unpublished data using pZP (pZP-22 and pZP incorporated in liposomes[i]) designed for long-acting contraceptive effects as well as a GnRH vaccine[b], have provided evidence of suboptimal and inconsistent results compared with those previously reported (Kane A, personal communication). Variations in results using similar or modified formulations of pZP-based agents may be attributable to a host of factors, such as delivery method (hand injection, jab-stick injection, remote delivery), alteration of formulations for long-term effects, adjuvant modifications, methods for monitoring, personnel administering of the vaccine, health and nutritional status of the mares, accurate identity of the vaccinated mares, and other uncontrollable factors in free-ranging horse management that may influence or limit the outcome of obtaining optimal and consistent results.

Many studies have emerged and have been reported in the literature on the successful use of liquid pZP,[h] as a fertility control agent. However, a major disadvantage of this pZP formulation is that it requires repeated injections on a yearly basis to maintain contraceptive effect in the mare. From a free-ranging, feral horse management perspective, the necessity to inoculate any mare on a yearly basis to achieve effective contraception on a long-term basis would likely be considered impractical. Liquid pZP[h] and pZP-22 have demonstrated safety for use in mares with no apparent short term, adverse effects with the exception of limited nodule formation at the injection site post-treatment.[38,39] Use of liquid pZP[h] after several consecutive years has also been reported to reduce ovarian estrogen production and ovulation in treated mares as mentioned previously. Despite these disadvantages, liquid pZP[h] and pZP-22 is safe for use in pregnant mares,[40] evidence for reversibility after inoculation has been reported,[41] pZP in inoculated animals will not pass along the food chain,[42] and there are no detrimental behavior changes in inoculated mares.[43,44] Additionally, it has been shown that mares inoculated with liquid pZP[h] on a yearly basis improve the overall body condition and longevity of the mares.[45]

Concerns that pZP inoculated mares may cause an extension of the breeding season have been raised. The treated mares' failure to conceive but continue to cycle normally, thereby causing excessive out-of-season activity of stallions and the production of out-of-season foals past the normal

physiological breeding and foaling season are controversial and not clearly defined. Photoperiod via the retinohypothalamic tract is a major regulator of the physiological breeding season in mares, as well as in stallions. Cyclicity and follicular development in approximately 70% to 80% of domestic mares and receptivity to the stallion decreases significantly as day length (northern hemisphere, 38–43° N) shortens toward the end of the physiological breeding season.[46] It is the author's opinion that it is unlikely that there are any physiological differences in photoperiod receptivity in feral, free-ranging mares, and thus, out-of-season breeding activity may not be a consistent finding among pZP-inoculated mares despite treatment. Other factors also play a role in influencing seasonality in mares such as temperature, age, nutrition, lactation, and stress. These modifiers of seasonality would seem to be more prominent and critical among free-ranging horse mares than domestic mares and may have a stronger influence or larger impact on seasonality of the estrous cycle in the majority of wild horse mares.

At this writing, while short-term contraceptive agents are available, there is no ideal contraceptive agent that meets the needs of controlling overpopulation of free-ranging horses. Although presumptively promising, limited information is available on long-term fertility control agents such as pZP-22, pZP incorporated into liposomes[i], GnRH and chemical vasectomies. Ongoing preliminary studies have indicated inconsistent findings among all fertility control agents tested to date. Critical to science is consistency in the repeatability of results, and as such, current usage of long-term contraceptive agents in large-scale population reduction of wild horses is not feasible. In view of the absence of an ideal contraception agent, free-ranging horse populations continue to increase at alarming rates—rates concerning enough to entertain the possibility of incorporating sterilization modalities in select groups within a population. If sterilization of select groups of mares and stallions is, in part, an end goal of reducing feral horse populations, formulations in the development of current contraception vaccines may require significant modifications, or other alternative methods will be required to achieve that end goal. From the author's perspective, a perspective that may not be shared by all, sterilization of a select group (older mares) of overpopulated wildlife may have several advantages. Firstly and although there are many anecdotal reports of exceptions, the fertility life of a domestic mare is generally accepted to decline at approximately 15 to 16 years of age. More than likely, the decline in fertility is at a younger age in free-ranging horses in view of the relative hostile environment a free-ranging mare is subjected to when compared with domestic mares. Pregnancy (at least in horses) takes a significant toll on a mare's well-being due to energy demands required to maintain pregnancy and lactation. Additionally, the incidence of uterine pathology in the mare increases with multiple births and age. Body condition scores and longevity increases in mares that have been contracepted for multiple years using yearly injections of a contraceptive agent.[45] However, body condition scores, whether contracepted or not, may be dependent upon available nutrition and the social environment of the area in which the mare resides. From a veterinary medical perspective, sterilization of mares eliminates or decreases the incidences of common, pregnancy-associated conditions such as chronic uterine infections, pyometras, cervical and uterine pathology, and most importantly, the life of a mare during dystocias. All together, the advantages of remaining "open" in older mares far outweigh the physiological disadvantages of being pregnant. Perhaps the combination of sterilization and contraception methods applied to select groups of mares and in select herd areas of management may potentially accelerate the reduction of population growth where there is critical need. Regardless of which method(s) are used, modeling studies are critical to accurately estimate the number of horses required to be treated in order to have any impact on population growth.

In summary, there are several potential fertility agents for the reduction of feral horse populations. However, based on current data available in the literature, long-acting fertility control agents such as pZP-based vaccines (pZP-22, pZP incorporated in liposomes[i]), GnRH-based vaccines[b], and chemical vasectomy require further development and investigation with consistent repeatable results in order to meet the needs and requirements of large-scale application for fertility control in feral horse populations. Essential criteria for a best-fit fertility control method when integrated with large-scale management of feral horse populations are based on a single injection protocol, long-term effectiveness, high percent effectiveness, and positive effect on health and behavior of the targeted animal and its environment.

Acknowledgments

Conflict of Interest

The Author declares no conflicts of interest.

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[a]Regumate, Intervet, Inc., Millsboro, DE 19966.

[b]Gonacon-K and Gonacon-B, National Wildlife Research Center, USDA/APHIS, Fort Collins, CO 80521.

[c]Adjuvac, National Wildlife Research Center, USDA/APHIS, Fort Collins, CO 80521.
[d]Improvac, Zoetis, Florham Parks, NJ 07932.
[e]Equity, Zoetis, Auckland, NZ.
[f]Ovuplant, Thorn Bioscience LLC, Louisville, KY 40204.
[g]Suprelorin, Peptech Animal Health/Virbac, Australia.
[h]Zonastat-H, The Science and Conservation Center, Billings, MT 59106.
[i]SpayVac, Terramar Environmental Research LLC, Ashland, OR 97520-1830.

Management of Feral Horses in the National Park Service

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Feral horses are considered non-native species within units of the National Park Service. Feral horse management actions depend heavily on whether they are considered desirable or detrimental within a particular locale. Public sentiment and population visibility has traditionally had substantial influence on actions taken. Author's address: National Park Service, Biological Resource Management Division, 1201 Oakridge Dr. #200, Fort Collins, CO 80525; e-mail: jenny_powers@nps.gov. © 2014 AAEP.

1. Introduction

The National Park Service (NPS) was established nearly a century ago, with the creation of the Organic Act (1916), as an agency with a primary mission "…to conserve the scenery and the natural and historic objects and the wild life therein and to provide for the enjoyment of the same in such manner and by such means as will leave them unimpaired for the enjoyment of future generations" (16 U.S.C. § 1). The National Park Service General Authorities Act (1970) further clarified that "though distinctive in character, [Parks] are united through their interrelated purposes and resources into one national park system as cumulative expressions of a single national heritage; … these areas derive increased national dignity and recognition of their superb environmental quality through their inclusion jointly with each other in one national park system preserved and managed for the benefit and inspiration of all people of the United States…" (16 U.S.C. § 1a-1). Finally, the Redwood amendment (1978) further strengthened the assertion that all management would be consistent with the Organic Act and should be for "the common benefit of all the people of the United States" and that management "…shall not be exercised in derogation of the values and the purposes for which these various areas have been established, except as may have been or shall be directly and specifically provided by Congress." (16 U.S.C. § 1a-1).

Quite possibly one of the strongest underlying values the NPS embraces is the idea of "naturalness." Although initially "naturalness" may appear to imply noninterference with the resource, active management is often necessary to address significant habitat and ecosystem changes associated with human influences. Sometimes intervention is necessary to restore natural processes. The NPS Management Policies (2006) directs managers to "…understand, maintain, restore, and protect the inherent integrity of the natural resources, processes, systems, and values of the parks while providing meaningful and appropriate opportunities to enjoy them. The Service recognizes that natural processes and species are evolving and the Service will allow this evolution to continue-minimally influenced by human actions." The "natural condition"

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is one that describes the condition of the resource "that would occur in the absence of human dominance over the landscape." Furthermore, "… in cases of uncertainty as to the impacts of activities on park natural resources, the protection of natural resources will predominate. The Service will reduce such uncertainty by facilitating and building a science-based understanding of park resources and the nature and extent of the impacts involved." Likewise, the "Service will not intervene in natural biological or physical processes, except when directed by Congress; in emergencies in which human life and property are at stake; to restore natural ecosystem functioning that has been disrupted by past or ongoing human activities; or when a park plan has identified the intervention as necessary to protect other park resources, human health and safety, or facilities. Any such intervention will be kept to the minimum necessary to achieve the stated management objectives." Finally, "All exotic [non-native] plant and animal species that are not maintained to meet an identified park purpose will be managed—up to and including eradication— if (1) control is prudent and feasible, and (2) the exotic species interferes with natural processes and the perpetuation of natural features, native species or natural habitats; damages cultural resources; or significantly hampers the management of park or adjacent lands…".[1]

Herein lies the conundrum. Equids evolved for millennia in North America but were extinct by the end of the Pleistocene, likely due to abrupt changes in climate and vegetation and coinciding with the immigration of humans to the continent.[2] Europeans re-introduced domesticated horses (*Equus caballus*) to North America in the late in the 15th century.[3] Descendants of these domesticated horses lived for hundreds of years in direct contact with humans, including Native Americans and European immigrants, and were instrumental in advancing agriculture and transportation throughout the nation. Donkeys or burros (*Equus asinus*) share a similar introduction to the country, likely coming with Spanish explorers into the desert southwest early in the 16th century.[3] As the country became more industrialized, horses were no longer relied upon to provide critical services for the developing country. Many horses were released or abandoned onto western range lands or remained as small remnant herds from early colonial exploration, homesteading, or ranching in areas around the United States.

Feral horses, ponies, and donkeys are found in several National Parks throughout the U.S. Currently, approximately 20 NPS units across the U.S. have horses or ponies and roughly 10 have burros. Population estimates on many NPS lands are unknown but likely total one to two thousand burros and approximately a thousand horses across the Service (all 401 NPS units within the U.S.) although historically there were substantially more.[3] Feral equids residing within NPS units across the Service typically fall into one of four categories.

The first category includes animals that are resident within an NPS unit and are not specifically maintained or managed as a cultural resource. Often, these populations existed in the area prior to the establishment of a park. They may have been released in the area or migrated from another extant population. They have typically been feral for many generations. Management ranges from attempting to eradicate these animals from within a park, to no management at all, often due to lack of funds or resources. Concerns regarding resource damage (e.g., vegetation grazing, damage to historic structures, soil erosion, competition with native wildlife, etc.) range from minimal to significant, depending on the park enabling legislation (e.g., the document that describes the purpose of the park), horse use and damage to available habitat, interaction with other wildlife species, interaction with humans, and interference with park operations. Tools to remove animals include capture and live removal from the park for sale or lethal removal, if necessary. Examples include burro populations at Virgin Islands Coral Reef National Monument, VI and Mojave National Preserve, CA.

The second category encompasses trespass animals from publically managed herds on neighboring lands. The NPS often agrees to comanage these animals on the edges of their herd management units and manages horses similarly to the Bureau of Land Management (BLM) or the U.S. Forest Service. Management activities may include round-up and removal for adoption or participation in fertility control trials. Examples of parks with this situation are Bighorn Canyon National Recreational Area, MT, which shares management of the famous Pryor Mountain horse herd with the BLM, or horses on the periphery of Death Valley National Park, CA, which are managed similarly to the BLM strategies.

The third category consists of trespass livestock from privately owned property or tribal reservation property. The first priority for park managers overseeing this category is establishing ownership and notifying the responsible party that their animals are trespassing. Once ownership has been established, owners notified, and the animals are considered abandoned, a full range of management options are available from fencing, to round-up and removal, to lethal removal. Examples of parks with these circumstances are Big Bend National Park, TX, with trespass livestock from Mexico and Mesa Verde National Park, CO and Glacier National Park, MT, with trespass horses from neighboring tribal lands.

Finally, the last category of animals is the most popular, celebrated, and often controversial. These are horses and ponies that are maintained as desirable feral species as part of the cultural landscape of the park. These herds tend to be small, highly visible, and with many interested stakeholders.

While park managers often identify significant resource damage due to overgrazing, trampling, or competition with native wildlife species, management options are often curtailed and subject to significant public scrutiny. A few NPS units have specific legislation or significant political pressure which mandates or drives horse management actions. Management actions include fertility control, genetic monitoring and manipulation, occasional veterinary care, capture for branding or microchip identification, round-up and sale, and partnering with non-NPS special interest groups to facilitate adoption. These herds are well-monitored and managers tend to understand not only herd demographics and dynamics but also can identify individual animals. These herds are generally geographically contained either on islands or within fences. Significant energy and resources are expended on maintaining physically and genetically healthy publically visible populations while, at the same time, balancing the needs of native wildlife species, habitat quality, and other park management priorities. Parks with this situation include Assateague Island National Seashore, MD and VA and Cape Lookout National Seashore, NC (16 U.S.C. § 459g-4) along with Theodore Roosevelt National Park, ND.

In those parks where horses are maintained as part of the cultural landscape, significant research has been conducted using immunocontraceptive fertility control agents. Some of the earliest work in free-ranging horses using immunocontraceptives occurred in National Park units, particularly Assateague Island.[4–6] At this time, both Assateague Island and Cape Lookout National Seashores use annual remotely delivered porcine zona pellucida (PZP) vaccination to manage their feral horse herds. Cape Lookout augments the use of PZP vaccination with occasional removal from the island and adoption though a non-profit foundation. Notable findings with the use of PZP vaccination on these islands has been an increase in mare body condition and an increase in longevity associated with treatment. This has been attributed to the absence of pregnancy and lactation.[7] Investigators found extended periods of vaccination increase efficacy and longevity of the vaccine, possibly to the point where mares are permanently infertile.[8] Additionally, the PZP vaccination extends reproductive cycling past the normal breeding season[9] and decreases harem fidelity.[10] Finally, for more than 20 years, managers from both Seashores have dedicated significant funds and personnel time to intensively manage these herds (n = ~100 horses per island) on an annual basis.

Similar to Assateague Island and Cape Lookout National Seashores, Theodore Roosevelt National Park in western North Dakota has free-ranging feral horses. These animals reportedly descended from ranch horses turned loose in the badlands area of ND prior to establishment of the park. They have received local, state, and national attention as culturally and historically important animals; some even suggest they are the founders of the "Nakota" breed. The park is fully fenced and the south unit where the horses reside encompasses approximately 46,000 acres. The park currently uses helicopter round-up and local sale as a means of limiting the number of horses. Horses share the range with other large ungulates including bison (*Bison bison*), elk (*Cervus elaphus nelsoni*), pronghorn (*Antilocapra americana*), mule deer (*Odocoileus hemionus*), and white-tailed deer (*Odocoileus virginianus*), necessitating careful resource allocation.

Park managers would like to limit the expense and safety liability of using helicopters to round-up animals and minimize the need to sell excess horses. This led to the current research project using an immunocontraceptive vaccine[a], directed towards the mare's own gonadotropin releasing hormone (GnRH) to limit fertility.[11] In October 2009 the park performed a round-up and removed approximately half of the herd. In a randomized complete block design, remaining mature adult females were blocked based on age, body condition, and reproductive status and assigned to a treatment (n = 29) or control (n = 28) group. Treatment animals received 2.0 ml of GnRH vaccine[a], which likely acts by antibody neutralization of endogenous GnRH and thereby decreases leuteinzing hormone and follicle stimulating hormone release from the anterior pituitary, in turn preventing ovulation. Control females received 2.0 ml physiologic saline. Most mares were pregnant at the time of treatment (~90% pregnant).

In the following 4 years mares were observed for evidence of foaling, foal survival, changes in body condition, changes in reproductive behaviors as well as time budgets (i.e., time spent eating, moving, resting, or doing other behaviors), and possible injection site reactions. During the first foaling season (April–July 2010) after treatment there was no statistical difference in foaling success (P = 0.65) or foal survival (P = 0.09) between groups; though not statistically significant foals born to mares treated with GnRH vaccine[a] were more likely to survive. Thus, it was concluded that there was no negative effect on foal survival of vaccination during mid-gestation. During the second foaling season (2011), or the first season treatment effects would be expected, the proportion of treated mares that foaled was 35% less than control mares (P = 0.04). In the third season, the proportion of treated mares that foaled was approximately 30% less than in control mares (P = 0.07). Three years post-treatment there was no difference (P = 0.59) in foaling proportions between experimental groups.[12]

There were minimal treatment effects on time budgets or reproductive behaviors; however, significant changes in herd dynamics and band fidelity were seen after removal of half of the herd at the time of round-up.[13] Approximately 80% of mares treated with GnRH vaccine[a] had evidence of swell-

ing at the injection site 1–4 years post-injection; whereas control mares did not have similar swelling. While most treatment mares had injection site reactions, only one had a draining abscess and none showed evidence of lameness. There were no differences in body condition between groups. The mean body condition scores for all study mares were between 4 and 6 throughout the study, indicating good nutritional status.[14]

The study concluded that a single vaccination with this GnRH vaccine offers a moderate decrease in fertility in free-ranging female horses for two years post-treatment, does not disrupt the current pregnancy, and is consistently associated with injection site reactions that do not appear to decrease fitness or animal welfare. A project is currently underway investigating the efficacy of revaccination four years after the initial immunization using the same product. The efficacy and persistence of the vaccine may be extended and behavioral effects may be more evident when a greater proportion of the mares are infertile.

Feral equids are currently managed as non-native species throughout the Service and may be considered either desirable or adverse, depending on the purpose and goals of the park unit in which they reside. Fertility control is a tool that may be appropriate to use in small intensively managed populations of horses and donkeys within the NPS to maintain cultural resource herds; however, a full range of management techniques, including lethal removal, remain necessary to meet management goals of more extensively managed populations. There is unlikely to be a "one size fits all" management approach to feral horses within the U.S. and flexibility as well as a full range of tools is necessary to allow managers to balance the competing needs of many natural and cultural resources.

Acknowledgments

Conflict of Interest

The Author declares no conflicts of interest.

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Management of Feral Horses on the Navajo Nation

Scott Bender, DVM

The Navajo Nation is actively working on the management and population reduction of feral horses, yet there are limitations of "ideal" population management, often due to outside interference, legislation, funding, and environment. The feral horse "problem" is NOT a horse problem, but a HUMAN problem. Author's address: PO Box 4889, Window Rock, AZ 86515; e-mail: scottbender@navajo-nsn.gov. © 2014 AAEP.

1. Introduction

The Navajo Nation is located in the four corners region of the United States, with lands within the states of Arizona, New Mexico, and Utah. The Reservation consists of slightly more than 18 million acres, making it larger than the state of West Virginia. There are 110 chapters, or communities, and these are also the local government system for the members that live in that area. The Bureau of Indian Affairs (BIA) grazing permit numbers for horses is around 24,000, while the current estimate has between 75 to 90,000 horses on the Navajo Nation.

2. Materials and Methods

Tribal Government Legislation was enacted as part of the drought emergency of 2013.[1–3] Included in the legislation was funding for feral horse depopulation and veterinary horse population management, due to the ecological damage being done by the rapidly growing horse population in the current drought. All unbranded feral horses on the Navajo Nation are Tribal property under Tribal code.[4] Additionally, there are over 12,000 permitted livestock owners, of which a majority have permits for one to five privately owned horses.[5,6] Under both BIA regulations and under Tribal code, stallions are not permitted to be free roaming on the grazing lands of the Nation.

Starting the first week of August 2013, local community governments were able to request the assistance of the Navajo Nation Department of Agriculture, for both community horse roundups and veterinary population management. Assistance for roundups and population management occurred only upon the local community chapter holding a vote and approving the request.[2,4] Following the approved chapter request, the Navajo Nation Department of Agriculture, in cooperation with the community members, provided staff, portable corrals, and horse transport to the impoundment center. Also on site were officers of the Navajo Nation Resource Enforcement, to inspect for brands and provide on-site law enforcement.

Veterinary services were provided for the local communities, independent of the depopulation program, in an effort to encourage people to attend, without fear that their horses would be impounded. Services included free stallion castration and free mare birth control vaccination (including first re-

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quired booster), utilizing the porcine zona pollucida (PZP) vaccine.[a] So far, with the exception of one mare, all mares and fillies have been vaccinated by hand.

Castrations are performed under general anesthesia, using xylazine[b] (1.5 mg/kg, IV) and ketamine (1.5 mg/kg, IV) mixed with diazepam (0.015 mg/kg, IV), with the ketamine-diazepam mixture administered after the xylazine has taken effect. The castrations are performed using the Henderson equine castrating tool.[c]

All horses castrated or vaccinated with PZP, are given a Microchip ID implant, a 5-way vaccination, and deworming at no additional cost.

Tribal emergency funding provided for 1000 castrations and 1500 mare PZP vaccinations. Additionally, funding was provided for field travel cost for staff to the end of the 2013 fiscal year (October 2013).

3. Results

In the months of the active 2013 program, a little over 8900 feral free roaming horses were impounded, of which about 250 were reclaimed by permitted owners, and the remainder sold,[7,8] with the requirement that they could not be returned to the Navajo Nation. In communities that held depopulation projects, forage conditions were markedly improved at the end of the 2013 grazing season.[8,12] To date, more than 700 horses have been castrated or vaccinated by the Navajo Nation Veterinary Program outreach, with a majority being castrations.

4. Discussion

The management of livestock, of which horses are included, has historically been left to the Bureau of Indian Affairs,[6] with the Nation slowly taking over the responsibilities as a sovereign Nation government.[4,9]

Much of this change was due to the John Collier approved reduction of livestock in the 1930s[10] and the "outbreak of Dorine in horses" at Canyon de Chelly National Monument. The Collier reductions resulted in mass euthanasia and burials of livestock. The Dorine outbreak also resulted in over 500 horses being euthanized by gun and buried on Tribal land by the USDA.[11] The aforementioned "waste of resources" has left a negative indelible mark on the attitude of the Navajo livestock owners towards the U.S. government.

The current horse issue is a direct result of the elimination of an outlet for surplus horses in 2006. After the closure of U.S. horse abattoirs, there was no local economic value to excess horses, so no depopulations occurred. It cost more to remove a horse that was received at auction. Thus, horses were left to breed without any human controls and in North America there is no longer any apex predator, other than humans for horses, to help control the population. One comment that is consistent at the field clinics and roundup has been "if it's ok to humanely euthanize a cow or a sheep, why is it wrong to use the same method for a horse?" Another comment often heard is "why is it better to put down a horse, and allow the animal be wasted?" which, for the Navajo, includes the cultural and traditional consumption of horses.

Following horse population reductions, many of the affected communities saw a marked improvement of range conditions.[8,12] One of the major activities of the Navajo Nation Fish and Wildlife is the protection of endangered species, with the Department's heritage program charged with the protection of endangered plants. Protection of these endangered plant species from horses has become a major activity, as they have been a major contributor of damage to the soil and surrounding plant communities. The reduction of horse populations has aided this effort by reducing the cost of installing physical barriers.

The response of the public to veterinary services has been positive. People have appreciated and recognize the value of the program, both personally for their horses and for the betterment of the community. The most-received comment was how "there are too many horses running free," and how "stallions caused so much injury and damage," While well received, the vaccination of mares generated two interesting points. First, the folks that got their mares vaccinated did it more to "keep stallions from being drawn to their horses" than to not have a foal and, second, there has been very poor follow-up booster vaccination, despite efforts to provide the booster vaccinations at the owner's convenience and after multiple efforts reminding them. Based upon the comments and poor owner compliance, we are investigating the use of the antigen GnRH vaccine,[d] as this would address both the stallion attraction issues, which PZP does not do, and the value and ease of a single vaccination, with a two to three year suppression of estrus.

One problematic issue has been off-reservation proponents of animal rights as "concerned citizens" wanting to know what is being done with the excess feral horses and where horse roundups are occurring. This was especially bad following articles covering the roundups and resulted in additional law enforcement presence with many of the local communities restricting noncommunity member access to the roundup locations.

In addition, high profile people, without firsthand knowledge of the localized damage being caused by the feral horses, have used their status to work to stop the depopulations with no alternatives or funding (at the time of writing this paper) to accomplish those goals being more than "paper" proposals. Interestingly, several of the proposals that have been put forward are to either "pen and feed" (who is going to pay the bill?) or to euthanize using drug overdose (which then requires burial or landfill disposal to prevent potential wildlife deaths). Currently, the Navajo Nation Department of Agricul-

ture has been restricted from assisting communities with their horse depopulation activities due to extended NGO agreements. However, the department continues to impound horses that are within the highway right-of-ways to protect public safety from feral horse collisions.

One of the growing concerns is the lack of any plans for donkeys or burros, many of which have come onto the Navajo Nation from the U.S. Park service and Bureau of Land Management lands. As their populations increase, and horse populations are being reduced, they are starting to replace horses in some areas due to local feral donkey reproduction. Unlike horses, there is no market or outlet for major numbers of this species, and they have been observed to be more behaviorally aggressive towards people in their grazing areas.

An interesting part of the Veterinary outreach is that all horses castrated or vaccinated are also microchip ID'd. This continues the microchip ID of horses on the Navajo Nation, originally started in 1997 and placed into law in 2006. To date, our program has placed microchip IDs in over 18,000 horses.

5. Conclusion

The Navajo Nation Veterinary program's horse population management outreach is slated to continue through 2014, until the goal of 1000 castrations and 1500 mare vaccinations is met. The problem of the horse population was caused by the elimination of markets for excess animals, coupled with the natural reproductive efficiency of both captive and feral horses and owners that allow reproduction without any viable use or market outlet. Through the activities of the Navajo Nation Veterinary Program and the Navajo Nation Department of Agriculture, we are attempting to address a small part of the problem by hopefully decreasing the recruitment of the feral population from the "captive" population and by the depopulation of feral populations at large. In addition, the Navajo Nation Veterinary Program and Department of Agriculture is working to educate horse owners in the value of reproduction control and through these efforts hope to moderate the increase in the feral horse population. However, until a stable and economically viable outlet for excess captive and feral horses is established, the population of feral horses and burros is expected to continue its growth.

Acknowledgments

Conflict of Interest

The Author declares no conflicts of interest and has not received any compensation from/for companies/materials discussed in this article.

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[a]PZP vaccine, The Science and Conservation Center, Billings, MT 59106.

[b]ZooPharm, Laramie, WY 82070.

[c]Stone Manufacturing and Supply Company, Kansas City, MO 64127.

[d]GonaCon™ vaccine, USDA-APHIS-WS- NWRC, Fort Collins, CO 80521.

How to Configure an Equine Facility to Prevent and Better Respond to Barn Fires

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1. Introduction

The tendency of most U.S. states and local fire jurisdictions to consider horse barns and veterinary treatment facilities as "agricultural buildings" has led to lax standards and reliance on traditional design, construction, and management practices that can contribute to the ability of fires to ignite and to spread quickly.

Many aspects of an equine facility can be considered as fire hazards (traditional wooden open interior aisle construction, use of hay and shavings and storage of these multiple potential ignition sources, lack of compartmentalization, etc.). Building a "fireproof" barn is not realistic; however, there are strong measures that can be taken to dramatically reduce the chance of an incident in your facility and that of clients. Prevention can be enhanced with updates to daily management practices, original property and facility design, and attendant emergency planning and practice. This paper will explore how to configure an equine facility to prevent barn fires based on new technologies and understanding of how fire behavior affects facilities for more efficient and effective response.

Few practitioners and horse industry stakeholders are aware that a great percentage of barn fires that occur are fully involved within 5 to 7 minutes of ignition (implying the total loss of the facility and everything within it and that the local—especially a rural—fire department will not have even had time to arrive within this time constraint.) Most facilities reach a dangerous to life level of smoke production at the 3 to 5 minute mark. Realistic reporting of these fires includes very few incidents where the animals are successfully salvaged from a burning building (Figs. 1 and 2) and even fewer where the facility is not completely destroyed. It is unfortunate that equestrians have a common idealistic and romanticized view of barn fires, which includes thinking that the fire department will arrive, put out the fire, and retrieve their horses from the smoke. It is far more common that the entire facility is fully involved and the animals are long since dead from smoke inhalation when the fire department arrives on scene. Even if they arrive in time to attempt rescue, horses presented with firefighters in full gear and breathing apparatus are slow to accept being caught, haltered, and led by what must appear to be an alien being (Fig. 3). Numerous training simulations across the country by one of our authors (Gimenez) has shown that it is very difficult for firefighters under realistically simulated conditions to find horses in dark smoky stalls with firefighting gear and remove them safely. Firefighting

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Fig. 1. Dogwood stables fire, Aiken, SC, 2013. All animals were successfully removed by a wedding party on the grounds when the fire started. Courtesy of George Hagan.[1]

Fig. 3. TLAER training event. Live demonstrator horse removed from a stall in training smoke and being walked out by a firefighter using an emergency rope halter (photo courtesy Gimenez).

gloves, masks, and clothing make it difficult to move, see, hear, and manipulate locks, halters, and even open latches in the dark and smoke.

There are numerous overlooked aspects to investigate when diagramming the layout of a future facility. Understand that most owners design a barn for its aesthetic appearance or efficiency, not necessarily for fire prevention, and rarely are veterinarians, fire prevention contractors, ventilation engineers, or fire marshals consulted by barn designers or builders. Barn fires occur all over the United States at various times of the year—there seems to be very little predictability to time of year, location, or facility type other than what would be expected (cold weather increases fires due to heating systems and hot weather increases fires due to fans and cooling systems.) Worse, the National Fire Incident Reporting System (NFIRS) is not formatted for and makes it difficult to extract data appropriate to learning about these fires. Adding to the problem, many equine facilities were established decades ago and need retrofitting to modern codes while others are newly constructed but may not have ascribed to national recommendations and codes. No matter what the age of the building is, there are some simple improvements that can be made to reduce the incidence of equestrian barn fires and improve safety for response when it does occur. Some of these improvements represent a small financial investment, and others are changes in management and design.

Fig. 2. Dogwood stables fire, Aiken, SC, 2013. All animals were successfully removed by a wedding party on the grounds when the fire started. Courtesy of George Hagan.[1]

The National Fire Protection Association (NFPA) has updated their standards for animal housing facilities in the 2013 version of NFPA 150[2], which includes horse facilities, although few local jurisdictions have adopted their guidelines. The NFPA recommendations remain the "best practices" reference for how to design, construct, and manage animal facilities for practitioners, clients, and owners. Their suggestions include numerous ways for both new construction and for retrofit of older facilities to reduce hazards to people and animals working and living within. Since many horse facilities are well known to increasingly feature human living spaces and electrical conveniences for both horses and people, we should not consider them just agricultural facilities. Improving standards for construction, especially for electrical service, would greatly decrease the number of electrical source fires.

It is important to recognize how much of a toll these incidents put on the equestrian industry. Some recently reported fires:[3–17]

December 2009—2 people and 43 horses dead at racetrack (Canada)

September 2010—27 horses killed at racetrack (WV)

September 2012—14 horses die (NH)
April 2011—13 horses dead and 3 people injured (TX)
May 2011—6 Olympic horses dead, 2 people injured (PA)
April 2013—12 polo horses die (FL)
July 2013—21 horses removed successfully, total loss of facility (SC)
October 2013—1 person, 3 horses killed (FL)
December 2013—1 person, 4 horses killed (NY)
February 2014—5 horses and a cow killed, vet clinic damaged (WV)
February 2014—18 horses killed (GA)
February 2014—0 horses killed, $260 000 value loss (MI)
February 2014—17 horses killed (OH)
February 2014—8 horses killed, vet clinic (KY)
February 2014—5 horses killed (LA)
February 2014—0 horses killed, barn total loss (Kansas City, MO)
February 2014—6 horses killed (CA)
March 2014—35 horses died at race stables (MI)
March 2014—2 horses died (MA)

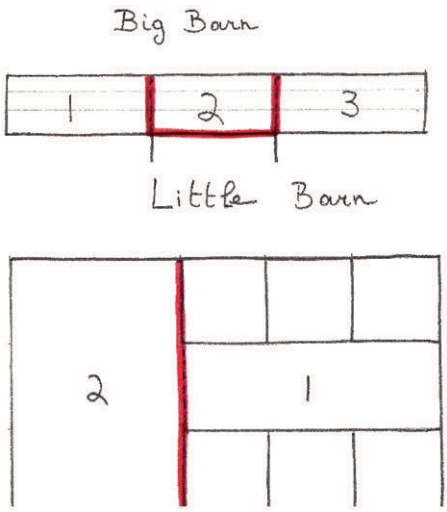

Fig. 4. Fire door and firewall use in barns (red lines) for compartmentalization. These ideas can be utilized in small or large facilities and only allow more time for removal of people and horses once the fire is detected.

Although there is no official statistics on barn fires, and this list represents only a few recent examples picked from the headlines of newspapers, there are numerous barn fires reported regularly across the country. Media reporting rarely gets the scope of the large dollar losses correct and often fails to follow up on these incidents.

2. Materials and Methods

Analysis of preventative measures into building and retrofitting equine facilities merits further clarification. The common methodologies that have been employed in so-called "modern" barn construction haven't really changed in over 400 years. However, there are numerous new measures available to increase the positive outcomes in both prevention and response to fires in equine facilities.

Layout

The overall layout of facilities is a key component. The primary thing to consider is compartmentalization of storage facilities and potential combustibles by physically separating each "type" of stored item (Fig. 4). As an example, fire doors may be practical in some facilities and can be as simple as a roll up door that separates the facility in half or thirds. Fire walls are built of heavy duty materials or fire retardants and separate different areas of the barn from each other (second floor living areas from the main barn on the first floor, one section of the barn from the other, etc.). Compartmentalization helps to separate combustibles from the main barn as much as possible and includes hay, farm equipment, appliances, electrical system, manure, and fuels in separate facilities (Fig. 5). Practically, this means a separate shed for hay, bedding, and straw; no "hay lofts"; a separate manure compost or storage area; and a separate shed for tractors and vehicles. Potential negative effects are increased property tax costs.

Manure, hay, and bedding are three major products related to or produced by an equine facility. All three are not only found in high quantities on a property, but they are highly combustible fuel and even ignition sources. The presence of stall bedding inside the barn cannot and should not be prevented, but storage away from the main barn of excess bedding is ideal, and some forms of bedding (shavings) have lower combustibility or dust ratings than others (hay, straw).

Forage Storage

In traditionally built barns, hay and grass forage was stored in the "hayloft." We now know that hay should never be stored overhead in a barn, as it only increases the fuel loading within the barn and speeds the spread of fires as well as sometimes contributing to ignition sources. Very few barns have used a fire wall to separate the hay from the rest of the barn, or detection, alarm, and suppression systems (Fig. 6). If it is critically necessary to store hay in the barn, consider storage of the majority in a separate facility and bringing in only a couple of days worth of feedings into the hay loft or storage area. Compartmentalization and proper handling is critical, especially with hay and straw. Green hay is highly combustible, particularly when it has just been baled in high humidity content and put into storage in the summer months. A probe should be used to determine the internal temperature of the bales stored in a location separate from the horse facility for at least 3 months after baling to determine if there is any immediate danger (Fig. 7).

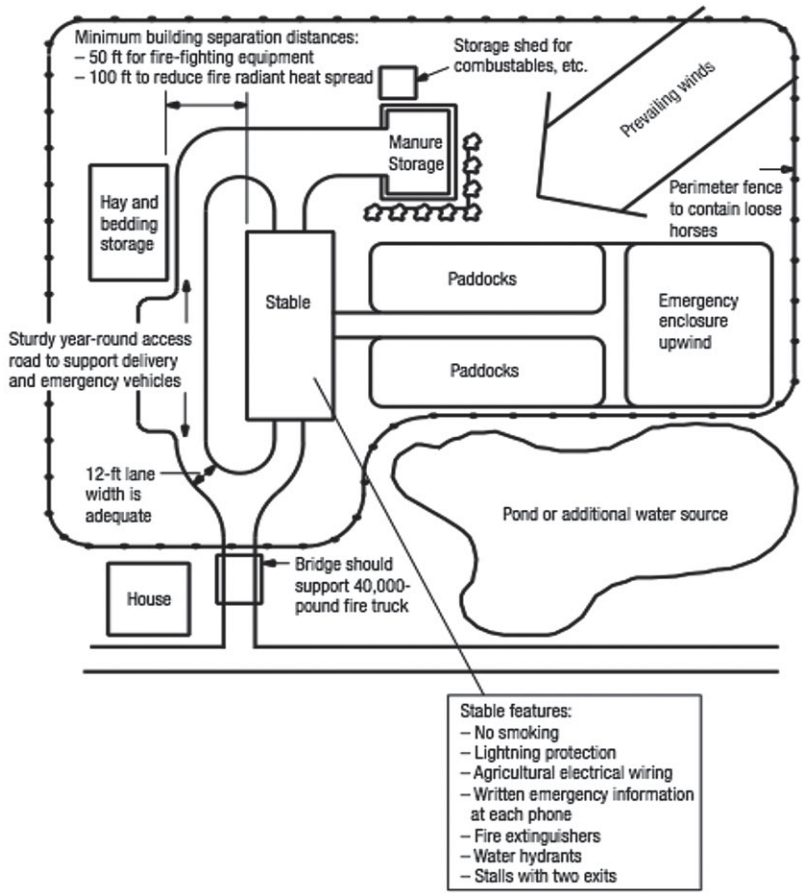

Fig. 5. Possible barn layout intended to maximize compartmentalization on a small acreage and provide security.[18]

Facility Access

Fire truck and first responder access and safety are topics for immediate consideration. The parking lot needs to be strong enough to hold the fire truck's weight—ideally a gravel or asphalt driveway. Many fire trucks will not advance on grass or

Fig. 6. Hayloft with installed detection, alarm, and sprinkler system. Notice that instead of filling the hay storage area completely, only a limited several days of hay is brought in to limit the fuel load (photo courtesy Gimenez).

Table 1. Determining Hay Temperatures with a Probe

You should use a probe and thermometer to accurately determine the temperature inside a stack of hay. Push or drive the probe into the stack and lower the thermometer to the end of the probe on a lightweight wire. If the probe is horizontal, use a heavier wire to push the thermometer into the probe. After about 15 minutes, retrieve the thermometer and read the temperature. Refer to the following temperature interpretations:

Below 130°F	No problem.
130 to 140°F	No problem yet. Temperature may go up or down. Recheck in a few hours.
150°F	Temperature will most likely continue to climb. Move the hay to circulate and cool the air. Monitor temperature often.
175 to 190°F	Fire is imminent or may be present a short distance from the probe. Call the fire department. Continue probing and monitoring the temperature.
200°F or above	Fire is present at or near the probe. Call the fire department. Inject water to cool hot spots before moving hay. Have a charged hose ready to control fire when moving hay.

[Source: Ogburn, 1995]

Fig. 7. Chart to assist with determining the fire danger in increasing stored hay temperatures. Freshly stored hay should be checked regularly until it is cured.[19]

Fig. 8. Trailers parked away from the main barn to allow easy access around the gravel drive that goes all the way around the facility (photo courtesy Gimenez).

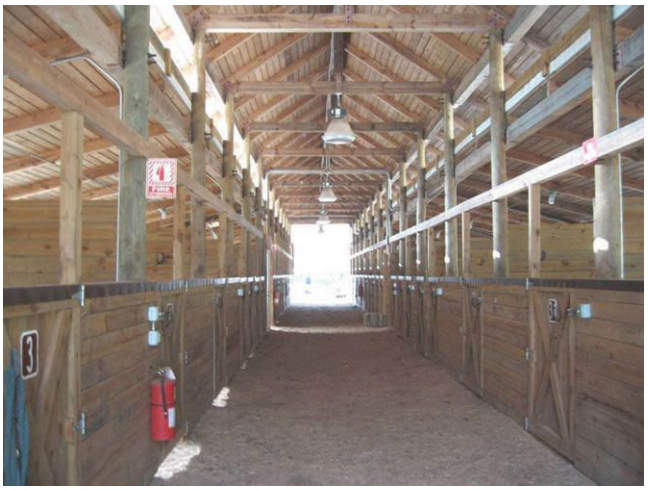

Fig. 9. Pole barn open barn design with fire retardant wood, excellent ventilation, electrical service to public facility code, appropriate egress, and preventative fire measures installed (photo courtesy Dr. Tomas Gimenez).

sand in fear of getting stuck and, therefore, should have maximal access to sources of water (ponds, fire plugs, pools, etc.). The first responders are there to help prevent spread of the fire, but will not risk their own safety to save animals or to stop the barn fire; if there is not sufficient room between structures they will not be able to maneuver close to the burning structure. Ensure that gates and fences at the entrance of the property have openings wide enough for the emergency trucks to enter through. "No Parking" signs and enforcement should be at every major entry to the barn to allow full access in case of an emergency. There is not time to get keys and move cars or horse trailers that are in the way when firefighters arrive on the scene of a barn fire (Fig. 8).

The arriving firefighters will first conduct a 360° hazard analysis and risk assessment. This includes looking for electrical sources to turn off, possible propane or gasoline hazards, while assessing the smoke/fire development and determining whether to make an inside attack to the facility (rare) or providing outside fire/smoke suppression and further exposure protection. Practically, this means that in most cases they will stay outside the facility and attempt to knock down the fire from a safer position instead of entering the building due to the well known dangers of agricultural fires (speed of spread, large fuel load, and lack of egress).

Building Materials

Many building construction and daily management materials used in an equine facility construction can fall in one of numerous product specific rating systems for materials, which give an idea of how fast they may allow smoke development, flame spread, and an overall fire rating. These ratings give the builder an idea of how the material will behave in a fire compared to some traditional materials such as concrete. When building a barn, consideration should be given to masonry, heavy timber, and fire-retardant woods since it takes longer for flames to spread across their surfaces; however, these are often more expensive. When substituting modern wood construction materials, consider the use of flame retardant soaked or sprayed wood to accomplish the same rating (Fig. 9).

Unfortunately, most common varnishes used in barns can accelerate the spread of fires because most of them are not fire retardant, although the fire retardant versions do exist. Existing facilities can be sprayed with these solutions as well. Talk with your builder and see what materials they offer that can withstand horses, weather, extensive use, and slow the spread of smoke and flame if a fire were to arise.

Electrical Service

Electrical failures along with human errors are the cause for an estimated 80% to 85% of barn fires.[20] Modern barns commonly have as many electrical appliances in them as a residential structure and sometimes even living quarters co-located. If building new construction, you should establish the best electrical blue print for the barn (Fig. 10) to provide sufficient service and minimize the need for extension cords. An electrician should understand what types of electrical appliances (bucket water heaters, clippers, heaters, infra-red heaters, hot water heater, microwave, washer/dryer, vacuum, etc.) that you might use in the "barn" to determine the kind of service panel you need and where the best location for it is. Ideally the electrical panel should be kept closed, easily accessible, and out of the way of dust, cobwebs, and humidity (Fig. 11).

If retrofitting a barn, an electrical review by a certified contractor is the best way to ensure safety

Fig. 10. Example of a clean and safe electrical panel (courtesy of Catherine Morauw).

of the electrical system and prevent potential fires. Walk through the barn together to look for issues (visible wires, lightning suppression systems (if present), solar electrical systems (if present), lack of conduit on wiring, incorrect or missing connections, lack of "outdoor" panels or plugs, sufficient panel service for the size and needs of the facility, etc.) that need to be addressed. While retrofits tend to be more expensive, it is a wise investment that may be reflected by contacting your insurance company and receiving a lower premium or higher coverage of the facility, and obviously is much cheaper than loss of the facility, contents, and live animals. Extra apparatus plugged into the electrical system need to be considered and either minimized (turned off when not in use) or ensure that they are rated for outdoor/agricultural use (infra-red radiators, heaters, air conditioners, microwaves, and fans). Small appliances have a great risk of becoming ignition sources to a barn fire when they are overloaded on a circuit, chewed electrical cords, or have a lot of dust get into their motors (Fig. 12).

Fig. 12. Horse with electrical box fan. Small appliances can be increased fire hazards and can become an ignition source. Purchasing an agricultural rated fan is recommended (photo courtesy Gimenez).

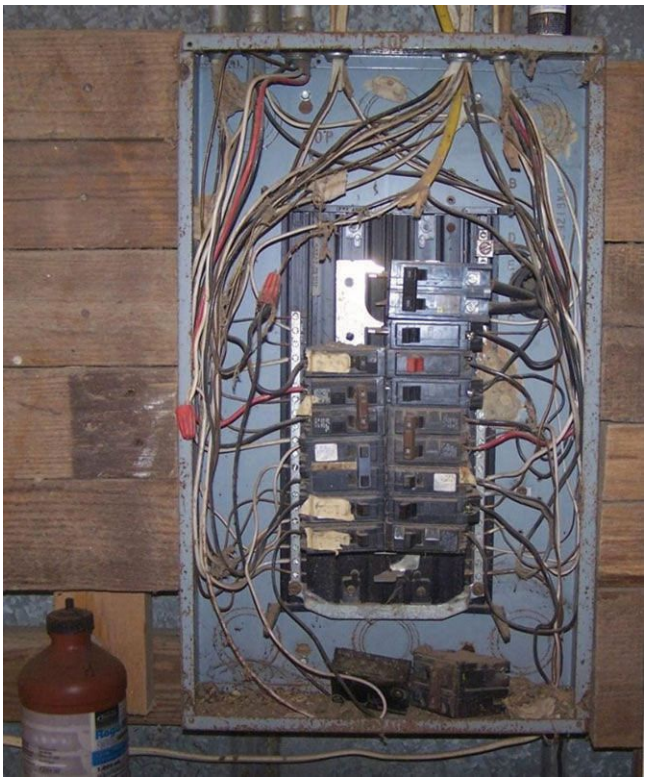

Fig. 11. Example of an unsafe electrical panel (courtesy of Justin Mcleod).

Another commonly overlooked aspect of animal buildings is thunderstorm security with lightning rods (Fig. 13). A well-grounded lightning system can reduce the occurrence of fires (and injury to animals and people in the barn) from a lightning strike by diverting the energy to ground. Lightning systems are easy to install in both current barns and new construction. It is critical to have the system installed by a professional and maintained, or else the system may do more harm than good.[19]

When building any kind of facility, local fire codes should always be consulted, and if they do not align with national fire prevention guidelines, consider the recommendations of NFPA Standard 150—Animal Facilities.[2] Even if it is considered an "agricultural structure," there are usually minimal codes

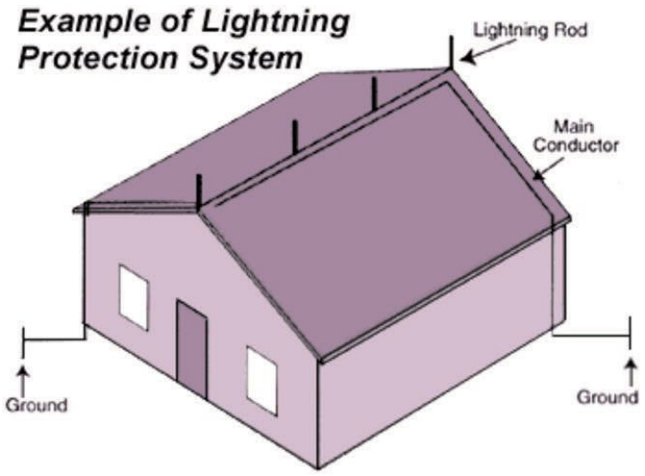

Fig. 13. Lightning rods installed on a barn.[21]

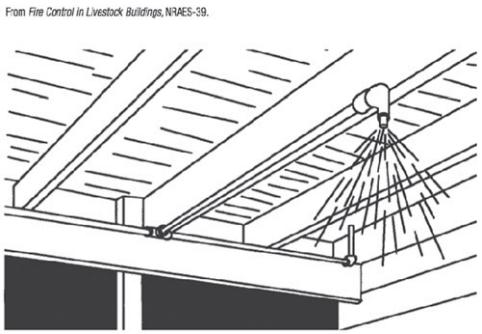

Fig. 14. Example of a Sprinkler suppression system.[18]

that need to be followed but consulting a fire marshal for information on improved standards will increase future success in preventing fires. Safety standards as set forth in local jurisdictions are created to ensure the safety of humans and animals but in most communities are a minimum protection level; for more valuable and insured animals, a higher safety standard should always be sought.

Detection, Alarm, and Suppression

Obviously, the best key to fire protection is prevention of ignition, but fires are inevitable and a detection system is of great consequence to a fast response. The first sign of a fire is often smoke which may smolder/burn for hours or even days without an open flame. Smoke detectors of several types (flame detectors, thermal rate of rise detectors, ionization detectors, etc. depending on barn design and expense) will need to be installed by a contractor in multiple open and closed areas of the barn. Having the detectors in direct communication with a local fire station or security system when they alarm dramatically reduces emergency response time to the location. Along with smoke and carbon monoxide detectors, consider a local security alarm installed to notify any local individuals of the intimate danger, which increases the chances of getting the horses out of the barn.

Suppression is vital to saving the horses trapped in a burning facility (Fig. 14). "Sprinklers in a barn at Plainridge Racecourse, a harness racetrack near Boston, saved some 35 horses when a fire broke out May 9. … The racetrack, located in Plainville, about 35 miles southwest of the Boston area, had a sprinkler system installed in the barns in 1999."[3] The question is not whether or not a sprinkler system needs to be installed, but what type of system is best. Insurance premiums are substantially reduced for facilities outfitted with a sprinkler system, but few equine builders or designers emphasize their use, which arises from few equestrians insisting on their use. Part of the challenge is initial expense for these systems can be up to 20% of the building costs, although components have been getting more reliable and cheaper over the last 20 years as contractors have installed more systems.

In residential and public buildings, numerous studies have shown that in facilities with automatic sprinkler systems, the ability of the fire department to respond and save people as well as the facility is substantially improved. Sprinkler systems can have an operational reliability, which means the system works as intended. It has been reported that a high of 93%[22] operational reliability can be reached. Performance reliability can also be measured—how adequate the system will perform once it is employed. This number is much lower indicating that sprinklers will control a fire but are not used to extinguish it, establishing again that detection and alarm at the local fire department are crucial to avoid loss of the facility and contents.

A major determining factor for sprinkler system choice is the climate and geographical location of the facility. Sub-zero temperatures will not allow for a "wet" system to be installed or pipes will freeze and burst in the winter months, rendering them useless. The most convenient is a dry sprinkler system where the pipes are filled with compressed air or nitrogen, which in turn holds a valve shut to hold the water out. The sprinkler head will open when there is a temperature variation, leading to the opening of the value and the rush of water flow into the pipes and system. Limitations include that a substantial water source and pump need to be available to provide enough pressure and water for the system (D Farr, oral communication, May 24, 2013). However, these challenges can be overcome with appropriate mitigation. Sprinklers are the single BEST method of suppression of a fire in any type of facility, providing enough time for the first responders to arrive and attempt rescue of trapped horses as well as preventing spread of the fire to prevent facility damage and insured dollar losses. Their lack of use in the horse industry can be attributed to lack of understanding and knowledge by the horse owning community.

Fig. 15. Classes of fire extinguishers.[23]

Fig. 16. Attempting horse rescue by firefighters in a training demonstration. In a real barn fire, there is not enough time to catch, halter, and lead out all the horses in the 3 to 5 minutes to full involvement of the barn (photo courtesy Tori McLeod).[20]

Another suppression method that is common but also misunderstood is fire extinguishers. There are three major classes of fire extinguishers: A, B, and C. Multiple designator extinguishers, Class ABC, are available and are most useful in barns due to their broader target range (Fig. 15). Located at every point of entry in the barn should be a fire extinguisher of a minimal 10 pound size, and these should be checked and maintained (turned upside down) on a monthly basis. Staff and patrons of the facility should all feel comfortable in handling and using a fire extinguisher; this is an easy training event to provide in concert with your local fire department.

Egress

Having multiple access/egress for humans and horses is an important factor to the success of evacuating animals and people (especially handicapped riding students at Professional Association of Therapeutic Horsemanship [PATH] facilities, etc.) A simple change to design of facilities allows initial on scene personnel to get horses out of burning barns more readily and safely. There should be a minimum 8-foot-wide and 8-foot-tall openings every 50 feet at a minimum along the outside wall of a facility preferably leading to a fire run-out lane so that animals can be released and chased out of the barn to a safe location.

Don't believe it? Try it. Time yourself. Run from your vehicle or location outside the facility to the inside, find a halter, catch a horse in a stall, lead it out to a paddock, close the gate, run back in to get the next horse, etc. Did it take you less than the 3–5 minutes that it would require for an ignited barn to reach deadly conditions where you could no longer enter the barn facility? At that point, in most rural areas, the fire department would just be pulling out from the station (Figs. 16 and 17).

As prey animals, in emergency situations stalled horses tend to stay in their stall even if the door is opened since it is normally an area of security for them, and their buddy horses are there with them. Furthermore, when a barn is full of smoke and firefighters geared up with their breathing apparatus on and approach a horse in a stall, most will not willingly approach a firefighter at the door of the stall, and entering the stall of a scared horse is not recommended either.

Ideally each stall will have an egress to both the inside aisle and outside paddock or run out lane. This allows easy and fast approach and egress for each individual horse with lesser risk of entrapping a human in a burning structure (Fig. 18). Advancing down the interior aisle of a burning barn can never be recommended, as it is known to be extremely dangerous for falling fuels and failure of the overhead roof structure due to the common use in

Fig. 17. Well-meaning owner attempting to rescue horses in a technical large animal emergency rescue (TLAER) barn fire simulation. Note horse is reluctant to leave the barn even when being led out (photo courtesy Gimenez).

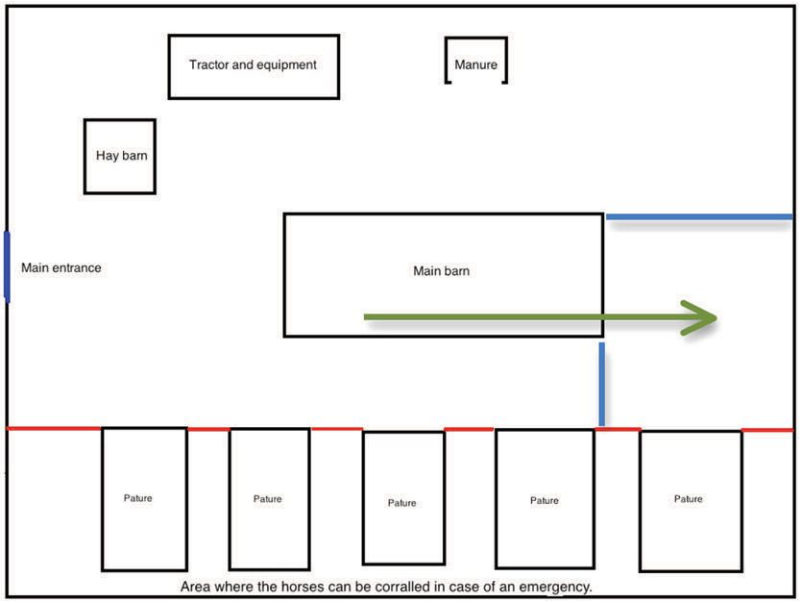

Fig. 18. Illustration of a fire lane in a barn. All the red accesses can be easily opened or closed in a case of an emergency and be used as a corral for the horses. Blue lines indicate permanent fences that can be used to direct released horses out away from the barn toward the corrals/pastures. Green line is direction of driving animals away from the barn as they are released from their stalls.

modern barns of lightweight wood construction. In some facilities, there may not be an attached paddock to every stall. Here a dedicated fire lane (where a few gates can be opened or closed) to provide safe egress as a group to corral the horses and drive them to an open paddock or pasture away from the barn. The ultimate goal is to save the horse from the burning building; however, if they are allowed to get loose and run down the road this will lead to tragic accidents with vehicles responding to the location. A holding area needs to be prepared in case of a fire, and this will allow a head count of both people and animals in a safe location.

Ingress to the property is crucial. First responders need easy access to the facility, preferably on gravel or paved roads that go around the barn. In case of an emergency the horses will need to be evacuated to paddocks or even alternative facilities requiring transport vehicles. Note: A gated property needs a plan established on how the rescue crew can enter. If it is a lock and key, then the fire station and police station should have a copy of that key, or use of a sound operated gate system can be implemented (Fig. 19). Any electrical gates should be supplemented by a solar system or with a battery backup. First responders will take appropriate action and demolish the gate in case of an emergency, but this is unnecessary with correct planning.

Cleanliness

The best fire retardant building materials, presence of a sprinkler system, and use of compartmentalization will not be maximally effective if the facility is not kept clean. Cobwebs and dust are substantial fuel sources for a fire, and are also easily combustible.[18] Cleanliness and maintenance of the electrical, immediate response, and detection systems are especially important (Fig. 20).

Emergency Plan

Lastly, an emergency plan should be written, implemented, and practiced. The facility's boarders, staff, local first responders, and any visitor should

Fig. 19. Siren operated sensor.

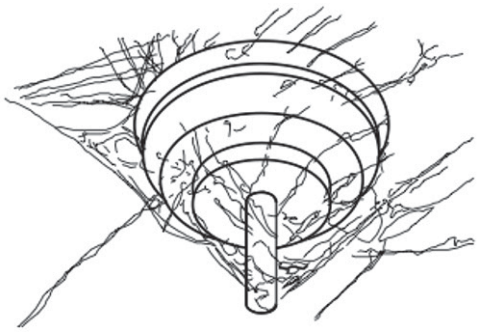

Fig. 20. Keep all detectors free of cobwebs and dust and maintained regularly. From Fire Control in Livestock Building, NRAES-39.[18]

be aware of the rescue strategies in case of an emergency. Emergency contact information sheets should be posted throughout the facility, along with first response equipment (fire extinguishers and hoses). Extra halters and lead ropes can be stored in a different building. Take advantage of the previously described compartmentalization and fire run-out plan for getting animals away from the burning building (Figs. 21 and 22).

3. Results

There are multitudes of simple to complex actions that can be taken in the quest to prevent a barn fire and improve the local response time and effectiveness of planning and prevention. As veterinarians and equestrians it is important to educate others and share our knowledge based on prior real losses and real response scenarios. Veterinarians will have the opportunity to walk into more different types of equine facilities (great, good, and bad) then most horse owners will in their lifetime. Using the information in this article and having better awareness of the problems will allow practitioners to make a difference within the industry by changing attitudes about fire losses.

Fig. 21. This new barn construction shows gates and pastures set up as a fire lane away from the barn in case of a fire. The original barn burned to the ground in 2008, but all of the horses were removed unharmed (photo courtesy of Windchase Farm).

Fig. 22. Windchase Barn Fire, 2008, approximately 24 minutes after lightning struck the barn. There is nothing left to salvage when a structure is fully involved. In this case personnel were on scene and saved all of the horses out of the barn (photo courtesy of Windchase Farm).[24]

The techniques and methods as discussed are intended to reduce the occurrence of barn fires and the associated losses of life (both equine and human) and facilities (invested dollars) that are related to them. For the best outcome, all components of prevention need to be implemented; however, even simple modifications to management and design can measurably reduce the incidence of fires and improve response by bystanders and emergency responders.

4. Conclusion

Barn fires are tragic events that cannot be completely eradicated but can be greatly reduced in occurrence. The number one priority should always be human safety and health, including for the veterinarian, bystanders, and first responders to the scene. Traditional building designs and management practices need to change to increase the success of detection, suppression, and response by both local fire departments and immediate responders. Many of the choices and aspects of building or retrofitting equine facilities affect the incidence of fires, and few traditional strategies are based on current research or technologies. Losses are not just limited to horses, but there are dramatic economic impacts on the loss of equestrian related facilities (including veterinary facilities) as well. Education of horse owning clients and the general public are vital to increased success of improved prevention and response methods being applied to our equestrian clinics and facilities.

Acknowledgments

Conflict of Interest

The Authors declare no conflicts of interest.

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Emergency Preparedness: A Fire Chief's Perspective

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1. Introduction

In a world of ever increasing specialization, it is important to remember that many occupations run parallel pathways. Veterinary medicine has influenced human medicine. Global Positioning Systems developed by the military have revolutionized civilian mapping, communications, and even vehicles. What connections could possibly be made between the fire service and veterinary medicine? Actually, many similarities exist. A paramount goal of both professions is to serve their clients through education and prevention programs in order to minimize emergencies and unexpected loss. Then, when something does go wrong, to provide capable response and help restore normalcy to the situation. Does it always work? No, but as professionals, it is our duty to try.

The 1970s ushered in the era of professionalizing the American Fire Service. Whether a career or volunteer department, responding to an emergency became a well thought out science. Lives saved through rapid Emergency Medical Services have steadily increased while property loss from fire has decreased. The ability to safely perform a wide range of technical rescue has improved as well. The 1990s challenged the fire service to respond to protracted emergencies, better termed *disasters*. Catastrophic acts of terrorism, hurricanes, wildfires, and hazardous materials incidents have caused the fire service to prepare and effectively respond to disasters. Forward thinking fire service leaders are now discussing how to prepare and respond to national *crises*, defined as widespread disasters protracted over months or years. Currently our nation is not well prepared to handle such extensive events.[1]

In much of the same way, veterinary medicine has seen profound changes in the industry over the last several decades. Medical research, technological advancements, and the demographic make up of the veterinary community have all contributed to advancements in equine care. At the same time, situations that veterinarians may find themselves and their clients in have grown more complex, and that is where our paths begin to cross.

2. Discussion

Education and prevention are important components of the fire service and the field of veterinary medicine. It is the basis of emergency preparedness as well. To put it in context, there are four phases of emergency management. It starts with *planning*. What are the risks, the likelihood of the risks, and the impact of the event should it occur in your clinic or community? The second phase of emergency management is the *mitigation* of the

NOTES

identified risk. What can be done to keep the risk from occurring, or at least reduce the severity of impact? As veterinarians desiring to prevent injury and illness, you can appreciate that practicing good medicine requires proactive education and prevention programs. Yet sometimes, bad things happen and a *response* is required. Timely implementation of a good plan, with appropriate resources that are well trained and equipped make for better outcomes than a response that does not have good preparedness as its foundation. Once the emergency is over, the phase of *recovery* begins. Whether rebuilding, healing, or attempting financial recovery, the greatest chance for a successful outcome is accomplished by those who have completed some level of preparation.

The fire service does many things well. A primary reason for this success comes from the practice of operating in organized teams. The foundation of the fire service is a team of two. Two teams make a company of four, which is the preferred staffing on a fire engine. Every firefighter on the engine knows their role, and is cross trained in each other's role, to provide depth to the team. Companies form Strike Teams and/or Task Forces, which in turn form larger Divisions and/or Groups. For very large incidents, another functional organization is a Branch. All of these subdivisions have an assignment, know who they work for and who works for them (Unity of Command), generally limit the number of subordinates to five people (span of control), know where they are to work, and how they are to communicate. All of this is managed by one (and only one) Incident Commander. What has just been described is the Incident Command System (ICS). Effective use of ICS comes from the fire service using it every day, on every incident. It is a flexible management model that provides for safety, accountability, and effectiveness. The systems approach capitalizes on the strengths of individuals working in teams, and can be applied to any field of business, including veterinary medicine. It also incorporates specialists, which is another arena veterinarians can expand into. If you anticipate ever operating with emergency services, it would be time well spent becoming familiar with the Incident Command System. A good overview of ICS and other preparedness classes can be found on-line at FEMA Emergency Management Institute's Independent Study.[2] The courses only take several hours to complete and provide the credentials required to work on federal incidents. Regardless of your interest in ICS, emergency preparedness still plays an important role in an equine professional's career.[3]

Planning

The following is a partial list of emergencies and disasters that could affect a veterinary clinic or their clients, precipitating interaction with emergency services. Determine what risks are probable and which are possible in your geographic area, factor in what the effect would be if they occurred, then prioritize which should be mitigated first.

- Clinic, barn, vehicle, home, or wildland fire
- Severe weather: tornado, snow, excessive prolonged heat or cold
- Flood
- Earthquake
- Vehicle, trailer, or entrapment accident: human and animal
- Serious personal injury or sickness
- Pandemic flu outbreak
- Man caused disaster: terrorism, financial system, or supply distribution breakdown
- Extended loss of water, electricity, gas, phone or internet communications.

Mitigation

For every risk identified, first attempt to implement prevention measures to reduce the chance of occurrence or lessen the severity if it should occur. A cursory look at the above risks could include some very basic remedies as well as some that may require a greater investment of time or financial resources. Remember, take care of your own family, home, and business first. By doing so, the veterinarian becomes a resource to assist others should a disaster occur. Often resolving the simple things (working batteries in flashlights and smoke detectors) creates momentum to tackle the bigger issues.

Fire

There is a plethora of information available on how to protect you from fire. Elimination of ignition sources—poor electrical wiring, overloaded circuits, improper storage of combustibles and flammables, and heat from open flame, lights, or equipment—are all easy ways to reduce fire risk. Working smoke detectors wired to provide notification and fire extinguishers in buildings and vehicles can provide early detection and allow for extinguishment when the fire is small. Sprinkler systems provide excellent fire protection. The National Fire Protection Association's *NFPA 150: Standard on Fire and Life Safety in Animal Housing* is the industry standard for fire prevention in these facilities.[4] Wildfire mitigation measures come from effective fuel separation and defensible space around structures as well as eliminating areas where wind blown embers could lodge into a combustible void space (Fig. 1). Information on wildfire prevention can be obtained from the Firewise website.[5]

Severe Weather, Floods, and Earthquakes

What type of weather/natural events does the region you live in experience? Minimally, you should be prepared to provide for your own needs for 72 hours with no public utilities. This includes shelter, water, food, and medical needs for people and animals. Make certain that insurance coverage is in place for any perils you face (Fig. 2).

Fig. 1. Wildfire threatening an equine facility.

Accidents

As an animal healthcare provider, have you expanded your knowledge to be able to provide adequate first aid and CPR to humans? Do you mandate seatbelt use, no texting, and defensive driver training? Do vehicles receive weekly checks and routine maintenance, including tires, lights, brake systems, and keeping the passenger compartment clear of flying objects of death (FODs)? Are facilities clear of entrapments for animals, with safe fencing and holding areas, and safe for people to work in close proximity with the animal? What local or regional resources exist for Large Animal Technical Rescue (LATR)? Many fire departments are now being trained to National Fire Protection Association's *NFPA Standard 1670, Annex K*, and are excellent resources for veterinarians facing a challenging equine rescue situation (Fig. 3).[6]

Fig. 2. Horses stranded in flood waters. AP Photo.

Fig. 3. Horse rescue from cattle guard with fire service assistance. Loveland Fire Rescue Photo.

Extended Personal Injury or Sickness/Pandemic Flu

For individual practitioners, what is your plan for keeping the practice covered through an extended injury or illness? Can your personal and business financial profile survive three to six months of your absence? Do you have Workers' Compensation coverage or some other type of insurance? If there is a pandemic flu outbreak, what is your plan for limiting exposure to infection or filling critical infrastructure needs with a reduced workforce? Remember that in such a situation, all regular services will be affected. To better understand the ramifications of pandemic flu, King County Washington has an excellent video that provides an overview of preparedness.[7]

Public Utility Infrastructure Breakdown

All public utilities and systems are at risk of failure, whether from natural disaster or a human causal factor.[8] It takes four gallons of potable water per person per day to provide for drinking and hygiene needs and ten gallons per horse. Can this be done without power or city water? How will communication with no internet or cell phones occur, or travel with limited fuel supplies? Can you operate a business with no communications, electricity, or natural gas for a week, a month? Can this be done in every month of the year or only seasonally?

Mitigating these risks requires advanced thought, a plan, and follow-through. Always confirm viable solutions by asking "what if" questions until an outcome is found that provides a feasible solution that requires no off site support. For example, what if the public water system fails for a week? The

thought of buying bottled water is limited as most likely there will be none in the stores within 12 hours. Stockpiling bottled water at 30 gallons/person/week creates storage and rotation issues. Boiling water requires time and fuel, and filtering requires the correct filtration system. For livestock drinking water, it may be appropriate to install a pond.

Response

Generally, you have up to 3 minutes to implement an effective response plan in an emergency. Having planned ahead for possible situations makes the response much more effective. This is primarily because you can focus on implementing a solution and not on trying to create one. Those that readily accept the situation as it is and respond accordingly have a substantially greater chance of surviving it well than those who cannot accept the reality of the situation. If you have built a relationship through interaction and training with your local fire department, your response will be much more effective.

Recovery

The process of recovery may be both physical and psychological. Working within a network of family, friends, and colleagues will be of great importance. Having retrievable back-up records is a must. A good relationship with your insurance, financial, and legal service providers and collaborative relationships with other veterinarians will pay huge dividends in the recovery process. Through education, your equine clients will receive invaluable information if guided through the same thought processes.

3. Summary

Ultimately, emergency preparedness is not difficult. It requires a little focused thought, a modest investment of time and finances, and the intentional development of a diverse and talented team. It is also not new. President Benjamin Franklin, founder of the American fire service and the insurance industry stated "A failure to plan is a plan to fail." Plan to protect your family and business, and then assist your clients to do the same.

Acknowledgments

Conflict of Interest

The Author declares no conflicts of interest.

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How to Perform an Oral Examination Utilizing a Five-Component Protocol

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1. Introduction

For years, the equine oral exam was a procedure in veterinary practice performed primarily to determine whether the horse's teeth were in need of "floating." As modern equine dentistry has advanced, our knowledge and understanding of equine oral health has greatly expanded, leading to a need for a more systematic and thorough examination approach.[1,2] Protocols in other species utilize specific components related to the oral cavity and teeth in order to systematically perform the examination.[3] Adapting similar principals to horses, the components of an oral examination include the following:

1. Extraoral examination of the head.
2. Evaluation of occlusion.
3. Evaluation of the periodontal tissues.
4. Evaluation of endodontic structures of the clinical crown.
5. Evaluation of the oral soft tissues.

The purpose of this paper is to outline a five-component protocol for oral examination. It is beyond the scope of this manuscript to address detailed descriptions of oral pathologies and detailed dental charting procedures. Further reading can be found in well-written texts for abbreviations and information regarding recordkeeping of oral findings.[1,4,5] Using a systematic approach to oral examination minimizes the risk of overlooking oral pathology and helps the veterinarian formulate an appropriate treatment plan for the patient.[6]

2. Materials and Methods

A thorough history and physical examination of the patient should be performed prior to administering sedation for dental procedures.[6] Instrumentation recommended for the oral examination includes a full mouth speculum, support for the head (headstand or dental halter), bright light source, oral dosing syringe, intraoral mirror, periodontal depth probe, and occlusal surface explorer. The author prepares an oral rinse solution for the instrument bucket by adding 10 ml to 20 ml of chlorhexidine gluconate (2% solution) per gallon of water. The presence of chlorhexidine solution in the rinse bucket also helps eliminate fogging of the dental mirror. Additional instrumentation that may aid the exam includes periodontal forceps, alligator forceps, and a dental scaler.

A dental chart is utilized to record findings. The chart utilized by the author (Fig. 1) has been designed to encompass all five components of the exam. The front page is used for recording pertinent oral findings for individual dental quadrants and the back page of the chart allows formulation of

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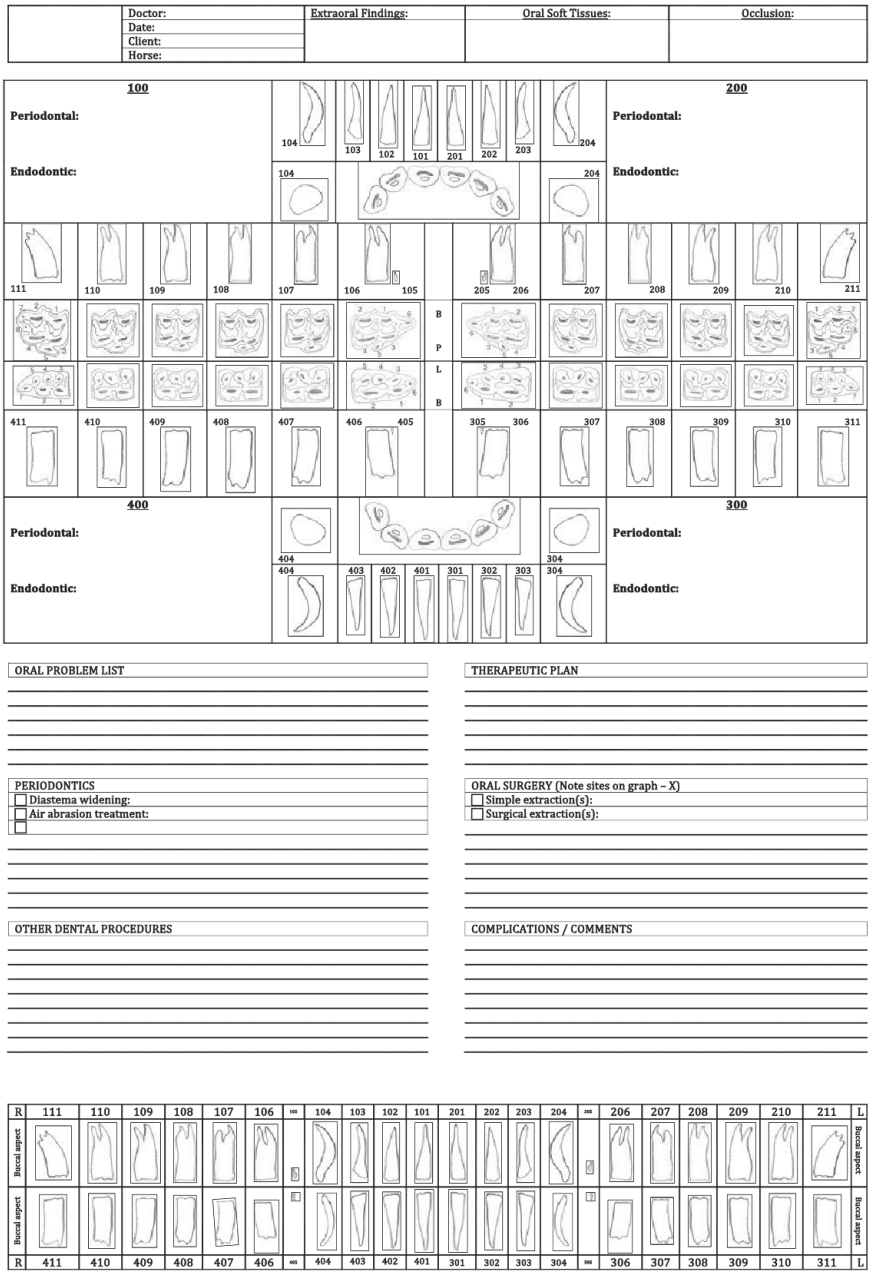

Fig. 1. Equine dental chart used by the author's practice. The front page is utilized for examination findings and back page for problem list, treatments, and complications/recommendations.

the oral problem list, treatments, and complications that were noted during procedures.

3. Results

Extraoral Examination

This component is usually performed prior to sedation and involves visual and tactile assessment of the head region. Performing the extraoral examination prior to sedation allows the practitioner to more accurately evaluate symmetry and whether the patient reacts painfully to palpation of different skull structures or abnormal areas of the head (this may be particularly important if attempting to rule-out jaw fracture prior to the mouth speculum being placed). Under normal conditions each of the ear pinnae, temporal muscles, temporomandibular joints, masseter muscles, orbits, globes, facial bones, and nostrils appear symmetrical in the horse. In some instances, if abnormalities of symmetry or abnormal swellings of the head are encountered, additional diagnostic testing may be indicated such as lab work, radiography, or computerized tomography.

Abnormal facial symmetry may result from the following conditions (Figs. 2A–2C):

Fig. 2. A, Extraoral finding: bony swelling associated with nonvital teeth (arrows). B, Extraoral finding: nasal discharge associated with secondary dental sinusitis. C, Extraoral finding: draining tract on a mandible associated with a nonvital Triadan 307.

- muscle atrophy
- soft tissue enlargement
- bony enlargement/indention
- neurological problems
- skull deformity

Abnormal swellings on the head may occur as a result of the following:

- tooth-root disease
- eruption cysts
- neoplasia
- sinus problems
- trauma
- guttural pouch enlargement
- lymph node enlargement
- salivary gland enlargement
- odontogenic cysts

Occlusion Examination

Evaluating Occlusion of the Incisor Teeth (Prior to Speculum Placement)

- When the horse's mandible is in a centric position, normal occlusion between upper and lower incisors when viewed frontally is a level bite.
- When the profile of the incisor teeth is examined, the labial edge of the upper and lower incisors meet evenly in normal occlusion; however, this is somewhat dependent upon head position in that the mandible retracts when the poll is extended and protrudes when the poll is flexed.
- Lateral excursion can be assessed to document the extent of molar occlusion using the methods of Rucker.[7]

Evaluating Occlusion of the Cheek Teeth

- The horse's anatomy results in a sloped chewing surface with enamel points on the buccal aspect of the upper cheek teeth and the lingual aspect of the lower cheek teeth.
- The mandible and maxilla have differing width (anisognathia), and the mandibular cheek teeth appear slightly more narrow than the maxillary cheek teeth.
- The rostral portion of the left and right upper rows of cheek teeth curve slightly toward midline.
- There is a normal upward curvature of the occlusal surface of the caudal aspect of the upper and lower dental quadrants (Curve of Spee).

Descriptions of Malocclusion

There are several descriptive terms for malocclusions in the horse. Malocclusions are often described when there are overlong portions of the

dentition involving the clinical crown due to a lack of attrition from opposing teeth. This situation is progressive and without intervention from the practitioner, can result in poor mastication, soft tissue injury, and overall poor health. It is important for one to identify why there is a lack of attrition as this is often the *primary* disease and the overlong portion of crown is a *secondary* response. Therefore, it is important to not only identify the overlong tooth/teeth but to identify the pathology causing the lack of attrition.

The nomenclature committee of the American Veterinary Dental College (AVDC) has divided malocclusions of dogs into two different types. They are described as skeletal malocclusions and dental malocclusions.[8]

Malocclusions can be described as *skeletal malocclusions* when they are related to an anatomical discrepancy of the jaws. *Dental malocclusions* are malocclusions that are related to positioning of individual teeth within normal jaws. Horses can have combinations of dental and skeletal malocclusions; an example would be an overbite in addition to a missing left mandibular first molar (Triadan 309) with an overlong left maxillary first molar (Triadan 209) representing a dental malocclusion. One must also have a working knowledge of equine dental anatomy pertaining to age and the eruption pattern of teeth as this will account for some malocclusions that can be noted in juvenile horses.[9] The scope of this paper is not to discuss every potential malocclusion but to have the reader understand the difference of skeletal versus dental malocclusions. This will allow the practitioner to formulate a dental treatment plan. When evaluating occlusion and planning treatment, the author keeps the following concepts in mind:

Skeletal malocclusions are teeth in an abnormal position as a result of asymmetry or deviations that exist between the bones that support the teeth. Skeletal malocclusions are noted when there is maxillary-mandibular asymmetry, which can be seen in a *rostro-caudal*, *side-to side*, or *dorso-ventral* directions. Examples of these types of malocclusions are what have previously been described as overbite, underbite, wry nose, and several other terms. Using overbite as an example, the horse has an abnormal conformation where the mandible occludes caudal to its normal position with the maxilla. This can cause malocclusions of the incisors, premolars, and molars. In general, skeletal malocclusions will have several teeth affected and be subject to ongoing occlusal adjustments to maintain the dental quadrants. Examples include mesial hooks, distal hook/ramps, excessive transverse ridges, and overlong maxillary and mandibular incisors that can be seen in horses with overbite or underbite.

Dental malocclusions are noted when specific teeth are out of normal position in the dental quadrant and the supporting bones have a normal relationship. Examples of a dental malocclusion would be an overlong supernumerary tooth lacking wear during mastication from an opposing tooth. This would result in a dental malocclusion. Other examples of dental malocclusions are situations where teeth are missing from a quadrant or where there is overcrowding and a tooth is not in a normal alignment with the rest of the teeth in the quadrant. When a malocclusion involves a tooth angled towards the tongue, the tooth is *linguoverted*. If the malocclusion involves a tooth angled towards the cheek, it is *buccoverted*. There are several other descriptive terms for teeth that the reader can find in the nomenclature section of the AVDC website (http://www.avdc.org/nomenclature.html#occlusion). Remember that a dental malocclusion is often related to a single tooth or a few teeth and can be secondary to other disease processes.

Classifications of Malocclusions

The nomenclature committee of the AVDC has further accepted a classification of *symmetrical* skeletal malocclusions which are described by the relationship of the mandible to the maxilla.[8] This classification system has also been described for use in the horse.[10]

Neutroclusion (Class 1 malocclusion; MAL/1): a normal rostral-caudal relationship of the maxillary and mandibular dental arches with malposition of one or more individual teeth. This would be an example of a dental malocclusion without a skeletal malocclusion. An example would be a horse with normal rostro-caudal relationship with an overlong left maxillary M1 (Triadan 209) as a result of a missing left mandibular first molar (Triadan 309) (Fig. 3A).

Mandibular distoclusion (Class 2 malocclusion; MAL/2): An abnormal rostral-caudal relationship between the dental arches in which the mandibular arch occludes caudal to its normal position relative to the maxillary arch. This was formally described as relative maxillary prognathia or parrot mouth. Dental malocclusions would be listed separately (Fig. 3B).

Mandibular mesioclusion (Class 3 malocclusion; MAL/3): An abnormal rostral-caudal relationship between the dental arches in which the mandibular arch occludes rostral to its normal position relative to the maxillary arch. This was formally described as relative mandibular prognathia or monkey mouth. Dental malocclusions would be listed separately (Fig. 3C).

The author's opinion is that identifying the type of malocclusion, whether skeletal or dental, brings greater understanding of how to address the overlong dentition. For instance, a skeletal malocclusion will never be fixed, but can be maintained through serial occlusal adjustments. Dental malocclusions, however, can be secondary to other pathology that the practitioner may need to treat along with correcting the malocclusion.

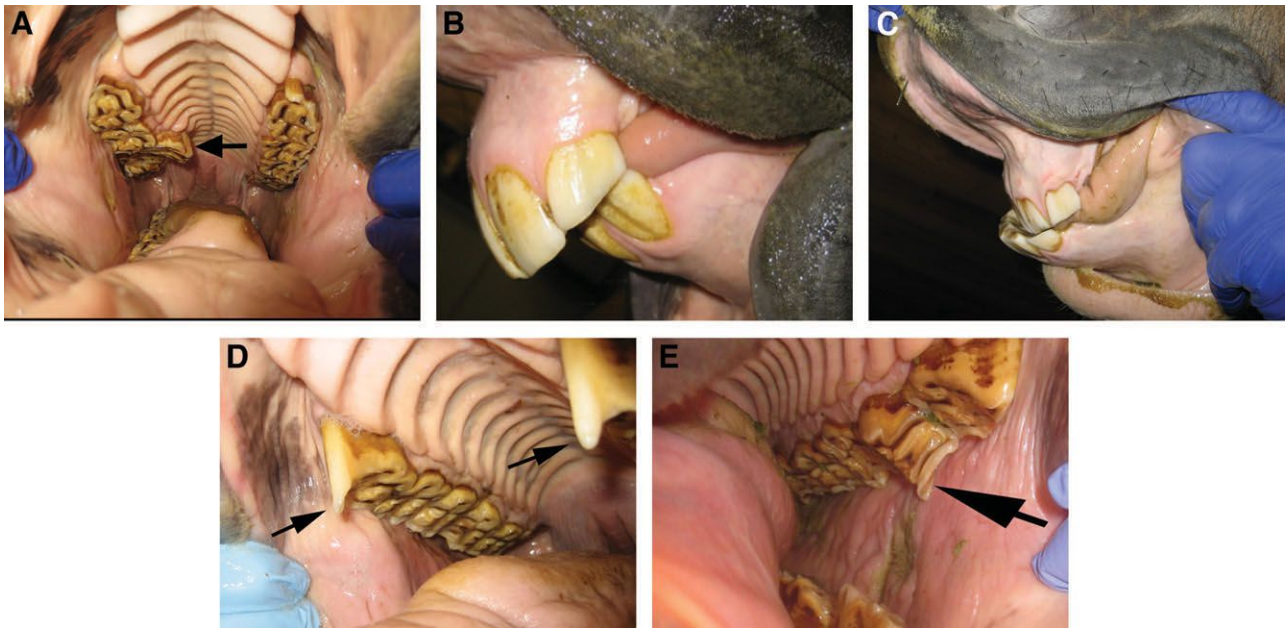

Fig. 3. A, Example of a dental malocclusion with linguoversion of the Triadan 108 (arrow) related to overcrowding in the dental quadrant. B, Example of Class 2 malocclusion (MAL/2): mandibular distoclusion, or relative maxillary prognathia. C, Example of Class 3 malocclusion (MAL/3): mandibular mesioclusion, or relative mandibular prognathia. D, Example showing a mesial hook of 106 that resulted in this case from skeletal malocclusion due to a Class 2 (Mal/2) malocclusion (overbite). E, Example of a dental malocclusion with an overlong crown of Triadan 207 due to a missing Triadan 307.

Oral Soft Tissue Examination

The oral soft tissues include the tongue, lips, cheek, mucosa, hard and soft palate, and interdental space. Examination of these structures may identify bleeding, abnormal growths, oral ulcerations (which are full thickness mucosal lesions; Fig. 4A), or abrasions (partial thickness lesions of the mucosa; Fig. 4B). The presence of mucosal lesions can often be associated with sharp enamel points or training hardware on the bars of the mandible and in the commissure of the lips. If an abnormal oral mass is identified, an incisional biopsy can be obtained at the time of the examination (Fig. 4C). Mucosal enlargement can be related to retained teeth, as in the case of retained maxillary first premolars (Triadan 05); if these are detected, radiographs should be obtained demonstrating the retained tooth. Lacerations also can be associated with any of the oral soft tissues, particularly the tongue, lips, hard palate and soft palate (Fig 4D). If an injury of the tongue is noted, a radiographic or ultrasound examination may be indicated to rule out a penetrating foreign body such as wire.

Examples of oral soft tissue problems are below:

- bleeding
- abnormal growths
- ulcerations
- abrasions
- lacerations

Important Note on Periodontal and Endodontic Examination

The periodontal tissues and endodontic structures of the clinical crowns of the incisor teeth and canine teeth are readily available for examination at the front of the mouth prior to speculum placement. An intraoral mirror or oral endoscope is utilized to better visualize the cheek teeth after opening the speculum. It is important to thoroughly rinse organic debris from the mouth so that the periodontal and endodontic components can be examined.

Periodontal Examination

Oral lesions associated with periodontal problems include the following:

- periodontal pocketing of roughage
- gingival enlargement
- gingivitis and bleeding
- pathologic diastemata
- gingival recession
- halitosis
- calculus accumulation

The structures relating to the periodontia are the gingiva, gingival sulcus, periodontal ligament, cementum of the tooth, and the alveolar bone. The normal depth of the gingival sulcus of the incisors, canine teeth, and cheek teeth in the horse is generally considered to be 3 mm to 5 mm. *Periodontal*

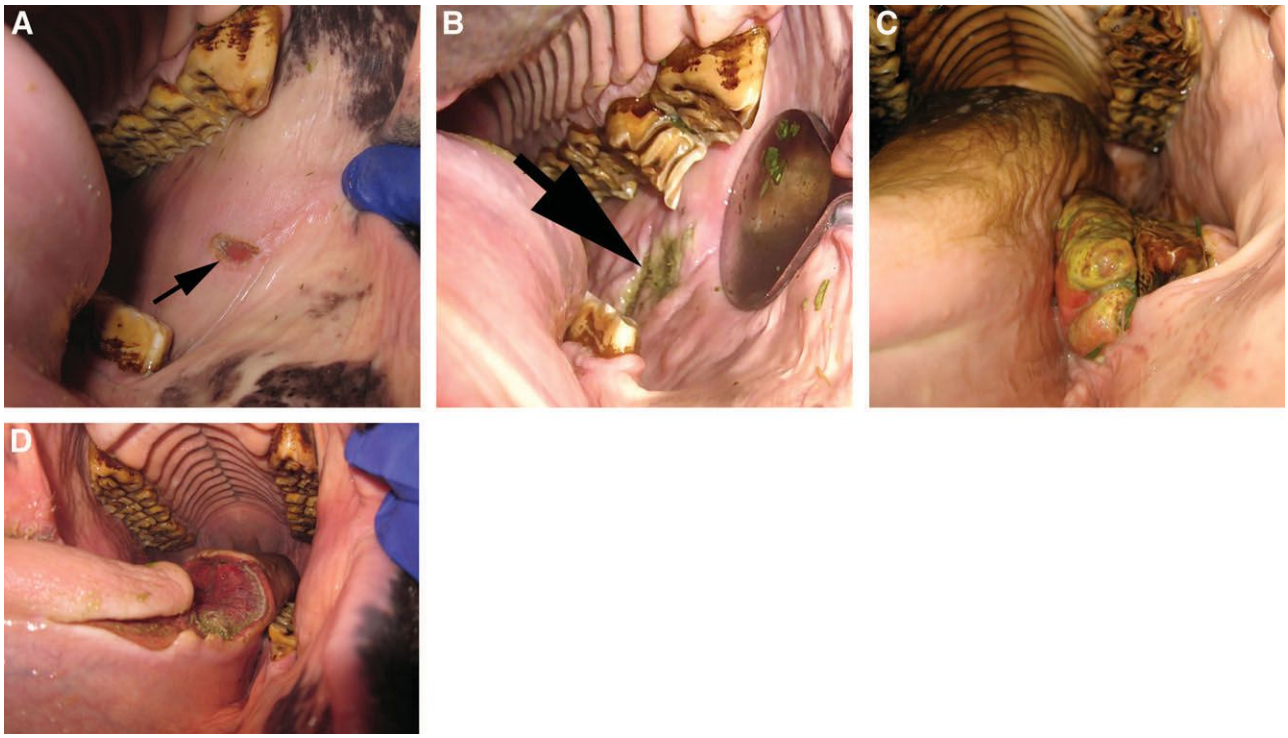

Fig. 4. A, Mucosal ulcer associated with overlong crown of 206. B, Mucosal abrasion (arrow) from overlong 207. C, A mass on the lingual aspect of the 300 dental quadrant was identified as an oral mandibular fibrosarcoma. D, Example of a severe tongue laceration.

disease is a progressive condition first recognized as gingivitis. If the alveolar bone becomes affected then the condition is termed *periodontitis*. The presence of periodontitis can only be determined by obtaining radiographs of the affected area and assessing the amount of bone loss[11](Figs. 5A and 5B).

Periodontal disease is often painful for patients and local anesthesia may be needed to complete the exam. Investigation of a periodontal problem includes removal of entrapped roughage using irrigation and instrumentation (i.e., alligator forceps and right angle perio forceps). The periodontal depth probe is utilized to explore the gingival sulcus and measure pocket depths. Tooth mobility is a function of periodontal attachment and, if compromised, an increase in tooth mobility will be noted[11] (Table 1). The author places the tip of a dental scaler on the occlusal surface of a tooth and manipulates the instrument in order to assess the mobility of individual teeth. It is the author's opinion that in all horses with a periodontal finding, radiographs should be obtained so that staging of the periodontal disease can be performed (Fig. 5C). Further information about evaluating periodontal tissues of the teeth can be found in another manuscript in this section.

Endodontic Examination

Structures to be examined include the occlusal aspect of the infundibula of the incisor teeth and maxillary cheek teeth, the occlusal surface of the pulp chambers of the incisor teeth, and pulp horns of the cheek teeth. Normal dentin associated with the occlusal aspect of the pulp has a characteristic tan-ivory color, depending, in part, on pigmentation from forage. More information regarding dentin classification and characteristics of equine dentin is covered in a different manuscript in this section. Areas where food is adhering to the surface of the teeth should be rinsed clean and an occlusal surface explorer used to determine if pathology is present. An abnormality of dentin over the occlusal aspect of the pulp may be associated with deeper endodontic problems and it is important during this component of the examination to attempt detection of irregularities such as fractures, caries, or necrotic pulp exposure. Radiography can be performed to better assess teeth with endodontic problems and to evaluate surrounding bone and sinus structures. Small fissure lines through occlusal dentin and enamel are frequently identified in cheek teeth, but the significance of these subtle lesions is not understood. When an irregular surface is noted, the occlusal surface explorer should be used to investigate the structure (Figs. 6A and 6B). Abnormal or necrotic areas of dentin over the occlusal end of the pulp will usually appear dark in color and contain feed material. The tip of the explorer will 'catch' or bury into the abnormal dentin or pulp tissue.

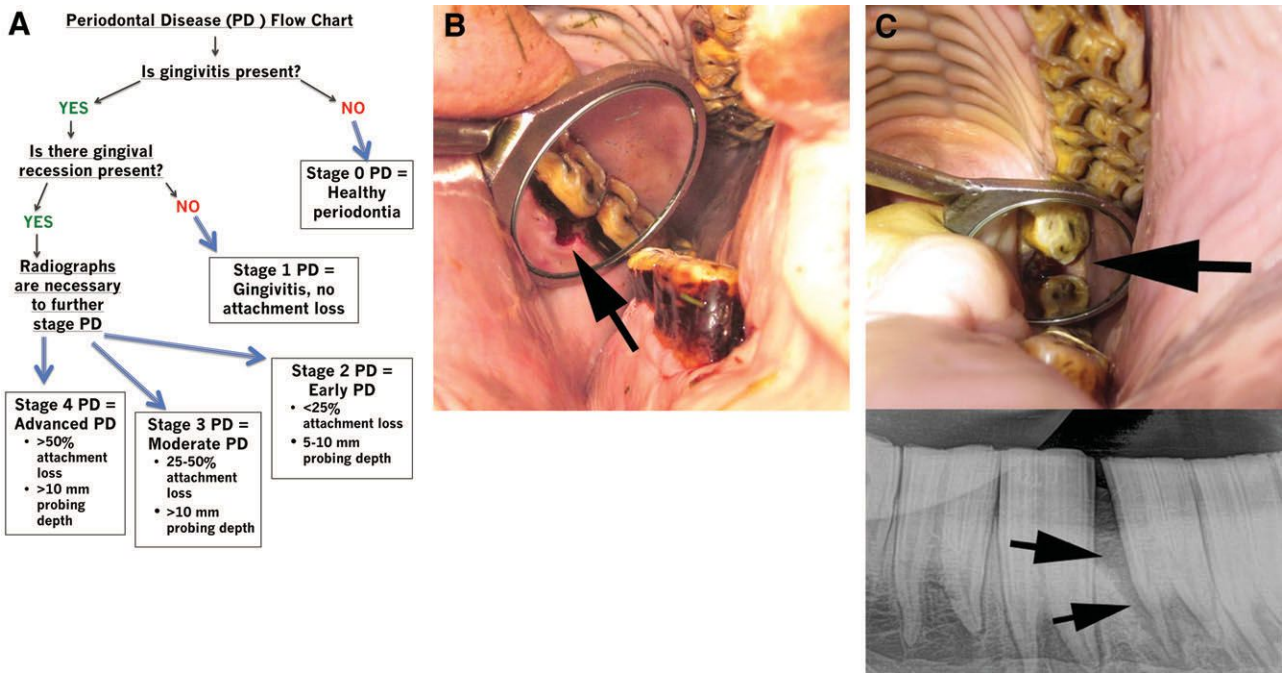

Fig. 5. A, Flow chart produced by the author for staging periodontal disease (adapted from Klugh[11]). B, A periodontal pocket and gingival recession is visible on the lingual aspect of the interproximal space at Triadan 307–308. C, Photograph of periodontal pocket at Triadan 308–309 and intraoral radiograph of the affected mandibular cheek teeth. There is severe (Stage 4) periodontal disease Triadan 309 (as evidenced by horizontal and vertical bone loss at the interproximal space of Triadan 308–309 with >50% attachment loss)

Tooth fractures are considered an important finding of the endodontic exam. When a fractured tooth is encountered, attempt to use the tip of the explorer to investigate the fracture line. One must determine if the pulp horn or chamber has been exposed and is devoid of dentin or if dentin exists within the pulp chamber or horn. The presence of dentin indicates a barrier that could be protective vital pulp tissue, preventing bacterial and food migration to the apex of the tooth. Classification of the fracture is based on involvement of specific tooth structures and whether pulp involvement is noted[12] (Table 2). Fractures above the gumline are considered *crown fractures*. A *complicated crown fracture* is a fracture of the clinical crown that involves the pulp horn or pulp chamber of the tooth, while an *uncomplicated fracture* does not involve the pulp. If the fracture incorporates the clinical crown and extends below the gumline, it is considered to involve the root of the tooth and is termed a *crown root fracture* (the horse has a reserve crown of tooth below the gumline which brings confusion to this classification; the reserve crown below the gumline in the horse has periodontal ligament attachment and functions similar to the root structure). *Root fractures* are fractures of the tooth only below the gumline and do not involve the clinical crown of the tooth.

An infundibulum is a blind invagination of enamel from the occlusal surface that is normally filled by cementum. Disorders of the infundibula of maxillary cheek teeth include incomplete cemental filling, which is thought to predispose to carious decay.[13] Infundibular caries are frequently noted in the infundibula of the maxillary cheek teeth and must be differentiated from areas of abnormal dentin over a pulp horn by the examiner. Infundibular caries are classified according to stage of their progression[14] (Fig. 6C); advanced infundibular disease may lead to development of fractures and periapical infection.[15]

Table 1. Tooth Mobility Scores

Mobility Score	Amount of tooth movement
0	Normal tooth and periodontium
1	First discernible sign of tooth movement
2	<3 mm tooth movement
3	>3 mm tooth movement; the tooth can be depressed into alveolus

4. Discussion

An oral examination that follows this approach results in the practitioner evaluating specific areas related to the oral cavity and facilitates association of lesions with specific disease entities (e.g., a valve diastema is a component of periodontal disease). The component-based exam essentially gives the

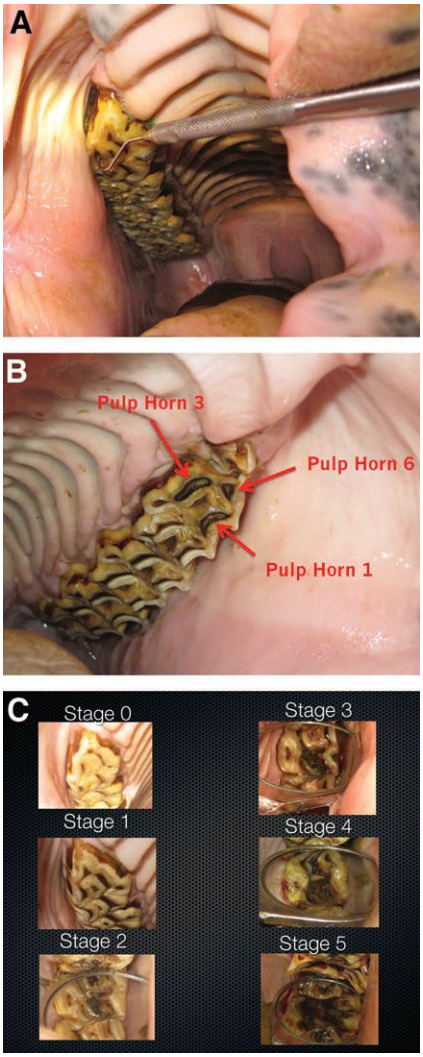

Fig. 6. A, Occlusal surface explorer is used to confirm the presence of exposed pulp horn #1 Triadan 106. B, Exposed pulp horns #1, 3, 6 of Triadan 206. Discoloration and trapping of roughage in the affected pulp horns is visible with close inspection. C, Infundibular caries staging system used by the author (adapted from Dacre[14]): Stage 0—Normal tooth; Stage 1—Caries involve cementum only; Stage 2—Caries involve cementum and infundibular enamel; Stage 3—Caries involve cementum, infundibular enamel, and surrounding dentin; Stage 4—Fracture of the tooth has occurred, extraction may be indicated; and Stage 5—Tooth has fractured such that it is nonvital and extraction is indicated.

practitioner a checklist to follow and decreases the temptation of identifying and focusing on a lesion without completing the entire examination, potentially missing other pertinent pathology. Studies have shown that equine dental disease can be present in 36% to 85% horses in first opinion practice.[16] It is the author's experience that many horses presenting for "routine" dentistry with no history of oral problems will have findings noted after thorough examination where further treatment and diagnostics are indicated.

Table 2. Tooth Fracture Classification

Tooth fracture classification	Description
Complicated fracture	Involves pulp
Crown fracture	Fracture of clinical crown that does not extend below gum line
Crown-root fracture	Fracture incorporates the clinical crown and extends below the gum line
Root fracture	Fracture of the tooth below the gum line not involving the clinical crown

5. Summary

The oral examination should result in a complete assessment of the patient's oral health and using a five-component protocol helps bring greater understanding to the veterinarian of oral lesions as they pertain to specific dental disease categories. Adhering to a systematic approach should improve the veterinarian's likelihood of identifying and managing equine oral pathologies prior to becoming advanced problems.

Acknowledgments

Conflict of Interest

The Author declares no conflicts of interest.

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How to Incorporate Oral Endoscopy Into an Equine Dental Examination

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1. Introduction

Accurate identification and documentation of pathology is very important for dental treatment planning. The interdental space, canine teeth, incisor teeth, and associated gingival tissues are positioned near the opening of the mouth and do not require sophisticated techniques for detailed examination and documentation. However, challenges exist in achieving adequate visualization of the caudal portion of the oral cavity due to a number of factors including the following:

- Relatively small mouth opening compared to the length of the oral cavity.
- Presence of a large, powerful tongue that obstructs visualization and impedes instrumentation.
- Restricted buccal space with tight adherence of the cheeks and skin to the skull.

Intraoral imaging systems can be utilized by veterinarians to augment dental examination and to provide intra-operative guidance during extraction or dental treatment procedures.[1–7] The magnified image generated by an intraoral imaging system greatly improves visualization and allows detailed examination (Fig. 1). Generally speaking, instrumentation for intraoral imaging is classified as follows[7]:

- *Oral endoscopic system*: consists of a rigid endoscope, light source, digital video camera, video monitor, and recording device. Rigid endoscopes for equine use typically have a diameter of 5 mm to 15 mm, working length of 50 cm, and an angled viewing lens of 60 to 115°.
- *Handheld intraoral camera*: adapted from human dentistry, these have a short, self-contained handpiece (approximately 22 cm long) coupled via USB to a computer with a monitor and software for viewing and recording. The device contains a built-in fixed focus camera with an LED light source and an image capture button located on the handle.

The purpose of this manuscript is to describe the author's approach using oral endoscopy as a component of dental examination and treatment. For a more comprehensive discussion on intraoral imaging systems and oral photography, the reader is referred to the manuscript by Galloway and Easley.[7]

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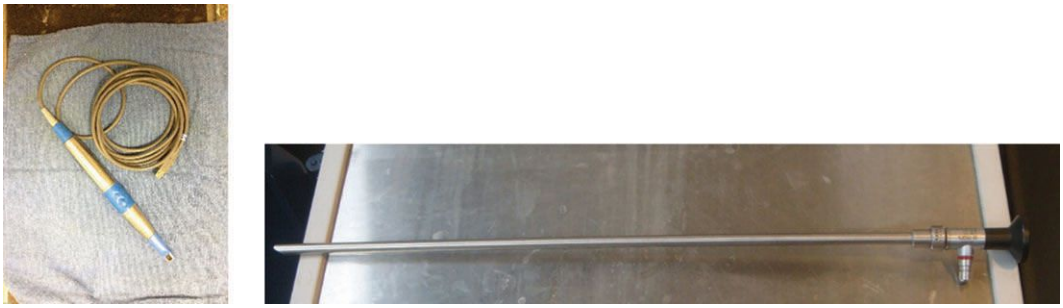

Fig. 1. Examples of a handheld intraoral camera (left; photograph courtesy of Travis Henry, DVM; Elkhorn WI) and a rigid endoscope for equine oral examination (right).

2. Materials and Methods

Endoscopic instrumentation (Fig. 2):

- Rigid endoscope (5.5 mm, 60°; working length 49.5 cm)[a]
- Protective sheath (7.9 mm, 5.8 mm capacity, working length 45.7 cm)[b]
- Video camera[c] and video camera control unit[d]
- Digital image capture device[e]
- Video monitor,[f] light source,[g] light cable,[h] and laparoscopy tower[i]

Oral examination procedure sequence:

- Discuss the primary complaint and clinical history with the client.
- Enter the patient information into the image capture device to create a folder for photographs and video obtained during the endoscopic examination.
- Perform an appropriate physical examination and external examination of the head.
- Administer sufficient sedation and analgesia to minimize chewing motion, tongue movement, and head movements (e.g., detomidine hydrochloride[j] 0.010–0.020 mg/kg IV with butorphanol tartrate[k] 0.005–0.010 mg/kg IV).
- Rinse the horse's mouth.
- Support the horse's head in a comfortable position for the horse.
- Perform initial oral examination of the incisor teeth, canine teeth, interdental space, associated soft tissues, and gingiva.
- Insert and open the dental speculum, lower the head and rinse any roughage, food, or foamy saliva accumulated on the occlusal surface of the cheek teeth to improve visualization.
- Elevate the head to continue the intraoral examination utilizing a bright light source and traditional methods.
- After initial intraoral examination, move on to the endoscopic exam.

Intraoral endoscopic examination:[2,5,6,7]

- Position the instrumentation tower and monitor for comfortable viewing by the examiner (Fig. 3).

Fig. 2. Components for intraoral endoscopy include a portable laparoscopic tower, video monitor, digital image capture device, video camera control unit, light source, video camera, and rigid endoscope (top). The video camera uncoupled from the endoscope (bottom).

Fig. 3. Performing intraoral endoscopic examination.

- Dip the oral end of the endoscope in warm water to eliminate fogging (repeat as needed throughout the examination).
- Ideally, assistants retract the ipsilateral cheek and gently stabilize the tongue to the side opposite. This improves visualization and minimizes unwanted movement of the scope.
- At any point during the examination, abnormalities of the clinical crowns or gingiva can be investigated under endoscopic guidance.
- During the course of the examination, photographic images and video can be shot using the capture device controls (the tower should be positioned to allow one assistant to use the capture device controls).
- The examiner places a hand on the lower bite plate of the dental speculum (generally, the author's right hand) to stabilize and guide the endoscope. The other hand is used to insert or withdraw the endoscope, rotate the instrument to the desired view, and adjust the focus ring and camera.
- Beginning on the right side of the horse's mouth, position the endoscope between the upper and lower dental quadrants and focus to view the occlusal surface of 106.
- Carefully advance the scope to the distal aspect of the last upper cheek tooth, viewing the magnified image of the occlusal surface, gingiva, and interproximal space of each tooth.
- At the distal aspect of 111, the endoscope can be rotated slightly to view in a vestibular (buccal) direction and positioned toward midline; the scope is slowly withdrawn to examine the cheek and palatal aspect of the 100 quadrant (gingiva, teeth, and interproximal spaces).
- To examine the vestibular (buccal) aspect of the dental quadrant, the scope is reinserted to the distal aspect of 111 and positioned to view in a palatal direction from the vestibule. The endoscope is slowly withdrawn to examine the gingiva, teeth, and interproximal spaces.
- After completing the examination of the 100 dental quadrant, the endoscope is rotated to view the 400 quadrant and the examination is carried out in a similar manner.
- For mandibular teeth, gently pressing the tongue away from the teeth with a long retractor improves visualization by the endoscope.
- After the 400 quadrant, the endoscope is moved to the left side of the mouth to evaluate the 200 and 300 dental quadrants in a similar fashion.
- For visual guidance with intraoral procedures (e.g., verifying correct placement of a molar spreader), an assistant holds the endoscope with the desired view while the veterinarian has both hands free to position or manipulate instruments.

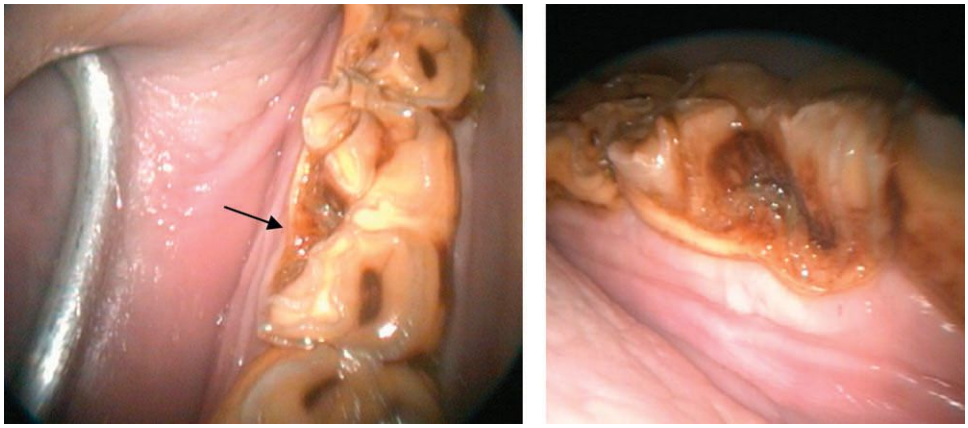

Fig. 4. Occlusal surface of fractured tooth 309 (arrow); the tongue is pressed gently with a long retractor to improve visualization (left). The endoscope has been rotated to view the lingual aspect of 309 (right).

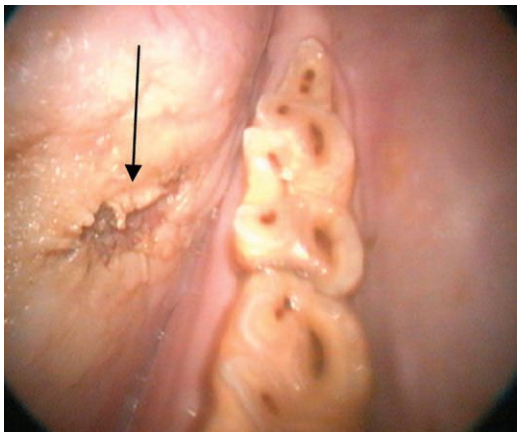

Fig. 5. Large abrasion on the side of tongue (arrow) associated with sharp enamel points of 300 dental quadrant.

Fig. 7. Necrotic pulp exposure of pulp horn #5 (PH#5) is confirmed and documented under endoscopic visualization.

- During the examination, an efficient method is to call-out the findings while an assistant records the information in the patient record.
- At the completion of the procedures, images on the capture device are copied to a flash drive and labeled with appropriate descriptions.
- Video and images are then copied from the flash drive to a storage drive on the local network.
- Between patients, remove the camera and disassemble the protective sheath from the endoscope, wipe the instruments with isopropyl alcohol 70%, and flush the chamber of the protective sheath with alcohol solution.

3. Results

It is important to know that excessive tongue motion, and especially chewing motion, create difficulty focusing and stabilizing the rigid endoscope.[5] Intravenous administration of detomidine hydrochloride and butorphanol tartrate works well, in the author's experience, to provide excellent sedation-analgesia and reduce chewing motion for oral examination and intraoral endoscopy. Having assistants available to gently stabilize the head and tongue and retract the ipsilateral cheek improves the steadiness of the endoscope. Using a long straight retractor to move the tongue away slightly from the mandibular teeth improves visualization[3,6]; however, use of a tongue retractor sometimes incites excessive chewing action and requires heavier sedation in some horses.

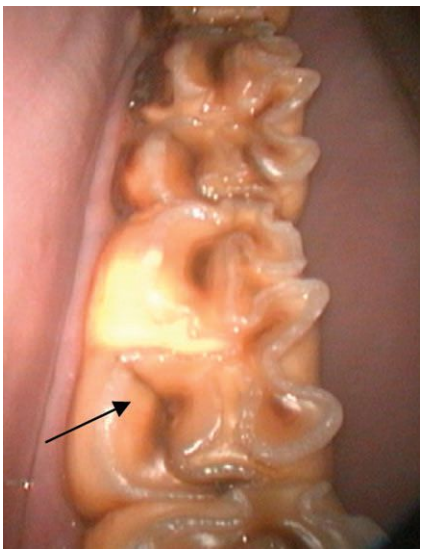

Fig. 6. A fissure is visible in the dentin associated with pulp horn #1 (PH#1) of a mandibular tooth (arrow).

Rigid oral endoscopy augments commonly used examination techniques and provides a detailed, magnified viewing of dental and soft tissue structures in the mouth. The improvement in visualization increases diagnostic confidence when confirming findings from the initial examination and the instrumentation facilitates the precise investigation and documentation of questionable or pathologic areas. The author finds that it can be more challenging to obtain the desired view of the vestibular (buccal) aspect of the upper cheek teeth compared to the other views, presumably due to the space restriction within the vestibule of the cheek. Oral endoscopy should not be viewed as a replacement for more commonly utilized examination procedures, but with it there is improved capability of detecting subtle changes that may be more difficult to visualize with a dental mirror, such as focal gingival recession or other lesions involving teeth located in the caudal portion of the mouth.[3] Examples of oral conditions identified with endoscopy (Figs. 4–7)) may include the following:

- Abrasions of the cheek and tongue
- Sharp enamel points
- Dental elongations
- Fissure lines involving dentin and enamel

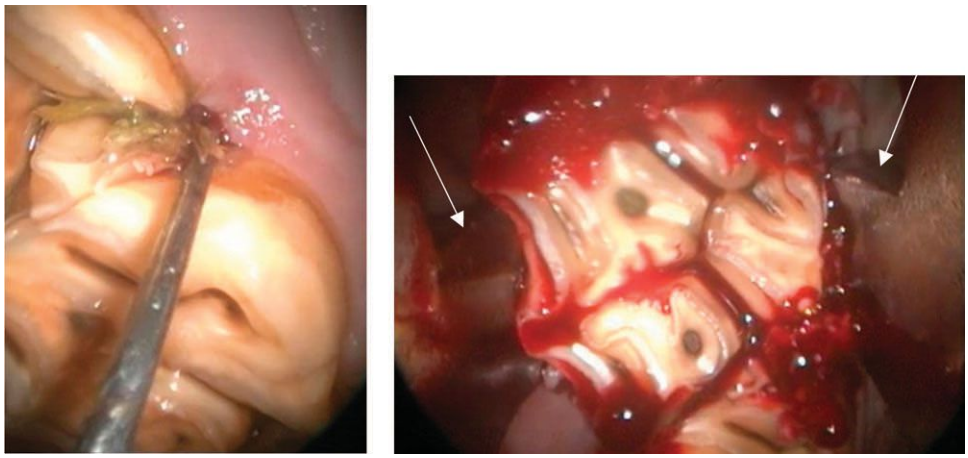

Fig. 8. Examples of intraoral guidance with the endoscope: investigating focal gum recession and periodontal pocketing (left); verifying placement of the extraction forceps (right; white arrows indicate forcep jaws).

- Diastemata
- Periodontal pockets
- Gingival recession
- Necrotic pulp exposure
- Crown fracture
- Infundibular changes
- Peripheral cemental abnormalities
- Supragingival calculus
- Parasitic larvae (*Gasterophilus* sp.)

In addition to oral examination, using the endoscope improves visualization and accuracy for other dental procedures (Fig. 8):

- Investigating periodontal and endodontic abnormalities involving the clinical crown.
- Positioning the molar spreader.[7]
- Positioning dental forceps and right-angle dental picks.[4]
- Positioning a drill bit.
- Intraoral guidance of extractions when utilizing minimally-invasive buccotomy instrumentation.
- Placing an alveolar packing.
- Inspecting the dental alveolus intra-operatively and post-operatively.

4. Discussion

Intraoral examination with rigid endoscopy appears to be safe and effective as previously reported by others. With the procedure, there is significant enhancement of visualization that allows detailed examination of the vestibular (buccal), occlusal, lingual, and palatal aspects of the cheek teeth quadrants. The result is an improvement in diagnostic confidence, but it is important to remember when viewing the magnified images not to over-interpret subtle findings or unresearched types of pathology (e.g., dentin fissures). For the author, endoscopy is an important teaching tool and an extremely valuable component of examination when working-up cases of suspected dental disease. The video monitor allows an owner, trainer, students, or consulting colleagues to view the real-time images. An added benefit is that video or photographs archived from the examination can be reviewed at a later time, including follow-up evaluations and discussions with other veterinarians.[5] When used for intraoral guidance, magnified viewing assists in the accurate placement of instruments during extractions and other procedures.[4,7]

The author's preferred examination technique with the endoscope is similar to previous reports. The technique is easier to perform with two assistants retracting soft tissues and stabilizing the head, but the examination can be carried out quite successfully with less support. Keep in mind that the initial investment for a system can be very expensive and most of the components described in the manuscript are not well-suited for ambulatory endoscopy. There are manufacturers with compact rigid endoscopic systems that utilize a battery operated LED light source, USB camera, laptop computer monitor, and wireless transmission technology to improve portability. Alternatively, less expensive handheld intraoral camera systems can provide excellent intraoral viewing and allow more flexibility for in-house or ambulatory cases. Among a host of factors, the veterinarian should always investigate the instrument's water-resistance specifications when considering purchase. Furthermore, it is advised to develop relationships with reputable manufacturers and to consult a trusted colleague familiar with the ins-and-outs of specific system components prior to purchase.[7]

Acknowledgments

Conflict of Interest

The Author declares no conflicts of interest.

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[a]Rigid endoscope, Richard WOLF GmbH, Knittlingen, Germany.
[b]Protective sheath, Richard WOLF GmbH, Knittlingen, Germany.
[c]Veterinary Video Camera III, Karl Storz & Co., Tuttlingen, Germany.
[d]Veterinary Video Camera III controls, 100–240 V, 50/60 Hz, Karl Storz & Co., Tuttlingen, Germany.
[e]Stryker SDC3 image capture, 100–240V, 50/60 Hz, Stryker Endoscopy, San Jose, CA 95138.
[f]Dell Corporation 21 in. monitor, Plano, TX 75075.
[g]Stryker X8000 light source, 300 W, 100–240 V, 50–60 Hz, Stryker Endoscopy, San Jose, CA 95138.
[h]Fiber optic cable 809590, Richard Wolf Medical Instruments Co., Vernon Hills, IL 60061.
[i]Standard video cart, Stryker Endoscopy, San Jose, CA 95138.
[j]Dormesedan, Zoetis, Florham Park, NJ 07932.
[k]Dolorex, Intervet/Merk Animal Health, Whitehouse Station, NJ 08889.

How to Assess the Periodontal and Endodontic Structures of Equine Teeth

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1. Introduction

The health of equine periodontal and endodontic structures is critical to evaluating the overall health of an equine tooth. The equine periodontium, comprised of the gingiva, alveolar bone, periodontal ligament, and cementum, is directly responsible for stabilizing and holding the tooth in place within the oral cavity. Periodontal disease is the inflammation and infection of the periodontium, and if left untreated, it can lead to extensive attachment loss of the tooth from surrounding hard and soft tissue structures. Mobility may even result if the attachment loss is greater than 25% to 50% of the remaining reserve crown and root. The equine endodontic system or endodontium is located within the root canals, common pulp chamber(s), and pulp horns of the teeth, and it is comprised of blood vessels, nerves, lymphatic structures, and connective tissue commonly referred to as dental pulp. The endodontic system is responsible for nourishing the majority of the cellular components that make up the main structure of the tooth via the dentin-pulp complex. Odontoblasts make up a significant portion of the dentin-pulp complex, and odontoblasts are responsible for creating and sustaining dentin. The continual generation of secondary and tertiary dentin by the odontoblasts is what protects the endodontium from exposure during the constant slow loss of dental structure during normal mastication.

Endodontic disease is inflammation and/or infection of the endodontium that may or may not result in pulpal injury, necrosis, and/or death. If the endodontium of a tooth is severely injured, the pulp will become necrotic and die. Without the pulp, the tooth is no longer able to develop or respond to normal attrition and abrasion (wear of the tooth from occlusal contact and feed material). If the initial injury did not already result in exposure of a pulp horn to the oral environment, then over time the normal continual wear of equine teeth will eventually expose the pulp due to nonresponsive odontoblasts. Once nonvital pulp horns are exposed to the oral cavity, feed packing and bacterial invasion into the endodontic space commences. The nonvital tooth harbors infection that slowly percolates into the surrounding bone via the apical portion of the roots and channels within dead dental structures. It is important to note that endodontically nonvital teeth can still have a vital, intact periodontium due to the fact the periodontium receives nourishment from surrounding vasculature.

NOTES

Disease of equine periodontal and endodontic tissue is well-documented in the literature.[1] The most commonly identified abnormality of incisors particularly in young horses (< 2 years of age) was transverse fracture with or without fracture of the surrounding bone and/or pulp exposure.[2] The incisor pulp horn is particularly vulnerable to exposure because of its position on the labial aspect of the tooth. Incisor primary periodontal disease and periodontal disease in relation to equine odontoclastic tooth resorption and hypercementosis is well-recognized.[3] Dental fracture, pulp exposure, apical infection, primary periodontal disease, and diastemata of cheek teeth have extensive reporting in the literature.[4] Prevalence of equine periodontal disease has been reported to be as high as 75% in some regions,[5] and 49.9% of horses in the general population have some level of disease.[6] Fractures commonly occur in cheek teeth especially on the maxillary teeth.[7] Endodontic disease can result from these fractures. Some additional causes of endodontic disease are excessive/rapid dental wear, severe infundibular carious lesions, severe periodontal disease, anachoresis, dental malformation, iatrogenic damage, regional fracture/past trauma, and unknown. In order to fully assess the health of the periodontium and the endodontium of equine teeth, a thorough physical and oral examination needs to be performed. If there is any suspicion that these disease processes are present, a radiographic evaluation should be performed of the suspect region.

2. Materials and Methods

Necessary Instrumentation

- Sedation
- Medical grade BRIGHT headlight (a camping style headlight will not provide adequate light to see subtle lesions)
- Full mouth speculum
- Oral irrigation preferably under pressure
- Periodontal probe—thin (1 mm width) with mm demarcations up to 2 to 3 cm in length (the tip of periodontal probes should be BLUNT not pointed to avoid gingival injury)
- Explorer number 23 on equine handle
- Dental mirror with defogger (alcohol, warm water, light layer soap, commercial defogger, etc.)
- Dental scalers, curettes, and picks
- Cheek retractor
- Rigid oral endoscope (if available)
- Extraoral radiographic equipment
- Intraoral radiographic equipment (if available)

Pre-Oral Examination

It is important to gather a thorough patient clinical history and perform a complete physical examination. Owners can provide ample information regarding onset of clinical signs, feeding behavior, progression of condition, systemic health, performance, and response to past therapies, all of which could be significant in the determination of pathology presence, diagnostics, treatment planning, and prioritization of the dental condition relative to the overall status of the patient. Observation of mastication pre-sedation can also enhance the clinician's ability to assess oral function and watch for signs of discomfort.

Sedated Oral Examination

A previous session in this series reported on how to perform a complete oral examination in the horse. This portion will focus solely on how to assess the endodontic and periodontal health of a tooth. Evaluation of the endodontic and periodontal health of a tooth should ALWAYS be part of a complete oral examination.

The maxillofacial region of the horse should be examined as part of a complete oral exam. If endodontic or periodontal health is compromised, extraoral examination findings might include enlarged regional lymph nodes, asymmetry of the muscles of mastication, bony or soft tissue swellings, regions of buccal feed packing, fistula formation, regions of sensitivity, and nasal discharge. Once the extraoral portion of the examination is complete, the incisor and bar region of the oral cavity is examined followed by speculum placement and examination of the oral cavity proper and cheek teeth. The following lists specific steps to performing a thorough examination of the periodontal and endodontic structures within the oral cavity. This comprises only a portion of a complete oral examination.

1. Water irrigation and dental scalers, curettes, and picks are used to completely remove debris, packed feed, and calculus from the oral environment and dental structures.
2. When inspecting intraoral periodontal and endodontic structures, ALL aspects of the tooth and surrounding structures must be examined. This includes the labial/buccal, palatal/lingual, mesial, distal, and occlusal aspects. Inspection of these structures in the rostral portion of the mouth can be performed by proper hand placement and digital manipulation of tissues. An incisor speculum is only necessary when an area of interest needs prolonged and careful inspection. A full-mouth speculum is necessary for examination of the premolar and molar teeth. The use of a dental mirror is absolutely necessary to be able to adequately examine all soft and hard tissue structures especially in the caudal portion of the oral cavity. Oral endoscopy, if available, provides even better visualization of all intraoral structures, and its use is becoming more common among practitioners with dental expertise. Palpation can assist the clinician in finding some pathology and it is encouraged, but palpation alone cannot

identify subtle pathology or minor defects between teeth.

3. With the lip and/or cheek retracted from the dental structures, the alveolar mucosa, mucogingival junction, and gingiva, are inspected for signs of inflammation, irregularities, fistulation, and underlying swellings. Palpation can be useful in detecting changes in juga definition or underlying bone; the juga is the bony contouring over the region of the reserve crown and root most distinct in equid incisors. Asymmetry or age inappropriate juga definition can be a sign of underlying pathology.
4. The relative height of surrounding bone at the clinical crown-reserve crown or clinical crown-root junction is examined. The height of the bone encasing the teeth on the mandible and maxilla is referred to as the alveolar ridge. Signs of potential attachment loss (loss of the structures of the periodontium) and decrease in alveolar ridge height include gingival recession, abnormal discoloration of gingiva, severe gingival inflammation, feed packing around or between teeth, subgingival calculus, decrease in height of the dental papillae (interproximal gingival tissue), malpositioning of teeth, increased space between teeth, age inappropriate incisive angle, bleeding from around the tooth after irrigation and debridement, and dental mobility.
5. Regions of abnormal gingival contour, gingival recession, gingival inflammation, decreased alveolar ridge height, feed packing, subgingival calculus, and overall suspect periodontal structure should be investigated with the periodontal probe. The periodontal probe is used to measure attachment loss of periodontal structure from the reserve crown and root. The periodontal probe is slid gently along the outer aspect of the tooth running parallel to the angle of the tooth in the region of the gingival sulcus and possible defect. The normal gingival sulcus depth has been reported to be 1 to 5 mm in the horse.[8] If the probe measures greater than 5 mm, an area of attachment loss has been identified, and if it runs along the dental structure, it is referred to as a periodontal pocket. Measurements of gingival sulcus depth in otherwise healthy looking tissue is not routine in the equid due to potential gingival sulcus trauma that may be caused by the probe in a moving patient.
6. After inspection of all the supporting structures of the tooth, the tooth itself is examined. Dental structure is examined from the periphery to the most central visible occlusal aspect of the structure. The condition of the peripheral cementum, enamel infolding, primary dentin, secondary dentin (overlying the pulp), infundibulum, and infundibular cementum are all examined closely for any signs of defect or loss. Knowing the normal structure and appearance of the clinical crown for all teeth at any age is critical to the ability to identify dental pathology.
7. The dental explorer is used to examine dentin for defects indicating pulp exposure and any portion of the hard dental structure that is in question. The tip of the dental explorer is sharply pointed in order to identify subtle defects. The tip of the explorer is properly used at a 90 degree angle to dental hard tissue, and the tip should never contact soft tissue. Using the explorer at a different angulation will decrease the sensitivity of the instrument.
8. If periodontal lesions or clinical crown defects involving the endodontium are identified, extraoral and intraoral (if available) radiographs are necessary to continue evaluation of the tooth and surrounding anatomy. Acquisition and interpretation of these radiographs has been well-described.[9,10] In cases where endodontic disease is suspected but no intraoral pathology identified, radiographs are critical to the evaluation of the health of the tooth. Computed tomography may even be necessary if both radiographs and oral examination do not reveal conclusive evidence of tooth health or disease.

3. Results

The use of adequate lightening, oral irrigation, a full-mouth speculum, dental mirror, periodontal probe, dental explorer, cheek retractor, and radiography are considered basic equipment for the adequate evaluation of periodontal and endodontic structures within the oral cavity. Radiographs are critical to evaluating dental structures for periodontal and endodontic health. These facts are well known and published in a vast array of textbooks, review articles, and research articles within and outside of the equine world.

4. Discussion

Periodontal and endodontic disease can affect teeth either independently or together. Depending on the age of the tooth and the extent of pathology, one disease can lead to the other. Severe periodontal disease extending to the tooth root and apex can lead to infection of the pulp, and endodontic disease that results in regional tissue destruction can cause extensive loss of the periodontium. These are called perio-endo and endo-perio lesions, respectively. Usually disease of this extent warrants the extraction of the tooth.

Explorers are commonly referred to inappropriately as picks in the equine literature. Explorers are meant to extend and enhance digital tactile sensitivity of the user. In order to perform this function, the explorer needs to be very thin and small. The number 23 sheppard's crook explorer has

proven to be rugged enough to withstand the motion of the equine oral cavity but sensitive enough to reveal small lesions. These explorers have been used in human and small animal dentistry for decades. Heavier equine dental picks cannot reveal small lesions like pulp exposures but they are useful for removing debris from and around dental structures. Dental explorers will catch or sink into regions of abnormal dental hard tissue. A dental explorer tip should slide smoothly over enamel and dentin, and cementum will feel either slightly roughened or smooth. Explorer tips that sink into softened dental hard tissue and defects indicate underlying potential pathology.

Evaluating dentin for changes in color and consistency will help determine the health of the endodontic system. Primary dentin, the dentin present within the tooth upon initial eruption, is highly mineralized and is roughly an off-white, eggshell color with a moderate degree of translucency. Secondary dentin, the dentin produced slowly by the odontoblasts on the periphery of the pulp horn over the lifetime of the tooth, is less mineralized and more prone to staining.[11] Therefore, secondary dentin can range in color from a light tan to a dark brown in color depending on the horse's forage. It is the secondary dentin that prevents pulp exposure over time due to normal attrition. A variation in secondary dentin coloration is considered normal. When regions of secondary dentin on the occlusal surface of the tooth lack shine, appear dull, or are deeply pigmented, a dental explorer should be used to examine the site closely for a defect. Secondary dentin should not stain black, and if a black, dull discoloration is identified with or without feed packing in the region of a pulp horn, then the health of the tooth is questionable and warrants further examination.

The use of the above technique and instrumentation for evaluation of the periodontal and endodontic structures of the teeth during oral examination is critical for recognizing and properly diagnosing disease of the structures. Although the tools and technique may require a minor investment of money and time, the reward of being able to repeatedly identify mild to severe pathology is beneficial to the horse and owner. The identification of pathology earlier in the course of disease allows for treatment and possible cessation of disease progression. The ability to visualize subtle dental defects creates a more direct path to appropriate diagnosis and treatment. Horse, owner, and veterinarian will benefit from the use of improved techniques for oral and dental assessment.

Acknowledgments

Conflict of Interest

The Author declares no conflicts of interest.

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How to Document an Equine Oral Examination Using a Dental Chart

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1. Introduction

Equine dental charts are an important component of adequate legally required record keeping. A dental chart allows detailed documentation and assists the practitioner with performing a consistent and methodical exam. *Charting is the process of recording the state of health or disease of the teeth and oral cavity.* It is an integral part of diagnosis, treatment planning, and monitoring.[1] Small animal dental charts allow for notation of diagnoses, pathology, and procedures performed in each quadrant on each side of each individual tooth.[2] The use of similar charting methods during detailed equine dental examinations and treatment allows for accurate annotation of the *comprehensive oral health assessment and treatment* of your equine patient[3] and ensures accurate communication with colleagues who may follow-up or to whom the case may be referred. For a comprehensive description of equine dental charting, the reader is referred to the previously published manuscript by Galloway.[4] The purpose of this manuscript is to demonstrate the author's preferred method of charting examination findings in equine dental patients.

2. Materials and Methods

Information about performing a detailed oral examination is covered by a different manuscript in this section. When filling out a dental chart standardized abbreviations and terminology are employed which make the process much more efficient than writing out exam findings in longhand. To this end, knowledge of appropriate dental nomenclature and abbreviations is necessary.[5]

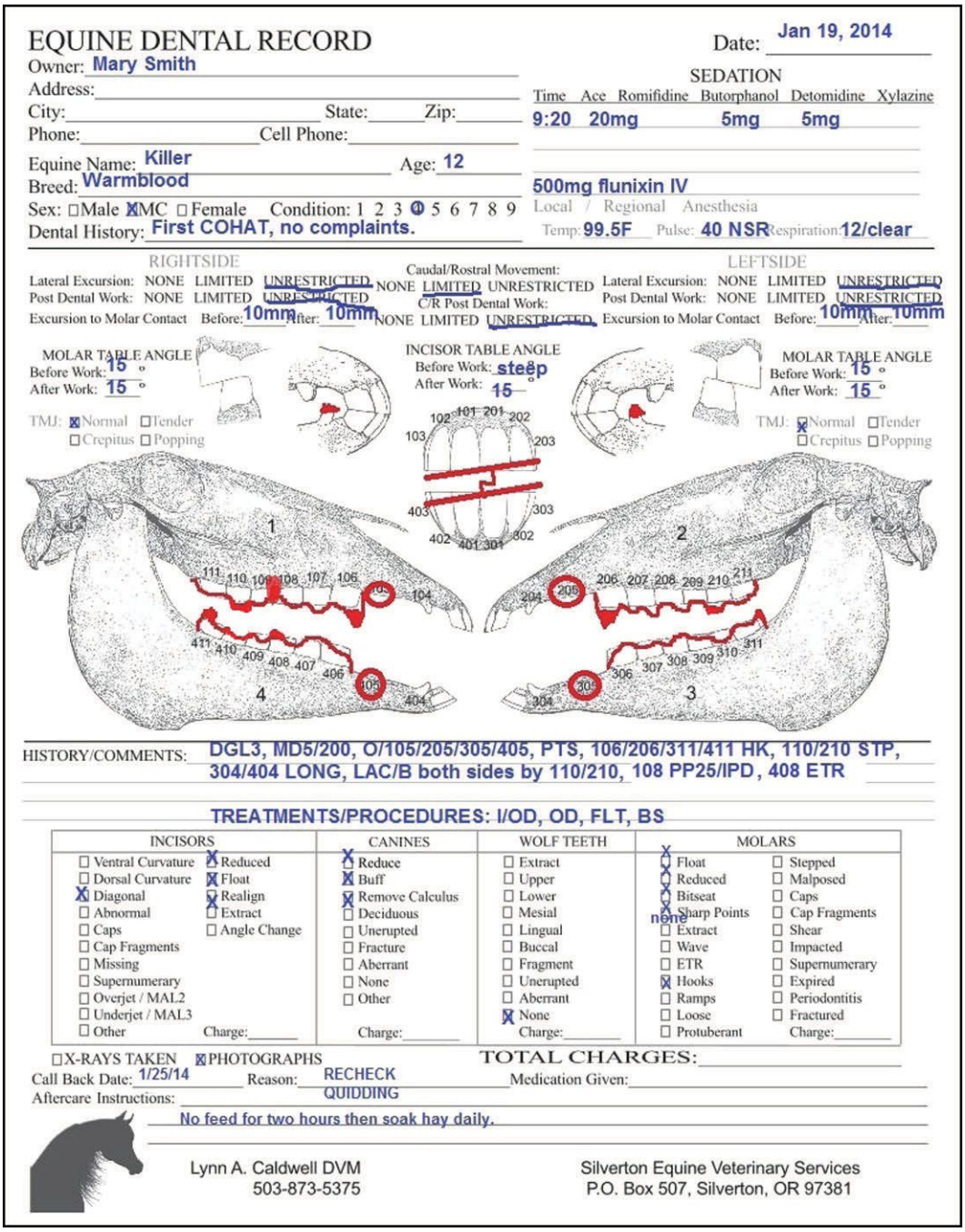
The Modified Triadan numbering system is the preferred system in veterinary medicine for annotating the specific teeth in the dental quadrants and has been in existence for quite some time in human and veterinary dentistry.[6] In addition to Triadan nomenclature, the Academy of Veterinary Dentistry (AVD) has a Dental Abbreviation Code List including an Equine Dental Abbreviation List that can be found on the AVD website (http://www.avdonline.org). The abbreviation list includes codes for diagnostic problems and treatment procedures that pertain to soft tissue, incisors, canine teeth, and cheek teeth. These abbreviations are intuitive and keep the chart from becoming cluttered. A list that includes AVD equine dental abbreviations is provided in Table 1.

To chart examination findings, a straightforward simple method is to record the Triadan tooth number followed by the appropriate diagnostic abbreviation (Triadan number may follow the diagnostic abbreviation, depending upon the practitioner's preference, with consistency being the most impor-

NOTES

Table 1. Diagnostic Abbreviations

Incisors:	
CV	Ventral curvature: upper central incisors extend beyond the level of the upper intermediate and corner incisors, "smile."
CD	Dorsal curvature: lower central incisors extend beyond the level of the lower intermediate and corner incisors, "frown."
DGL	Diagonal: lower incisors longer on either the left side or right side. Defined with respect to mandibular incisors longer on arcade number 300 or 400. DGL/4 (400 arcade longer), DGL/3 (300 arcade longer)
MAL2	Class II malocclusion, overbite, brachygnathism, mandibular brachygnathism: extension of upper teeth vertically beyond lower teeth.[1] Defined by the term "distoclusion," where some or all of the mandibular teeth are distal in relationship to their maxillary counterparts.
MAL3	Class III malocclusion, underbite, prognathism, mandibular prognathism: defined by the term "mesioclusion," where some or all of the mandibular teeth are mesial in their relationship to their maxillary counterparts.
Cheek Teeth:	
HK	Hook: excess crown longer than wide.
RMP	Ramp: excess tooth wider than long.
WV	Wave: more than one tooth with excess crown.
STP	Step: one tooth only with excess crown.
ETR	Excessive transverse ridges: ridges in excess of 3 mm in height.
PTS	Sharp enamel points: buccal cusps on maxillary cheek teeth and lingual cusps on mandibular cheek teeth sharpened from wear (attrition).
CUPD	Cupped: crown worn past infundibulum. Still has crown above gingival margin. Can also be seen in lower teeth.
EXP	Expired: attrition to gingival margin with crown connecting all roots.
EXP/RTR	Expired/retained tooth root: attrition to gingival margin with no crown present
O	Missing/absent
RD	Retained deciduous: caps
FX	Fracture
FX/SAG	Sagittal: below gum line (subgingival) through infundibulum.
FX/WDG	Wedge: outside infundibulum.
FX/CHIP	Chip: occlusal margin only. Not fractured down to gingiva.
IPM or D	Interproximal: between teeth. Mesial or distal.
B	Buccal
P	Palatal
L	Lingual
TI	"Tooth impacted," "blind": not completely erupted. Partially or fully covered by bone or soft tissue. Commonly seen with wolf teeth.
RRT	Retained root tip: portion of root or tip retained.
RTR	Retained tooth root.
Soft tissue:	
LAC/B	Buccal laceration
LAC/L	Lingual laceration
AB	Abrasion
PD	Periodontal disease stage 1–4
PP	Periodontal pocket
Other	
INF/CA	Infundibular cavity
CA	Caries
SN	Supernumerary
Treatment and procedure abbreviations include:	
OD	Odontoplasty: reduction of excessive crown of occlusal surface.
I/OD	Incisor odontoplasty: incisor reduction
FLT	Float: reduction of lingual and buccal enamel points.
BS	Bit seat: rounding of rostral margins of mandibular and maxillary 2nd premolars.
IFO	Infra-orbital nerve block
MAX	Maxillary nerve block
IFA	Inferior alveolar nerve block
MEN	Mental nerve block
LIP	Local infiltration of anesthetic
X	Extraction, simple
XS	Extraction, sectioned
XSS	Surgical extraction
506X, 606X, etc.	Cap extraction or retained deciduous extraction
105X, etc.	Wolf tooth extraction



EQUINE DENTAL RECORD

Date: Jan 19, 2014

Owner: Mary Smith
Address:
City: State: Zip:
Phone: Cell Phone:
Equine Name: Killer Age: 12
Breed: Warmblood
Sex: ☐Male ☒MC ☐Female Condition: 1 2 3 ❹ 5 6 7 8 9
Dental History: First COHAT, no complaints.

SEDATION

Time	Ace	Romifidine	Butorphanol	Detomidine	Xylazine
9:20	20mg		5mg	5mg	

500mg flunixin IV
Local / Regional Anesthesia
Temp: 99.5F Pulse: 40 NSR Respiration: 12/clear

RIGHTSIDE
Lateral Excursion: NONE LIMITED UNRESTRICTED
Post Dental Work: NONE LIMITED UNRESTRICTED
Excursion to Molar Contact Before: 10mm After: 10mm

Caudal/Rostral Movement: NONE LIMITED UNRESTRICTED
C/R Post Dental Work: NONE LIMITED UNRESTRICTED

LEFTSIDE
Lateral Excursion: NONE LIMITED UNRESTRICTED
Post Dental Work: NONE LIMITED UNRESTRICTED
Excursion to Molar Contact Before: 10mm After: 10mm

MOLAR TABLE ANGLE
Before Work: 15 °
After Work: 15 °
TMJ: ☒Normal ☐Tender ☐Crepitus ☐Popping

INCISOR TABLE ANGLE
Before Work: steep
After Work: 15 °

MOLAR TABLE ANGLE
Before Work: 15 °
After Work: 15 °
TMJ: ☒Normal ☐Tender ☐Crepitus ☐Popping

HISTORY/COMMENTS: DGL3, MD5/200, O/105/205/305/405, PTS, 106/206/311/411 HK, 110/210 STP, 304/404 LONG, LAC/B both sides by 110/210, 108 PP25/IPD, 408 ETR

TREATMENTS/PROCEDURES: I/OD, OD, FLT, BS

INCISORS	CANINES	WOLF TEETH	MOLARS
☐ Ventral Curvature	☒ Reduce	☐ Extract	☒ Float
☐ Dorsal Curvature	☒ Buff	☐ Upper	☐ Reduced
☒ Diagonal	☒ Remove Calculus	☐ Lower	☐ Bitseat
☐ Abnormal	☐ Deciduous	☐ Mesial	☐ Sharp Points
☐ Caps	☐ Unerupted	☐ Lingual	☐ Extract
☐ Cap Fragments	☐ Fracture	☐ Buccal	☐ Wave
☐ Missing	☐ Aberrant	☐ Fragment	☐ ETR
☐ Supernumerary	☐ None	☐ Unerupted	☒ Hooks
☐ Overjet / MAL2	☐ Other	☐ Aberrant	☐ Ramps
☐ Underjet / MAL3		☒ None	☐ Loose
☐ Other			☐ Protuberant
☒ Reduced			☐ Stepped
☒ Float			☐ Malposed
☒ Realign			☐ Caps
☐ Extract			☐ Cap Fragments
☐ Angle Change			☐ Shear
			☐ Impacted
			☐ Supernumerary
			☐ Expired
			☐ Periodontitis
			☐ Fractured
Charge:	Charge:	Charge:	Charge:

none

☐X-RAYS TAKEN ☒PHOTOGRAPHS TOTAL CHARGES:
Call Back Date: 1/25/14 Reason: RECHECK QUIDDING Medication Given:
Aftercare Instructions:
No feed for two hours then soak hay daily.

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Fig. 1. Completed chart from the author's practice.

tant feature of the chart). For example, a finding of hooks of the maxillary second premolars would be abbreviated by the author "106/206 HK." Other examples of diagnostic abbreviations include the following:

- sharp enamel points (PTS)
- absent canine teeth (O/104/204/304/404)
- incisor bite deviation, ventral curvature (CV)
- laceration of buccal mucosa (LAC/B)

If the diagnostic codes are not adequately descriptive or intuitive for the knowledge level of the practitioner, an excellent pictorial aide in assigning diagnostic names and codes to various dental conformations can be found in Klugh's *Principles of Equine Dentistry*[7]. Consistency of diagnostic abbreviations is important so that another veterinarian can read the chart and understand the diagnosis and treatment plan. Dental conditions that affect the cheek teeth such as hooks (HK), ramps (RMP),

Key to Fig. 1.
Diagnoses:

- Diagonal incisors (DGL3)
- Midline discrepancy (MD) of 5 mm with mandibular 301/401 IP space shifted to maxillary 200 side
- Long canines 304/404
- Wolf teeth 105/205/305/405 not present (O/)
- Sharp enamel points (PTS)
- Hooks 106/206/311/411 (HK)
- Steps 110/210 (STP)
- Excessive transverse ridge 408 (ETR)
- Buccal lacerations/irritation associated with enamel points of 110/210 (LAC/B)
- Perio pocket 25 mm distal side of 108 (PP/IPD)

Treatments:

- Odontoplasty of incisors (I/OD)
- Odontoplasty of cheek teeth and canines as represented by solid red over tooth structure removed (OD)
- "Float" all sharp enamel points smooth (FLT)
- Minor contouring of mesial surfaces of 106/206/306/406 (BS)

Instructions to client:

- Recheck in one week to determine if quidding has ceased
- No feed for two hours after the procedure, then soft feed

Step-by step dental chart instructions used by the author:

1. Name, date and physical exam findings recorded on the chart.
2. Presenting complaint, if any.
3. Specific drugs, exact amounts, and times of administration.
4. Gross examination findings of the head, including any nasal discharge or foul odors.
5. Observations concerning lips, gingiva, incisors, and canine teeth.
6. Distance of excursion to molar contact, before any treatment.
7. Observations concerning intra-oral condition of gingiva, lips, and cheeks.
8. Observations concerning cheek teeth and periodontium.
9. Any procedures and treatments performed such as nerve blocks, odontoplasty, extractions.
10. Indicate if any photographs and radiographs were taken.
11. Distance of excursion to molar contact after treatment.
12. Aftercare instructions and next exam/treatment time frame.

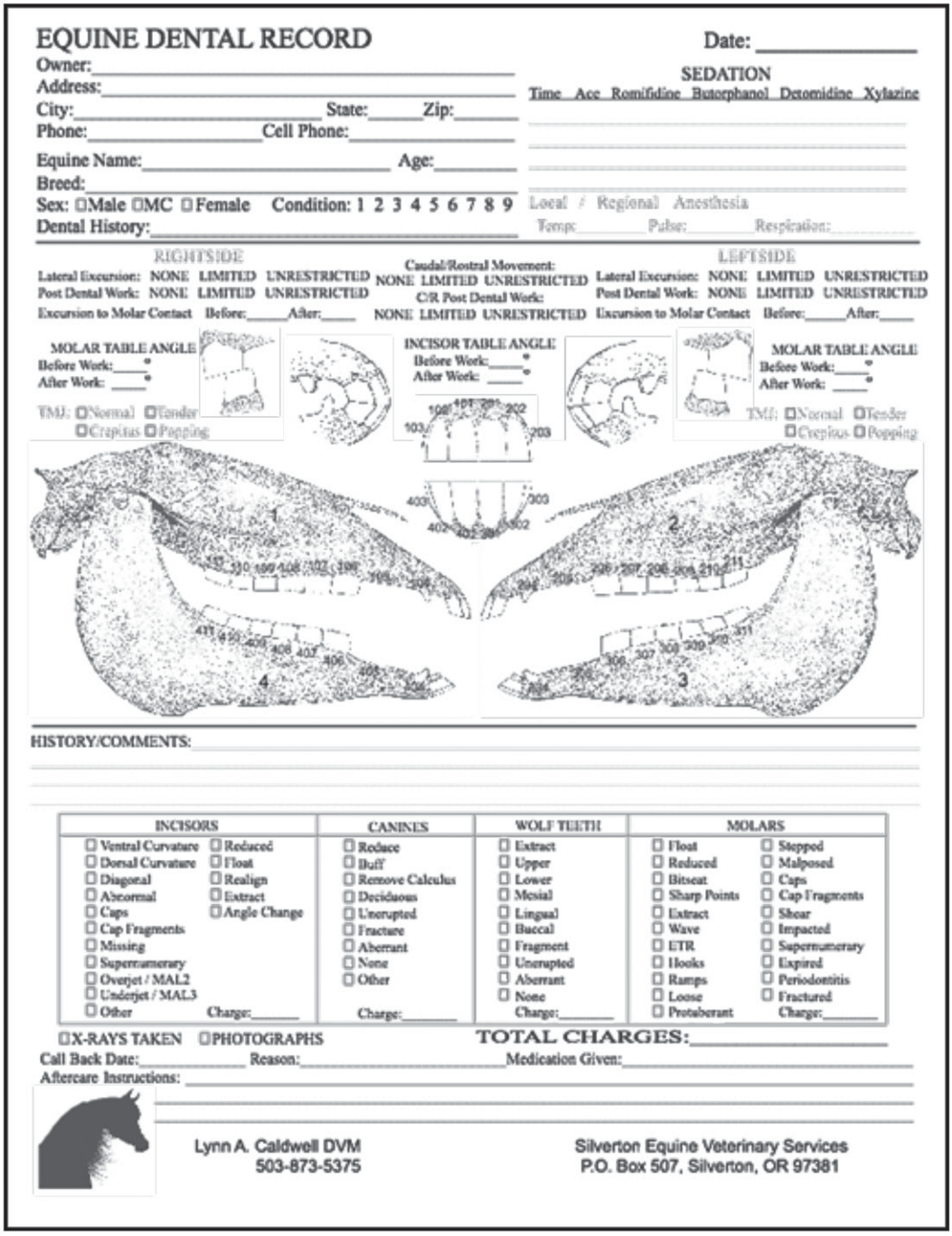
waves (WV), steps (STP), or excessive transverse ridges (ETR) can also be diagrammed on the dental chart by drawing the lateral profile of the teeth on a diagram.[4] Likewise, deviations from normal incisor occlusion such as diagonal bite (DGL) can be drawn on a frontal diagram of the incisor teeth. When diagramming findings and treatments, the author prefers to draw in red ink in order to make it easier to see what has been charted. Absent teeth are circled on the diagram, extraction is indicated by placing an "X" over the extracted tooth, and occlusal adjustment reductions are recorded on the diagram by shading the portion of tooth removed. The author also writes diagnostic and treatment abbreviations in the chart to clarify findings and treatment that were drawn on the diagram. A completed example of a primary chart used in the author's practice is included (Fig. 1).

The author finds it beneficial to use dental charts made from NCR paper (no carbon required) so that the client can have a copy. Computer-generated charts may be printed or e-mailed to the client and/or referral veterinarian. The primary chart that the author uses for routine documentation includes a profile image of the entire skull and teeth with close-up diagrams of the incisors and canine teeth (Fig. 2). For more detailed annotation an ideal chart would include tooth drawings similar to those used in small animal dentistry to allow diagramming of any tooth surface. The secondary chart used by the author for more detailed annotation is taken from Baker and Easley's *Equine Dentistry*, second edition[8] (Fig. 3). Dacre's rendering of the occlusal surfaces of the cheek teeth allows annotation of problems such as pulp exposure, infundibular decay, and periodontal problems involving any surface of the teeth, as well as various fracture conformations and misaligned or deformed teeth. The author prefers a modified version of the original published chart that depicts both maxillary and mandibular arcades, as viewed occlusally.

Charts can be handwritten or computerized. Computerized records obviate the use of paper and storage space and can be electronically transmitted to the client or referral equine dental specialist. There are a number of styles and layouts of equine dental charts that are commercially available, or you can develop your own version of a computerized or print chart. The basic template can be stored within a tablet device, filled out during the exam, and saved as a unique patient file.

3. Summary

It is the author's opinion that a comprehensive oral health assessment and treatment plan should be part of the minimum standard of annual health care of all horses. The recording of this exam, procedures, and treatments employed are a legally



EQUINE DENTAL RECORD

Date: _____

Owner: _____

Address: _____

City: _____ State: _____ Zip: _____

Phone: _____ Cell Phone: _____

Equine Name: _____ Age: _____

Breed: _____

Sex: ☐Male ☐MC ☐Female Condition: 1 2 3 4 5 6 7 8 9

Dental History: _____

SEDATION

Time Acc Romifidine Butorphanol Detomidine Xylazine

Local / Regional Anesthesia

Temp: _____ Pulse: _____ Respiration: _____

RIGHTSIDE

Lateral Excursion: NONE LIMITED UNRESTRICTED

Post Dental Work: NONE LIMITED UNRESTRICTED

Excursion to Molar Contact Before: _____ After: _____

Caudal/Rostral Movement:

NONE LIMITED UNRESTRICTED

C/R Post Dental Work:

NONE LIMITED UNRESTRICTED

LEFTSIDE

Lateral Excursion: NONE LIMITED UNRESTRICTED

Post Dental Work: NONE LIMITED UNRESTRICTED

Excursion to Molar Contact Before: _____ After: _____

MOLAR TABLE ANGLE

Before Work: _____°

After Work: _____°

TMJ: ☐Normal ☐Tender ☐Crepitus ☐Popping

INCISOR TABLE ANGLE

Before Work: _____°

After Work: _____°

MOLAR TABLE ANGLE

Before Work: _____°

After Work: _____°

TMJ: ☐Normal ☐Tender ☐Crepitus ☐Popping

HISTORY/COMMENTS: _____

INCISORS		CANINES	WOLF TEETH	MOLARS	
☐ Ventral Curvature	☐ Reduced	☐ Reduce	☐ Extract	☐ Float	☐ Stepped
☐ Dorsal Curvature	☐ Float	☐ Buff	☐ Upper	☐ Reduced	☐ Malposed
☐ Diagonal	☐ Realign	☐ Remove Calculus	☐ Lower	☐ Bitseat	☐ Caps
☐ Abnormal	☐ Extract	☐ Deciduous	☐ Mesial	☐ Sharp Points	☐ Cap Fragments
☐ Caps	☐ Angle Change	☐ Unerupted	☐ Lingual	☐ Extract	☐ Shear
☐ Cap Fragments		☐ Fracture	☐ Buccal	☐ Wave	☐ Impacted
☐ Missing		☐ Aberrant	☐ Fragment	☐ ETR	☐ Supernumerary
☐ Supernumerary		☐ None	☐ Unerupted	☐ Hooks	☐ Expired
☐ Overjet / MAL2		☐ Other	☐ Aberrant	☐ Ramps	☐ Periodontitis
☐ Underjet / MAL3			☐ None	☐ Loose	☐ Fractured
☐ Other	Charge: _____	Charge: _____	Charge: _____	☐ Protuberant	Charge: _____

☐X-RAYS TAKEN ☐PHOTOGRAPHS TOTAL CHARGES: _____

Call Back Date: _____ Reason: _____ Medication Given: _____

Aftercare Instructions: _____

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Fig. 2. Author's basic dental chart.

required part of the patient's medical records. Knowledge of appropriate dental abbreviations and the Triadan nomenclature system of identifying individual teeth within the stomatognathic system are a required minimum for this process. The consistent and repetitious use of standardized abbreviations and terminology upon a form within a medical record system allows for quick concise record keeping and the ability of any medical care provider to understand the specific health conditions, diagnoses, and treatment plan for each individual patient. The equine dental chart can be filled out patient-side in only a few moments to prevent confusion when treating more than one patient during any time frame. Computerized versions can even generate reminders for follow-up care, potentially increasing practice revenue, while at the same time ensuring compliance with state or country practice acts.

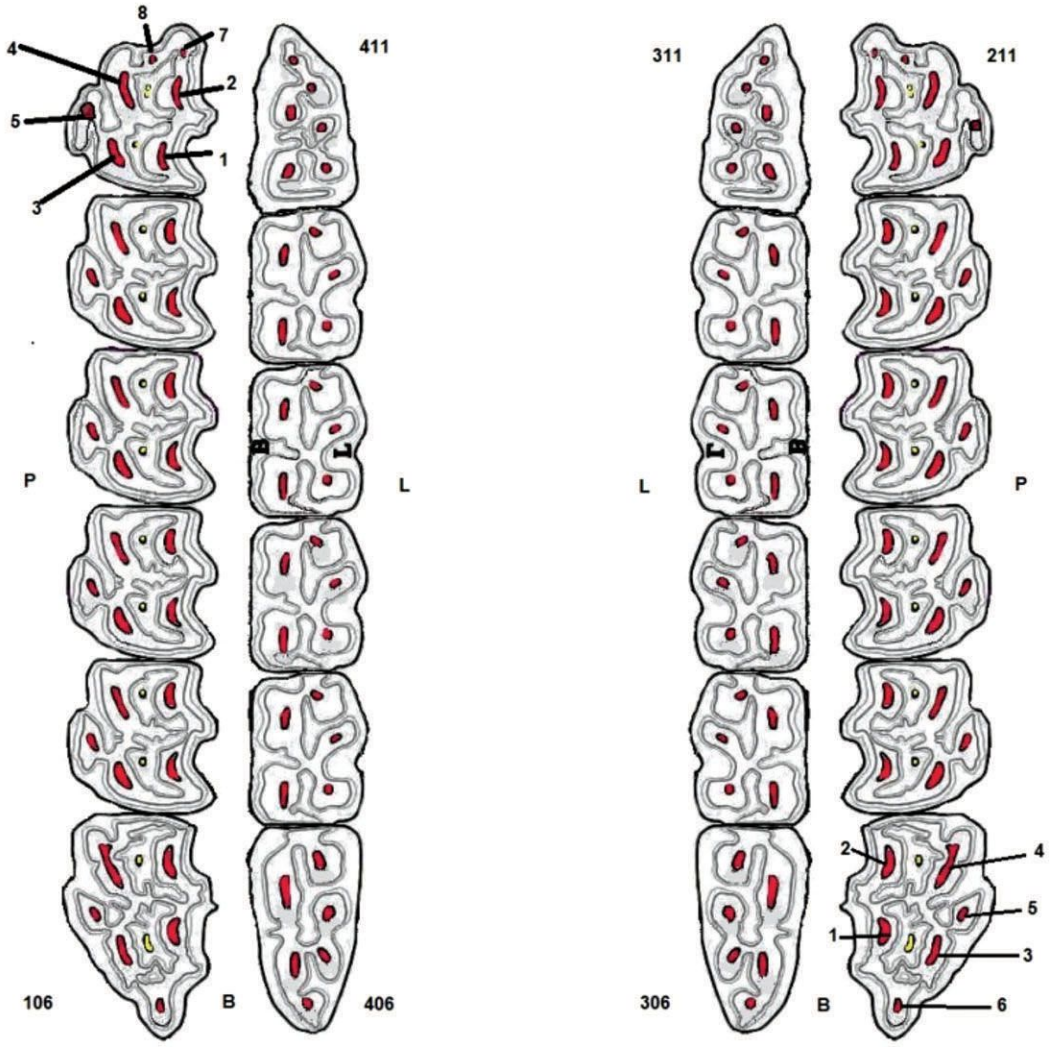

Fig. 3. Supplemental occlusal chart on which periodontal pockets, fractures, cupped teeth, and endodontic problems may be charted. All dental quadrants are shown from the occlusal surface and surfaces are coded as B for buccal, P for palatal, and L for lingual. Pulp horns and maxillary infundibulae are represented.

Acknowledgments

Conflict of Interest

The Author declares no conflicts of interest.

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How to Obtain a Diagnostic Radiographic Study of the Equine Cheek Teeth

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1. Introduction

The use of standardized radiographic views of the equine skull greatly facilitates interpretation of dental pathology. Digital radiographic systems are rapidly replacing traditional radiographic film in clinical equine practice. The methods for obtaining diagnostic radiographs of the equine cheek teeth with digital radiographic systems are presented. Standardized labeling and presentation are important for interpretation of skull radiographs.

2. Materials and Methods

Images presented were obtained with a digital system.[a]

The American Veterinary Dental College and the American College of Veterinary Radiologists (ACVR) have differing conventions for radiographic presentation of extraoral dental radiographs. For the purposes of this paper, the ACVR presentation and labeling conventions are used.[1] With regard to the head, lateral images are always presented with the horse's nose facing the viewer's left. The plane of the hard palate is used as the reference when considering angles of obliquity to the dorsal plane. Radiographic projections are described using the direction of travel of the primary beam, and for the purposes of this manuscript, the following terms and abbreviations have been utilized when naming radiographic views: left (L), right (R), ventral (V), dorsal (D), and oblique (O). A series of radiographs of the skull that enables evaluation of the entire dentition includes:

- Latero-lateral view: The right lateral view is obtained with the X-ray detector on the right side of the head (and the generator on the left side) and presented with the horse's nose to the viewer's left. A RIGHT label would be used.
- Dorsoventral (DV) views: The X-ray detector is positioned on the ventral aspect of the mandibles, and the central X-ray beam is directed perpendicular to the dorsal plane (the palate). The rostral aspect of the image is on the bottom, with the horse's right side to the viewer's left.
- Oblique views:
  - Left dorsal-right ventral oblique (LD-RVO) and right dorsal-left ventral oblique (RD-LVO). A LD30RVO is obtained with the X-ray generator on the horse's left side and the X-ray detector on the right. The X-ray beam is directed in a left dorsolateral to right ventrolateral fashion, 30° to the dor-

NOTES

sal plane. For comparison, the opposite oblique image should be obtained. This view will project the apical aspect of the maxillary cheek teeth closest to the X-ray detector. For example, the RD30LVO will project the apices of the left maxillary cheek teeth. For this view a LEFT marker would be used (and placed dorsal to the image). With the horse's mouth opened for the RD30LVO view, the crowns of the left mandibular cheek teeth will be projected.

- Left ventral-right dorsal oblique (LV-RDO) and right ventral-left dorsal oblique (RV-LDO). A LV45RDO is obtained with the detector on the right side of the horse's head and the generator on the left. In this image, the reserve crown-roots of the right mandibular cheek teeth would be highlighted. A RIGHT marker would be placed on the ventral aspect of the image. When the horse's mouth is opened for the LV45RDO view, the apical portion of the right maxillary cheek teeth is projected.

- Intra-oral (occlusal) images of the maxillary and mandibular incisors are presented with the maxillary crowns down, mandibular crowns up, and the horse's right to the viewer's left.[2]

While the straight lateral and DV views are obtained with the mouth closed, the lateral oblique views are obtained with the mouth held wide open with a speculum or a bite block. The speculum[b] used by the author does not significantly interfere with imaging of the cheek teeth. The open-mouth positioning of the oblique projections eliminates much of the superimpositioning of the cheek teeth. More importantly, these projections of the apical aspect of the maxillary cheek teeth and the coronal aspects of the mandibular cheek teeth in the space between the maxillary and mandibular arcades provide excellent dental detail, comparable to the images obtained with intraoral placement of the detector using a computed radiography (CR) system (Figs. 2 and 3). The increased apical detail of the maxillary cheek teeth with the open-mouth oblique views (LV45RDO and RV45LDO) compared with the other oblique views (RD30LVO and LD30RVO) is due to the fact that the X-ray beam is passing through only one maxilla in the former, as compared with both maxillae in the latter.

3. Results

The lateral image (Fig. 1) is used primarily to evaluate the paradental anatomy. The paired sinus structures are superimposed in the lateral image; therefore, laterality of the sinus pathologic change is not possible. However, fluid lines and soft tissue masses within the sinus spaces are readily appreciated (Fig. 2). In the oblique views (LD30RVO and RD30LVO), images of the right and left sinuses are separated which facilitates evaluation of these structures and localization of pathology (Fig. 3).

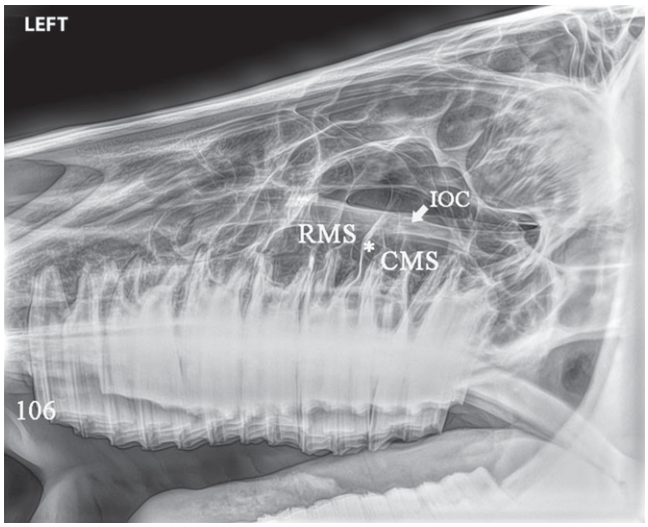

Fig. 1. Left latero-lateral image of the skull of a 10-year-old Quarter Horse mare (cadaver). The X-ray beam is centered on the rostral end of the facial crest. The maxillary cheek teeth arcades are superimposed. The right maxillary 2nd premolar (106) is projected rostrally. In this view the mouth is open, which is not necessary for the straight lateral projection. Note that the infraorbital canals (IOC) are superimposed. The RMS is separated from the CMS by a thin bony septum (*). This septum is continuous with the bulla of the ventral conchal sinus which forms the "roof" of the dorsal aspect of the ventral conchal sinus and the RMS where they form a single sinus space dorsal to the IOC.

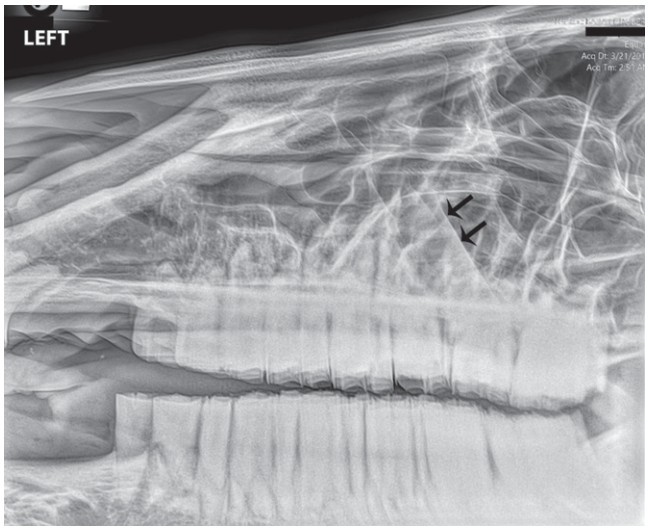

Fig. 2. Lateral image of a 21-year-old Missouri Fox Trotter gelding with a fetid, mucopurulent, left nasal discharge. A fluid line (arrows) is readily identified. The laterality of the affected sinuses cannot be identified.

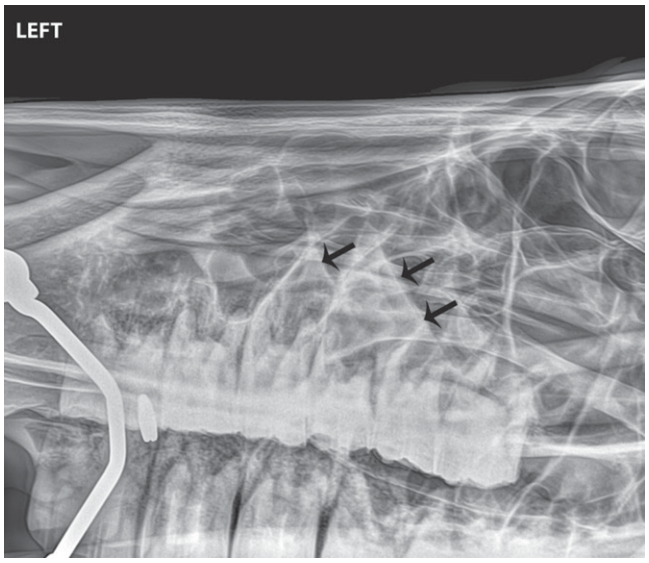

Fig. 3. RD30LVO view of the same horse imaged in Fig. 2. Fluid lines (arrows) can be appreciated in both the RMS and CMS in this image.

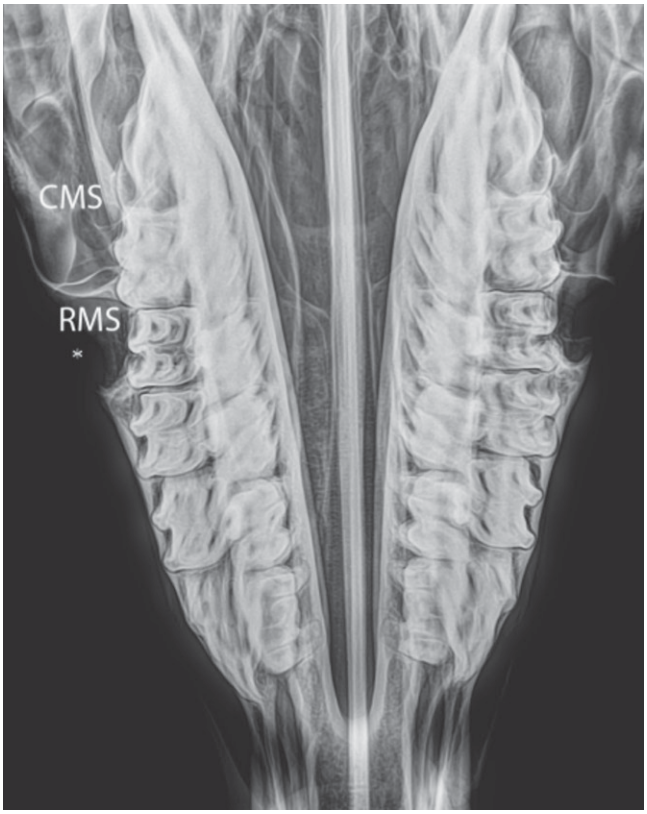

Fig. 4. DV view. The technique has resulted in saturation artifact (overexposure). The lateral wall of the rostral maxillary sinus (*) is not visible due to this artifact.

The DV view can also be used to evaluate the sinuses, as the most lateral aspects of the rostral maxillary sinus (RMS) and the caudal maxillary sinus (CMS) project laterally of the maxillary molars (Fig. 4). Sinus cysts, ethmoidal hematomas, and neoplasms within the sinuses may also be evident as soft tissue densities between the mandibles in the DV views, often with displacement of the vomer. The offset mandible DV will provide improved separation of the mandibular and maxillary arcades (Fig. 5). Due to the angulation of the rostral and caudal cheek teeth, when the central-ray beam is directed perpendicular to the dorsal plane, it will be parallel to the long axis of only the middle 4 cheek teeth. This results in loss of detail of the rostral and caudal cheek teeth in this image.

Evaluation of the cheek teeth requires multiple projections, as the crown and reserve crown-root often cannot be evaluated well in a single image. The three roots of the maxillary cheek teeth are generally difficult to separate and identify radiographically. In the oblique view (LD30RVO or RD30LVO), the buccal roots can usually be identified, but the palatal root is often obscured (Fig. 6). The opposite oblique image of the maxillary apices, the open-mouth oblique views (LV45RDO or RV45LDO), will sometimes isolate the palatal root (Fig. 7). In young horses, the length of the reserve crown may make it difficult to isolate the maxillary cheek teeth apices in the interdental space in the ventrodorsal oblique (LV45RDO or RVLDO) image.

The radiographic appearance of the roots of the cheek teeth vary significantly with the horse's age. The mandibular cheek tooth roots lengthen with age, whereas the maxillary cheek tooth roots usually do not lengthen as significantly. Periodontal disease clinically associated with senile diastemata, feed packing, and tooth mobility may be accompanied by radiographic signs such as widened periodontal ligament space, apical alveolar bone lysis ("halo"), periapical alveolar bone sclerosis (condensing osteitis) and tooth root blunting (Fig. 8). Similar radiographic signs are also evident with endodontic disease in the young adult horse (Fig. 9).

Idiopathic crown, reserve crown, and crown-root fractures are seen in both young and older horses. While the etiology often is undetermined,[3,4] radiographic evaluation is important in treatment planning.[5] As an example, consider the 17-year-old Quarter Horse mare presented for evaluation of crown fractures of the left mandibular 4th premolar (308) and the right mandibular 2nd molar (410) (Fig. 10). Oral examination revealed a slab fracture of 308, through pulp horns #1 and #2, with buccal displacement of the fragment, and a slab fracture (missing the fragment) of 410, also through pulp horns #1 and #2. The oblique views (LD30RVO and RD30LVO) were useful for imaging the crowns of the fractured mandibular teeth (Figs. 10A and 10B), while the LV45RDO and RV45LDO views were necessary for evaluating the reserve crown-roots of these teeth (Figs. 10C and 10D). The benefit of open-mouth lateral oblique positioning is well-demonstrated by this case.

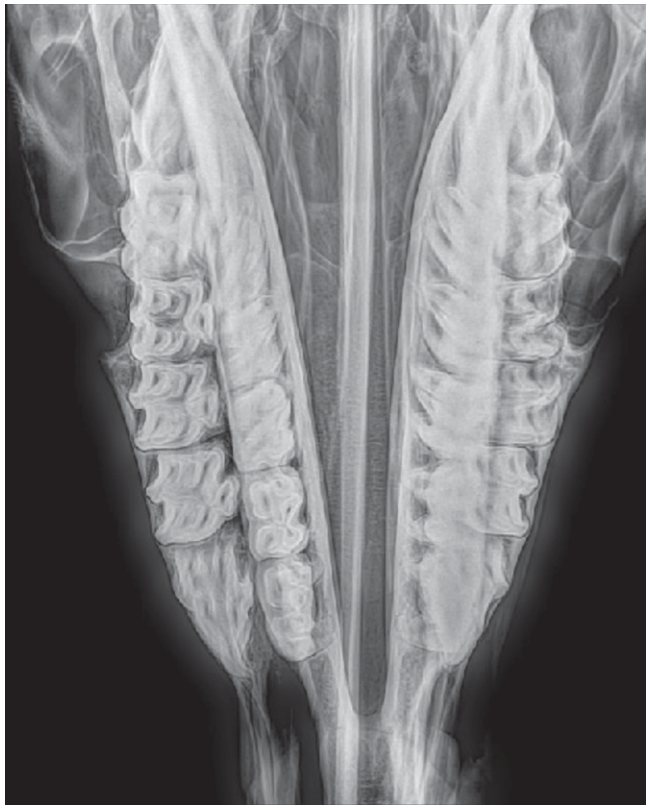

Fig. 5. DV image with mandible offset to the left (10-year-old Quarter Horse mare, cadaver). This patient positioning allows better visualization of the right maxillary and mandibular arcades.

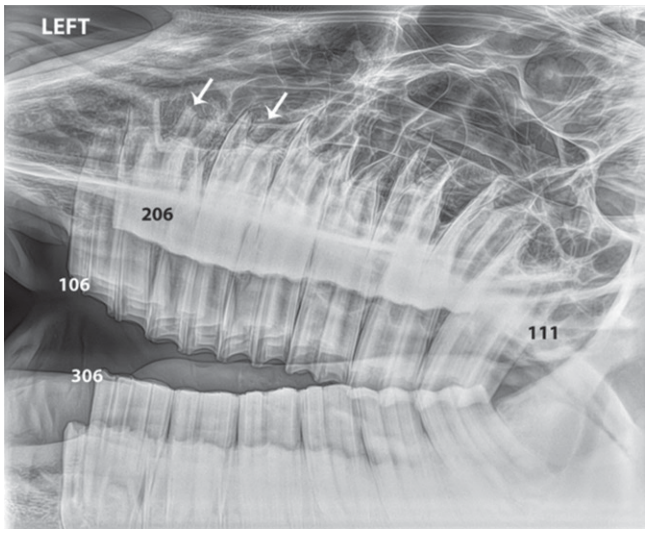

Fig. 6. The RD30LVO projection is used to image the apices of the left maxillary and the crowns of the left mandibular cheek teeth (10-year-old Quarter Horse mare, cadaver). Note that the right maxillary crowns are also imaged in the interarcade space and that in this image the right maxillary 3rd molar (111) is projected caudal to the left maxillary 3rd molar. All three roots of the left maxillary cheek teeth can be readily identified only for the 2nd and 3rd premolars (arrows).

Figs. 1–6 assume the use of a 10 by 12 inch imaging plate/sensor which, if positioned correctly, will capture all 4 arcades in each view. Supplemental views will be necessary with smaller plates/sensors

4. Discussion

The radiographic anatomy of the head is complex with superimposed symmetrical anatomy. Radiographic evaluation of the teeth is often difficult with subtle findings correlating to clinical disease. The widespread use of digital radiography and higher kilovoltage (kVp), milliamperage (mA) portable generators has enabled the practitioner to obtain images with good radiographic technique. However, many practitioners have little experience with the radiographic and patient positioning for studies of the cheek teeth. Correct radiographic positioning is paramount for the interpretation of dental and paradental disease. A full study of the skull is required to make a diagnosis. This includes:

- Dorsoventral view
- Latero-lateral view
- Right dorsal-left ventral oblique (RD30LVO), open-mouth
- Left dorsal-right ventral oblique (LD30RVO), open-mouth
- Right ventral-left dorsal oblique (RV45LDO), open-mouth
- Left ventral-right dorsal oblique (LV45RDO), open-mouth

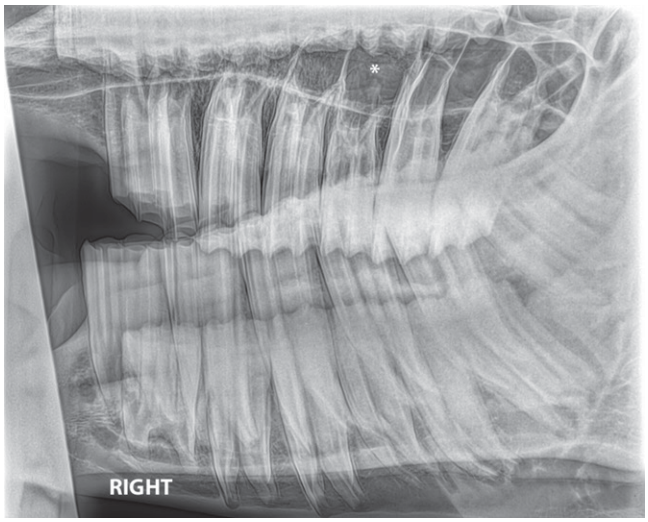

Fig. 7. The LV45RDO view of a 10-year-old Quarter Horse mare (cadaver). With the mouth wide open, the right maxillary cheek teeth are isolated in the space between the left maxillary and mandibular arcades, which gives apical detail similar in quality to that obtained with intraoral projections only possible with some CR systems. In this view, the broad, thin, palatal root (*) of the right maxillary 1st molar (109) is projected dorsal to the buccal roots. The same positioning is used to image the apical portion of the right mandibular cheek teeth (406–411).

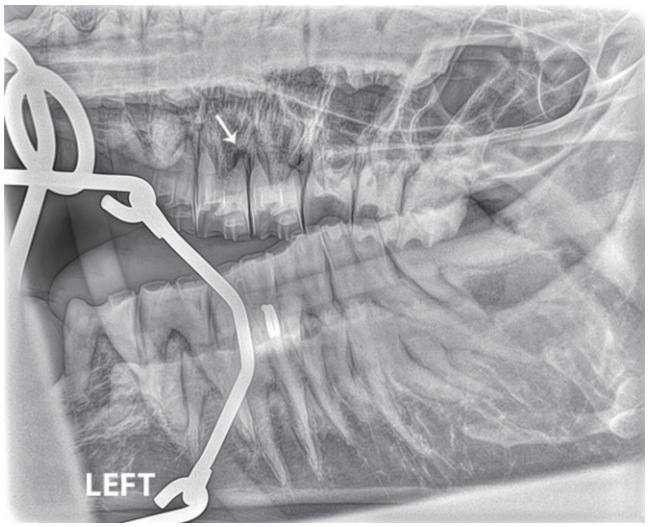

Fig. 8. RV45LDO radiograph of a 22-year-old Tennessee Walking Horse mare. There was feed packing in diastemata between the left and right maxillary 3rd and 4th premolars and 1st molars. Periapical bone lysis (arrow) is evident on the distal buccal root of the left maxillary 3rd premolar (207). Note the short roots of the maxillary cheek teeth in comparison to the mandibular cheek teeth.

or if positioning is not optimal. The addition of offset mandible and intraoral views of the incisors and canine teeth is case-dependent.[1]

The use of all 6 of these views allows for evaluation of each cheek tooth from several angles and is analogous to obtaining 4 to 5 views in a fetlock joint study with the same views of the contralateral limb for comparison. Standardization of the study has the benefit of permitting the radiologist to readily recognize when the view is appropriately positioned, speeds up the radiographic examination, and enhances the ability to obtain a diagnosis or seek a consultation from a specialist. Digital systems allow the radiologist to obtain this study quickly. The author recommends presenting this service as a *study* billed similarly to a distal extremity study in a lameness examination (Equine dental radiographic study: $200–300) rather than billing by the image, which tends to result in the client requesting fewer images than are needed for a complete assessment and treatment planning.

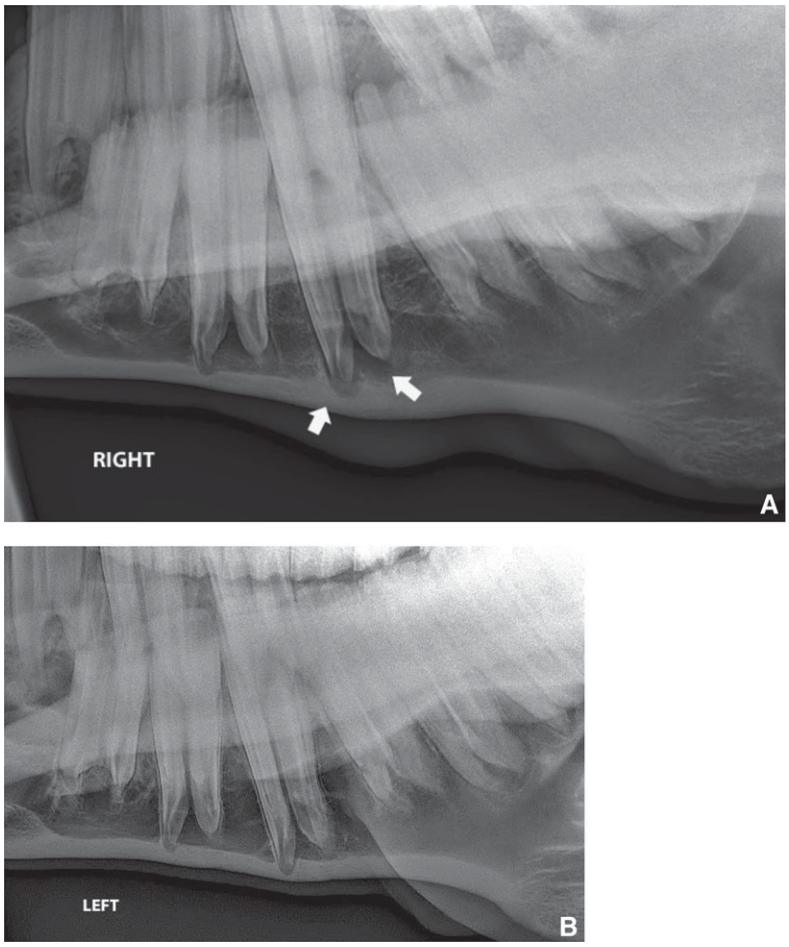

Fig. 9. An 8-year-old Holsteiner gelding with a complicated crown fracture of 408 associated with right ventral mandibular swelling and a draining tract. There is periapical sclerosis and apical bone lysis (arrows) associated with the fractured 408 (A) as compared with the contralateral 308 (B). Courtesy of Leah Limone, DVM.

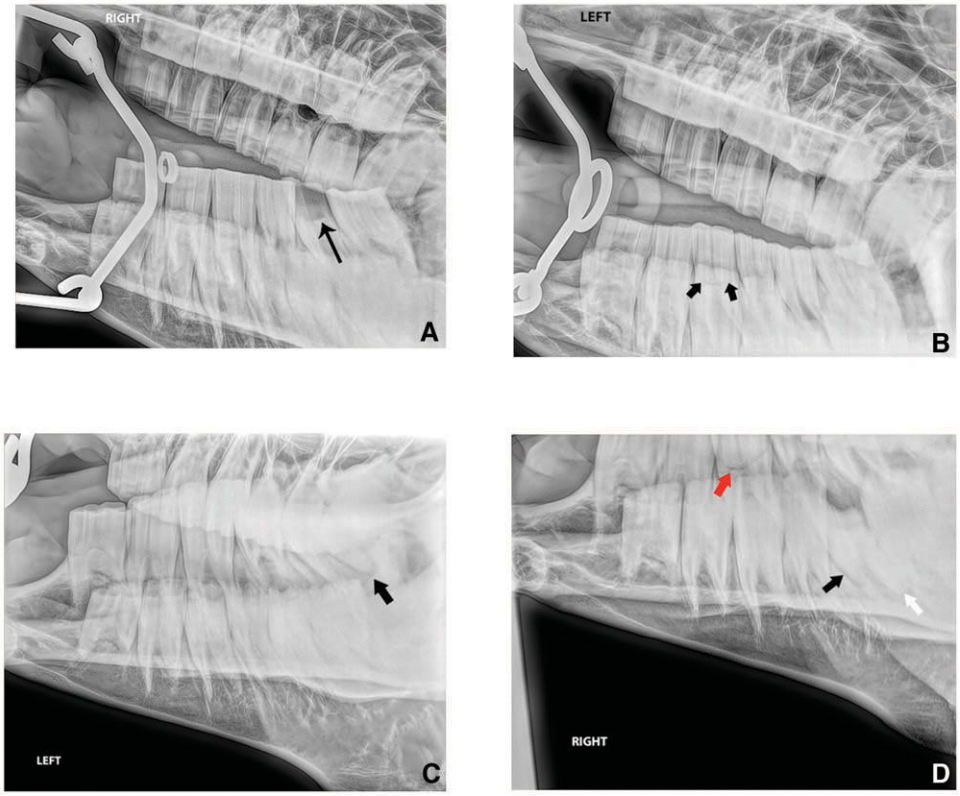

Fig. 10. In the clinical examination of a 17-year-old Quarter Horse mare, a complicated crown (buccal slab) fracture of the left mandibular 4th premolar (308) and the right mandibular 2nd molar (410) were identified. Multiple views were required to obtain a complete radiographic assessment. (A) LD30RVO open-mouth lateral view demonstrates the fracture of 410 (arrow). In the RD30LVO view (B), the transverse fracture of 308 is evident (arrows). In the RV45LDO view (C) the distal root of 410, which was poorly imaged in the LV45RDO view (D), is isolated and exhibits a widened periodontal ligament space (arrow). (D) the LV45RDO view reveals the widened periodontal ligament space of the mesial root of 410 (black arrow). The distal root of 410 (white arrow) lacks adequate detail in this view. Note that 308, isolated in the interarcade space, also nicely exhibits the transvers reserve crown fracture (red arrow). There is no radiographic evidence of periodontal disease associated with the 308 reserve crown fracture in this study.

Acknowledgments

Conflict of Interest

The Author declares no conflicts of interest.

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How to Utilize Advanced Imaging in the Management of Equine Dental Cases

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1. Introduction

Equine dental cases are challenging. All cases require a complete and thorough oral examination that often leads to routine radiographic imaging in order to establish a diagnosis. In selective cases, more advanced diagnostic imaging such as nuclear scintigraphy (NM), computed tomography (CT), or magnetic resonance (MR) imaging enhance the clinician/surgeon's ability to clearly define the problem and plan treatment. Sensitivity and specificity of radiography for dental disease ranges from 52 to 69% and 70 to 90%, respectively.[1,2] While radiographs are the most common and easily accessible imaging modality utilized in equine dentistry, accurate interpretation is difficult due to the complexity and summation of anatomic structures. Radiography is often the only imaging modality available and frequently yields an immediate diagnosis. On the contrary, further advanced imaging may be required in patients that are recalcitrant to medical therapy, require extensive surgical intervention, or have a disease that involves more than one tooth. As a general practitioner, it is important to understand the limitations of radiography to clearly diagnose equine dental disease. Advanced imaging may be employed to better characterize the extent and exact location of the abnormality, thus allowing a more effective treatment.

2. Materials and Methods

Advanced Diagnostic Modalities

A complete physical and oral examination must be completed prior to performing any imaging in patients with suspected dental disease. A proper oral examination must include the use of a mouth speculum, a light source, and a dental mirror or oroscope on a properly restrained and sedated horse. It is nearly impossible to have an accurate diagnosis from an oral examination without the appropriate instrumentation. If dental-related disease is suspected, a complete series of skull radiographs is the next step in the course of confirming a diagnosis prior to advanced imaging.[3]

When deciding whether to perform advanced imaging in dental cases, it is important to realize that NM, CT, and MR do not guarantee a precise diagnosis. On the contrary, advanced imaging is acceptable to perform even on cases where a fairly confident diagnosis has been made through other modalities. Dental disease can be perplexing to diagnose and treat with the potential for complications. Performing invasive dental surgery without

NOTES

Table 1. Comparison of Nuclear Scintigraphy (NM), Computed Tomography (CT), and Magnetic Resonance (MR)

	NM	CT	MR
Expense	$400–700	$500–1200	$2000–2500
Requires anesthesia	No	Yes/No	Yes
Multiplanar images	No	Yes	Yes
Anatomic bone detail	Variable	High	Moderate to high
Anatomic soft tissue detail	Variable	Minimal	High
Physiologic information	Yes	No	Variable to yes
		High (1024 × 1024 matrix)	Moderate (320 × 290 matrix)
Spatial resolution	Low (256 × 256)	~1 mm slices	~3 mm slices
Contrast resolution	N/A	Moderate	High

an accurate diagnosis and complete understanding of the pathological process can lead to long-term complications such as chronic oro-nasal, -sinus, and -cutaneous fistulas that are extremely difficult to resolve. It is the authors' opinion that invasive surgical treatment of dental related disorders not be performed until there is confidence in the diagnosis. Advanced imaging can only strengthen a clinician's confidence in a diagnosis, resulting in a well-constructed and precise treatment regime for the horse. While advanced imaging is significantly more expensive compared to other diagnostic tools, the financial cost of inaccurate diagnoses and/or treatment can oftentimes result in higher overall costs to the owner and morbidity to the horse.

Prior to referral for advanced diagnostic imaging, it is important to understand the differences between NM, CT, and MR and their advantages and disadvantages (Table 1).

3. Results

Nuclear Scintigraphy

Nuclear scintigraphy images reflect physiologic rather than anatomic structure and are highly sensitive to bone turnover.[4] The sensitivity and specificity of scintigraphy has been reported at 95% and 86%, respectively.[1] When radiographs and scintigrams were concurrently evaluated, the sensitivity and specificity for detection of dental disorders increased to 97.7% and 100%, respectively.[1] One advantage to NM is the ability to perform imaging in the sedated standing horse. This is obviously better for the horse by avoiding general anesthesia. However, since NM does not provide multiplanar images, anatomical understanding is challenging and fails to improve the diagnosis of secondary disease such as sinusitis or fistulas. At $400 to 700 for a focused exam of the skull in cases of suspected dental disease, the authors feel that NM should be reserved for cases where CT or MR imaging are not available, general anesthesia must be avoided, or where an accurate diagnosis is unobtainable by either modality.

Computed Tomography

Computed tomography has played a pivotal role in the diagnosis and effective treatment of equine dental and sinonasal disorders.[5–9] Computed tomography acquires radiographic skull images in the transverse plane and a complete examination can be performed in approximately 15 min. Postacquisition reconstructed images can then be made in any plane or into three-dimensional images for surgical planning and owner/client education. New software can even allow 3-D printers to construct scaled down versions of the skull or dental tissues. Computed tomography allows for evaluation of the skull without anatomic superimposition and provides excellent contrast and spatial resolution. For these reasons, CT provides a more accurate assessment of the extent and physical features of diseases of the skull than conventional radiographs. Both soft tissues and bone can be imaged with CT. However, the soft tissue contrast resolution of MR imaging is superior to CT. An important function of CT is the measure of tissue attenuation (radiopacity) in Hounsfield units (Table 2). This can help differentiate pure fluids from soft tissue masses. Common CT features of dental disease include widening of the periodontal space, tooth root lysis/blunting, tooth crown or root fragmentation, alveolar bone sclerosis, and apical tooth root gas.[5,8]

Computed tomography imaging has traditionally been performed with the horse under general anesthesia. More recently, a handful of imaging facilities around the world allow CT examinations to be performed in the standing sedated horse. This technique is becoming increasingly popular. Standing CT will allow veterinarians and owners the opportunity to perform advanced imaging safely

Table 2. Hounsfield Units of Tissues

Tissue	Hounsfield unit
Enamel	2400–2600
Dentin	1600–1800
Cementum	1200–1500
Bone	1000
Soft tissue	40–60
Brain	30–40
Water	0
Fat	−100
Air	−1000

Table 3. MR Tissue Appearance on Common Imaging Sequences

	T1 Weighted	T2 Weighted	STIR
Bone Cortex	Very low signal (black)	Very low signal (black)	Very low signal (black)
Bone Marrow	High signal (white)	High signal (white)	Low signal (grey-black)
Fluid (low protein)	Iso signal (grey)	High signal (white)	High signal (white)
Fluid (high protein)	High signal (white)	Low signal (grey)	High signal (white)
Fat	High signal (white)	High signal (white)	Low signal (grey-black)
Dentin	No signal	No signal	No signal
Enamel	No signal	No signal	No signal
Cementum	No signal	No signal	No signal
Pulp	Iso to high signal (grey to white)	Iso to high signal (grey to white)	Iso to high signal (grey to white)

with decreased costs and risks. In the authors' opinion CT imaging is the gold standard advanced imaging modality in equine dentistry and will continue to increase in its importance and value as standing CT becomes more popular and accessible.

Magnetic Resonance Imaging

While MR imaging is more commonly utilized for the diagnosis of soft tissue and musculoskeletal injuries, it can be advantageous in the diagnosis of dental disease. Unlike CT and radiographs that base images on attenuation, MR images are based on the signal measured from spinning magnetic moments of the hydrogen nuclei. When describing MR images, findings are referred to by signal intensity, often relative to muscle or normal areas of soft tissue. Low signal intensity is black and consistent with cortical bone, teeth, air, tendons, ligaments, and some stages of hemorrhage. High signal intensity is white and generally consistent with fat on sequences that do not have fat suppression. Other soft tissues are shades of grey, depending on their magnetic properties (Table 3).

There are few publications specific to MR findings in equine dental disease. Most literature focuses on the normal anatomy of structures of the skull and pathologic changes in the brain and pharynx.[10–13] As previously mentioned, MR imaging provides great soft tissue contrast (Fig. 1). However, teeth are void of signal in conventional MR imaging. More recently, human studies are utilizing new techniques to allow both the solid and soft tissue component of teeth to be imaged for dental and maxillofacial applications.[14] These new techniques may eventually make a place for MR imaging in equine advanced imaging for dentistry. Gerlach et al describes equine MR pulp abnormalities as blurred with a lower signal intensity.[15]

An MR examination is considered relatively expensive at $2500/scan. Examinations of the skull are performed under general anesthesia and scan

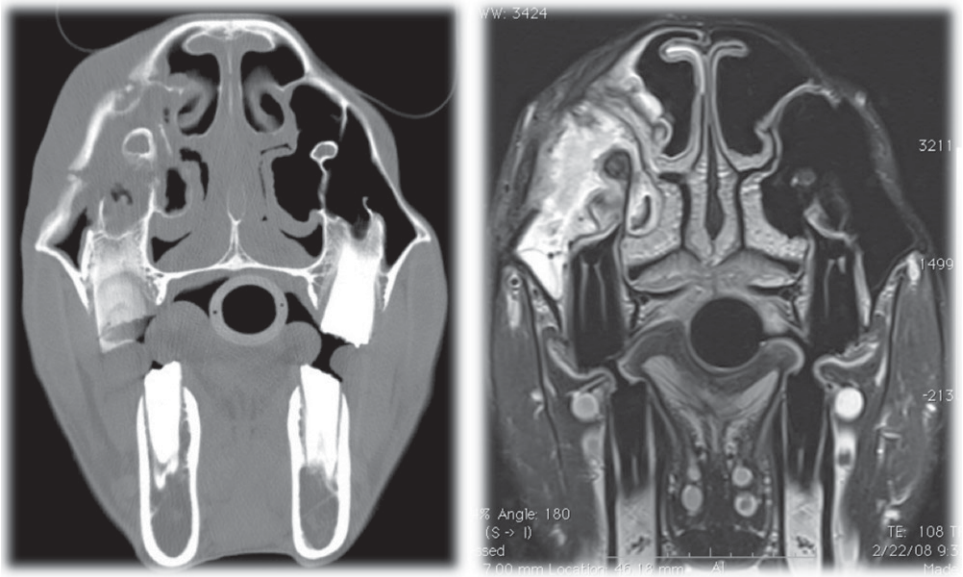

Fig. 1. Transverse CT (left) and MR (right) image of the same horse at the level of the 1st molar. Notice the range of contrast in the MR image compared to the CT.

times range from 45 to 120 min. Magnetic resonance imaging should be considered an alternative imaging modality when CT imaging is unavailable. The authors have found this modality to be beneficial in the diagnosis of dental disease, but less accurate in challenging cases of dental disease than CT due to the decreased spatial resolution and void of signal within teeth.

4. Discussion and Summary

Nuclear scintigraphy is considered an advanced imaging modality that has been shown to improve the ability to accurately diagnose equine dental disease. It should be reserved to cases where general anesthesia must be avoided or CT and MR imaging are not available.

Unlike NM, multiplanar imaging, such as CT and MR, have advanced our understanding of functional anatomy not only in the skull, but throughout the entire equine body. The ability to noninvasively "look inside" anatomical structures has revolutionized veterinarians' ability to diagnose and treat equine diseases. The equine skull is one of the most complex anatomical structures and intercommunication between the teeth, bones, maxillary sinuses, and nasal cavities make disorders of the equine skull difficult to diagnose and successfully treat.

In the authors' opinion, CT is the most valuable advanced imaging modality in the diagnosis of dental disease. With the advent of standing CT, this modality is becoming increasingly more valuable, less expensive, and safer for the horse. If you consider the cost of a complete radiographic series of the skull to be approximately $300, a standing CT examination of $600 is extremely beneficial and, when available, could potentially replace radiographic examination in cases where financial constraints exist. The additional cost of the CT examination outweighs the risk of potential misdiagnosis and inappropriate treatment. Magnetic resonance imaging can often provide similar diagnostic capabilities in equine dental disease, but should be reserved for cases where CT is nondiagnostic and/or unavailable, or improved soft tissue contrast resolution is required.

The decision to utilize advanced diagnostic imaging in cases of dental disease should be similar to the decision process of referring a colicky horse for surgery. Veterinarians consider referral based on the severity and complexity of disease, financial constraints of the owner, experience/comfort level of the clinician, and the risks of an inaccurate diagnosis/treatment plan. An inaccurate diagnosis or treatment plan can lead to increased morbidity or even mortality. As with referral of colics, advanced diagnostic imaging in cases of dental disease can only lead to greater understanding of the disease, resulting in improved outcomes for the horse and client.

Acknowledgments

Conflict of Interest

The Authors declare no conflicts of interest.

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How to Incorporate Nutritional Discussions and Nutritional Alterations As Components of Equine Dental Care

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1. Introduction

The purpose of this paper is to outline an approach that incorporates both nutritional assessment and dietary modification into a program of regular equine dental care. The annual oral examination provides the equine practitioner with the opportunity to not only identify common dental abnormalities (periodontal, endodontic, and occlusal) but also to document the loss of cheek-teeth chewing surface area that occurs with age as a product of normal dental eruption and wear.[1,2]

A complete oral examination begins with taking a dental history. This is followed by patient observation, an extra-oral physical examination, and finally the oral examination.[3] Findings are documented on the dental record. Important nutritional information can be obtained at each phase to give the equine practitioner a clear picture of how the horse is handling its current diet, especially hay. Such monitoring of this progressive process gives the equine owner early awareness of impending dental and nutritional issues and allows implementation of dietary modifications prior to any significant weight loss or more serious choke or impaction events.

2. Materials and Methods

The dental history should focus on determining what the horse is fed, how the food is delivered, and how it appears to be meeting the horse's nutritional needs; in particular, its caloric requirements. Questions should include:

- What do you feed your horse? How often? Is there access to pasture?
- Is your horse still able to eat hay? Does the horse leave any undigested boluses of hay?
- Where is the horse fed? Is the horse separated from other horses when it feeds?
- How long does it take for the horse to finish eating?
- Has the horse gained or lost weight over the past year?
- What do the fecal balls look like? Formed and moist? Dry? Presence of undigested hay?
- Is your horse on any medication(s)?

Observing the horse allows the equine practitioner to assess the animal's body condition, eating habits (i.e., the ability to prehend food and any evidence of quidding hay), and fecal consistency.

NOTES

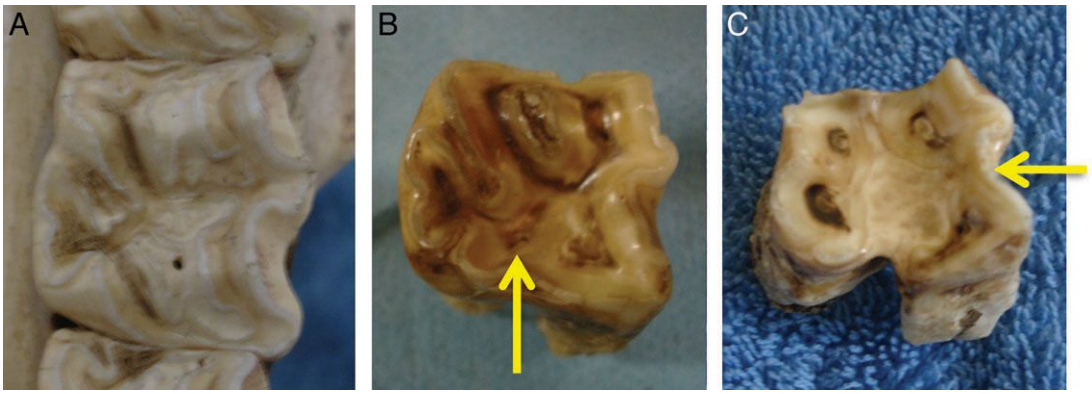

Fig. 1. Occlusal view of maxillary cheek teeth showing progressive smoothing of the surface with age. A, Young mature horse. All occlusal enamel is present. B, Loss of occlusal enamel in mesial infundibulum (yellow arrow). The dental chart should identify this tooth as cupped (CUPD). C, Loss of occlusal enamel in both mesial and distal infundibulae. Only a small portion of the peripheral enamel remains along the vestibular (buccal) aspect of the tooth (yellow arrow). The dental chart should identify the tooth as expired (EXP).

The results of the initial extra-oral physical examination can provide another, albeit superficial, indication of possible underlying issues (i.e., heart murmurs, anemia, long hair coat suggestive of pituitary dysfunction) that may warrant further workup.[1,2] The diets of many senior animals are high in molasses (for palatability) and protein, both of which may be contraindicated for horses with pituitary dysfunction or advanced liver abnormalities.[4]

The oral examination, from a nutritional perspective, should focus on the evaluation of the occlusal surface of the cheek teeth to identify the progressive loss of infundibular enamel in the maxillary cheek teeth and enamel infolding in the mandibular cheek teeth. Enamel loss decreases the chewing surface area used to masticate hay (Fig. 1).[5,6] The term "smooth mouth" describes teeth experiencing this process: teeth without the infundibular or central enamel.[1,2,5,6] Maxillary cheek teeth are referred to as "cupped teeth" when their clinical (exposed) crown is worn below the apical extent of the infundibulae, but still have clinical crown above the gingival margin. Mandibular cheek teeth similarly lose the normal enamel infolding in the center of the tooth, but there is no specific term for this. The occlusal surface of the center of both maxillary and mandibular teeth is smooth and concave in appearance. The loss of enamel exposes the underlying cementum and dentin, both dental tissues with little wear resistance, so these teeth quickly undergo fur-

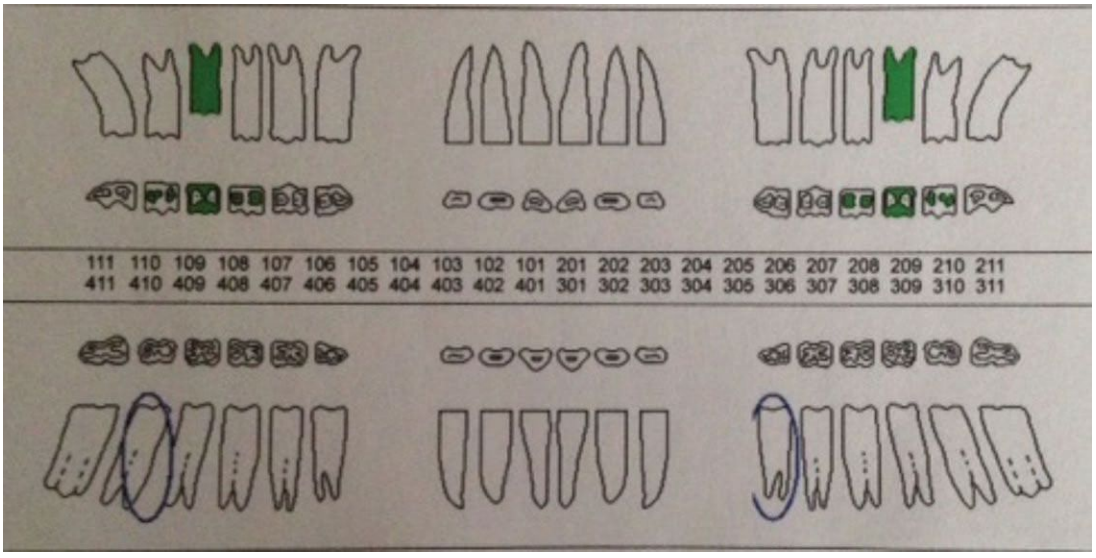

Fig. 2. Example of a dental chart showing the use of colors to depict occlusal wear. The green on the surface of the upper 08s and 10s corresponds to cupped teeth while the solid and shortened upper 09s correspond to expired teeth. 306 and 410 were also missing in this horse (blue circles).

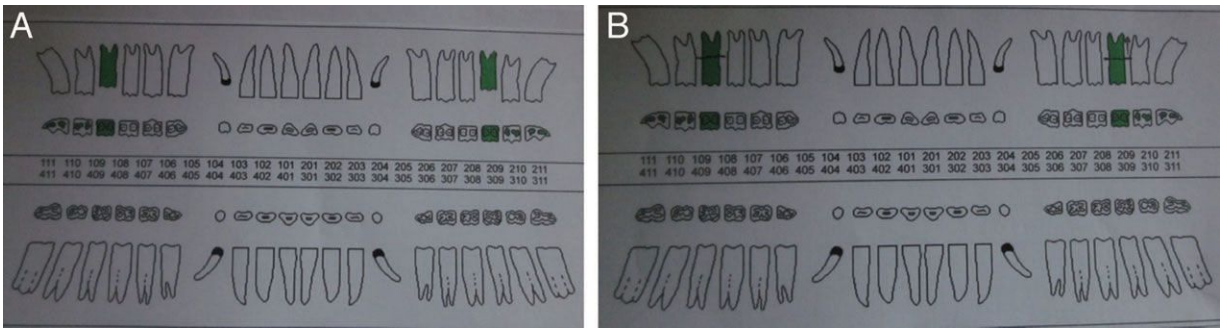

Fig. 3. Dental charts showing alternative ways to document expired teeth. A, Expired maxillary 09s are a solid color and are shortened. B, Expired maxillary 09s are a solid color and a line is drawn through it to show the loss of clinical crown.

ther attrition. "Expired" describes maxillary or mandibular cheek teeth at the end stage of smooth mouth, where the teeth are worn down to the cemental root remnants.[5,6]

All levels of attrition need to be documented in the dental record. Adding drawings and color to the written record is useful to visually reinforce the loss of surface area (Fig. 2). The next important step is to educate the owner by showing them the changes observed during the examination. Intra-oral photos and drawings can also be used to reinforce the findings for both present and absentee owners.

On the dental record the entire tooth is filled in when it is expired (end-stage smooth mouth). The author's computer program allows for manipulation of the tooth height so the loss of clinical crown can also be depicted (Fig. 3A). The tooth could instead be marked with a line, as shown in Fig. 3B, to denote this same loss (Fig. 3).

3. Discussion

With most equine practitioners seeing a growing number of geriatric patients, it can be valuable to incorporate proactive nutritional discussions into the examination routine. Nevertheless, appropriate nutritional advice is not possible until all aspects of the clinical examination (dental history through oral examination) have been completed.

Completion of the dental history in combination with a look at conditions at the stable or private residence can often indicate the immediate cause of observed weight loss. For example, the thin geriatric horse scheduled for examination may be the one seen standing out in a paddock with a round hay bale along with the other younger and fatter horses. That older horse's teeth are simply unable to chew the hay and, thus, that horse can only stand aside as the others feed. Another common scenario is that the owner has called for an appointment because, despite eating 20 lb of a senior feed each day, the horse is still thin. In the latter situation, it may be useful to find out how long it is taking the horse to eat each feeding. A good rule of thumb to use is that if a horse takes longer than 45 min to finish each meal, switching to a more calorically dense feed is warranted.

The observation and extra-oral physical examination is also an opportunity to determine if further diagnostics may be warranted either before or after the horse is sedated for the oral examination. A routine complete blood count (CBC), chemistry, and other specific endocrine testing could identify the presence of anemia or liver, kidney, or pituitary dysfunctions that may influence the final nutritional recommendations.

The oral examination is the most valuable tool for evaluating how well the horse, as it ages, can chew hay. Documenting the level of wear present in a horse's mouth is important because many horse owners have the false belief that horse's teeth grow

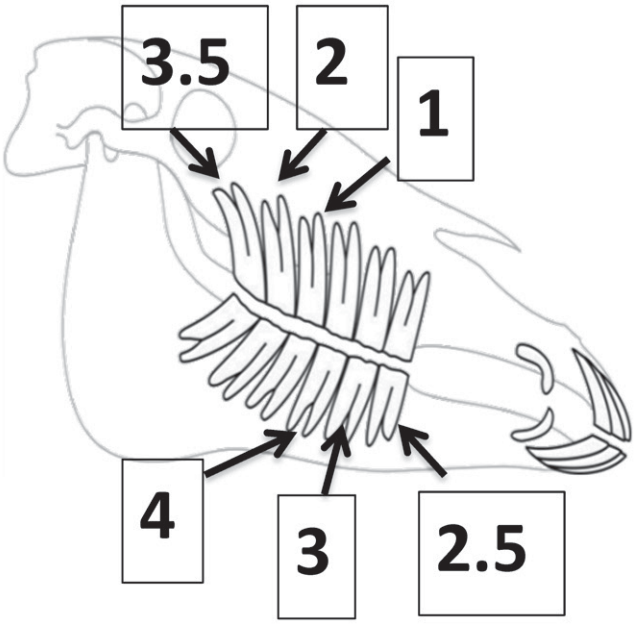

Fig. 4. Schematic showing the sequence of permanent cheek teeth eruption in the horse. Numbers represent the horse's age in years.[7]

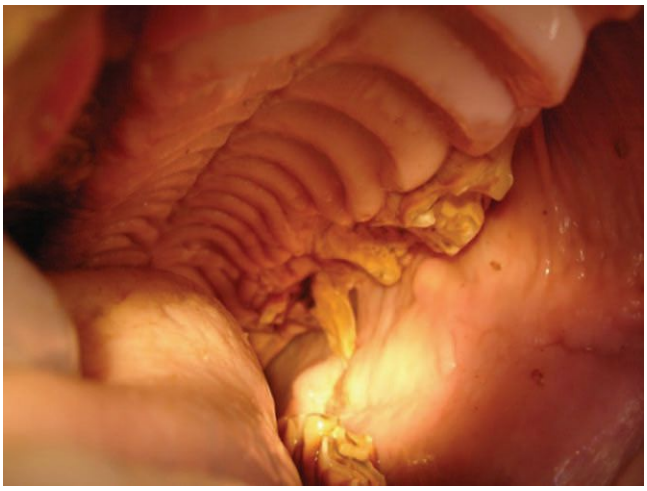

Fig. 5. Intra-oral picture of the 200 arcade in a geriatric horse showing advanced wear.

over time and since many geriatric horses require minimal dental corrections, owners often have an unjustified sense of security regarding their horses' nutritional needs. This misconception can be corrected by talking to owners about eruption and wear once cupping is identified in the maxillary first molars. The progressive nature and pattern of the wear can be emphasized by using dental eruption charts as visuals (Fig. 4). Dietary recommendations should be a part of these conversations before the horse has lost weight and its mouth looks like that depicted in Fig. 5. Owners should be asked to start watching for signs of undigested hay (quidding) when the upper 09s and 10s are cupped. When all the maxillary 09s, 10s, and 11s are either cupped or expired a long-stem hay alternative should be found, especially if pasture is not readily available. For senior horses with greatly diminished chewing function such as the horse in Fig. 5 (assuming lab work has precluded problems such as renal or hepatic dysfunction), chopped or soaked grass and grass/alfalfa-mix hay cubes fed at a rate of 1.0 to 2.0% of body weight per day; a senior feed (crude protein: 12–14%; crude fat: 8–20%; crude fiber: 5–10%),[4] and possibly the use of edible oil up to 1 cup/day or rice bran (0.5–1.0 lb/day), again assuming normal hepatic function, to increase the digestible energy if the horse needs to gain weight.[1,2]

4. Summary

The routine oral examination presents an opportunity to improve client awareness of the significance of age-related tooth wear and to discuss proactive dietary modifications to prevent cases of choke, impaction, and dramatic weight loss as the horse ages. In most instances, proper nutritional advice can be obtained by performing a thorough dental history, examining the horse, evaluating the stable environment, observing the horse's eating habits, and analyzing a fecal sample. Lab work may be indicated as part of the evaluation to rule out underlying disease conditions.

Acknowledgments

Conflict of Interest

The Author declares no conflicts of interest.

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How to Understand Regenerative Medicine—What Is It?

Ashlee E. Watts, DVM, PhD, DACVS

There is still much to learn about the optimal treatment paradigm in regenerative therapies including indications, technique, route, dose, timing, and frequency. In order to fully assess therapies, the treating clinician must have a solid understanding of regenerative medicine techniques. Author's address: Large Animal Surgery, Department of Large Animal Clinical Sciences, College of Veterinary Medicine and Biomedical Sciences, Texas A&M University, College Station, TX 77843; e-mail: awatts@cvm.tamu.edu. © 2014 AAEP.

1. Introduction

Regenerative medicine is the process of harnessing natural healing processes to improve upon tissue repair for a more functional healed tissue. The holy grail of regenerative medicine would be to recapitulate development, resulting in healed tissues that cannot be distinguished from uninjured tissue. Although to date this has not been achieved in musculoskeletal tissues, the potential to substantially improve outcomes with regenerative techniques is considerable. Subsequently, there has been much activity in research and widespread clinical use of regenerative therapies for equine orthopedic applications. Some of the tools for regenerative medicine in orthopedics include stem cells, platelet rich plasma, autologous conditioned serum, growth factors, and gene therapy. Regenerative therapies can be applied by intralesional, perilesional, intraarticular, or intravenous injections.

2. Stem Cells—Defined

Stem cells, unlike their somatic cell counterpart, are self-renewing, highly proliferative, and capable of multilineage differentiation. The ultimate stem cell is made at conception. After fertilization, the zygote consists of totipotent stem cells that are able to form all 3 germ layers as well as placental tissue. Once the zygote becomes a pre-implantation blastocyst, the inner cell mass consists of pluripotent stem cells that will give rise to all 3 germ layers: the ectoderm, mesoderm, and endoderm and can no longer form placental tissues. At this stage the stem cells are embryonic. After day eight, the cells will become either somatic cells (terminally differentiated) or stem cells committed to a specific lineage (multipotent). Subsequently, the stem cells are considered adult-derived, despite their presence in fetal tissues. Local niches of lineage committed multipotent stem cells remain in adult tissue throughout life for normal tissue remodeling and repair. With increasing age, the number, expansion potential, differentiation potential, and so-called potency of stem cells declines; therefore, there is increasing interest in allogeneic embryonic and fetal derived stem cells as well as banking of autologous stem cells from post-natal samples.

Modifications to these classifications of stem cells (embryonic and adult-derived) are also being inves-

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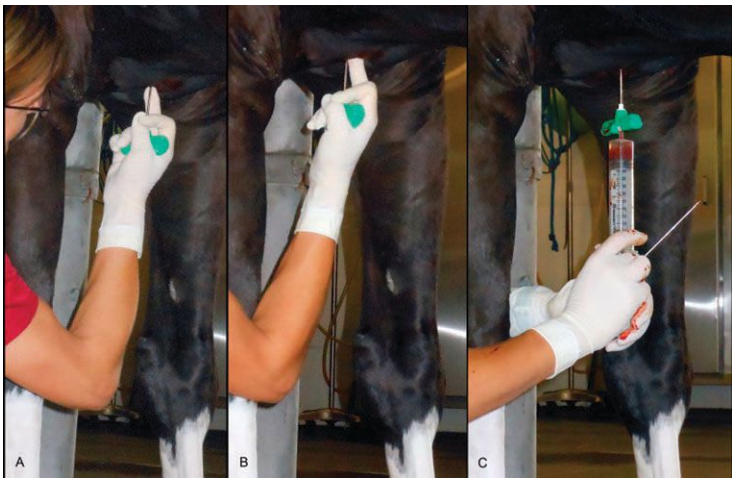

Fig. 1. Placement of an 11 gauge 110 mm biopsy needle for the aspiration of bone marrow from the sternum. The horse is sedated and there is local subcutaneous anesthesia. A, The needle is grasped with the middle finger extended along the length of the needle and placed against the skin at the level of the elbow. B, The needle is advanced with a twisting motion until the tip of the middle finger is touching the skin (~ 2 cm advancement) and the needle is firmly seated in the bone. C, The stylet is removed and bone marrow is aspirated.

tigated and will be briefly discussed. One modification is a fetal-derived stem cell that has been manipulated in vitro to act more like an embryonic stem cell. One such product has been developed and tested in the horse but clinical availability is pending FDA approval.[1] An important benefit of this type of stem cell is its immediate availability as an 'off-the-shelf' product and its increased potency due to a pluripotent-like (embryonic-like) state. Another modification is the induced pluripotent stem cell, where in vitro manipulations are applied to adult somatic cells, such as skin fibroblasts, to de-differentiate them and induce a stem cell-like state. The induced pluripotent stem cell is currently being investigated by several equine research groups.

Because of their broad overlap with other cell populations, mesenchymal stem cells (MSCs) cannot yet be accurately sorted by cell surface markers. Therefore, many labs select and isolate MSCs by expanding the tissue culture plastic adherent population of colony forming cells. This translates to a culture period of 2 to 3 weeks, in vitro, to isolate and expand MSCs from clinical samples for autologous therapy. In the horse, MSCs have been isolated from bone marrow, adipose, tendon, muscle, umbilical cord blood and tissue, gingiva and periodontal ligament, amniotic fluid, and blood (Figs. 1–3). The different tissue sources vary in the ease of harvest, expansion potential, and differentiation capacity. Several academic and commercial laboratories provide for the isolation, expansion, and cryopreservation of stem cells from several different tissue sources; namely, bone marrow, fat and umbilical cord, or blood. Directions for collection and shipping procedures are available from each lab. To date, bone marrow derived MSCs from both the horse and human have been the most thoroughly studied and have the most evidence for the ability to undergo chondrogenesis, tenogenesis, and osteogenesis and might contribute to cartilage, tendon, and bone repair as well as modulate inflammation and soft tissue repair within the joint.

3. Stem Cells—Autologous or Allogeneic

Autologous (self) therapy has been used most in horses to date. Autologous cells are considered safe with minimal risk for disease transmission. A major disadvantage of autologous cells is that unless cells have been banked prior to injury, their use dictates a delay of 2 to 3 weeks for isolation and expansion. Although many labs are offering banking of autologous MSCs, the long-term viability of cryopreserved MSCs has not been fully elucidated. One way to avoid the culture delay for autologous MSCs is to use patient side kits to concentrate stem cells.[2] Several commercial kits are available that enrich for the nucleated cellular portion resulting in a higher concentration of MSCs in a small volume from bone marrow and fat. Another method to avoid delay would be to use allogeneic (nonself) cells. Because MSCs are immune privileged, allogeneic cells can be used in nonrelated individuals and without immune testing. Although this has been demonstrated in most species, it has not yet been thoroughly reported in the horse. Use of an allogeneic stem cell line would allow use of an 'off-the-shelf' stem cell product and would have several advantages: reduced variability, allow same day treatment, allow use of younger stem cells, and use in aged horses. Finally, it may reduce costs. However, allogeneic stem cells are considered a drug by the Food and Drug Administration and, as such, are required to undergo the same safety and efficacy

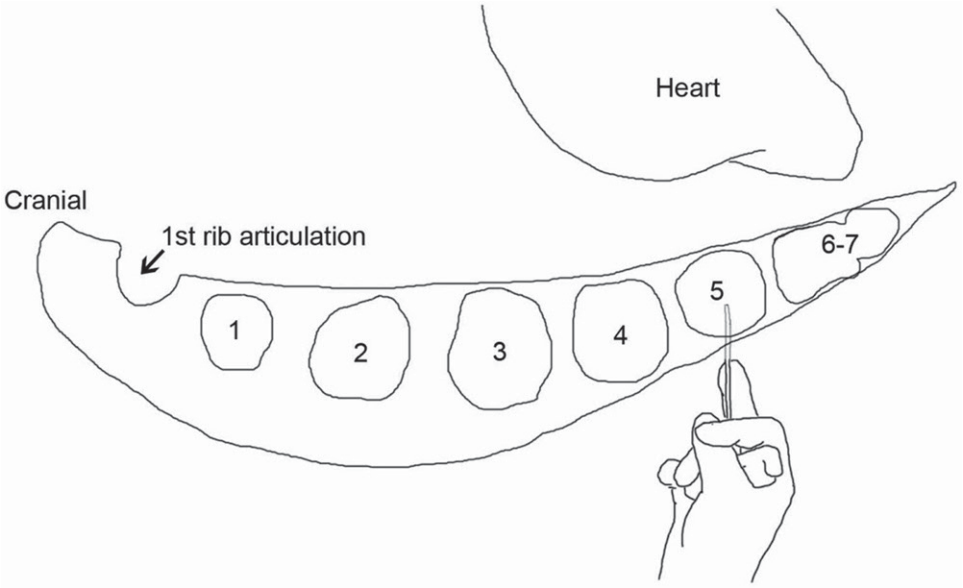

Fig. 2. Line drawing of needle placement in the sternum for bone marrow aspiration. The needle is placed in the fifth sternebra as in Fig.1. The dorsal to ventral height of the marrow space in this location is approximately 5 cm. The heart lies dorsal to the sternum and is closest to the sixth and seventh sternebrae.

trials and manufacturing processes that are required of pharmaceuticals. Such trials are expensive and time consuming; therefore, commercial allogeneic stem cells are not yet available. In contrast, the use of autologous stem cells in veterinary patients is not currently regulated by the FDA.

4. Platelet Rich Plasma (PRP)

Following wounding, circulating platelets accumulate and become activated when exposed to a basement membrane. Activation causes platelet degranulation and release of many bioactive substances that promote healing, stimulate angiogenesis, recruit endogenous stem cells, and regulate inflammation. Specific growth factors released from activated platelets at high concentrations include platelet derived growth factor (PDGF), transforming growth factor beta (TGF-β), fibroblast growth factor (FGF), epidermal growth factor (EGF), insulin-like growth factor (IGF), and vascular endothelial growth factor (VEGF). Platelet rich plasma (PRP) is a fraction of blood with an increased platelet concentration above baseline and is used largely for its anabolic properties. Other components of PRPs are plasma proteins dissolved in water (adhesive proteins, clotting factors, fibrinolytic factors, proteases and antiproteases, basic proteins, and membrane glycoproteins), varying concentrations of leukocytes, and sporadic erythrocytes and stem cells.

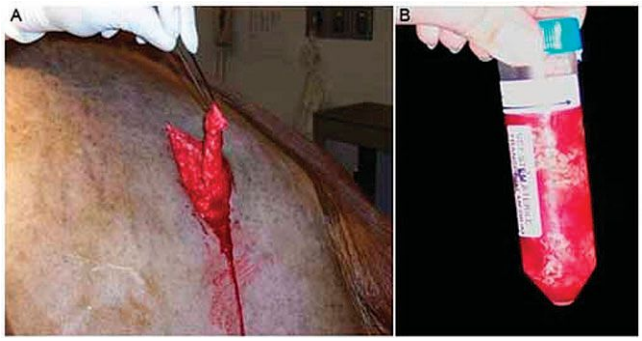

Fig. 3. Surgical incision for collection of adipose from the gluteal region. A, A longitudinal incision is made. B, Twenty grams of adipose tissue are collected and placed in 50 ml conical tube. Photo courtesy of Dr. Alan Nixon, Cornell University.

The principal advantage of PRP is that production can be performed patient-side for immediate use, relatively inexpensively. The PRP is produced through centrifugation or filtration of venous blood and can generally be accomplished within 15 min. The blood collection and preparation procedure varies from manufacturer to manufacturer and will influence the composition and volume of the product (platelet and leukocyte fold change, for example). It is likely that the varying reports of efficacy in clinical outcomes are influenced by the PRP composition and the ideal concentration of platelets and leukocytes within PRP remains undefined. Certainly, as platelet concentration increases, so does growth factor concentration. Therefore, higher platelet concentrations may be desirable. In support of this view, in vitro tendon explant data shows

that tendon and ligament gene expression was improved with increasing platelet concentration. In the same study, increasing leukocyte concentration increased gene expression of collagen type III, the protein composition of scar tissue, which is undesirable.[3]

Extra doses of PRP can be stored frozen (−20 °C) for later use. It is important to note that leukocytes within the PRP will be lysed and platelets will be activated by this storage process. While some have recommended platelet activation of fresh PRP with varying additives (calcium chloride, thrombin) or by freezing, it is probably not necessary as the local environment should be sufficient to activate platelets for growth factor release. If PRP is to be used as a clot, the addition of thrombin and calcium is required. Clinical anecdotes suggests that PRP is useful for acute tendon and ligament injury when injected intralesionally under ultrasound guidance to acute to subacute lesions. Platelet rich plasma has also been utilized for arthropathies and delayed bone healing. Anecdotally, joint flares have been reported following PRP injection and may be related to the platelet to leukocyte ratio and leukocyte concentration.

5. Autologous Conditioned Serum

Autologous conditioned serum (ACS) therapy was developed to counteract the inflammatory mediator, interleukin-1, with a naturally occurring antagonist protein, interleukin-1 receptor antagonist protein (IL-1Ra; IRAP). Inhibition of interleukin-1 provides an analgesic as well as an anti-inflammatory effect and, thus, ACS is used for its anticatabolic properties. Commercially available kits for the production of ACS are available in which blood is incubated overnight in the presence of medical-grade glass beads. This incubation leads to the de novo synthesis and release of stored endogenous substances, including IL-1Ra, by leukocytes and platelets within the blood. Twenty-four hours later, the sample is centrifuged and the supernatant (serum) is collected, sterile filtered (0.2 μm filter), and separated into several aliquots. A portion (usually about 2 ml) of the ACS is injected to the affected region and the extra doses can be stored in a freezer (−20°C). Although IL-1Ra is the target protein made in this process, there is probably a large and diverse set of factors present in ACS that makes it effective.

There has been widespread use of ACS in horses, primarily via intra-articular injection in the treatment of joint disease, osteoarthritis, or synovitis. Joint flare, or serious adverse reaction to joint injection, appears to be infrequent but has occurred. Practitioners have also used ACS for intra- and perilesional tendon or ligament injection. Anecdotal evidence suggests that the majority of horses that receive and respond to ACS are those that have become refractory to intra-articular corticosteroid, except on a very frequent re-injection schedule. Timetables employed for ACS therapy vary among practitioners. Some administer each injection weekly for 3 to 4 treatments and others administer each injection monthly.

6. Growth Factors and Gene Therapy

The addition of growth factors, either directly as proteins or indirectly through gene therapy techniques to stimulate their production, has been used in several orthopedic applications. Compared to the injection of protein, which has a very short half-life, gene therapy would allow for continued expression of the transgene, increasing the duration of growth factor exposure. Members of the TGF family of growth factors and IGF have been used via proteins or gene therapy in the joint to stimulate synthesis of hyaline cartilage, improve subchondral bone architecture, and inhibit inflammatory responses, bone morphogenic protein (BMP) and gene therapy has been used in fractures and cystlike lesions to stimulate bone production, IGF protein and gene therapy has been used in tendon lesions to stimulate repair, growth-hormone-releasing hormone (GHRH) gene therapy has been used in the treatment of laminitis, and IL-1Ra gene therapy has been used in the joint to minimize inflammation.

Many methods for gene transfer are available. For satisfactory transduction efficiency and effective protein expression, the best-described gene therapy procedures involve viral vectors such as retrovirus, adeno-associated virus, adenovirus, and many others. In the horse, adeno-associated virus and adenovirus have been reported. Nonviral methods are also available but have been less studied. Of the virally-mediated gene therapy techniques, there is great variability in the genomic integration and, subsequently, the duration of transgene expression. It is unknown whether a long duration of transgene expression or permanent transgene expression would be required in orthopedic applications. Gene therapy techniques are not yet available for clinical application but may become a routine part of practice in the future.

7. Conclusion

There is still much to learn about the optimal treatment paradigm in regenerative therapies including indications, technique, route, dose, timing, and frequency. There are several factors that contribute to the lack of evidence. First, most regenerative techniques are unencumbered by federal regulations and, as such, are being employed for a variety of conditions, by differing manufacturing processes, and with differing treatment regimens. Second, autologous regenerative products have differing compositions both between patients and even within the same patient from different collections. Such widespread use of a variable product makes it increasingly difficult to make sound conclusions. Based on the anti-inflammatory effects and the ability of regenerative techniques to orchestrate tissue

repair and regeneration via endogenous cell recruitment and trophic factors, early treatment and possibly repeated treatments may be advantageous.

Acknowledgments

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Conflict of Interest

The Author declares no conflicts of interest.

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How to Use Stem Cells in Clinical Laminitis Cases

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Stem cell therapy as an adjunctive treatment of laminitis in clinical cases has shown promise. In our experience, the use of stem cells in the laminitic patient prior to the development of hyperplastic undifferentiated lamella or the "lamellar wedge" has been clinically beneficial. The use of stem cells does not eliminate the need for traditional supportive care of laminitic patients. Authors' address: Rood & Riddle Equine Hospital, 2150 Georgetown Road, Lexington, KY 40511; e-mail: smorrison@roodandriddle.com. *Corresponding and presenting author. © 2014 AAEP.

1. Introduction

Feet affected by chronic laminitis suffer from detachment of the dermal-epidermal interface and various patterns of pedal bone displacement.[1] Once separation of this interface occurs, horses heal with varying degrees of stability. Healing in the chronic laminitic foot is primarily through lamellar epidermal cell hyperplasia or lamellar wedge formation.[2,3] The lamellar wedge is composed of hyperplastic/dysplastic epidermal tissue with variation of tissue keratinization.[3] The degree of stability is indirectly related to the thickness of the lamellar wedge.[4,5] That is, the more hyperplastic undifferentiated thickened tissue is less stable. This tissue is often referred to as "rubbery" and does not secure the pedal bone to the hoof wall and would be considered "unstable." The more stable laminitic foot heals back with a tighter horn to pedal bone junction with less lamellar wedge formation. Factors which are believed to affect the degree of healing are: the extent of initial lamellar insult, subsequent structural damage, and the magnitude of distractive forces placed on the lamina. Reducing distractive forces by providing proper digital support (wedging heels, providing axial support, foot casts), and by reducing the load placed on the affected limbs (slings, weight displacement with water, recumbency) early in the course of the disease will likely contribute to the degree of healing.

In spite of the most aggressive treatment and management, many cases heal with inferior tissue quality and chronic instability. Unfortunately, it can take several months to realize the integrity of tissue that heals the damaged lamellar interface. The laminitic horse may spend months enduring painful rehabilitation, only to be euthanized because the quality of tissue regenerated is found to be inferior. These horses' feet suffer frequent setbacks from sepsis and digital instability. This all too common scenario is extremely frustrating and costly to the owner. Mesenchymal stem cell (MSC) therapy has recently become an attractive treatment modality because of its potential to influence the quality of tissue that repairs a lesion. Mesenchymal stem cell therapy has been shown to improve

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the quality of healing in various orthopedic lesions.[6–12] To date, no studies have been completed to determine the effects MSC therapy may have on healing or improvement in success rates when used adjunctively to treat laminitic cases. Stem cell activity in a foot damaged by laminitis may play a role in the type of healing. Decreased stem cell expression has been observed in lamellar tissue samples from feet with chronic laminitis.[13] Stem cell therapy has also been shown to have an anti-inflammatory effect[14] and, therefore, may have a temporary palliative effect on chronic laminitic cases after treatment. This study looks at laminitic cases treated with mesenchymal stem cells as an adjunctive therapy between 2010 and 2013. Success rates are compared to previously published data in cases treated (without stem cell therapy) from our practice.[15,16]

Fig. 1. Severe unilateral laminitis case with rotation and medial sinking.

2. Materials and Methods

Case Selection

Cases with laminitis categorized as uncompensated/unstable were selected to be treated with stem cell therapy. Uncompensated laminitis cases are defined as having severe damage to one or more of the growth centers (coronary corium or sole corium) of the foot. These cases have no evidence of sole and/or wall growth over time. In more acute cases, venograms show perfusion deficits, indicated by a lack of contrast material in the vasculature in weight bearing and unweighted images. Uncompensated cases often have shear lesion of the coronary band, seroma and abscess formation, and sometimes more serious deeper infections of the pedal bone.

Cases treated for chronic unstable laminitis were divided into 3 groups: acute rotation only (<30 days), sinkers, and chronic cases with advanced bone disease. Success rates for each category treated at the authors' clinic have previously been published.[15,16] Records from cases with laminitis and adjunctive treatment with mesenchymal stem cells since August 2010 were evaluated. The cases treated with MSCs were classified according to the specific type of laminitis existing in each. Cases were placed into three categories: (1) severe rotation with mild to no bone disease, (2) chronic laminitis with significant bone disease, (3) sinking. Success rates were compared to the previously published data.

Stem cell therapy was performed either by retrograde venous digital perfusion or intra-arterial injection (Fig. 1) at the level of the palmar/plantar digital vein/artery. Each patient was sedated with detomidine. Perineural anesthesia was performed at the basisesamoid level with 2% mepivicaine hydrochloride. For intravenous perfusion, surgical tubing tourniquet was place around the metacarpal phalangeal joint. The medial or lateral palmar/plantar digital vein site was scrubbed and prepped. A 26-g ¾ inch butterfly catheter is placed into the digital vein and the limb slowly perfused with a solution containing 20 to 30 million cells suspended in saline (Fig. 2). The foot was unweighted during at least half of the injection process. The tourniquet and perfusate was left in place for 25 to 30 minutes before removing the catheter and tourniquet. For intra-arterial injection, the medial or lateral palmar digital artery site was scrubbed and prepped. The palmar digital artery was palpated and isolated between the thumb and index finger. A 25-g ¾ inch needle was inserted into the palmar digital artery in a proximal to distal direction, once in the artery a pulsating stream of arterial blood is seen from the needle. The syringe is gently attached to the needle hub, and the stem cell dose is slowly injected.

Treated limbs were then bandaged following the procedure. Each case received an allogenic umbilical cord blood derived mesenchymal cell dose as the first treatment. Bone marrow from the sternum was harvested, cultured, and expanded for subsequent treatments. All cases were treated at monthly intervals, and the average case received 3 to 4 treatments per affected foot.

3. Results

There is a relationship between time to first treatment and success.

The median time to first treatment was 71.5 days. For horses who were first treated sooner than 71.5 days, 87% (13/15) were successful. For horses whose first treatment was more than 71.5 days, 53% (8/15) were successful ($P = 0.0464$).

Although not quite statistically significant, age is also related to success. The median age of the horses was 11. For horses less than 11, 82% (14/17)

Fig. 2. A, Left limb receiving intravenous stem cell perfusion; B, same foot 30 days later; C, same foot 3 months later.

were successful. While for older horses, 50% (6/12) were successful ($P = 0.0636$).

Is it age or time to first treatment that impacts success? It's both. For horses that are older than 11 and treatment after more than 71.5 days, only 33% (3/9) were successful, while for all other horses 86% (18/21) were successful ($P = 0.0041$)

HLZ improvement was defined as the average HLZ measurement at time 1 minus the average HLZ at the last treatment.

For horses that had time to first treatment more than 90 days, 17% (2/12) had improved HLZ, while for horses with first treatment up to 90 days, 69% (11/16) improved ($P = 0.0062$).

Among the variables investigated, only severe bone disease was related to improved HLZ. For horses with severe bone disease, 0% (0/6) had improved HLZ, but 59% (13/22) of others had improved HLZ. The authors think that this result would be expected.

It might be interesting to point out that HLZ improvement does not appear to be related to age.

The time from onset of laminitis to first injection of stem cells was as follows: (<30 days, 10 cases; 30–60 days, 4 cases; 60–90 days, 2 cases; >90 days, 14 cases). Each case received between 1 and 4 doses of 20 to 30 million cells per foot. Of the 30 cases, 21 (70%) were successful. Deep digital flexor tenotomy (DDFT) was performed on at least one of the affected limbs in 15 (50%) of the cases. Of those cases receiving stem cells <30 days from onset of laminitis, 100% were successful. Cases receiving stem cells >90 days from onset of laminitis showed a 50% success rate (Figs. 3 and 4).

4. Discussion

Umbilical cord blood derived MSC treatment has been shown to be safe with no adverse reactions when injected intradermally in horses.[17] This allows for a readily available dose to be injected.

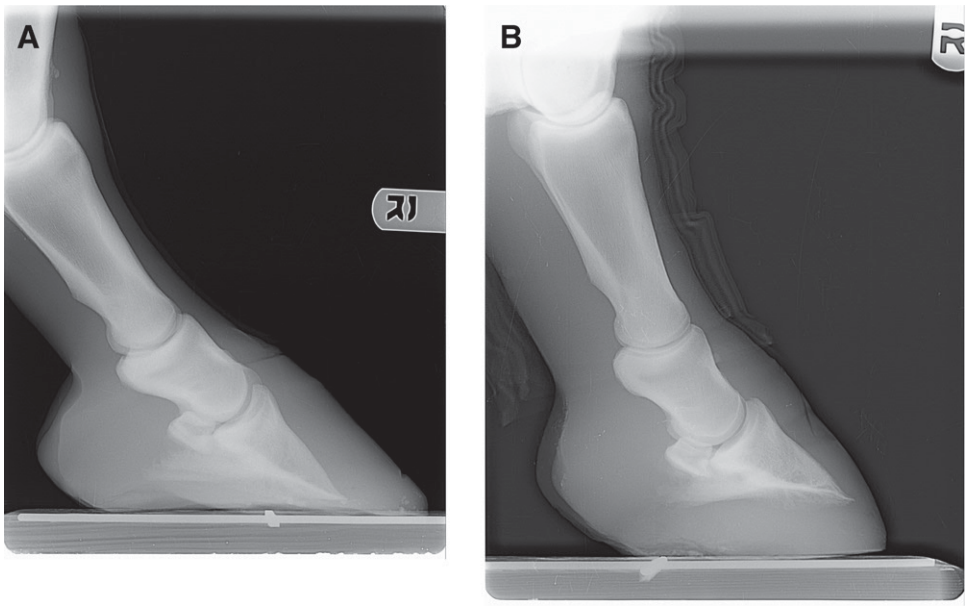

Fig. 3. Thoroughbred mare with bilateral sinking and rotation. Pre (A) and post (B) treatment with stem cells, tenotomy, and shoeing.

Intra-arterial and venous regional limb perfusion has been shown to be an effective method of delivering MSC to the digit.[18] Intra-arterial perfusion has been shown to result in a more reliable distribution to the foot. The venous route was used in most of these cases. It is currently not known when the ideal timing of the first dose should be instituted. Reason would justify that for the benefits during the repair phase of the disease, stem therapy would be most useful before lamellar wedge or epidermal cell hyperplasia occurs. This has been shown to take place within the first 30 days of pedal bone displacement.[5] In chronic advanced cases with bone disease and lamellar wedge formation, stem cell therapy had no effect on prognosis. These cases still maintained a thick lamellar wedge, which did not change in appearance on radiographs. In one case, with bone disease and a thick lamellar wedge, the dorsal hoof wall and lamellar wedge was resected prior to stem cell therapy. This was done to strip the unstable tissue in hopes of reseeding the interface with more patent tissue. This case continued to suffer from abscesses, instability, and continued discomfort and was euthanized. There is an intricate relationship between the health of the parietal surface of the pedal bone and the epidermal lamellae, lamellar, and sublamellar dermis.[19] This microenvironment has an effect on the regenerative

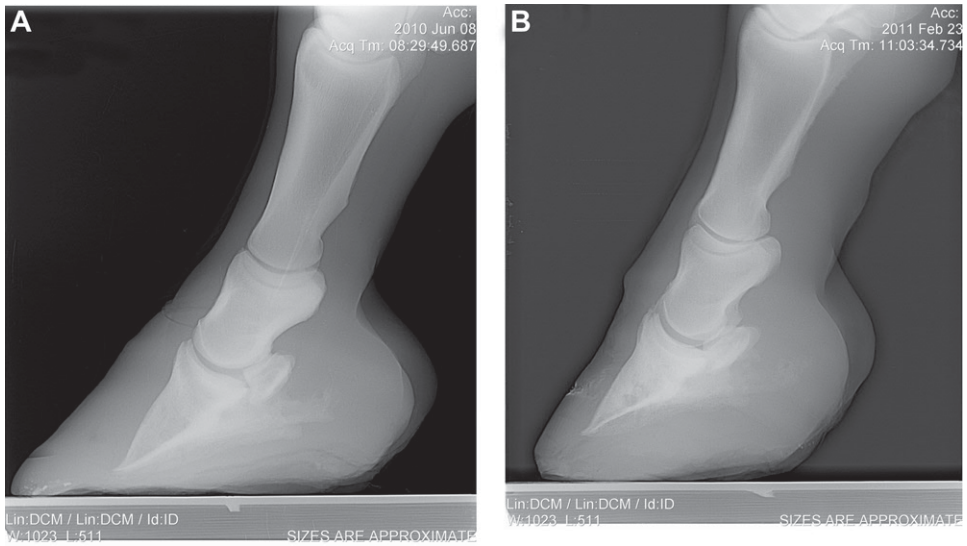

Fig. 4. Another horse before (A) and 8 months after (B) treatment with stem cells, tenotomy, and shoeing.

potential of the lamellar interface. Simply stated, for good quality tissue to be produced and maintained requires a healthy osseous foundation. Stem cell therapy had a significant effect on prognosis in cases treated early, within 30 days. The majority of these cases healed with a decreased distance between the bone and hoof wall (see Figs. 3 and 4). Studies are currently underway to evaluate lamellar biopsy samples from cases treated with MSCs to further evaluate the quality of tissue which repairs this interface.

Acknowledgments

Conflict of Interest

The Authors declare no conflicts of interest.

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How to Select Cases and Use Platelet Rich Plasma for Tendon and Joint Injuries

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1. Introduction

Regenerative medicine is a commonly used term that broadly applies to the use of biologics to enhance tissue repair. In the context of equine medicine, regenerative therapies are more commonly used to enhance the quality of repair in musculoskeletal tissues following injury. The most commonly used biologics include platelet rich plasma (PRP), stem cells, and interleukin-1 receptor antagonist protein (IRAP). The popularity of biologics is increasing, with owners looking for more "natural" therapies and their reluctance to treat their horses with drugs such as corticosteroids. Traditionally, PRP was used for tendons and ligaments, IRAP for joint injections, and stem cells for either soft tissue or joint injections. However, there is evidence for use of PRP for tendon or joint injuries. It should be remembered that all biologics are heterogeneous in their final composition because they are based in biology, not chemistry. Biologics are not drugs and there are many subtypes of each biologic. For example, PRP preparations vary considerably in the concentration of platelets and leukocytes. There is tremendous controversy over which type of biologic (PRP, stem cell, or IRAP) is best for a specific tissue or injury and then what specific type of PRP, etc, is best for a given application. Current evidence suggests that leukocyte-low PRP preparations with platelet concentrations 2 to 4-fold over baseline are optimal for increased tissue repair. There are no head-to-head comparison studies to answer those questions, thus some extrapolation between studies is required to formulate a recommendation for clients. Recent reviews on regenerative medicine in horses are cited in the following text for further reading.[1–6]

2. Methods and Results

PRP for Joint Pain

There is evidence that PRP can increase hyaluronic acid (HA) synthesis by synoviocytes and decrease joint pain and inflammation, which should help enhance early rehabilitation.[1] Platelet rich plasma is not just for tendons and can be considered as a first line of joint therapy. Case selection is those horses with mild to moderate joint disease. Like all other joint medications, PRP is not effective in end stage arthritis cases. Many practitioners use PRP as a postoperative adjunctive therapy following arthroscopic chip removal or fracture repair. The first injection is given 3 weeks postoperatively with a second injection 2 weeks later.

Platelet rich plasma is generated using sterile principles per a manufacturer's directions. This can

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be by centrifugation of blood or by gravity filtration using a bag system. Approximately 2 to 4 mL/fetlock or carpus or 5 to 8 mL/stifle is injected twice at 10 to 14 day intervals. Based on evidence in the human literature, a third injection is not necessary. In long-term outcome data regarding the use of PRP for treatment of human knee arthritis, PRP is as, or more, effective as HA in restoring function and decreasing pain, but the effect lasts longer.[2] The effects of HA typically last 6 months, while the effects of PRP are reported to last for a year or longer.

No activation step such as thrombin is necessary. When platelets in PRP are exposed to joint tissue, they are activated and degranulate, thereby releasing their growth factor contents. Residual PRP can be frozen and used at a later date without loss of growth factor activity (unpublished data). There is no need for rest after a PRP injection. One of the major benefits of intra-articular PRP injections is diminished pain signaling, which should allow for aggressive rehabilitation in cases of mild to moderate arthritis.[3] If there is no response within a week after a second injection, it is unlikely that a third injection will help. If there is a response, then routine joint maintenance (with 2 injections, as previously described), or treatment as needed can be performed, according to your practice philosophy. There are no published "flare rates" following PRP injections, but they have been anecdotally reported.

PRP for Tendon or Ligament Injury

Platelet rich plasma injections into tendons and ligaments increases normal tendon matrix synthesis, such as collagen type I, and decreases cytokines associated with matrix degradation.[4] If PRP is administered into a lesion within a few days of injury, it can stop the progression and growth of a lesion.[5,6] The effectiveness of PRP for tendonitis appears to be related to the chronicity and severity of the lesion. Therapeutic intervention with PRP should be considered as soon as a lesion is diagnosed, not after weeks or months of failed rehabilitation. Once scar tissue has formed and remodeled, the regenerative capacity of tissues is limited. This time frame for successful intervention depends on the site and severity of the injury, but is likely limited to those injuries less than 3 to 6 months old.

Ultrasound guidance should be performed for injections, so two sets of hands, one to run the ultrasound and one to do the injection, is ideal. A convex probe is best for ultrasound guided injections. A sterile probe cover should be used. The volume of PRP for injection is simply enough to fill the lesion, which can be visualized under ultrasonography. A small 23 gauge needle can be used to minimize damage to normal tendon during injection.

After PRP treatment, the horse resumes active rest with a minimum of 30 min in hand walking per day. A recheck examination in 2 to 3 weeks and a second injection can be considered if the lesion has not significantly improved with a 50% improvement in palpation, lameness, and ultrasound appearance of the lesion. Platelet rich plasma injections into bowed tendons appear to result in a more rapid resolution of lameness and early return to linear fiber orientation in the injured region.[5,6] It is unclear if the ultimate time to return to performance is diminished by the injection of PRP.

3. Discussion

For tendon and joint injections, PRP appears to both enhance tissue repair and to decrease pain. The diminished pain allows for a faster return to rehabilitation, which is particularly important in middle-aged horses that are prone to secondary lameness issues with extended rest. The use of PRP is an attractive method of therapy because of the growth factors that are delivered, but it must be remembered that none of the biologics are drugs and, therefore, each preparation will vary, as will the clinical outcome. It is unclear which biologic is best for any given situation, but given the devastating loss of time and performance associated with musculoskeletal injuries in horses, a dual PRP/stem cell approach is commonly employed. Regenerative therapies should be applied early after injury, within a week if possible, so that they can change the healing environment before scar tissue begins to form.

Acknowledgments

Conflict of Interest

The Author is a consultant to Arthrex, Inc.

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How to Choose Cases and Utilize Mesenchymal Stem Cells in Joint Injury

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1. Introduction

The use of "stem cells" for treating orthopedic disease is rapidly growing. We are currently experiencing an exponential growth in the number of peer reviewed and Medline indexed publications (Fig. 1). This means that the equine practitioner is faced with greater information to help in the best selection of cases in which to utilize stem cells. This does not mean that unproven sources (i.e., commercial sources) or indications of mesenchymal stem cells (MSCs) does not exist but this manuscript will provide the author's opinion on current available information and case selection as it pertains to stem cell use for joint disease.

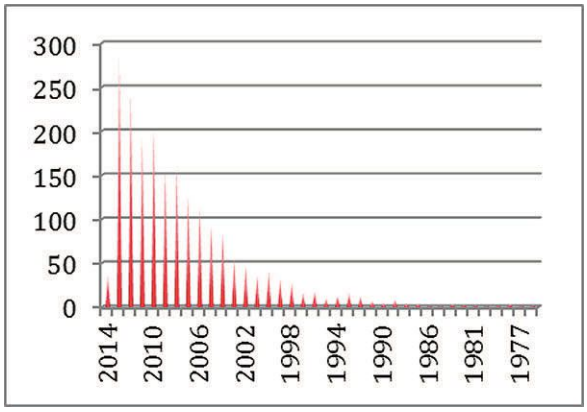

Fig. 1. A frequency table by publication year from a PubMed search on February 11, 2014 using the key words "stem cell" and "joint."

2. Treating With MSC: Points to Consider

The first point of consideration is the tissue source of stem cells. Multiple studies have been published comparing various tissue sources of mesenchymal stem cells (MSCs) and current thought suggests bone marrow-derived cells provide the best source for joint-related treatments.[1] Specific locations and volumes of aspirates have also been assessed and a low volume (5 mL) aspirate from the ilium has been shown to have significantly better results as it relates to cartilage matrix production.[2] This is not to say that bone marrow draws from the sternum do not produce usable MSCs, it is more to say that the ilium draws produce cells which may be more desirable. Specific studies assessing the dose of MSCs to be used in joint tissues have not been published; however, the range of doses being used in studies

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with successful outcomes is 10 to 50 million cells in roughly a 10 to 50 mL joint.[1] A recent clinical study in human patients with medial meniscectomy suggested 50 million MSCs performed as good, if not better, long-term compared to 150 million;[3] this agrees with the author's clinical experience as well. The timing of the treatment, as well as the use of multiple treatments, has also not yet been definitively worked out. The best evidence exists in a study looking at MSC treatment/timing in tendon injury. This work demonstrated significantly better long-term outcomes by delaying MSC treatment past the inflammatory phase of the injury.[4] This is also consistent with the author's experience in clinical cases. Finally, most of the published reports in treating joint related injuries have, to date, utilized a single treatment; thus, compelling clinical results to pursue multiple treatments is lacking.

3. Case Selection

In joint disease there are four main tissue types that become injured: (1) the articular cartilage, (2) fibrous joint capsule/synovial membrane, (3) soft tissue structures such as the meniscus or collateral ligaments, and (4) subchondral bone. In many cases of joint disease, some or all of these tissues are involved to some extent and the severity and extent are what should dictate the treatment protocol. Currently, in cases with generalized osteoarthritis and no or minimal soft tissue involvement, the MSC treatment is not the primary mode of therapy utilized by the author. Rather, other types of medical treatments such as corticosteroids, hyaluronic acid, polysulfated glycosaminoglycans, or biologics such as interleukin-1 receptor antagonist are the first line of treatment. However, in cases that are refractory to such treatments and no surgical options exist, there is evidence of significant beneficial effects of administration of intra-articular (IA) stem cells free in the joint space. This benefit is mainly through a reduction in inflammatory mediators such as prostaglandin E2 but this can have a significant long-term effect on overall joint health and the author has had palliative success.[5]

In cases where the primary issue is cartilage loss, i.e., a focal cartilage lesion, numerous studies have shown the lack of effectiveness of MSCs contained in a scaffold or fibrin gel for direct repair of an articular cartilage defect.[a,6] Conversely, administration of IA MSCs free within the joint following a cartilage resurfacing technique, such as subchondral bone microfracture, has been shown to provide added long-term benefit over cartilage resurfacing alone.[7] This combination of surgical debridement followed by MSC therapy 30+ days postoperatively is utilized by the author in cases with cartilage lesions.

In cases where soft tissues within the joint cavity are injured, published studies have shown a significant improvement in soft tissue healing as well as a decrease in osteoarthritis progression following the utilization of MSCs free within the joint. Specifically, building on experiential studies,[8,9] a recent double blind randomized placebo-controlled study in human patients demonstrated long-term significant benefit following administration of 50 million bone derived culture expanded stem cells post medial meniscectomy.[3] These significant improvements were not only in the regeneration of meniscus but also in pain for the patient two years post-treatment. A recent equine study has been published with long-term follow up of horses undergoing stifle surgery that were diagnosed with meniscal disease. Surgical debridement was performed followed by treatment with IA bone derived MSCs postsurgery. The study demonstrated an improved ability of these horses to return to performance when compared to similar published cases undergoing surgery alone.[10]

Cases with significant subchondral bone disease have been treated using a regional perfusion of MSCs but published scientific evidence of efficacy is currently not available. Thus, the author reserves MSC therapy as a last resort in such cases. In these cases the vein supplying the area is aseptically prepared with a tourniquet above and/or below, depending on the region. The author uses 10 to 20 million MSC diluted in a 20 mL volume of a buffered polyionic solution. The horses are typically sedated and the tourniquet left in place for 20 to 30 min.

4. Summary

Strong evidence exists for the use of bone-derived culture expanded stem cells in cases with cartilage or other soft tissue lesions within the joint in various species. Similar evidence has been reported specific to the horse. The dose typically administered after the inflammatory phase has subsided (which for the author is 4–6 weeks postinjury or surgery) is a one-time treatment (usually in the presence of a 20 mg dose of hyaluronan). The dose is in the range of 20 to 50 million cells for 10 to 50 mL joints with a nonsteroidal antiinflammatory drug administered parenterally at the time of injection. The dose is administered free in the joint, i.e., not within a restrictive scaffold, and other routine surgical options can be employed in addition to the stem cell treatment. The author avoids the use of corticosteroid post IA MSC injection for 6 months or greater but the use of hyaluronic acid-based products are encouraged. Routine rehabilitation protocols are utilized in most cases but typically include 4 months out of full work or competition. In general, hand walking commences within days of the MSC treatment and continues for 4 to 6 weeks with pony-exercise at a trot beginning for an additional 4 to 6 weeks. This is followed by work with a rider increasing intensity and duration of exercise over the next 4 to 6 weeks.

Acknowledgments

Conflict of Interest

The Author is a shareholder of Advance Regenerative Therapies, Fort Collins, CO.

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[a]Frisbie DD, unpublished data, February 2014.

How to Select Cases and Utilize Stem Cells in Tendon Injury

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1. Introduction

The use of stem cells for the treatment of tendon injuries has gained acceptance over the past decade. While there has been no randomized controlled trials for the use of mesenchymal stem cells (MSCs) in the treatment of tendon overstrain injuries, an increasing amount of experimental[1–3] and clinical data[4] have mostly demonstrated improved quality of equine tendon healing with cellular treatments.

2. Case Selection

The cells are most commonly delivered by intratendinous injection and therefore require a defect within the tendon that is surrounded by an intact paratenon to retain the cells after implantation. The size of the lesion is not critical, although small lesions are more difficult to inject and generally carry a better prognosis and therefore cellular therapies are less indicated for the smaller lesions. Cell retention is very poor if the paratenon is not intact (unpublished data). Hence, percutaneous lesions, where this is not the case, are not appropriate for treatment unless at the stage when a granulation bed is present that can act as this retaining layer.

Analysis of the clinical data shows improved outcome with earlier implantation although not always significantly different statistically. For racehorses, there was an average of 46 days between injury and implantation for successful cases compared to 54 days for cases that subsequently re-injured[4]; for sports horses, the re-injury rate was lower for horses treated within 1 month of the injury compared to those treated after a month (unpublished data). Autologous treatments are a two-step procedure requiring first the aspiration of bone marrow (or other tissue) to obtain the MSCs and second, after a variable time depending on the preparation used, to implant the cells. In the intervening time, the horse is either maintained on box-rest (if lame or severely injured) or given up to 10 minutes handwalking per day. For bone marrow, a period of approximately 3 weeks for culture is needed, which limits any earlier implantation, and so bone marrow should be aspirated as soon as is possible after injury. The use of banked or allogenic cells allows even earlier implantation although it is logical to target the cell implantation to when there is a vascular supply established to support the cells after implantation—normally at least 1 to 2 weeks after injury.

3. Cell Choice

There are a number of options with respect to cell choice. The most commonly used preparations are

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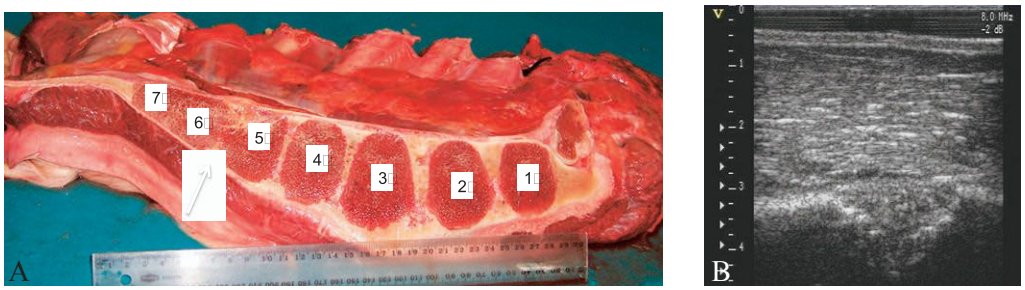

Fig. 1. A, longitudinal section of the equine sternum (caudal to the left). Usually the two most caudal sternebrae (6 and 7) are fused and the first intersternebral space encountered when scanning from caudal to cranial is between sternebrae 5 and 6 (arrow). The easiest sternebra to aspirate is the 5th. Either the 4th (further cranial between the forelimbs but complicated by the presence of the ventral 'keel') or 6th (more caudal but with less dorsoventral thickness so care should be taken to avoid inserting the Jamshidi needle too deep) can also be aspirated. Care should be taken to avoid the caudal border of the sternum, which is thin and close to the apex of the heart. B, the ultrasonographic appearance of the intersternebral space between sternebrae 5 and 6. This space is usually level to the caudal aspect of the elbow.

MSCs recovered from bone marrow or fat, with more recent additions of umbilical cord cells[5] and fetal tendon cells.[6] The major differences between these cell products are more related to the preparation methods rather than the source. Of the autologous treatments, cells can either be 'minimally manipulated,' when cells are released from tissue by enzymes and a cell mixture returned to the veterinarian for implantation (often the case when fat is chosen as the tissue source), or MSC populations are expanded by culture in a laboratory. Culture and expansion is necessary if it is deemed important to produce an abundant and homogenous stem cell preparation because of the low numbers of MSCs in the source material (e.g., only 1 in 100,000 of the nucleated cells in bone marrow is an MSC). Allogenic cells simplify the process and may become more commonly used, providing legislation allows, as it avoids the first step and is easier and cheaper. Allogenic cells do not appear to cause immunological reactions because of the immunosuppressive effects and appear to be well tolerated (unpublished data). However, such products have not been extensively researched or well characterized to date to ensure quality and effectiveness and, therefore, should be used with caution.

4. Dose

There have been no good studies documenting a dose response. Initially, 10 million cells (suspended in bone marrow supernatant at a concentration of 5 million cells/mL) were used for an 'average-sized' lesion occupying up to 30% of the cross-sectional area of the superficial digital flexor tendon. More recent studies have shown that there is limited retention of cells after implantation[7–11] and an analysis of treated horses showed a reduced re-injury rate in horses treated with 20 million or more cells when compared to the original empirical does of 10 million cells/lesion. Therefore this 'routine' dose has been increased to 20 million cells (at the same concentration of 5–10 million cells/mL) with the option to increase this twofold for larger lesions.

Step 1–How to Obtain a Bone Marrow Aspirate

Equipment Needed

- Ultrasound machine with 7.5 MHz or above linear transducer
- Sedative
- 2 mL syringe (for local analgesia)
- Local anaesthetic (mepivicaine)
- Heparin (stock solution concentration: 5000 IU/mL)
- 11 scalpel blade
- 11 G 10 cm Jamshidi needle
- Two 10 mL or one 20 mL syringe
- At least two 5 mL syringes
- Sterile swabs
- Sterile containers and transport pack (to ship the bone marrow to the laboratory for culture).

Technique: Sternum

As the bone marrow has to be transported to the laboratory after it is obtained, aspirations should only be done on a Monday to Thursday to avoid prolonged delivery over weekends.

The horse is first sedated with a combination of an $\alpha 2$ agonist and opiate (e.g., detomidine HCl and butorphanol tartate). A 10 cm wide band overlying the sternum is clipped and scrubbed with surgical scrub (e.g., chlorhexidine) and surgical spirit.

While some don't advocate ultrasound, ultrasound is advised to ensure accurate aspiration and therefore minimize the risks of inadvertent penetration of the heart. The sternum is examined ultrasonographically to identify the three most caudal sternebrae by the appearance of their intersternebral spaces (Fig. 1). The most caudal one is avoided as it is thinner and hence penetration of its deeper surface is easier.

Fig. 2. Marking the intersternebral space between the two sternebrae to be aspirated with a marker pen.

The position of the most caudal aspect of the sternum and the intersternebral spaces between 5 and 6 and 4 and 5 are marked on the adjacent hair with a marker pen (or white correction fluid for a dark-coated horse; Fig 2). As a double check, the space between 5 and 6 is usually level with the caudal aspect of the forearm.

The area of the sternum is prepared aseptically and local anesthetic is placed at the predicted aspiration entry points to aspirate bone marrow from sternebrae 5 and 4 (or 6), as shown in Fig. 3.

Prior to aspiration, syringes are aseptically preloaded with heparin (to give a final concentration of 250 IU/mL bone marrow). Commonly, a 10 mL syringe is preloaded with 0.5 mL of a 5000 IU/mL heparin solution into which 9.5 mL bone marrow is aspirated.

The area is then scrubbed a final time before a small stab incision is made through the skin with a No. 11 scalpel blade. The Jamshidi needle is introduced through the stab incision and advanced until it contacts with the ventral surface of the sternebra on mid-line (Fig. 3A). The index finger is placed 2 cm from the skin surface on the needle shaft and the needle is gradually advanced using rotating movements until the index finger is against the skin surface. This ensures that the needle does not penetrate the deep surface of the sternebrae.

The central trocar is removed from the Jamshidi needle. Typically, bone marrow does not initially flow spontaneously from the needle but requires gentle aspiration with an attached syringe. This can be, but only initially, associated with a small amount of discomfort to the horse, usually manifested by a slight guarding of the abdomen. Thereafter, bone marrow flows easily into the syringe (Fig. 3B) and it spontaneously drains from the needle when the needle is disconnected.

At least two more 4 mL samples are also obtained without heparin and transferred immediately to sodium citrate glass blood tubes. Care should be taken when transferring bone marrow to citrated tubes or universal containers to avoid the possibility of contamination. These samples are used to derive the bone marrow supernatant used to re-suspend mesenchymal stem cells for implantation. The tubes should be vigorously agitated to prevent clotting, with thumbs over the lids. For very large lesions (over 30% cross-sectional area) more than two sodium citrate tubes should be submitted (two more per 10 million additional cells required). Alternatively, a jugular blood sample can be substituted for the bone marrow for later resuspension of the cells.

The second sternebra is then aspirated in the same fashion (or a second insertion of the Jamshidi needle into a separate site in the same sternebra to provide a back-up sample for culture in case of clotting or failed cell recovery).

Once the Jamshidi needle is withdrawn, the portals can continue to bleed but pressure is usually all that is necessary to stop this hemorrhage. Closure is unnecessary.

Technique: Tuber Coxae

A 10 cm square area overlying the tuber coxae is clipped. In an average-sized horse the aspiration

Fig. 3. A, insertion of the Jamshidi needle. The index finger should be placed 2 cm from the skin surface once the Jamshidi needle makes contact with the ventral surface of the bone to prevent overinsertion of the needle. Note that the entry point is close to the caudal aspect of the forearm. B, successful aspiration of bone marrow.

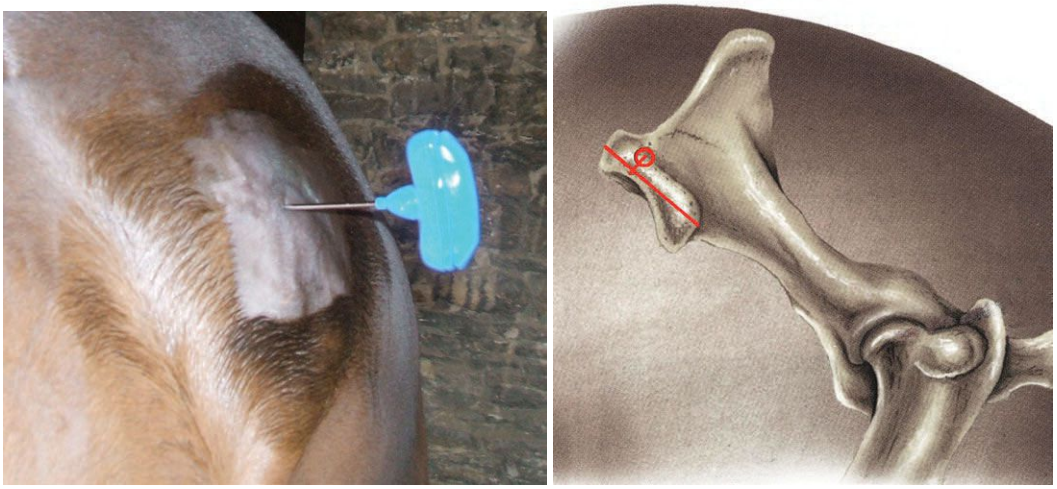

Fig. 4. Insertion of the Jamshidi needle. Once the Jamshidi needle makes contact with the caudolateral surface of the bone, advance the needle ~5 cm, in a horizontal direction.

site is a point 2 cm caudal to the ridge running from craniodorsal to caudoventral and approximately one quarter to one half dorsoventrally.

The area is prepared aseptically and 5 mL local anesthetic is placed subcutaneously along the aspiration path and deeper over the periosteum.

Prior to aspiration, the 20 mL syringe is preloaded with 1 mL of 5000 IU/mL heparin. A larger syringe can be used to allow greater negative pressure during aspiration (required for tuber coxae aspirations compared to sternum).

The area is scrubbed a final time before a small stab incision is made through the skin at the aspiration site with a No. 11 scalpel blade.

The Jamshidi needle is introduced (horizontally) perpendicular to the skin through the stab incision and advanced until it contacts the surface of the tuber coxae. The needle is then gradually advanced approximately 5 cm into the bone using rotating movements (Fig. 4). The needle can alternatively be introduced more caudally to enter the wing of the ilium 2 to 3 cm from the tip of the tuber coxae. The latter technique offers the advantage of avoiding the dense outer cortex of the tuber coxae, which can be difficult to penetrate with the Jamshidi needle.

The central trocar is removed from the Jamshidi needle. Bone marrow does not initially flow spontaneously from the needle; aspiration with an attached syringe is required. The preloaded syringe is attached to the Jamshidi needle and full negative pressure is applied in order to aspirate 19 mL of bone marrow (making a total volume of 20 mL).

The bone marrow sample is gently agitated in the syringe to ensure adequate mixing of the anticoagulant with the marrow (bone marrow clots extremely quickly). The sample is then transferred into the two universal plastic containers provided, divided into two 10 mL aliquots. Continue to gently mix the samples once in these containers.

An additional sample, without heparin, is then taken and transferred immediately to two or more sodium citrate glass blood tubes (4 mL bone marrow into each tube).

Once the Jamshidi needle is withdrawn, the portal can continue to bleed but pressure is usually all that needed to stop the hemorrhage. Closure is unnecessary.

Step 2–How to Implant Stem Cells Into Injured Tendons or Ligaments

Equipment Needed

- Ultrasound machine with 7.5 MHz or above linear transducer
- One sterile drape
- Two 2 mL syringes and 20 G or 19 G, 1.5, or 2 inch needle (for stem cells)
- Four 2 mL syringes and 23 G 1 inch needles (for local analgesia)
- One arthroscopic camera sleeve (or sterile ultrasound probe sleeve)
- One intrasite gel (or sterile ultrasound gel)
- Robert Jones bandage material
- Local anesthetic (mepivicaine)
- Sedative

Technique

The cell delivery time and place should first be arranged. It is important that the cells are implanted as soon as possible after arrival; therefore, implantation will normally need to be carried out in the

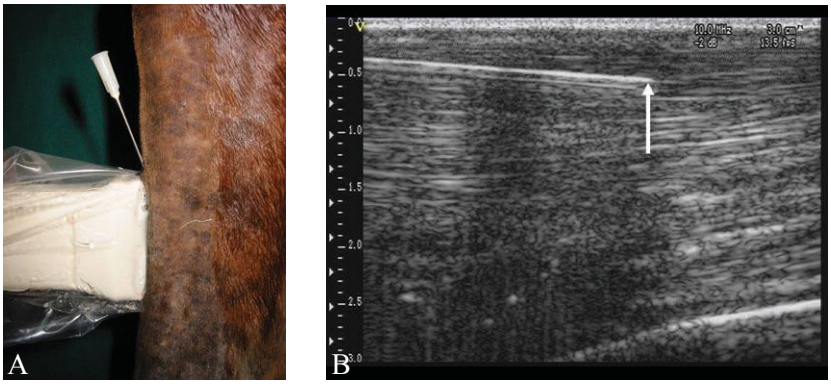

Fig. 5. Localizing needle in a core lesion. A, triangulation technique with the needle and ultrasound transducer in line on the palmar aspect of the limb, and B, ultrasound appearance of the needle as it is advanced into the tendon (arrow).

morning and only on a Tuesday to Friday (to avoid delayed transit of the cells over a weekend).

The horse is restrained in stocks and sedated with a combination of $\alpha 2$ agonist and opiate (e.g., detomidine HCl and butorphanol tartate).

The leg to be implanted is clipped to include the subcarpal local anesthetic sites. The area is then cleaned with surgical scrub (e.g., chlorhexidine) and surgical spirit (alcohol).

An ultrasonographic examination is used to identify the core lesion, its extent, and the appropriate site for stem cell implantation.

The site for local analgesia is aseptically prepared. To ensure complete desensitization of the skin overlying the superficial digital flexor tendon (containing the lesion to be treated), both the palmar nerves deep to the metacarpal fascia and the subcutaneous nerve supply superficial to the fascia have to be 'blocked' on both sides of the limb at the subcarpal site. If the accessory ligament of the deep digital flexor tendon or suspensory ligament is being treated, the palmar metacarpal nerves should also be 'blocked.' A uniaxial suspensory branch injury would necessitate only uniaxial blocks.

The palmar metacarpal region should then be prepared aseptically.

In a sterile fashion, at least two separate syringes are loaded with stem cell aliquots. The cells are resuspended in the supernatant within the transport vial by gently shaking the vial prior to aspirating its contents into the 2 mL syringes. Usually, only 2 mL (10 million cells/mL) is injected into an superficial digital flexor tendon (SDFT) lesion—larger volumes can be damaging to the tendon—although larger volumes with the same concentration of cells can be used to deliver higher doses (up to 40 million cells) for very large lesions.

The ultrasound transducer is placed into a sterile sleeve (a sterile arthroscopic camera sleeve with the end sealed can be used for this purpose). Contact between the transducer and the skin is optimized with the use of scanning gel within the sleeve and intrasite gel or sterile ultrasound gel, or alcohol on the skin.

With the ultrasound transducer oriented in longitudinal fashion, triangulation of a 20 G 1.5 or 2 inch needle is visualized entering the tendon (Fig. 5) in the weight-bearing limb. Care should be taken to ensure that the whole needle is visible ultrasonographically so that the end does not penetrate the deep surface of the tendon.

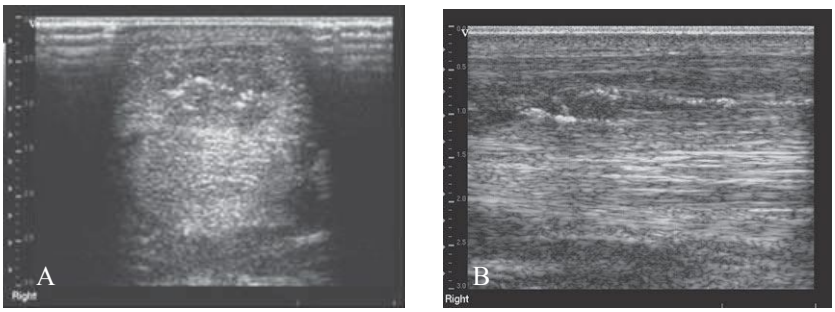

Fig. 6. After injection, air bubbles from the injection are present with the core lesion in both transverse, A and longitudinal, B images.

Table 1. Recommended Standardised Rehabilitation Programme for Stem Cell Treatment of a Superficial Digital Flexor Tendon Injury

Exercise level	Week	Duration and nature of exercise
Box rest	**0**	**Implant cells**
	1	Box rest; maintain clinical bandage
Walk	2	10 minutes walking; replace clinical bandage with stable bandage; maintain stable bandaging
	3	15 minutes walking; maintain stable bandaging
	4	20 minutes walking; maintain stable bandaging; **Repeat ultrasound examination**
	5–6	25 minutes walking
	7–8	30 minutes walking
	9–10	35 minutes walking
	11–12	40 minutes walking
	12	**Repeat ultrasound examination**
Trot	13–16	35 minutes walking and 5 minutes trotting daily
	17–20	30 minutes walking and 10 minutes trotting daily
	21–24	25 minutes walking and 15 minutes trotting daily
	24	**Repeat ultrasound examination**
	25–28	20 minutes walking and 20 minutes trotting daily
	29–32	15 minutes walking and 25 minutes trotting daily
	32	**Repeat ultrasound examination**
Canter	33–48	Introduction of canter work; gradual return to full work
	48	**Repeat ultrasound examination**
Full work	48+	Treat as normal

The stem cells are then injected into 1 to 5 sites depending on the nature of the core lesion. The first site of injection is usually the midpoint of the lesion as there is often good spread of the cells throughout the lesion after implantation when the core lesion is still very hypoechoic (e.g., within 2 months of injury). More advanced healing (e.g., when the core lesion has filled with echogenic tissue— usually around 3 months post injury) requires more injection sites due to less spread. Accurate placement is confirmed by the presence of air bubbles within (and only within) the core lesion (Fig. 6), which will also indicate the degree of spread.

The limb should be bandaged immediately with a Robert Jones style bandage to minimize subcutaneous hemorrhage and loss of injected cells from the tendon. The bandage should be left in place for 5 to 7 days. Thereafter, stable bandages can be used to maintain the temperature of the distal limb (to maximize healing).

Peri-operative antibiotics and nonsteroidal anti-inflammatory drugs are unnecessary.

Repeated delivery of cells is a logical approach to obviate the relatively poor retention of cells after implantation although, often, expense precludes this approach. Some have advocated initial early treatment with allogenic cells (legislation allowing) while others propose a second autologous dose given by regional perfusion 2 to 4 weeks after the first dose. Regional limb perfusions seem to be a less efficient delivery route[7,10,11] but do avoid repeated injections into an injured tendon, which can potentially cause further damage.

After implantation, the horse commences a rehabilitation program, which is not specific for stem cell treatment (Table 1). There is no current evidence that stem cell treatment shortens the rehabilitation phase, which is consistent with the current hypothesized mechanism of action of improving tendon healing by modulating the inflammatory response. This programme is approximately 12 months in length for superficial and deep digital flexor tendon injury (Table 1) but shortened for suspensory ligament desmitis (to 6–9 months) and desmitis of the accessory ligament of the deep digital flexor tendon (to 3–6 months).

Acknowledgments

Conflict of Interest

The Author was formerly a Director and Technical Advisor of VetCell Bioscience, Ltd.

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How to Select Cases and Use Autologous Conditioned Serum to Treat Proximal Suspensory Desmitis

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1. Introduction

The suspensory ligament (SL) can be divided into 3 regions subject to injury: the proximal region, the body, and the medial and lateral branches. In this presentation, only treatment of the proximal region is discussed.

Proximal suspensory desmitis (PSD) is a common injury of athletic horses causing pain and lameness. It can occur unilaterally or bilaterally and can affect the hind limbs, the fore limbs, or both.[1] Clinical signs displayed by horses with PSD have been well documented[1] and pain causing lameness can be localized to the proximal aspect of the suspensory ligament (PSL) by using a variety of local and regional anesthetic techniques.[1] The PSL and adjacent bone is usually imaged by using ultrasonography and radiography. Nuclear scintigraphic examination of horses affected with PSD can be helpful, but magnetic resonance imaging (MRI) is the most defining method by which the ligament and surrounding structures can be imaged.[1]

Most horses lame because of acute PSD of the fore limb, respond favorably to stall confinement and controlled walking exercise for 3 months,[1,2] but for the 3-year-old Western performance horse, 3 months of confinement may end the horse's show career, thereby significantly reducing the horse's worth. The prognosis for return to soundness for horses lame because of PSD of one or both hind limbs is poor when affected horses are treated by confinement and incremental increase in exercise.[3,4]

Besides stall rest, a myriad of adjunctive therapies have been recommended for horses lame because of PSD, including systemic and topical administration of an NSAID; periligamentous injection of a corticosteroid, hyaluronan, polysulfated glycosaminoglycans, extract of the pitcher plant[a], or a combination of these drugs; topical application of dimethyl sulfoxide (DMSO), with or without a corticosteroid or other drugs; extracorporeal shockwave therapy; and intralesional injection of 2% iodine in almond oil, extracellular matrix[b], mesenchymal stem cells, or platelet rich plasma.[1]

NOTES

Surgical treatments of horses for PSD include splitting the ligament, lateral palmar/plantar neurectomy, neurectomy of the deep branch of the lateral palmar or plantar nerve, ulnar or tibial neurectomy, fasciotomy of the fascia overlying the ligament, and combinations of these surgical treatments.[1]

In this presentation, we describe periligamentous injection of autologous conditioned serum (ACS)[c] for the treatment of horses lame because of PSD. Autologous conditioned serum is made by collecting the patient's blood in a proprietary syringe containing glass beads soaked in chromium sulfate. The blood is incubated in the syringe for approximately 24 hours (18–26 h) and then centrifuged, filtered, and divided into dose-sized aliquots, which can be injected immediately or frozen for future use. The processing procedure is designed to specifically harvest anti-inflammatory cytokines,[5] but anabolic cytokines and morphogenic proteins may be harvested, as well.[6]

Commercial veterinary systems are available for the horse for patient-side production of ACS.[c,d] Autologous, conditioned serum has been used in the horse for several years to modulate synovitis and has become an accepted mode of equine joint therapy.[1,7–13] Autologous conditioned serum has also been investigated for modulation of the post-breeding inflammatory response in the mare's uterus.[14] Autologous, conditioned serum is used to treat humans for a variety of synovial and non-synovial orthopedic maladies,[15,16] and experimental studies using rats have demonstrated improved tendon healing after ACS therapy.[17,18]

The purpose of this report is to describe how we select horses lame because of PSD for treatment with ACS using the irap[c] system. Our criteria for selection of horses for this treatment are based on our experience in treating 271 horses lame because of PSD.

2. Materials and Methods

Case Selection

Horses in this study were those determined to be lame solely because of PSD and that were treated with ACS by one of the authors[e] between September 2004 and September 2013. All horses included in this study were lame at the time of treatment, showed signs of pain during palpation of the PSL, and had the pain causing lameness localized to the PSL by performing various techniques of diagnostic analgesia using 2% mepivacaine hydrochloride (HCl). Only horses with a minimum follow-up time of 3 months, after the initiation of treatment were included in the study.

Fore Limb Blocks

Pain in the distal portion of the fore limb(s) was excluded as a cause of lameness by observing no improvement in lameness after administering a low four-point nerve block or after administering an abaxial sesamoid nerve block in combination with intrasynovial anesthesia of the fetlock joint and, occasionally, intrasynovial anesthesia of the digital flexor tendon sheath. Pain was isolated to the PSL by observing substantial improvement in lameness after desensitizing the PSL by anesthetizing the lateral palmar nerve at the level of the accessory carpal bone.

Hind Limb Blocks

Pain in the distal portion of the hind limb(s) was excluded as a cause of lameness by observing no improvement in lameness after administering a low four-point nerve block or after administering an abaxial sesamoid nerve block in combination with intrasynovial analgesia of the fetlock and, occasionally, intrasynovial analgesia of the digital flexor tendon sheath. Pain was isolated to the PSL by observing substantial improvement in lameness after desensitizing the PSL by anesthetizing the tibial nerve or by locally infiltrating the PSL with local anesthetic solution. For some horses, pain originating from the distal joints of the hock was excluded as a cause of lameness by observing little or no improvement in lameness after intrasynovial analgesia of the distal intertarsal and tarsometatarsal joints during lameness exam performed after the effects of local or regional analgesia had dissipated.

Imaging

The metacarpus (Mc III) or metatarsus (Mt III) of all horses were radiographically examined to exclude horses with substantial bone disease at the proximal aspect of Mc/Mt III, and all were examined sonographically in the palmar/plantar metacarpal/metatarsal region. Horses with a stress fracture of the proximal palmar/plantar Mc/Mt III were excluded from the study. Horses with substantial tissue disruption of the PSL or damage to other structures in the area such as the inferior check ligament were excluded. Horses that had enlargement of a PSL or edema of a PSL, as compared to the contralateral limb, were included (Fig. 1). For horses with bilateral PSD, assessment of enlargement and edema of the PSL was based on published guidelines.[4]

ACS Harvest and Processing

Whole blood harvested from each horse was incubated in the proprietary syringe in which it was collected and processed according to the manufacture's guidelines. Some aliquots were used immediately after processing whereas others were stored in a −20°C freezer for future use. Frozen aliquots were thawed in a water bath immediately prior to use, and the ACS was filtered through a 0.2 μm disk filter[f] prior to injection.

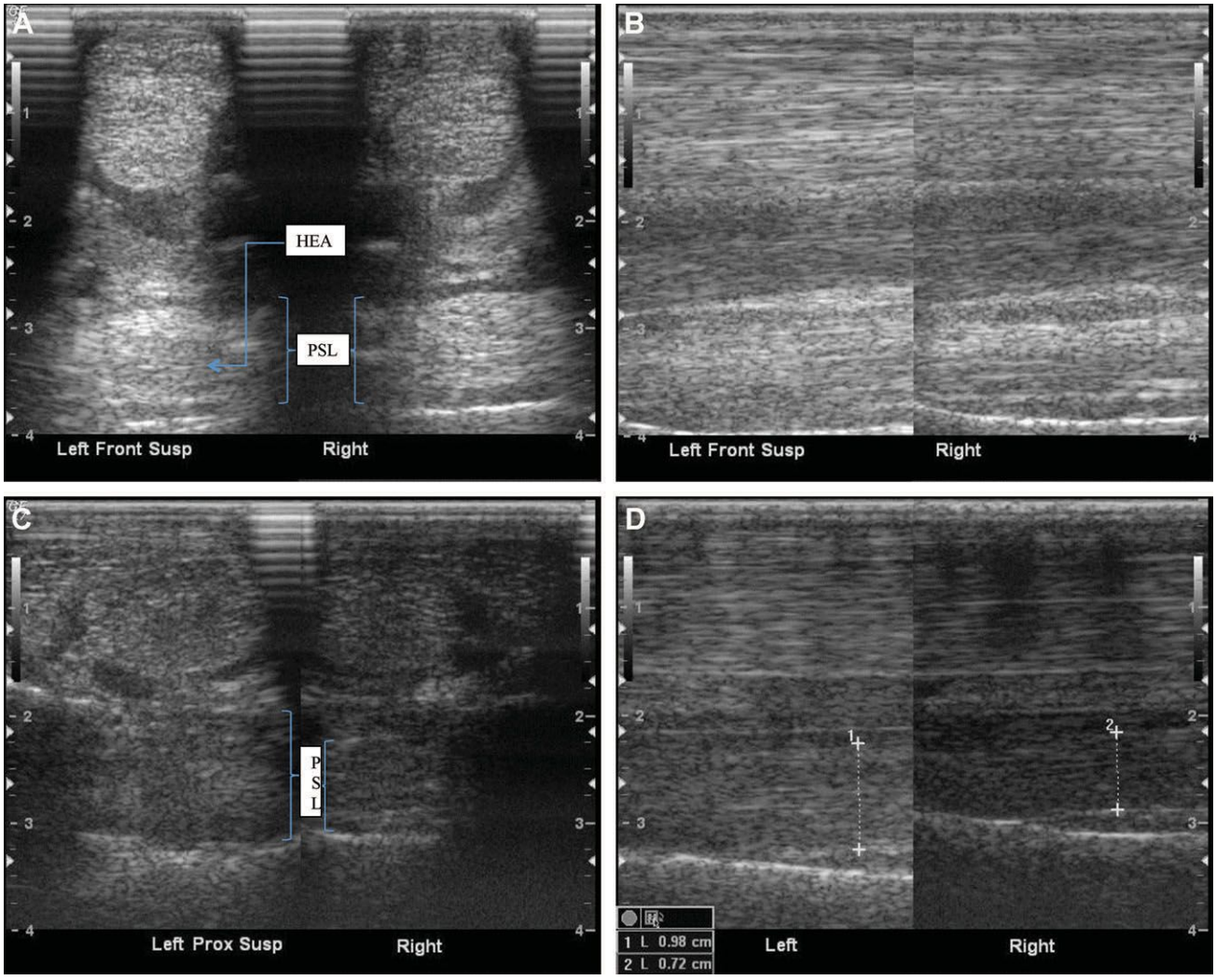

Fig. 1. These sonograms of forelimbs (1A and 1B) and hindlimbs (1C and 1D) are examples of sonograms of horses described in this report. In these examples, the left ligament is from the limb exhibiting lameness, and the right is from the sound contralateral limb. Sonograms of the diseased left ligaments demonstrate increased cross-sectional area (swelling), poor definitions of margins, decreased echogenicity (edema), and lack of striations (i.e., loss of normal fiber pattern), whereas the right ligaments have a more normal appearance. A, Cross-sectional image of an injured (left) and non-injured (right) forelimb PSL. Note the poorly defined central hypoechoic area (HEA) denoted by arrow. B, Longitudinal image of the same forelimb PSL. Note: 1. Enlargement of PSL in left as compared to right. 2. Poor definition of dorsal and palmar margins in left as compared to right. 3. Loss of linear striation. C, Cross-sectional image of an injured (left) and non-injured (right) hindlimb PSL. Note: 1. Enlargement of PSL in left as compared to right. 2. Poor definition of peripheral margins in left as compared to right. D, Longitudinal image of the same hindlimb PSL. Note enlargement of PSL in left as compared to right.

Treatment

Injection Technique

All injection sites were prepared for aseptic injection, and the injector wore sterile gloves. The horse was sedated, and the affected fore limb or hind limb was held in flexion. A 20-gauge, 1.5 in needle, attached to a 6 mL Luer-lock syringe containing 5 mL of ACS, was inserted through the skin approximately 4.5 cm distal to the proximal end of the head of the lateral splint-bone. The needle was directed proximomedially and inserted deep to the PSL until its tip engaged the palmar/plantar cortex of Mc/Mt III axial to the axial border of the lateral splint bone in the space between Mc/Mt III and the PSL. The ACS was injected with little or no resistance if the needle was properly placed. If resistance to injection was encountered, the bevel of the needle was assumed to be within the periosteum of the Mc/Mt III, and the needle was withdrawn until the ACS could be injected with ease.

All horses received a single dose (1 mg/kg) of flunixin meglumine intravenously after injection of ACS. The limbs were not bandaged after injection.

Rest, Follow-Up Examination, and Retreatment Protocol

All horses were discharged to the owner/trainers care immediately after the injection was completed.

The owner or trainer was instructed to confine the horse to a stall and to walk the horse in hand for 20 minutes daily for 14 days. The horses were reexamined for lameness at 14 days, and degree of the lameness was recorded. The limb(s) affected with PSD were re-injected with ACS in similar fashion, the horse was discharged, and the owner or trainer was instructed to confine the horse to a stall and to walk the horse in hand for 20 minutes daily for another 14 days. At 28 days after the initial injection, the horse was reexamined for lameness, and structures palmar/plantar to the Mc/Mt III were examined ultrasonographically. The horse was then administered a third treatment with ACS. If the horse was judged to be sound during examination and sonographic quality of the PSL was within normal limits or if the edema and/or cross-sectional area of the PSL were reduced, the owner or trainer was instructed to continue confining the horse to a stall but to walk the horse in hand for 20 minutes daily for 5 days and then, on the sixth day, to ride the horse using a regime of gradually increasing, controlled exercise. The owner or trainer of those horses that did not meet the criteria to enter a controlled, ridden exercise program was instructed to continue to confine the horse and to walk it in hand until the horse became sound and had no sonographic abnormalities, or an alternate treatment plan was instituted.

Rehabilitation Exercise Program

During the first week of controlled, ridden exercise, the horse was walked daily for 15 minutes and then trotted for 5 minutes. For each subsequent week, the daily trotting time was increased by 5 minutes until the horse was able to trot without lameness for 15 minutes for 14 consecutive days (2 weeks). Beginning the fifth week, if the horse was not lame, loping/cantering was added to the exercise regimen. The time of loping/cantering initially was 2 ½ minutes daily, and increased weekly by increments of 2 ½ minutes. After the horse was able to walk for 15 minutes, trot for 15 minutes, and lope for 15 minutes daily for 2 weeks without lameness, the horse was reintroduced to its normal training regimen at the same level of training it had been receiving prior to injury. The treatment protocol was considered successful if the horse entered controlled, ridden exercise at approximately 33 days from initial injection, maintained soundness without deviation from the described protocol of controlled, ridden exercise, entered full training by at least 110 days, and trained at least 1 month without recurrence of lameness.

3. Results

Two hundred seventy-one horses met the criteria required for inclusion in the study, and of these, 47% (127/271) were lame because of PSD of one or both fore limbs, and 53% (144/271) were lame because of PSD of one or both hind limbs. A total of 352 limbs were injected with ACS. No horse suffered an adverse reaction to any treatment with ACS.

Horses Treated for PSD of One or Both Fore Limbs (127/271)

Of horses lame because of PSD of one or both fore limbs, 83% (105/127) were affected unilaterally, and 17% (22/127) were affected bilaterally. Failure of a horse affected bilaterally to return to soundness in either limb was considered a failure of treatment.

Horses Treated for PSD of One Fore Limb (105/127/271)

Of the horses treated for PSD of one fore limb (105), 85% (89/105) were judged to have become sound, able to complete the exercise schedule prescribed, and to have maintained soundness for at least 30 days after reentering full training.

Horses Treated for PSD of Both Fore Limbs (22/127/271)

Of the horses treated for PSD of both fore limbs (22), 82% (18/22) were judged to have become sound, able to complete the exercise schedule prescribed, and to have maintained soundness for at least 30 days after reentering full training.

Horses Treated for PSD of One or Both Hind Limbs (144/271 Total)

Of horses lame because of PSD of one or both hind limbs, 59% (85/144) were affected unilaterally, and 41% (59/144) were affected bilaterally. Failure of a horse affected bilaterally to return to soundness in either limb was considered a failure of treatment.

Horses Treated for PSD of One Hind Limb (85/144/271)

Of the horses treated for PSD of one hind limb (85), 78% (66/85) were judged to have become sound, able to complete the exercise schedule prescribed, and to have maintained soundness for at least 30 days after reentering full training.

Horses Treated for PSD of Both Hind Limbs (59/144/271 Total)

Of the horses treated for PSD of both hind limbs (59), 71% (42/59) were judged to have become sound, able to complete the exercise schedule prescribed, and to have maintained soundness for at least 30 days after reentering full training.

Confinement coupled with controlled exercise was continued for many of the horses for which treatment was considered to have failed (i.e., those horses unable to sustain the scheduled rehabilitation and train without lameness for at least month), and although many of these horses eventually became sound and were trained successfully, we have not determined the percentage that did.

4. Discussion

Proximal suspensory desmitis is a common cause of lameness of performance horses of many disciplines

and other categories of horses as well.[19] In the practice of one of the authors,[e] PSD is the number one cause of fore limb lameness of young, Western performance horses that precludes a horse from competing in a major event for which it has trained and ranks in the top 3 causes of fore limb lameness overall. In the same practice, PSD is also one of the 3 most common causes of lameness in the hind limb of the Western performance horse.

Treatment of horses for PSD typically includes a long, costly period of confinement and inactivity (i.e., 3–12 months)[1] and is often the cause of missing a major event for which they were bought and trained. When a young, Western performance horse misses a major event such as the discipline's major futurity, the worth of the horse is reduced. Horses with PSD of one or both hind limbs have a guarded prognosis for return to full athletic function at all.[1]

Treatment of horses for acute or chronic PSD has encompassed a vast array of therapies,[1] perhaps because no single therapy is effective for most horses affected with PSD. Almost every therapy used to combat pain and inflammation or stimulate healing in any other portion of the body has been used to treat horses for PSD.[1] The authors believe that although some traditional therapies, such as regional injections of a corticosteroid, initially reduce pain, swelling, and inflammation, they inhibit healing by slowing metabolism, growth, and replication of fibroblasts important in repair of the injured ligament. Some therapies, such as internal blisters of 2% iodine in almond oil or intralesional injection of extracellular matrix[b] may lead to production of nonelastic scar tissue within or around the PSL. Other therapies, such as extracorporeal shock wave therapy, may only reduce ligamentous pain, rather than stimulate healing, thereby potentially perpetuating damage to the ligament when the horse is returned to training.

Our goal for using ACS to treat horses lame because of PSD was to not only rapidly reduce pain, and therefore lameness, by reducing inflammation in the ligament but also to augment healing to return the ligament to its original form and function. Treatment with ACS shortened the time of lay-up of horses lame because of PSD as compared to other published reports,[1] thereby allowing many horses to compete in events they otherwise would have missed, thus preserving the horse's worth and reducing expense to the owner. Numerous cases that the authors considered failures for this report continued to have further treatments of ACS and controlled exercise and many of them too eventually reentered training.

Autologous conditioned serum has been administered most commonly intrasynovially to reduce inflammation of synovial structures,[5] but this study and others[17,18,20] have shown that ACS can be used effectively to modulate the repair phase of non-synovial structures. Meier, et al theorized that cytokines, such as interleukin-1 (IL-1), played an important role in degenerative musculoskeletal diseases and originally attributed the therapeutic qualities of ACS to the production of anti-inflammatory cytokines in the serum, particularly interleukin-l-receptor antagonist protein (IL-1ra).[5] Hraha, et al[6] determined that other cytokines besides IL-1ra, such as interleukin-10 (IL-10), insulin-like growth factor-1 (IGF-1), transforming growth factor-beta (TGF-β), tumor necrosis factor-alpha (TNF-α), and interleukin-1b (IL-1b) were present in higher concentrations in ACS than in control serum. They theorized that these cytokines may play a previously understated role in clinical improvements seen when ACS has been used to alleviate clinical signs of osteoarthritis and suggested that these cytokines, rather than IL-1ra alone, might be involved in ACS's efficacy.[6]

Autologous conditioned serum has historically been considered to be an anti-catabolic treatment by virtue of its ability to block the inflammatory cascade initiated by IL-1. By blocking inflammation, ACS might have an indirect, anabolic, pro-healing effect. However, ACS also contains the growth factors listed above, and perhaps others, that are released by platelets activated during clotting while whole blood is incubated with the glass beads. These growth factors may be directly beneficial in healing, and, therefore, ACS may also have a direct, anabolic effect on healing. Numerous studies have documented the beneficial effects of individual growth factors found in ACS, such as vascular endothelial growth factor (VEGF), TGF-β, platelet-derived growth factor (PDGF), growth and differentiation factors, IGF-1, basic fibroblast growth factor (FGF-2, also bFGF), and bone morphogenetic proteins (BMP-12 and BMP-13), on tendon healing using animal models.[18] Exposure, in vitro, of patellar tendon fibroblasts to bFGF was found to affect cellular proliferation and collagen type III expression, both early events in tendon healing.[18] In addition, bFGF was found to stimulate angiogenesis.

Majewski, et al showed that ACS, when injected into the rat Achilles tendon, accelerated the rate of organization of repair tissue.[18] Specifically, it increased collagen mRNA expression, collagen deposition, as reflected by tendon thickness, and accelerated collagen fiber maturation, an aspect the authors believe may be important in treating horses for PSD. Majewski, et al also showed that stiffness of the ACS-treated tendons improved significantly over that of controls by week 4 post-treatment and theorized that this effect was likely due to reduction in time required for collagen cross-linking and remodeling of the repair site.[18] This time frame concurred with our time frame of implementing controlled work in horses post ACS treatment of PSD. Majewski, et al noted that remodeling of tendinous and ligamentous tissue is a slow process as evidenced by their finding that tendon thickness remained elevated even by week 8, but PSD of the horses in our study was accompanied by minimal

loss of fiber. Majewski, et al observed that ACS treatment might shorten recovery time because ACS-aided tendon repair is characterized by repair with well-organized, strong collagen fibers, suggesting that the healed tendon should contain fewer imperfections than in repair tissue formed after spontaneous healing. They believed such tissue would be less prone to re-injury, and they acknowledged that the biologically active component(s) in ACS that were responsible for the accelerated healing of the injured Achilles tendons remain to be identified.[18]

The authors believe the horses selected to treat with ACS for PSD suffered from acute or chronic SL strains rather than major tearing of the SL. Strain is damage to elastic fibers from stretching of the fibers beyond their elastic limit without visual disruption of fibers. This results in pain, swelling, and inflammation within the strained structure as well as potentially causing compressive damage to adjacent nerves.[21] Tóth, et al[21] clearly showed that horses with PSD of the hind limbs could also have pathologic changes of the deep branch of the lateral plantar nerve caused by compression from swelling of the PSL. They theorized that this nerve damage could be one potential reason for the poor long-term prognosis of PSD in the hind limbs of horses.[21] While ACS could have an indirect effect on nerve pathology by reducing compression on the nerve by reducing inflammation and thus swelling of the PSL, there is evidence that ACS can have a direct effect on pain from compressed nerves. Becker, et al demonstrated that epidural injections of ACS to treat lumbar radicular compression in humans were associated with clinically remarkable positive outcomes, potentially superior to the same treatment method using triamcinolone.[20]

The authors believe the acutely lame horses in this study were ideal candidates for treatment with ACS because the ligaments of these horses had no ultrasonographically discernible major disruption of fibers. Lameness of some horses may go undiagnosed causing it to become chronic if lameness of a fore limb is mild, if the lameness involves both fore limbs, or if the lameness affects one or both hind limbs. The authors believe that as long as major disruption of fibers is not ultrasonographically evident, even horses chronically lame because of PSD are good candidates for treatment with ACS, as it may be possible to return the tissue to normal size and function and heal or treat pain from potential neuropathies. Periligamentous injection, as described in this report, rather than intralesional injection, is indicated for horses without a core lesion. Forcing ACS into ligamentous or tendinous tissue in which there is no tissue void forces fibers apart, disrupting important cross-linking, this may worsen lameness and lengthen time of healing.

Short-comings of this study are the short follow-up time and lack of control groups. However, it has been the experience of one of the authors[e], in many cases of PSD in the young Western performance horse, that if we can get a horse to train a month without immediately getting lame, we have a chance of successfully keeping that horse in training long-term. With that stated, however, many of the horses will get lame again at some point from the same cause albeit usually less severely. When this occurs, we reevaluate sonographically, and unless we have tissue disruption of some sort (core lesion, etc.), we have been able to re-inject most of those and only give them short lay-offs of 5 to 7 days and resume training. Many do this several times in their careers and get into a routine with a fairly consistent interval. As these young Western performance horses mature, however, the intervals between flare-up usually widens until they reach 6 years of age in which many seem to mature out of the syndrome. We hypothesize that this is partly due to a reduced training schedule and amount of work needed to stay in top form (i.e., the horse knows its job) but also because the horse begins adaptive remodeling, which becomes complete by the time it is 5 to 6 years old. Occasionally, it was observed that the ligament begins to fail requiring that the horse be removed from training and subjected to a different treatment type or prolonged rest period.

An important consideration when using regenerative products, such as ACS, is that differences may occur between commercially available products. The concentration of IL-1 receptor antagonist protein and IL-1 itself, as well as anabolic growth factor concentrations, may vary among manufacturers. The concentrations may also vary both between and within patients. Hraha, et al[6] tested 2 different commercially available veterinary systems, irap[c,d] and Arthrex IRAP II[d], using whole blood in a clot tube as a control. They compared each system using the blood of 5 horses and found that IL-1ra was up-regulated by both systems[c,d] and within the control, clot tube, but that the IL-1 to IL-1ra ratio was only increased compared to serum in Arthrex IRAP II,[d] thus illustrating differences. The authors used one of the systems[c] and did not measure IL-1ra or other factors of interest. The authors believe caution should be used when extrapolating this data to other systems used to produce ACS.

The authors believe that the large number of horses with PSD treated with ACS demonstrates the safety and effectiveness of this treatment when horses are selected appropriately. There were no control groups of horses with PSD that received other therapies to which can be compared to the effects of treatment with ACS, but this experience with other treatments of horses for PSD as well as the published results of other clinicians[1] indicates that horses treated with ACS are able to be returned successfully to training much sooner than horses receiving other treatment. Treatment with ACS has allowed many young Western performance horses to resume training in time to complete their

schedule of competitions. Further, the authors believe that this treatment should be evaluated in blinded clinical trials and that work should be done to define the exact mechanism of action.

Acknowledgments

Conflict of Interest

The Authors declare no conflicts of interest.

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[a]Sarapin® High Chemical Company, Levittown, PA 19056.
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[d]Arthrex IRAP II® Arthrex Vet Systems, Naples, FL 34108.
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Review of Vascular Administration of Mesenchymal Stem Cells in the Equine Distal Limb

Mathieu Spriet, DVM, MS, DACVR, DECVDI*; and Larry D. Galuppo, DVM, DACVS

Intravenous regional limb perfusions result in heterogeneous and asymmetric distribution of stem cells. Furthermore, serious limitations related to poor foot perfusion and tourniquet failure are identified in standing horses. Intra-arterial injections should not be performed with a tourniquet due to a risk of arterial thrombosis. Intra-arterial injections without a tourniquet lead to homogeneous and diffuse distribution and are considered the preferred technique. Although more challenging, intra-arterial injections can be performed in the standing horse. Authors' address: School of Veterinary Medicine, University of California, Davis, One Shields Ave, Davis, CA 95616; e-mail: mspriet@ucdavis.edu. *Corresponding and presenting author. © 2014 AAEP.

1. Introduction

Vascular administration of mesenchymal stem cells (MSCs) to the equine distal limb has been proposed as an alternative to direct injection into lesions. The main advantages of such techniques are the treatment of lesions that cannot be reached directly with a needle (for example, lesions within the hoof) or diffuse lesions that would require multiple injection sites. Another advantage of the technique is to avoid iatrogenic damage created by needles in the structures adjacent to lesions. Both intravenous and intra-arterial injection techniques have been used in laboratory animals[1,2] and in human patients.[3] The intra-arterial technique seems beneficial regarding the perfusion of peripheral tissues.[1–3] In the horse, techniques of vascular administration can be subdivided according to the type of vessel used (vein or artery), the exact site of injection, and the use or not of a tourniquet. The outcome might also be affected by the use of standing sedation or general anesthesia.

The use of an intracellular radioactive label (^{99m}Tc-HMPAO) allows scintigraphic tracking of the MSCs after injection. Different injection techniques have recently been evaluated with scintigraphy. The goal of this review is to summarize the pros and cons of each technique and issue a recommendation regarding the clinical use of vascular administration of MSCs. The different techniques that have been investigated include cephalic vein and palmar digital vein regional limb perfusion (RLP) and median artery injection. The arterial injections have been assessed both with and without the use of a tourniquet. All techniques have been performed both in anesthetized and in standing horses.

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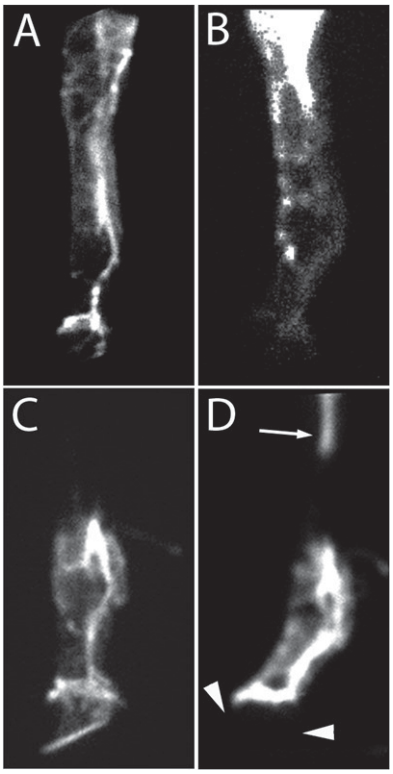

Fig. 1. Lateral scintigraphic images of the equine distal limb acquired after administration of radiolabeled mesenchymal stem cells using different intravenous injection techniques. A, Cephalic vein regional limb perfusion in the anesthetized horse. The radioactive signal is mostly located in the area of the main vessels and the coronary band. B, Cephalic vein regional limb perfusion in the standing horse. The radioactive signal is mostly located proximally with poor perfusion of the distal limb. C, Lateral palmar digital vein regional limb perfusion in the anesthetized horse. Radioactive signal is present within the hoof. D, Lateral palmar digital vein regional limb perfusion in the standing horse. Radioactive signal is identified migrating proximally to the tourniquet (arrow), while the tourniquet is still in place. No radioactive signal can be observed within the hoof.

2. Cephalic Vein Regional Limb Perfusion in Anesthetized Horses

Cephalic vein RLP under general anesthesia was the first technique investigated.[4,5] A pneumatic tourniquet inflated to 400 to 500 mm Hg was placed on the forearm just proximal to the cephalic vein catheter for 30 min. Twenty-five to 45 million radiolabeled MSCs suspended in 2 mL of saline were injected and flushed with 20 mL of saline. All of the radiolabeled MSCs remained in the limb while the tourniquet was in place and no major loss of radioactive signal was observed immediately after removal of the tourniquet. Only 3 of the 6 injected limbs had radioactive signal in the entire distal limb, whereas the other 3 limbs only had uptake in the carpal and proximal metacarpal areas.[4] The maximal signal intensity was mostly in the area of the larger vessels (Fig. 1A). In horses with induced lesions in the superficial digital flexor tendon, the distribution and quantification of the radioactive signal was not different from the control horses.[5]

3. Lateral Palmar Digital Vein Regional Limb Perfusion in Anesthetized Horses

The lateral palmar digital vein RLP was assessed in a later study with the main objective to improve distribution of MSCs to the foot when compared with cephalic vein RLP.[6] A pneumatic tourniquet inflated at 450 mm Hg was placed for 30 min in the metacarpal region, proximal to the injection site. Approximately 35 million MSCs suspended in 10 mL of saline and flushed with an additional 10 mL of saline were injected. Similar to the cephalic vein RLP in the anesthetized patient, the tourniquet effectively retained all radioactivity within the limb. The radioactive signal was present within the foot in all 6 injected limbs; however, a marked asymmetry was identified with low to absent radioactive signal at the medial aspect of the foot and pastern. Most of the radioactive signal was located in the area of the lateral palmar digital vein and coronary band vascular plexus (Fig. 1C).[6]

4. Cephalic Vein Regional Limb Perfusion in Standing Sedated Horses

For this study, horses were sedated with 0.01 mg/kg detomidine IV and 0.01 mg/kg butorphanol IV and a pneumatic tourniquet inflated at 225 mm Hg was placed on the forearm proximal to the injection site for 30 min.[7] Approximately 40 million radiolabeled MSCs suspended in 2 mL of saline were injected and flushed with 20 mL of saline. Only 3 of 6 horses showed a radioactive signal distal to the proximal metacarpus (Fig. 1B). The quantification of the signal revealed a much lower uptake than on the initial study under general anesthesia, suggesting failure of the tourniquet.

5. Lateral Palmar Digital Vein Regional Limb Perfusion in Standing Sedated Horses

Horses were sedated similarly as for the cephalic vein RLP study.[7] A pneumatic tourniquet inflated to 450 mm Hg was placed on the metacarpus for 20 min. Forty million radiolabeled MSCs in 10 mL of saline and flushed with an additional 10 mL of saline were used.[7] Despite all horses tolerating the tourniquet well, very little radioactive signal was present distal to the injection site in 3 of the horses. The quantification of the signal revealed a wide range of uptake. The dynamic acquisition at the time of injection demonstrated failure of the tourniquet with the radioactive signal proximal to the tourniquet. In the 3 horses with a better distal radioactive signal, uptake within the hoof was lower than in the anesthetized horses, suggesting poor perfusion related to the weight-bearing position (Fig. 1D).[7]

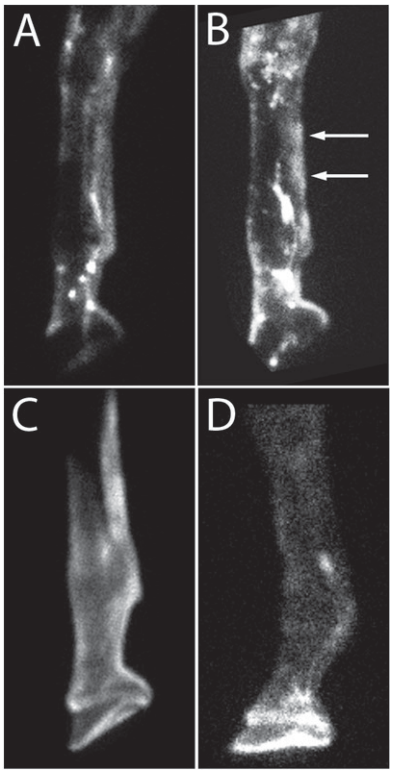

Fig. 2. Lateral scintigraphic images of the equine distal limb acquired after administration of radiolabeled mesenchymal stem cells using different intra-arterial injection techniques. A, Median artery regional limb perfusion in the anesthetized horse (normal limb). The entire limb is perfused but the distribution of radioactive signal is heterogeneous, mostly centered on the larger vessels. B, Median artery regional limb perfusion in the anesthetized horse with mechanically induced lesions in the superficial digital flexor tendon (arrows). Increased radioactive signal is identified in the lesion area. C, Median artery injection without a tourniquet in the anesthetized horse. Homogeneous diffuse radioactive signal is present in the distal limb. D, Median artery injection without a tourniquet in the standing horse. Radioactive signal is present diffusely in the distal limb, with a stronger signal within the hoof.

6. Median Artery Regional Limb Perfusion in Anesthetized Horses

Ultrasound guidance was used to place an 18 gauge catheter in the median artery at the level of the chestnut. A pneumatic tourniquet inflated to 400 to 500 mm Hg was placed on the forearm just proximal to the catheter for 30 min.[4,5] Twenty-five to 45 million radiolabeled MSCs suspended in 2 mL of saline were injected and flushed with 20 mL of saline. The radioactive signal was present in the entire distal limb in all 12 horses injected with this technique, demonstrating better perfusion than with the cephalic vein RLP. The radioactive signal distribution mainly followed the path of the arteries (Fig. 2A). Two out of the 12 horses developed clinical signs (lameness and skin necrosis in one horse) that were attributed to complete thrombosis of the medial palmar artery.[4,5] In 2 horses with 10-day-old induced superficial digital flexor tendon lesions, an increased uptake of radiolabeled MSCs in the lesion area was observed when compared to control horses and 3-day-old lesions (Fig. 2B).[5]

7. Median Artery Injection Without a Tourniquet in Anesthetized Horses

A catheter was placed similarly as for the median artery RLP. Thirty-five million radiolabeled MSCs suspended in 2 mL of saline were injected in the median artery without the use of a tourniquet.[6] A diffuse homogeneous distribution of the radioactive signal was observed through the entire distal limb (Fig. 2C). Quantification of the signal suggested that about half of the radiolabeled MSCs initially remained in the distal limb despite the absence of a tourniquet. No evidence of thrombosis was observed.[6]

8. Median Artery Injection Without a Tourniquet in Standing Sedated Horses

The feasibility of injecting the median artery in standing horses was first assessed using radiographic contrast material.[8] The use of a catheter versus direct needle injection was assessed in 6 horses. Ultrasound guidance was used for both techniques. Catheter placement was more challenging and induced a higher risk of arterial spasm, precluding injection. Furthermore, due to the deep location of the artery, motion of the limb after catheter placement led to retraction of the catheter from the artery. For these reasons, despite a risk of peri-arterial extravasation, direct needle injection was considered the preferred technique.[8]

Approximately 35 million radiolabeled MSCs were administered in the median arteries of 3 horses via direct needle injection.[9] Five out of 6 of the injections were successful; one of the injection led to partial peri-arterial injection. Diffuse symmetric uptake was present in all distal limbs with a strong radioactive signal in the hoof (Fig. 2D). No arterial thrombosis was observed; however, partial venous thrombosis was present in 3 of the horses. No lameness was associated with the partial venous thrombosis.[9]

9. Discussion

The intravenous RLP techniques performed under general anesthesia demonstrated good efficiency of the tourniquet and retention of MSCs in the limb after removal of the tourniquet.[4–6] However, the distribution pattern was not optimal. With cephalic vein RLP, the inconsistent perfusion of the distal limb was questioned as being related to too small of a perfusion volume.[4] In a later study, we investigated cephalic vein RLP using 22 mL of iodinated contrast material for radiographic evaluation of the distribution. The authors observed radiographic contrast in the entire distal limb (including the foot) in all cases (unpublished data).

It was concluded that the incomplete perfusion of the distal limb with radiolabeled MSCs was potentially due to obstruction of the venous valves by the MSCs. The lateral palmar digital vein RLP improved the perfusion of the foot compared to the cephalic vein RLP but the asymmetric distribution, with lower or absent radioactive signal medially, suggested that both the lateral and medial palmar digital veins should be injected in order to perfuse the entire foot.[6]

When performed in the standing horse, an additional limitation was the much lower signal observed in the limb.[7] Failure of the tourniquet was clearly demonstrated with the lateral palmar digital vein RLP when the radioactive signal was seen migrating proximal to the tourniquet on the dynamic acquisition at the time of injection.[7] Although similar dynamic data were not available for the cephalic vein RLP study, based on the limited distribution and poor signal observed on static acquisitions while the tourniquet was still in place, failure of the tourniquet likely also happened. The lower pressure (225 mm Hg) used in standing horses for the cephalic vein RLP can be blamed for tourniquet failure; however, for the lateral palmar digital vein RLP, the same pressure (450 mm Hg) was used for the tourniquet in anesthetized and standing horses. The tension in the tendons in the weight-bearing limbs likely explains the lower efficiency of the tourniquet in standing horses.[7]

The lower uptake within the hoof in standing horses compared with anesthetized horses is another significant limitation of the standing technique. This is likely related to the difference in pressure in the venous system in the weight-bearing foot.[7]

Another limitation to all intravenous RLP techniques was the heterogeneity of the signal distribution with higher uptake matching the location of the larger vessels, suggesting that the MSCs mostly remain in these larger vessels.[4,6]

The intra-arterial RLP (with a tourniquet) was advantageous compared with the intravenous RLP in terms of distribution and uptake in lesion areas; however, due to the severe arterial thrombosis that occurred in two out of twelve horses, this technique could not be recommended for clinical use.[4,5] Intra-arterial injection without a tourniquet resolved the thrombosis issue.[6] The retention of MSCs within the distal limb can be explained by trapping of the cells in the capillary beds. Some MSCs are lost to the general circulation due to the absence of the tourniquet but better perfusion and more homogeneous distribution of the MSCs can be appreciated. For these reasons, the intra-arterial technique was considered the preferred technique for vascular administration of MSCs. A limitation of this technique was the technical difficulty of placing a catheter in the median artery in a standing horse. The use of direct needle injection of the median artery under ultrasound guidance appeared to be an interesting alternative to the use of a catheter to facilitate the use of this procedure in the standing sedated horse.[8] Peri-arterial injection remains an inherent risk of this technique; however, this risk can be minimized in the hands of an operator experienced with ultrasound guidance. We have now performed over 30 such injections and peri-aterial leakage occurred in only 2 occurrences.

The difference in the pattern of distribution between intra-arterial injections in anesthetized and standing horses with a higher accumulation of MSCs in the foot in standing horses was an unexpected finding.[9] Interestingly, intravenous RLP led to a lower accumulation of MSCs in the foot of standing horses compared with anesthetized horses.[6] These findings are most likely related to the difference in pressure and perfusion at the capillaries of the lamina between weight-bearing and non-weight-bearing positions. The presence of partial subclinical thrombosis of the veins following arterial injections in standing horses was surprising since this did not happen in anesthetized patients. Again, differences in the capillary circulation with probable higher stress on the MSCs in the standing horses might explain this phenomenon. Partial venous thrombosis observed in the scintigraphic study[9] might also be related to higher stress on the MSCs from the labeling manipulation. We have now performed this technique with non-labeled MSCs in over 30 limbs and have only occasionally observed mild transient swelling of the distal limb without further complications.

10. Conclusion

Scintigraphic tracking has been extremely useful in comparing different vascular injection techniques for MSC administration to the equine distal limbs. As could be expected, the results in the anesthetized horses were different from the standing horses.

As performed in these studies, the intravenous RLPs were not reliable in the standing horses. The use of different tourniquets could be considered to try to improve the intravenous RLP; however, the intra-arterial injection technique without a tourniquet is our preferred technique as it provides better distribution of the MSCs.

Acknowledgments

Conflict of Interest

The Authors declare no conflicts of interest.

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Treating the Difficult Horse and Mule

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1. Introduction

The principles of equine behavior apply to both the horse and the mule. Mules are just like horses but more so, meaning that if the mule is afraid, he is extremely afraid or if the mule is disrespectful, he is extremely disrespectful. Having a basic understanding of equine behavior can make our work half as difficult and twice as safe. This is especially true when we are presented with an uncooperative horse. We can formulate a plan to approach the horse by modifying his behavior. In most circumstances, a horse's behavior can be modified safely in a short amount of time by reducing the fear, instilling the respect, and preparing him to accept the veterinary procedure. There are infinite degrees of fear, disrespect, and confusion. We counter fear with security, disrespect with discipline, and confusion with clarity. A horses' self-preservation is as strong as ours and when all parties involved feel safe and confident, the desired outcome will usually prevail. The purpose of this paper is to discuss the basic fundamentals of equine behavior and then how these fundamentals are used in developing a protocol for modifying the horse's behavior.[1]

2. General Information About the Horse-Human Relationship

Many difficult situations between horses and humans can be explained by the differences between prey and predators. Horses are prey and humans are predators and each think differently. Horses are not afraid of predators, but are afraid of predatory behavior. Most difficult-to-handle horses are too fearful of the human. Some difficult-to-handle horses are too disrespectful to the human. Horses can switch from one type of behavior to the other in a moment's time. When this happens, the human has to change the approach strategy accordingly. Difficulties will also arise if the horse is inadequately prepared to accept the veterinary procedure. Such problems dealing with horses can be greatly minimized by an appropriate approach and skillful handling. A cooperative horse is not afraid of the human, yet is willing to do what is asked.

3. Basic Information of Equine Behavior

Natural Instincts of the Horse

Horses have several important natural instincts. The most important instinct is self-preservation. A second important instinct is perception of changes to their environment—especially movement and sound. A third important instinct is flight from fear. This is the horse's first reaction to a situation that he perceives as unsafe. The horse is a herd animal, dependent upon the protection of the herd. There is a dominance hierarchy in the herd determined by the leader's ability to control the movement of his peers.

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How the Horse's Natural Instincts Plays a Role in Behavior Modification

The horse's perceptive instinct allows the horse to perceive cues during training. The horse's flight from fear instinct enables the horse to move his feet, especially when being ridden. The responsibility of the human is to become a trusted and respected leader of the herd.

Characteristics of the Horse

Horses have monocular vision. Eyes are located on the side of the head to better see predators sneaking up on him. Horses have very little cross-over reasoning from left to right and vice versa.[1] This essentially creates two horses—a left horse and a right horse. Anything taught on the left side of the horse needs to be repeated on the right side. Horses are claustrophobic. Horses know they are much more susceptible to being caught and/or killed in small enclosed areas. Horses have excellent memories.[1] They never forget any experience, positive or negative.

Wants of the Horse

Horses want safety, comfort, food, and play, in that order. Being aware of these desires and the order in which they are listed are important concepts when modifying a horse's behavior.

4. Analyzing the Horse's Present State of Mind

Most of the time, the horse is in a peaceful state of mind until a human invades their environment. Once the horse's view of the situation is determined, the approach can be adjusted in order to safely handle the horse. I think horses see the world along a spectrum ranging at one end, which is life-threatening fear, to the other end, which is complete disrespect for the human. There are important milestones along the spectrum, as illustrated below.

Fear ↔ Curiosity ↔ Friendly ↔ Respectful ↔
Pushy ↔ Bold ↔ Biting ↔ Total Disrespect

The ideal place on the spectrum for the horse-human relationship is where friendly meets respect.

5. Recognizing and Modifying Fear

The fearful or scared horse will be in the flight-from-fear state of mind. The sympathetic response will activate the adrenal medullae to release epinephrine. This will cause the horse to move away from the handler with a raised head, eyes wide open, and muscles hard. This horse will be emotionally unconfident and very reactive. If this type of horse feels trapped, he may become defensive. There are two subtypes of fearful horses: one type is high energy and the other is low energy.[2]

The high energy group of fearful horses has trouble standing still, has a tendency to run, may rear, and is very quick in reaction time. This horse needs reassurance of his safety by taking a softer approach.

Use a lot of approach and retreat techniques. These techniques utilize applying pressure, waiting for acceptance, and then removing the pressure by taking a step backwards.

Types of pressure would be invading the horse's space and then touching the horse. The next level of pressure would be manipulating some anatomical part such as opening the mouth, invading a body cavity, or lifting a foot. Pressure motivates the horse to change his behavior and release teaches the horse the correct choice was made. Signs of acceptance are relaxation of the eye and blinking, relaxation of the muscles, ear movement, looking at the human, lowering the head, chewing and licking the lips, sighing, blowing out, and yawning. The amount of retreat will range from taking several steps backwards for the very fearful horse to only a lean backwards for the suspicious horse. The sequence of steps using approach and retreat is important. For example, when catching a horse, the first step is to get as close to the horse as possible without causing the horse to move his feet. Wait until the horse relaxes. At the first moment the horse relaxes, take a step backwards, which will reassure the horse that the correct choice was made and he is safe. Well-timed releases from pressure will give progression in modifying the horse's behavior. Repeat this sequence until the horse is willing to touch the handler. It is important for the horse to touch the handler before the handler touches the horse. Touch the horse towards the base of his neck or withers. After the horse is caught, he may want to move his feet. Let the horse move in a circle, but frequently change directions. This will change the behavior from reacting to responding in order to get his feet under control. Once the horse's feet are under control, disengage the hind quarters in order to establish some respect. Disengaging the hind quarters is done by getting the horse to step medially with the inside hind leg, crossing over in front of the outside hind leg. Disengaging the hind quarters of the fearful horse will be more important than disengaging the fore quarters at the beginning. Once a balance between fear and respect is established, start preparing the horse to accept the veterinary procedure.

The low energy fearful group is even more afraid than the high energy group. This type of horse will most likely quiver and shake as you approach him. These horses are also under the influence of epinephrine and are high-headed, wide-eyed, and hard-muscled. They may not even blink or move their ears. These horses are tense and want to freeze up. If not allowed time to relax, these horses will explode with an extremely fast reaction time. This group of horses doesn't move their feet because of obedience, but are very distrustful. The low energy type horse will be more likely to kick than rear. A few of these types of horses will become so with-

drawn from their environment that they will become catatonic. This is the horse that falls asleep in the cross-ties while being saddled. This condition is not narcolepsy but adaptive behavior to deal with too much pressure during previous training sessions. This type of horse needs to be more trusting and this is accomplished by making the horse feel more comfortable. Again, use approach and retreat techniques. The approach will be soft. Move slowly but assertively. These horses will not acknowledge the approach and will stare straight ahead. The smallest try is a blink or movement of the ear. Compared to the first group, approach these horses using less pressure. The release from pressure is at a further distance from the horse and the wait is a longer time before re-approaching the patient. The waiting time in the beginning is a good investment for future visits. Taking the time now means taking less time in the future.

6. Recognizing and Modifying Disrespect

A disrespectful horse invades the space of the handler. This group of horses is emotionally confident and physically pushy. These horses are usually quite tolerant. The disrespectful type of horses can also be divided into two groups: a high energy group and a low energy group.

The disrespectful high energy group of horses can be the most difficult type for the owner and veterinarian to manage. These horses can be the most dominant and even dangerous. This group of horses will take a more firm approach. They are smart, exuberant, very playful, and easily bored. They chew on everything, including lead ropes, saddles, and the handler. These horses have a tendency to bite and strike. Be aware of all head shaking, tail wringing, ears back, and feet striking or kicking the air. These are all threats and warnings that if the horse's behavior is not adjusted, you might be injured. This horse needs to be more obedient. This can be accomplished by getting the horse to yield, especially his head and fore quarters. Ask the horse to back up and then to move his fore quarters left and right away from the handler. The approach to this type of horse will be directed more toward his head than his withers. Wait until the horse willingly yields before starting to prepare him for treatment.

The disrespectful low energy horse is usually pretty easy to treat and work around. This group of horses is gentle, lazy, stubborn, easily bored, but can be defiant. This type of horse is the most likely to hurt you, because you think he is gentle. The defiance in this type of horse will show up when asking him to move, like longing for a lameness exam. This horse will lay his ears back, poke his nose at you, and be reluctant to move forward with any impulsion. When more pressure is applied to this horse, he may rear high into the air, nearly falling over backwards and then when he comes back down, will kick at you. When asking this horse to move forward with a rope or whip, make sure it is long enough so that you can touch him, but he cannot touch you. This group of horses needs to be more motivated and this can be accomplished by using increasing or decreasing levels of comfort. More comfort might be something such as following a cow, rest, rubbing, or food. If food is used, make sure it is used as a reward and not a bribe. Motivating these horses using less comfort would be making the wrong thing difficult and the right thing easy. This is done by applying more pressure. Apply this pressure using increasing levels of pressure. Start with low pressure and increase the pressure as you go. This gives the horse an option and will decrease the odds that he will become aggressive towards you. Remember spanking is a two-way street.

7. Preparing the Horse to Accept Veterinary Procedures

The preparation is a series of simple steps—one at a time. These steps will be using the fundamentals of removing fear and instilling respect. Removing fear from the horse will allow the area of treatment to be touched. Establishing respect will allow the area of treatment to be moved. An example would be touching the leg and then picking up the foot. Next, make a plan based on what the horse needs to do physically in order to for the task to be accomplished.

8. Steps in Preparing the Horse for Veterinary Procedures

The following lists are steps the author has worked out over the years for preparing the horse to accept the different procedures.

Steps in Preparation for Getting Into the Mouth

1. Wait for the horse to lower his head or apply pressure to the poll.
2. Rub the outside of his lips.
3. Introduce your finger into the corner of the mouth.
4. Touch his tongue with your finger.
5. Get the horse to open his mouth.
6. Ready to flush mouth or deliver medication.

Steps in Preparation for Intramuscular Injections

1. Touch them.
2. Stroke them with firm pressure.
3. Stroke them with deep pressure.
4. Poke them with the tip of your finger using constant deep pressure.
5. Pinch with the meat of your finger and thump using mild pressure and a large fold of his skin.
6. Pinch with your fingertips.
7. Pinch with your fingernails for about a second.
8. Ready for injection.

Steps in Preparation for Intravenous Injections

1. Same as intramuscular injections but along the jugular groove.
2. For the really difficult horse, use a lip chain for distraction and place local anesthetic subcutaneously at the injection site.
3. Wait 2 to 3 min and enter the vein through the blocked area of the skin.

Steps in Preparation for Intranasal Vaccine

1. Touch the outside of the nostril.
2. Touch the inside of the nostril.
3. Touch the mucous membrane inside his nose—you may be able to touch only momentarily at first.
4. Repeat several times until the horse can accept having his mucous membrane touched for about three seconds or more.
5. Ready to insert catheter into his nose.

Steps in Preparation for Taking Temperature

1. Rub tail head area until horse willingly raises his tail.
2. Lift on tail until the horse relaxes.
3. Touch the perineal area.
4. Touch anal sphincter.
5. Ready to insert the thermometer.

9. Conclusion

Considering the horse's natural tendencies when making a plan to handle the difficult horse will produce the most time efficient, effective, and safest results. When done properly, there is increased harmony between the horse and human. This technique for handling horses works successfully on every horse, no matter the breed or the gender.

Acknowledgments

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Conflict of Interest

The Author declares no conflicts of interest.

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Using "One Step Horsemanship" in Handling the Difficult Horse

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1. Introduction

In our daily veterinary practice we occasionally have to deal with a difficult horse. One difficult horse can certainly have an impact on that day's schedule. However, I now find that I have fewer and fewer of these horses for several reasons. Some of them grow old and die, some are sold out of the area by their owners, some just mellow, and some, I would like to think, have changed because I have changed.

The highly acclaimed horseman, author, and advocate of the horse, Tom Dorrance, was once paraphrased as saying, "If you take care of the little problems the big problems will take care of themselves." I understand this to mean that we are responsible for all our actions around the horse. If we can modify our behavior around the horse in such a way that the horse views us as a companion or friend, the horse will then more than likely allow us to work with them to everyone's benefit.

Before explaining a technique of administering injections to unruly horses it may be useful to understand the basic concepts of "*One Step Horsemanship.*" This is an applied working horsemanship philosophy that I developed from experience, study, and understanding during my many years as an equine practitioner. It is so utterly simple that it is all too frequently overlooked. When applied in everyday veterinary practice "*One Step Horsemanship*" will shorten that list of "difficult horses" by taking care of those little problems and often resolving the big ones.

There are a few basic concepts about horses, of which we should all remind ourselves. Horses typically outweigh us 6:1, frequently 8:1, and sometimes even 10:1. Horses are 20 to 40 times more powerful, and react 3 to 4 times faster than us. Veterinarians are usually not able to out-muscle horses. However, the human brain is approximately 6 times larger than the horse's brain and, frequently, somewhat more intelligent.

Visualize the horse as a brain, much like our own, and a body merely dressed in a horse skin. Honor the horse as an animal which thinks, feels, understands, and has emotions. This is paramount to understanding the horse.

Relate to a horse just as you would to another person. You have friends you enjoy. You like being with them. You also know people who upset you, who "push your buttons." You don't like being with them. If given a choice, you always choose the people who make you feel good.

It is the same with horses. Give the horse the opportunity to choose you as a friend; give him a reason to be with you. You should treat your

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horses as you would your friends. Always be honest, always tell the truth, never be rude, let the horse know when he has done something unacceptable and, especially, tell him when he has done a good job. Praise and reward for a job well done. Build a solid relationship based on praise, good feeling, and honesty. This is the key to "*One Step Horsemanship*."

Envision a horse contained within an imaginary frame or box. The horse is relaxed; his head, neck, and spine are longitudinally straight and in "neutral." He is comfortable. Then consider the spine of a horse much like a long spring (analogous to the long thin spring attached to the old-fashioned screen doors.) The spring is always seeking straightness. If you put pressure on one end of the spring by pushing or pulling it out of straightness, it pulls or pushes against you. By placing the spring in an abnormal position you create energy within the spring and that energy seeks straightness.

Thus it is with a horse. If you move a horse out of its neutral position, out of that imaginary frame, and change the position of the spine out of straightness (bending the spring), energy is created that wants to regain the "neutral" straightness. "*One Step Horsemanship*" is based on this concept.

It can be challenging when you ask a horse to take a step forward, and the horse is not at that level of education or due to previous experience puts up resistance. It seems a horse's natural tendency, based on survival instincts, is to be cautious and resist when being pulled. The challenge for you is to show the horse that by taking a step forward he will be rewarded and find comfort.

2. Stocks

Let's assume you are attempting to get a balky horse into a set of exam stocks. The horse doesn't want to go; he is being difficult.

Before starting, it is good to make sure your adjustable rope halter fits properly, the throat-latch is snug and centered, the noseband lies just below the facial crest, and the cheek pieces avoid the eyes. The medium-sized rope halters with a balanced long enough lead rope attached without a snap works well for the average-sized horse.

Initial correction and education of the horse should not be associated with the stocks, so move away from the stocks to a neutral area. Stand a couple of yards from the horse and slowly pull on the lead rope, either straight ahead or to the side, until you move the head and neck "out of the box." The spine is now "out of straightness." Do not continue to pull, but merely hold the rope in that position and wait. And wait some more. (This is a true test of patience.)

If this request of walking either forward or to one side is either new to the horse or he is just resistant, he may not understand or cooperate. The horse may pull on the rope, twist his neck, stick his nose out, roll his eyes, and maybe even jerk backwards. But maybe, just maybe, the horse steps forward. Because you are only holding the horse out of position, not continuing to pull, the forward step loosens the rope. The horse has found relief in the release and that relief initiates understanding.

To better this lesson, you do the same again, only this time you respond more quickly. Your goal should be to provide release not merely when the horse's foot has moved and hits the ground, but before, when the hoof is leaving the ground. Even better, you should be giving release when the horse mentally decides to move the foot. Movement is initiated in the mind and the faster the relief is given, the better the lesson.

You are merely asking for a step. One step, and only one step. You are helping the horse seek the reward of release. Release is the initial reward, but better yet, is the praise you give after the release. Verbal praise combined with stroking of the horse's head and neck reinforces the success. Make the accomplishment a big deal for the horse and show him praise and love.

Then ask the horse again. Move his head out of position and hold. Wait for the desired movement. When done correctly, the hold and waiting period will become shorter than before. The horse is beginning to understand and is searching for that good feeling you provide. When releasing on initiation of the step, you might even "push" the rope back to the horse, making the release even faster. The faster you respond, the faster the horse understands.

Allow the horse a short time between your requests. Let the horse process what has just happened. Give the horse time to think and relax. The horse will often lick his lips, soften his muzzle, or lower his head in understanding.

Then ask again. A few steps forward, maybe one step back, a few steps forward, always waiting, always rewarding. With each succeeding step you may be able to lighten your request. Instead of asking with 5 pounds of pull and holding, you ask with only 2 pounds, and now with only 1 pound of pull. Now with merely half a pound, then hardly with a feel. Finally, maybe with only a thought!

You are instilling a desire in the horse to respond to the lightest possible request by putting praise and good feeling into the end. You ask for nothing more at this time. This is his reward and the horse soon begins seeking ways to find this reward. The horse is now working with you, he is now thinking with you, he wants to be your friend. He is now your partner, not your slave.

Using his newfound understanding and realization of comfort and praise, introduce the horse to the exam stocks. Be patient. The horse may stop before reaching the stocks. That's acceptable. Now ask the horse for just one step towards the stocks. When the horse gives it to you, lavishly praise him. Now ask again. Remember, patience and praise. Then ask for another step, always just one step. Up to the stocks, now a few steps into the stocks.

Stop and praise the horse again. You may even back the horse out a few steps. Then back in. Show the horse that the stocks aren't scary. Show the horse that praise and comfort come with every step. This process can take some time but pays great dividends in the end.

"*One Step Horsemanship*" is applicable to all forms and levels of equine education. The concept is applicable to many uses whether it is picking up a hoof, leading into an exam stock, or trotting out for a lameness exam. It is based on one step, nothing more, only one step, and then another. And then another. It is about your relationship with the horse. It is about your own personal learning curve and your own relationship with life. It is all about taking one good step, then feeling good about it. Then doing it again…

Pull, hold (patience-wait), release (reward and praise).

3. The Difficult Horse

The philosophy and use of "*One Step Horsemanship*" is useful in all phases of veterinary practice, every day. It requires observation, patience, and understanding of the horse, not merely as an object of our livelihood, but rather recognition of the horse as an individual who benefits from our respect. It is the basis of all good horse handling.

The difficult horse you occasionally meet has a reason for being obstinate. The horse may not understand what you are asking, or he may be fearful from previous experiences, or he may be reacting to his innate instinct of fight or flight for survival. The horse will usually present somewhere within the spectrum of being very dominant or being extremely fearful (of which both extremes can be very dangerous). There are many ways for you to handle each type of horse. Your action will be determined by your interpretation of the horse and the handler.

Difficult horses may be presented to you by the owner, a groom, a trainer, or anyone else assigned to the job. The handler may be very good, or may not have the horsemanship skills to cope with that particular horse, or may not be mentally engaged with the handling of the horse. The handlers themselves may be fearful. They can often hide their fear by acting overly dominant of the horse. Observation and assessment of the handler is important. It will determine how you approach and examine the horse.

Knowing that the horse is very skilled and capable of reading your actions, it is helpful to pay close attention to how you move around the horse. Instead of the usual veterinary time-conserving style of going directly to the horse, giving an injection, and quickly walking away, it may be more respectful and helpful to first introduce yourself to the horse.

By slowly approaching and stopping a few feet from the horse and waiting for recognition by the horse you become less threatening. Once the horse's attention is on you, introduce yourself by extending your hand, giving the horse time to smell and touch you. Often, touching or softly rubbing the horse's forehead is a welcome and effective action of introduction.

Before any examination of the horse it is best to have both his mental and physical attention. The horse must be present. If the horse is mentally off in the distance looking for his buddies or focused on a nearby barn cat, he is probably not thinking of you. Take the time to refocus the horse onto you so that he is aware of what you are doing. This prevents any surprises. It is also very helpful to have the horse standing squarely and balanced on all four feet, if possible (it's especially important when attempting to pick up a foot.)

4. Vaccinations

Now, suppose you are asked to vaccinate a horse who you've been told doesn't like vets. The horse presents with his head held high and slightly tilted away from you, his eyes "showing some white," and so tense and tight it looks like if you "pricked" him with a needle, he'd explode. His whole body feels like a rock. What is your approach?

A method that is useful when dealing with this type of high-headed mentally distracted dominant-type horse begins with your own personal self-confidence, calmness, skills, and determination.

It's usually best if you handle the horse by yourself so the horse has only you to contend with. Taking extra time for the introduction and mental focusing of the horse is the initial step. This is frequently best obtained by merely first stroking and rubbing the shoulder and neck and then, if possible, later progressing to his cheek and face. This may take some time and patience from you. Calming and trust is exhibited when the horse allows you this intimacy. A slight sigh or licking of lips indicates some degree of mental refocusing.

While softly but firmly holding the halter's left cheek-piece with your left hand, begin massaging the horse's neck with your right hand, initially on the right poll, then over to the left. Continue down the neck, softly asking the horse to flex his head down and towards you, arching his neck around the point of contact of your right hand.

When asking the horse to flex avoid a constant pull from your left hand, but rather use an ask-and-release technique; ask for a little flex, then allow the horse to straighten slightly, then ask for a little more flex, then again give it back to him. Continue this until the horse has relaxed throughout his neck and lowered his head. Typically, the horse's body will also relax when his neck relaxes.

This minor exercise seems to achieve several objectives. By first taking hold of the left cheek-piece you have obtained a direct contact with the horse and you have gained better control of his head and are better able to move with the horse if necessary. You are standing just in front of the left shoulder and inside the arc of his body, which is typically a

relatively safe position. By asking the horse to flex his neck with a non-confrontational manner of asking-and-releasing, the horse feels less threatened than if you were to demand that he flex his neck. You are gaining some respect and trust from the horse. You are also prepping the horse for an intramuscular injection.

Once this level of relaxation is obtained, if for only a short time, it can be helpful to back away from the horse and give him a break (a 'recess' from class). Go find something else to do for several minutes. This gives the horse time to think about what has just occurred. The horse may return to his far-off unfocused thoughts, but typically he feels pretty good about what just happened and what he has learned. You can then return, repeat the exercise, and find the horse to be more at ease, supple, and relaxed. The horse is now ready for the injection.

There are many techniques of administering injections. There is no exclusively right way; maybe just some ways that are better than others. The techniques you use will depend on the situation and your own personal preference.

With the difficult horse that has just learned to flex his neck with your request, a one- handed approach may work well. Once again, hold the halter's left cheek-piece close to the horse's left eye with your left hand. With your right hand lay the loaded syringe across your palm as if you were holding a screwdriver, but with your index and middle fingers extended beyond the tip of the needle, the syringe secured by your thumb.

Now begin asking the horse to lower and flex his neck as you did before, but this time place your syringe-holding right hand in the area you choose to inject. Your leading index and middle fingers create the main pivot point. As the horse flexes and the neck softens, maneuver the syringe so that the needle tip is just next to the skin alongside your pressing finger tips.

Now instead of pushing the needle in through the skin, ask the neck to flex onto and over the needle point. You are moving the neck onto the needle instead of pushing the needle into the neck. Once you have penetrated the skin, stop, wait for a split second, and then continue inserting the needle to its hub. The rest of the injection is as usual: aspirate, push plunger, etc.

If possible take your time removing the needle. It may be comforting to the horse, but especially to the owner if you take some additional time to gently massage the area. As a reminder, don't forget to breathe through the procedure!

The combination of your left hand possibly blocking some of the horse's peripheral vision, the left hand causing some mild distraction by soft shaking of the halter cheek-piece lateral to the eye, the neck musculature being relaxed and soft, and the movement of the neck onto the needle typically allows for a nonconfrontation injection. If you feel you need just a bit more distraction, a brief coughing spasm by you at the time of injection can be helpful.

The benefits of this particular one-handed technique includes safety, working with the horse to focus his attention, lowering his head, softening his musculature, working slowly, avoiding the "jab" of an injection, and building a foundation of trust and calmness for the future.

Taking time with this horse now will pay big benefits in the future. Spend time now to save time later.

5. Picking up a Hoof

In school we all learned the process of picking up a horse's hoof. Most of the horses were cooperative, some better than others, some a little more difficult, and most of them being school horses with limited concerns. Then we stepped out into the real world and realized not all horses were willing and cooperative. In fact, some were very resolute about not picking up their feet because of a lack of training, lack of understanding, discomfort, pain, or maybe the practitioner's lack of preparation, approach, and technique.

The approach when asking a horse to pick up his foot is similar to the approach described when giving a difficult horse an injection. As with most procedures dealing with horses, the problems are usually not always about the procedure itself but rather about the horse's prior experiences, his natural instincts of survival, and the practitioner's own skill level, initial presentation, and confidence.

Two objectives must be reached before a horse is able to pick up his hoof and keep it up. The horse must be able to stand in "neutral," that is, stand quietly. He must also be able to balance on the three legs left on the ground. If he is unable to stand quietly and balance on three legs there will not be enough time for any examination or farriery procedure.

If excessive sedation is used to put the horse in neutral it can cause an unstable ataxia and increase the difficulty of holding the hoof off the ground. Additional training is a better long-term solution than is chemical sedation for the uncooperative horse.

The horse must be mentally present and focused. He has to take an active part.

It is nearly impossible to pick up a horse's hoof without his help. In fact, I have never been able to do it. However, I have become quite proficient at asking the horse to lift up his own hoof.

There are several methods veterinarians use to ask the horse to lift its hoof: these may include pinching the chestnut, rolling or twisting the skin over the flexor bundle, gently tapping the fetlock with a shoeing tool, pulling up on the fetlock feathers, or pulling the fetlock forward. It is also helpful to lean against the horse in such a manner to encourage the horse to shift his weight away from that leg. Actually, all of these methods are merely ask-

ing the horse to unload the limb and rebalance on the other three legs. Once that happens the lifting part may be up to you.

With the front hoof loosely held between your legs or the hind hoof laid across your lap it is helpful to adjust your stance to position the hoof as low and in parallel alignment with the horse's spine as possible. This will minimize any discomfort to the horse from the leg being held in an awkward position.

The difficult horse may be categorized into the totally unhandled, the untrained, the spoiled, the bully, or the horse that is painful such as the bilateral laminitic horse.

The horse painful from laminitis, sub-solar abscesses, fracture, or soft tissue issues can be difficult to work with. Frequently the affected hoof is able to be raised when the sound hoof is on the ground, but raising the sound hoof with the horse bearing weight on the painful hoof typically is not so successful. General or local analgesics may be necessary when these conditions are bilateral.

6. Taking the Temperature of a Difficult Horse

When doing a physical examination on a horse it is common to do a TPR, noting the temperature, pulse, and respiration. The pulse is generally heard with a stethoscope or felt by light palpation and the respiration is counted visually or listened to with a stethoscope, with all methods being very noninvasive. However, taking the temperature usually involves inserting a well-lubricated thermometer through the anus. Most horses accept these procedures very well; however, there will be the occasional horse that lacks the training to accept the thermometer.

The recommended technique for inserting the thermometer, as learned in veterinary school, involved standing facing the left hind leg, sliding the left hand over the rump down to the top of the tail, then grasping and lifting the tail, then with the right hand inserting the well lubricated shook down thermometer under the tail, through the anus, and slowly lowering the tail back to its original position.

The horse who objects typically will clamp its tail tightly against its perineum, swing his butt into or away from you, or swiftly kick you. Recognizing your necessary position, and acknowledging the danger you are putting yourself in, it is strongly recommended using an examination stock to contain the horse. There is no temperature worth risking your health or life.

All live horses have a temperature; few have a fever.

7. Additional Considerations

Each horse must be approached and dealt with according to the individual situation and the skill of the handlers, farriers, and practitioners.

It should not be the veterinarian's responsibility to train the horse; however, there are times when a horse does not have the understanding and will require additional time and patience from the veterinarian to complete the job.

Since most difficult horses are scared and, therefore, unpredictable, it is always wise to have a personal escape route as well as an available avenue of escape for the horse (which hopefully does not include running through or over you.)

It is important to have a skilled handler that you know and trust. Often on the big farms and ranches the horse-holder responsibility is delegated to the lowest person on the labor list, usually someone of low skill, of short attention span, and limited aptitude.

If a skilled horse-handler is not available it may be more effective to work alone, encouraging the horse to focus his attention on you rather than being distracted by the handler.

It is best to not tie a horse securely to a post, tie ring, or rail, but rather tie in such a fashion that if the horse pulls back, the lead rope will slip a little but still be contained. If the horse is not familiar with cross-ties it might be better to secure them with a single lead rope since some horses get scared when their head is too confined and will tend to blow up with fright.

Any activity that involves picking up a horse's hoof and holding it up for any period of time requires you to have some skill, some strength, and lots of patience. To make it even easier, the horse must be present, understand, and be capable of standing still and be able to balance on three legs. It is rarely easy.

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The Author declares no conflicts of interest.

Safety Tips for Working Around Horses

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1. Introduction

Any veterinarian who engages in equine practice must be vigilant of safety. Whether on a farm, at a stable, or in a hospital environment all parties involved in the examination and treatment of equine patients can be at risk. This includes, but is not limited to, the owner, their horse, the veterinarian, and his/her employees.

Equine practitioners need to develop and use good horsemanship skills and "savvy" when working with horses to minimize the chance of injury to any of the previously mentioned parties. It is not the purpose or scope of this paper to discuss legal ramifications or reference numbers regarding injuries as they relate to equine practice. The author merely will attempt to highlight certain areas of concern that come to mind when discussing this subject.

Being aware that the horse is a prey animal helps explain why horses are such a highly perceptive species and often are described as "missing nothing" by knowledgeable individuals. The horse has a propensity for "leaving" when threatened. However, when surprised or when they feel trapped this large and potentially explosive species can and will defend themselves. To this end, it behooves the practitioner to constantly be alert and use sound preventative measures to minimize the risk of injury.

In the following paragraphs the author will break down specific areas of concern in regards to handling the horse.

2. Areas of Most Sensitivity

Two of the most sensitive and, therefore, potentially dangerous areas of a horse are the muzzle/mouth and the tail/rear end. Both the very front and the very back (rear) should be approached with caution, respect, and in a slow deliberate fashion. Experienced practitioners can testify that quick abrupt movements around the equine patient, especially in these two areas, can lead to an unfavorable reaction. Both of these areas can be easily desensitized in the naïve horse. Unfortunately, because the horse is very communicative with its muzzle/mouth this area of its anatomy can be a nuisance or, even worse, a hazard if the patient has been allowed or encouraged to be disrespectful. It is the author's opinion that there is no good reason to indiscriminately touch, tickle, stroke, or kiss a horse below the level of the noseband on a halter. Absolutely no good can come of this practice! This area can easily be examined with the proper approach and purpose. One can also make the case that handling the muzzle with an ungloved hand by anyone could be the first step in facilitating the spread of respiratory disease and should not be done in a casual manner.

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The rear of a horse is intuitively a dangerous area and most mature humans have that knowledge imbedded in their genes. With a slow approach and using careful deliberate stroking (similar to brushing) most horses will allow touching the tail, perineum, and lower hind limbs in a relaxed manner. They must not be surprised in this area though as it could elicit an explosive and, therefore, very dangerous reaction which could be life-threatening to the examiner. Also using multiple "points of contact" to keep the horse aware of your presence is highly encouraged, especially when it may be difficult for the horse to easily see where the examiner is located.

3. Haltering/Leading

When haltering, the generally accepted approach is to enter the horse's space from the left side toward the base of the neck/shoulder area. Regardless of where haltering is taking place a horse should be encouraged to go forward and not backward. Backing away from or toward the handler is a more dangerous situation than having the horse move forward. If a horse thinks they can leave they tend to not feel as threatened, whereas if they feel trapped they are more likely to flee or fight.

Holding or leading a horse can be challenging if the animal's ground manners are less than ideal. The author prefers to use either a nylon or leather halter on the horse to facilitate maximum control and safety. In a standing position the side of the halter or varying lengths of the lead rope can be held. When instructing an inexperienced person the author uses illustrations to describe the location where the animal should be held (Figs. 1–3).

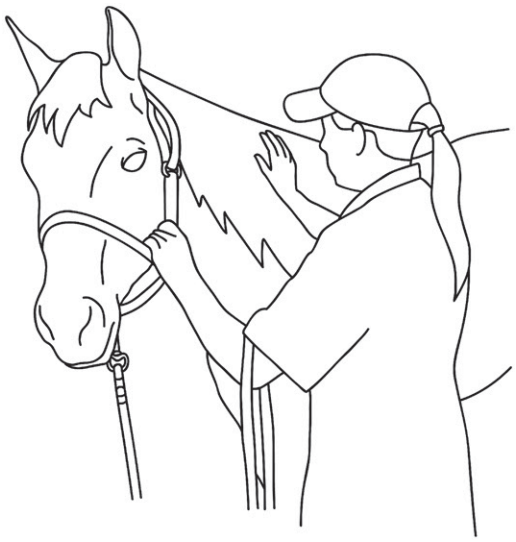

Fig. 1. Standing on the left or right side at the head or throatlatch, the handler holds the side of the halter, preferably on the noseband where it connects with the vertical strap. With the back of the hand up and the fingers held lightly over the horizontal nose strap the author feels this is the safest and most effective approach to directing the horse's nose toward the handler while keeping the forearm above the muzzle. This position is especially helpful when the examiner is approaching or working around the back half of the horse. Ideally, the nose should be tucked toward the handler slightly while the free hand can hold the lead rope and either stroke the neck or hold the mane near the withers. This allows the horse to move forward, away from, and around both the handler and the examiner in a safer manner.

4. Helpful Tips for Practitioners When Interacting With Equine Patients

- Few equine practitioners are or want to be horse trainers. However, we should strive to leave our patients "better than we found them" to facilitate cooperation in our exam/treatment regimes in future encounters.
- For the most part, the practitioner (or handler) is either sensitizing or desensitizing the equine patient every time we interact. Both are necessary and effective tools to encourage cooperation from the horse but care should be taken to not use the wrong technique when the opposite approach might be more likely to produce the desired result.
- When leading a horse, always change direction by turning the animal away from the handler. This is a safer technique and maintains the horse in a straighter body position and encourages the horse to yield to the handler's space.
- If presented with a horse that has a tendency to nibble or bite when being held or led, the author places a dog collar over the nose band of the halter and around the muzzle (similar to a cavesson) to discourage this rude behavior.
- Only one person should be doing an exam on a horse at one time. This basic safety rule is frequently broken by inexperienced individuals.
- Stocks do not provide complete protection for the handler, observer, or practitioner, regardless of where one might be standing.
- Sedated horses should not give the handler/examiner false security. A sedated horse may be more easily surprised by movement or noise and react violently.
- Generally, once past mid-thorax of the equine patient the examiner would be better served to face the rear of the horse with one's back toward the head.
- A good rule of thumb as a veterinarian is to never examine or attempt any treatment procedure on a horse that is tied.
- There is a saying among horsemen, "there's a time to work and there's a time to wait." It is best not to get the two confused!!
- One should try not to reward a horse at any time for doing the wrong thing. Reward (release) should only come when the desired reaction takes place. As humans, we can easily

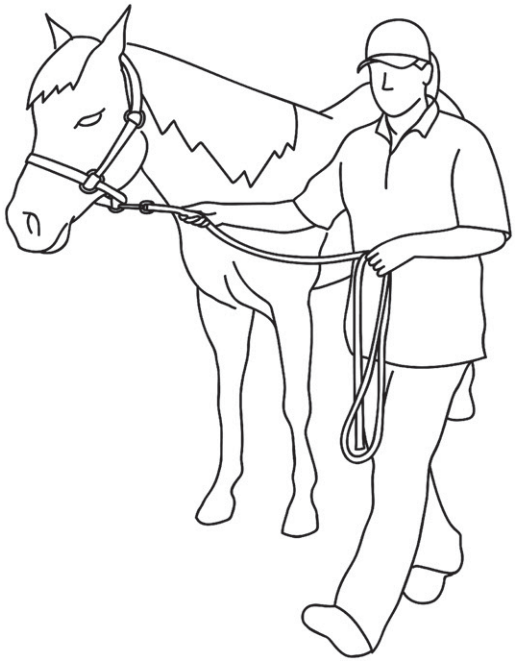

Fig. 2. When being led, most horses are accustomed to the handler holding the shank (lead rope) approximately 6 to 12 in. from the halter. The handler is in the safest position if the horse moves forward while the handler maintains a location approximately even with the throatlatch on the left side. If the patient is unaccustomed to being led in this manner then the handler should let the shank slide through his/her hand to the third position.

become confused and unknowingly reward an inappropriate response. To master this challenge requires focus, timing, patience, and experience but it makes for much more relaxing and enjoyable days in equine practice.

Acknowledgments

Conflict of Interest

The Author declares no conflicts of interest.

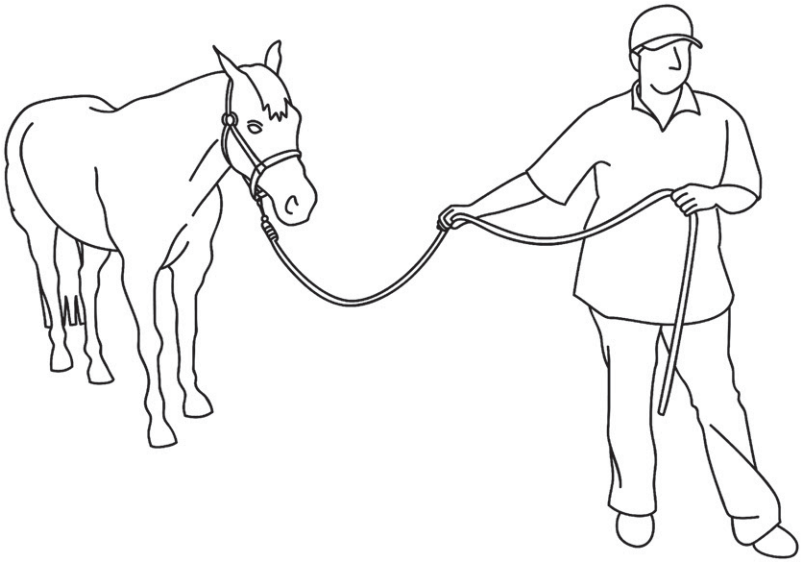

Fig. 3. This location is variable but, generally, the idea is for the handler to walk/trot out in front of the horse, encouraging the animal to follow. The distance down the shank can be relatively short (3–4 feet) or much longer, if necessary, to achieve the desired result. There is no substitute for practice and experience when leading a horse safely at a walk or trot.

Treating the Difficult Equine Patient

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1. Introduction

There is an important saying in the growing mule industry: "Mules *must* be trained the way horses *should* be trained." There is great truth in this saying. The mule will test your horsemanship.

No longer primarily a draft, pack, or agricultural work animal, the mule is quickly becoming a popular recreational equine, widely used for trail riding and in competitive Western, English, and Dressage disciplines. In recent decades, mule shows have increased from two major events to dozens, held all over the country. While registrations have decreased in nearly all major horse breeds due to the poor national economy, mule breeding and sales have escalated to numbers not seen since the early 20th century.

In order to understand the mule, one must understand the behavior of its sire, the donkey. The horse evolved on the open grasslands, also inhabited by hungry predators, mostly of the canine and feline species.

The most effective defense was flight. Not equipped with horns or tusks as are many prey species, the horse's anatomy, physiology, and behavior are designed for high speed escape from any discerned or potential threat.

The donkey evolved in harsh, arid, steep, and precipitous terrain, where blind flight could be fatal. Therefore, when a potentially threatening stimulus is perceived, it does one of three things:

1. It will run like a horse.
2. It will freeze, feeling safe when it stands, even in the presence of danger. Hence, the reputation for stubbornness.
3. It will attack. That is why donkeys are being used to protect sheep against coyotes and why their hybrid offspring, mules, have been known to kill predatory species such as dogs and pumas.

The choice of the above three options can be made in a *millisecond*. Thus, mules are judgmental. They make decisions. They choose their reaction. This makes them more challenging as patients than horses, which are more predictable.

Having owned, bred, and trained mules for more than forty years I have learned to understand them. When people ask me why I ride mules more often than horses, I tell them "I love horses. I respect mules. Mules challenge my horsemanship."

Mules are, in general, less domineering than horses. They will usually defer leadership to a horse. If treated kindly and wisely they can be even more sociable and friendly to humans than many horses. If mistreated they can be vengeful and more dangerous than horses.

If the training of newborn horse foals, which I have done and advocated for over half a century (that is, handling them extensively as soon as possible after birth and during their imprinting period)

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is done properly with mules, it will guarantee a docile and well-behaved mule later on. Imprint training (training *during* the postnatal imprinting period) is now universally popular in the mule industry and is, I have been told, a major reason for the current popularity of mules.

The mules I have raised, all imprint-trained, have, without exception, had flawless behavior and successfully performed in both English and Western events, even beating horses. They have been perfect patients, accepting worming, vaccinations, dentistry, and farriery with no resistance.

On the contrary, the mules I have owned which were born elsewhere and handled conventionally as foals, all had behavior quirks which were undesirable and problematic.

2. The Quick Injection Technique

Some horses, a minority, are completely oblivious to routine injections. This is probably due to the variation in pain thresholds. A larger majority of horses do not like injections, show visible discomfort when injected, but, nevertheless, accept them without undue resistance. Then there are those horses, all too common, who fear and resist injections. A majority of these can be coped with using several techniques.

1. Manipulating and massaging a fold of skin at the injection site and then sneaking the injection in.
2. Popping the needle in alone and then attaching the syringe afterward. This method, although popular, is not what the author prefers.
3. Vigorously rubbing the injection site, as with an alcohol-impregnated gauze sponge, in order to desensitize the area. This is effective for many difficult horses. The rubbing should be firm and rapid, for as many as 50 to 100 strokes and the injection given the instant the final stroke is finished.
4. Distraction, as with a twitch or a lip chain. Using a lip chain, it is often not necessary to put pressure on it, but simply to vibrate it before and during the injection.

Then there are those horses that are violently "needle shy." Rarely, some are so phobic as to be nearly impossible to inject. I have been able to train some of these phobic horses to stand quietly for injections using the Rarey leg strap. However, this technique, although it has been consistently effective in curing the phobia, is only for the most extreme cases. I probably have used it on less than 30 horses during my entire career.

The most practical technique I have found for the "needle shy" phobic horse, in order to do a simple vaccination, is the "quick injection" method, which most veterinarians who do a lot zoo practice use. With it, I have been able to inject countless lions, tigers, and other dangerous zoo species. It is done very rapidly through the cage bars as the patient walks past them.

Before I describe the technique, I must state that this is not an ideal injection technique. Aspiration is not done prior to injection so if we were to inadvertently inject a blood vessel, the consequences could be dire. However, having given thousands of such "quick injections" to zoo animals, many truly phobic horses, and even to difficult dogs on leashes at public rabies clinics, I have never had an adverse reaction. Therefore, I regard it as an acceptable modality. I must emphasize that it is a technique I have rarely used on horses. For example, at community equine vaccination clinics, where we vaccinate great numbers of horses, we occasionally encounter the truly phobic "needle shy" horse, which cannot even be approached to inject.

With the "quick injection" technique, we conceal the syringe behind our back, very casually approach the horse, stand near it for a while, looking away from it (a nonpredatory posture), and then inject as we walk away from the horse. Again, the retreat is non-predatory and although the horse reacts to the injection with a violent start, the cause (me) is walking away.

In order to do this you have to be *fast*. Vaccinating big cats in zoos and giving immobilizing injections to chimps and gorillas in their forearms as they reach for a banana through the cage bars, *you learn to give a fast injection*. The technique is as follows:

1. The volume of the injection should be no more than 2 cc, ideally less.
2. Use a disposable plastic syringe. I have had the best results with the Monoject 2 cc syringe and a 20-gauge needle.
3. The syringe is held between the second and third fingers. The thumb is on the top of the plunger. It is the thumb that causes the injection.
4. The arm is propelled so quickly towards the patient that the syringe is emptied and the force of the thrust causes our arm to bounce back still holding the syringe.

Used on horses, I combine this thrust, simultaneously stepping *away* from the horse. This is important in order to prevent one of two reactions from the truly phobic horse.

1. Immediate escape behavior if they identify you as the cause of the injection. They do not if you are looking away and moving away.
2. Immediate defense behavior which can include bolting, striking, or whirling to kick.

Obviously, we do not seek opportunities to use the fast injection technique. If, for example, the client offers to put the horse in a chute or stocks, we concur. However, the fast injection method, often

expedient on zoo species, can be useful for the rare extremely phobic horse.

3. The Lip Chain

When I graduated from veterinary school in 1956, tube worming was the "bread and butter" of equine practice and the use of the twitch was constant for that and many other procedures.

During the last two decades of my practice career, I used the twitch less than once a week. I had learned that using time, patience, and persistence to reassure my patients and get them to accept many procedures such as gastric intubation and floating the teeth, without resorting to physical restraints.

Second, a variety of tranquilizers, hypnotics, and immobilizing drugs have become available to facilitate many procedures in difficult equine patients.

Lastly, I found that a chain lead shank, placed under the upper lip of the horse could serve in three ways to restrain it:

1. The weight of the chain under the lip has a sedating effect. Acupuncturists describe that area as a location for sedation. I learned that soft gentle massage with one finger under the lip can sedate any horse. If they fear my finger I insert it at the corner of the mouth where most horses are used to bit pressure. Then I begin a massage of the upper gum. Most horses quickly relax. Then I advance the massaging finger to the front of the mouth. Once the head drops and the eye softens, I gently place the chain shank under the lip, then step back and wait. The horse will usually move its lips and soon relax, and the head will drop. Again, the eyes soften and at this point I can often proceed with whatever it is that I want to do. Let us use a jugular intravenous injection as an example.
2. If the preceding procedure is inadequate, the next step is to vibrate the chain. This will divert a majority of horses, allowing the injection with no further diversion. If I have a well-trained assistant, I will have that person vibrate the chain. If not, I do it myself. I obstruct the jugular vein with the thumb of my left hand. The other fingers hold the chain and vibrate it. I hold the syringe and give the injection with the other hand. Obviously, many horses can be given an intravenous injection with no restraint whatsoever. But the vibrating chain is a sufficient diversion to most (not all) "needle shy" horses to make injection easier.
3. Lastly, if vibration of the chain is inadequate, strong downward pressure on the chain works exactly as a twitch does. That is, as I was taught in school, "inhibition through pain" I do not accept the concept expressed in later decades that the twitch (or "earing" a horse, or twisting a fold of skin at the shoulder) immobilizes a horse because of the elaboration of endorphins.

Of course, pain causes endorphin release, but endorphins take time to alleviate pain. They are the reason that twitching or "earing" or lip chain pressure after a few minutes usually stop working. However, before the endorphins take effect, these pain evoking methods temporarily immobilize by causing immediate paralyzing pain.

Because they are painful, they should be used as a last resort. This includes the preceding method, number three, using the lip chain.

Most fractious horses can be handled using the lip chain, as described in method numbers one or two.

As stated earlier, by simply using patience, diligence, and understanding the innate behavior of this species, a majority of equine patients can be taught to accept most routine veterinary procedures (injections, simple dentistry, nasogastric intubation, sheath cleaning, working on the feet, nonpainful invasion of any body opening, deworming, medicating the eyes, etc.) with no restraint at all. By rarely "fighting" my patients, I enjoyed my practice much more. Most of my regular patients liked me and respected me and, importantly, my clients liked my "bedside manner." This enhanced the success of our practice.

Another method of diversion that does not involve the use of a lip chain might be mentioned here, although I did not discover it until after I retired from practice and became a client. One of my mules was moderately needle shy. She was never aggressive but was very fearful. I called one of my former practice partners to do an annual workup on my horses and mules, which included vaccination.

I reminded him of the one mule's fear. He said, "We have a technique for her." His tech fed the mule a very hard biscuit. When she finished eating it, the tech offered her a second biscuit, but this time she hung on to it. While a tug-of-war went on between the tech and the patient, the doctor quickly gave two injections and the mule was oblivious to them.

When I expressed admiration for the techniques, my colleague said, "They can only think of one thing at a time."

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The Author declares no conflicts of interest.

Managing A Resistant Horse: The Right Mixture of Sweetness and Strength

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1. Introduction

This presentation addresses the issues of unpredictabilities in horses which can make them dangerous as the inherent risk law supports. As veterinarians, we need to respect this because all horses should be considered potentially dangerous regardless of the package. This is true whether they appear bold and rude or quiet and docile. However, as we become more sensitive to them and grow in our own personal awareness, the horse becomes more predictable and we find more options for our safety. My lifelong study of recognizing the behavioral characteristics of the horse motivates me to share with you how to remain safe and manage your environment. As practicing veterinarians, we know many horses show a negative connotation towards us that may not be present for the owner. This is true whether it is in a clinical setting or in the field. Our approach to manage the behavior of the horse with finesse not only keeps us safe, but allows us to maintain clientele, despite their horses' attitude towards us.

Multiple factors may trigger this negativity. Whether it is the sound of our truck, the scent we bring in, or the fact that we are outsiders, the responsibility of managing this negative behavior becomes incumbent upon us. In many cases it is not the horse trainer or the owner's responsibility to change the viewpoint of the horse. We must recognize this responsibility as we take ownership in building this interaction with the horse. It is not to be taken personally but responsibly. If we can get the horse to behave for us, then chances are the horse will be cooperative with follow up treatments, which will encourage the owner to be more compliant. This results in the better health of the horse.

Patience is an important element to the horse's healthy recovery as well as our safety as veterinarians. We must recognize when to work and when to wait. The horse needs to know when we need them to move and when we need them to be still. If the horse is being resistant during a procedure, then we need to use our intelligence as humans and manage their sensitivity. This begins with learning how to best manage the environment that will enable the horse to accept and trust us. This is accomplished by our increasing insight of how the horse lines up for or against us. An example of this negative response would be an energy pushing against us or blocking and shutting us out. Our efforts can be more productive by redirecting the negative behaviors rather than trying to use ineffective brute force. Reward the smallest change and the slightest try from the horse. Reach out to them in a calm and

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passive manner to see if they will reach back with a change of expression. This gives them more reason to seek out a more preferred response which is the foundation of how they start to fill in for us.

Filling in is the dance, the closing of the gap between our efforts and the horse's in our mutual interaction. There will always be a constant balance between us and the horse: the yin and yang of our interaction. I have observed veterinarians that have the ability to perform procedures on horses without raising their stress level or anxiety when the horse appeared to be non-compliant. These natural abilities to fill in for the horse come from experience and being less abrasive; however, these skills can also be learned. When performing a procedure, if the horse is willing to accept it, then they are willing to accept the balance between us. If they show a resistance, it is our responsibility to help them. They should be allowed to work through things they view as troublesome. As they identify what is non-productive for them, they will pause and weigh the outcome. The desired outcome is a horse with a non-defensive posture towards us or the procedure. The sensitivity of the horse allows them to find their non-defensive posture when we use a rhythm, through an observation, and through perseverance on our part. We need to block the movement we want to discourage and encourage the movement we do want, which is a non-defensive movement. As this is achieved, the horse is neither lined up to leave nor pushes against us.

There are two common defensive movements in creating a body position for the horse: one is the inside hind leg not reaching up under their belly. The other is the inside front leg being pushed from the outside hock and loin through the front leg into us. We need to encourage the inside hind leg to reach further up under their belly by asking the outside front leg to move more lateral than forward. This is effective because the outside front leg draws the inside hind leg. The other defensive movement would be countered by encouraging the inside front leg to step back up under their girth. This will naturally break the push from the opposite hind leg because the backup is a two-beat diagonal gate. Once this is executed, it is critical to give the horse a chance to digest what was just presented. They will develop a respect toward us and start to reach out. They will understand their boundaries more clearly.

Boundaries are important as we proceed and advance behind their eye. This is a vulnerable place for them and where the support becomes so crucial. Once we obtain acceptance to go past their eye, it is important that we do not challenge them. If getting past the eye becomes an issue for them, then we need to address it. Once we have acceptance of advancing behind the eye, it is now an appropriate time to withdraw. We know how we positioned them the first time so we can achieve this again. Taking an opportunity to change sides will balance the horse's mental capacity and increase our safety by continuing the balance on both sides of the horse. If they attempt to block us again, we reinitiate the inside hind leg stepping up and forward under their belly. Then the outside front leg will step out in a forward motion around us. This shows that they have allowed us in behind their eye and now it is appropriate to change sides again. If the horse accepts this, then they are neither lined up to leave nor lined up to push. True feel, timing, and balance is critical to understand when we need to change our angle, have good rhythm, and movement. Be confident but relaxed and always ready to leave. If the trouble is too great, then we need to eliminate it. This is true even if we are the cause of the trouble. This will keep us safe and the horse more predictable.

To continue the horse's predictable behavior, something as simple as how we use the coordination of our upper and lower body movement can assure the horse that they are safe to be still. The coordination of this movement can facilitate a quality response so the horse can regain its balance or maintain a non-defensive posture. It is important to the horse's state of mind that they remain confident that it is alright to move or stay. The horse needs to recognize there is a need to move only when both our upper and lower body is engaged in the request for movement. Otherwise, there is no need for the horse to move as we advance toward our position for the procedure as long as either our upper body is moving while the lower body is quiet and always ready to withdraw, or the upper body is quiet and ready to withdraw while our lower body is advancing. How we regulate the quality of our touch is as vital as this understanding.

If the horse is unsure of a touch to a new area, this touch needs to be very quiet with your lower joints ready to withdraw, but the contact attempts to remain. Once the horse accepts the contact, the veterinarian builds from a quiet touch to a more direct touch that includes movement. We would first move their skin with this touch, then move this contact over their skin, proceeding on to a new spot and then repeat. This is a preparation to the position for the transition. This approach can be applied to all aspects of veterinary procedures.

Managing our safety includes educating our horse handlers to the proper way of taking over control of the horse. Those helping are critical to our success as we transition that role to them and move into the task of completing the medical procedure. Their energy needs to be sympathetic to our needs as well as that of the horse and aware of their concerns, keeping us safe while we do our job. We need to coordinate with them and make sure they know what to do when the horse objects to a procedure that causes them discomfort. We are dealing with a shifting energy within the horse stimulated by the procedure. Our horse handlers need to direct that energy by maintaining a non-defensive posture.

They should have slack in the lead rope but be mindful of the distance, so they can always maintain the direction of the horse's movement. This can be achieved with a hand on the halter rather than a tight grip on the lead rope and possibly an appropriate contact.

By engaging with the horse, our handler can quickly identify the first sign of trouble. They can determine if we should wait, retreat, or allow them to stay. If we miss the signs, the horse should be redirected, as we've already discussed, and repositioned. It is best to identify the trouble before it escalates. Hold off on the procedure until the horse is calm. The worst thing we can do is hurry the injection or procedure as this will make them less trusting the next time. Despite our greater intelligence, we must remember the horse's sensitivity is keen and bright and they usually have us figured out before we can figure them out.

With this being said, we have to make the right choice regarding our ability to manage the horse's behavior. At any point, if their resistance is unmerited according to the procedure, the proper use of a lip chain and/or the use of sedation tools may be appropriate. If used appropriately, this will reduce the risk of injury to everyone involved without negative repercussions. The purpose of the lip chain is to teach the horse to take direction that they otherwise were struggling to learn. Once the chain is in place under the upper lip, you must be very careful because their first impulse may be to use their hock and loin to push against this pressure, causing them to rear. It is necessary to have them move both forward and backward, to eliminate their need to rear. This method encourages obedience and aids the handler in directing the horse to a non-defensive posture. In keeping the horse in a non-defensive posture, we continue to reiterate to the horse an environment for our own safety and their well-being. With the use of these tools we still must remain considerate for owner compliance and, in the end, we have still created a positive interaction with this difficult horse.

2. Oral Medications and Deworming

When approaching a horse that is objecting to oral medications or being dewormed, I will set them up in the same manner as previously discussed. I will find places to remove braces either from breaking the push, or move them forward and around me to a place they can become still again. Then, I will advance on by moving their skin before I move my hand over their skin; this is done at any troubled place as a method of checking them out, or consoling them, before I go on. Continuing on, I will make sure the halter is tight so their movement can be directed as I attempt to place my left thumb in the left side of their mouth. My effort in doing this is to condition a response for them to open their mouth on cue with my thumb. If they object as I am attempting this, I will stay with them, and be willing to move my feet. When they stop objecting, I will let them know the correct response was simply to open their mouth. Sometimes we may struggle with our understanding of how to prepare the horse to the position. However, I think that more often we have the horse well-prepared physically, but not mentally. Therefore, the horse is working from a place where they are not confident enough to complete the procedure the way we intended. We need to prepare to position, then withdraw, and reprepare, however many times it is necessary until their mind is right for what we intend. Pet the horse on the forehead, reposition their feet if needed, and then repeat until satisfied that they are conditioned to open their mouth upon approach. It can be an advantage to repeat this in a couple of different places, or move them a few feet to a new location, since this will better prepare them mentally and they will become more predictable. Next, I will use the syringe or dewormer in place of my thumb, with my left hand still on the bridge of their nose and halter, directing their feet and supporting their head movement.

3. Intranasal Tubing and Vaccine

This is similar to how I prepare a horse to receive an intranasal vaccine or a nasogastric tube. Again, I will have a hand on the halter over the bridge of their nose directing them as they get comfortable with having my thumb in their nose. Once I feel they are willing to accept this, I pet them with the tubing of the vaccine down their face, from their poll to their nose. Once they are no longer suspicious of the tubing, I will hold their left nostril out with my thumb and my fingers on the halter as I direct the vaccine up their left nostril with my right hand. As the vaccine is quickly pushed in, I encourage the horse's head to go up since this is where the vaccine needs to go. As for the nasogastric tube, preparaing the horse to comply when making contact with the tube to the nostril is accomplished through techniques that have already been applied. Once we have made and maintained contact, it becomes important to how we advance, particularly in two areas. First is that contact until the tube has advanced to a distance of approximately 4 in., where both the horse and the veterinarian are confident that a sudden movement of the head would not dislodge the tube. It is critical that the tube and the nostril are properly lubricated and that the tube is around our neck so we can maintain control if we need to move. Our ability to maintain control of the tube is paramount because we may need to either steady it, withdraw it, or advance it. As we make these decisions, we search for a comfortable, quiet spot for the horse before we advance further. Conditioning them to seek this quiet spot becomes the barometer of when we can advance. We use a slight movement of the tube but no advancement as a presignal to the advancement, stopping again before their suspicions are aroused then repeat or

withdraw. As long as we have the horse's best interest in mind, making mistakes is acceptable as long as we learn how to improve their acceptance of the procedure. I had a coach help me find these subtle spots; without a coach, it can be frustrating getting past some of these unfavored conditioned responses. It is appropriate to sedate these horses, so we can maintain our confidence and continue to search for these subtleties. Once the tube has been advanced to a place where we are confident the horse has accepted it and it is no longer vulnerable to slipping out, then we need to be aware as we approach the esophageal opening. We do not want to activate the gag reflex and shut down the swallow reflex. If this happens and you cannot get the horse to swallow the tube, have someone take a dose syringe full of water and slowly squirt it into the horse's mouth. This will cause them to use their swallow reflex and allow you to advance the tube into the esophagus.

4. Foals

I take a slightly different approach when handling foals, in comparison to adult horses, since their fight or flight response is much stronger. They might regard humans as predators and their instinct will tell them to fight or flee. We might be able to outmuscle a young foal, but in their struggle to get free, may cause an injury. Most serious injuries are caused when foals rear up and flip over backwards. Foals from birth to 4 weeks are generally addressed with the aid of some physical restraint, regardless of a specific technique used. As we proceed, imprint training techniques should be employed, whether the foal has or has not been imprinted. With a firm restraint and gentle effort to imprint, we help them avoid undesirable behavior.

Sometimes when working on smaller foals, it is easier to lay them down to accomplish certain procedures. There are a couple of good techniques to laying foals down. The first one is described by Dr. Madigan and uses a rope around the chest, using a squeezing technique. This is best performed on foals a week old or younger. The second one is used on slightly older foals; this technique brings their nose back to the hip and folds them down into lateral recumbency. Once down on proper matting or bedding, it is helpful to cover the foal's eyes. They are much more vulnerable to learn the wrong thing and their negative response to get away can be dangerous to us since they are more flexible and agile. These techniques have limitations as the foal gets older and stronger and it becomes less likely that someone will be able to physically restrain the foal. In these cases, sedation is necessary.

There are many factors to consider: the foal's age, how much handling they have had, or if they have been halter broke. From a newborn to approximately 8 weeks old, it is best to have a technician wrap one arm around the chest while the other hand is at the base of the foal's tail and press the foal against a wall for additional support, if necessary. This way they can hold them while directing their movement both vertically and horizontally. There is always give and take lifting the tail up and down. Remember the restraint needs to be firm and the attempt to examine is gentle following imprint training protocols. As the foals get bigger and older, two people may be required to hold them: one at the head and one at the tail. If the foal is used to the halter and lead rope then we can use the lead rope around their buttocks and possibly eliminate the need for two people. It is important to keep in mind as we restrain these foals that we are not conditioning them to learn negative responses.

Once a foal has reached a size where they cannot be physically restrained, it is time to use horsemanship skills, as previously described. Foals are going to come with a wide variety of previous exposures and tolerances. We understand that we are not in the horse training business; however, if we spike their adrenaline and ignite the flight instinct in an effort to sedate, it is counterproductive to improving their learning skills and ours. Let our idea become their idea. Listed below are several mannerisms that foals have and how we can use them to our benefit.

Nativity: the negative aspect of their nativity is that they do not even know how to give us a correct response. The positive aspect is that they are a clean slate and are teachable.

Flexibility: the negative aspect of their flexibility is resistance to a procedure which can result in bodily harm. The positive aspect is with any horse as they get rid of their braces, softness and flexibility are the things we look for. This is an asset in younger more flexible horses. They are not as strong, so forcing their compliance is lessened, and allows us the freedom to drift with them while they find a way to release the brace, restoring their flexion. With naive foals we need to be cautious not to trigger the negative side of their suspicion. If we present these options to get the horse to find the response that most satisfies their safety and our need, then the options we have discussed result in development of their learning center and they will become more predictable.

Using their mother as a shield: the negative aspect with this is that the foal will try and hide behind the mare and eventually become very evasive. The positive aspect comes when transporting them to a different area. The fact that they can use their mother as a shield will give them less anxiety and they will be more willing to go forward to this new area. If we work at keeping ourselves between the mare and the foal, by moving the mare not the foal, their suspicion will lessen and their curiosity will grow towards us allowing us a leadership role.

Curiosity: the negative side is that their curiosity can get them into trouble. We need to discourage curiosity (e.g., nipping or becoming interested in our equipment and then getting tangled up in it).

The positive side is that they seek out all possible sources of needs such as food and nurturing, which supports leadership.

Suspicion: the negative aspect to suspicion is that it leads to fear and adrenaline spikes, which causes the mannerisms listed above. The positive aspect is that their suspicious nature can be used to discourage them from being overly curious and search for what this new feel from us is about. We can use both this suspicion and the search to our advantage as we move along with them and change their thought, which accomplishes performing our task.

Separation anxiety: the negative aspect of separation anxiety is a lack of focus and they lose all respect. The positive aspect is that it is easy to bring the life up in them, which gives us an opportunity to redirect this energy and make them more responsive to the end of the lead rope.

Resistance: foals that show more resistance and react in a negative way tend to fire off and be more hazardous to themselves and us. Be careful not to cause resistance and get sucked into the argument.

5. Summary

In this presentation, I have discussed:

- Knowing and understanding the balance between the veterinarian, the horse, and our handlers
- Body positioning
- Non-defensive posturing of our body and the horse
- Driving the proper behavior during a procedure and effectively using your handlers
- When using sedation or a lip chain, it is still incumbent upon us to leave them with a positive interaction
- The fact that the human is more intelligent and the horse is more sensitive and how to use their sensitivity to our advantage
- Techniques for getting into the mouth and up the nose
- A few differences in handling foals versus adult horses

In conclusion, I am going to quote the late horseman Ray Hunt:

> "They feel, they think, they make decisions, they change their minds, they get hot, they get cold, they get sick, they get well, and if you cut them the same thing runs out of the them as you and I, life."

They do think, they do make decisions, and they do change their minds. If we understand the ever-changing energy in our interactions, which is caused by all of the predictable parts of being a veterinarian, then we can make adjustments. We can make better decisions, which keeps us safe and the horses healthy. It is our job to create the environment and encourage the body positioning so we can achieve an ideal balance between sweetness and strength. This allows us to make predictable an environment otherwise thought to be unpredictable.

Acknowledgments

Conflict of Interest

The Author declares no conflicts of interest.

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