Proceedings of the 61st Annual Convention of the American Association of Equine Practitioners

Las Vegas, Nevada
December 5–9

Program Chair: Kathleen M. Anderson, DVM

ACKNOWLEDGMENTS
P.O. Eric Mueller, DVM, PhD, 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.

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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

Dear AAEP Members and Guests:

Welcome to Las Vegas and the AAEP 61st Annual Convention! You have come here expecting the very best in equine veterinary continuing education and I am certain you will not be disappointed. Las Vegas has in the past proven to be an outstanding location for our convention and I am confident you will have a great time again this year.

The AAEP strives to present the best CE anywhere for the equine practitioner. For this meeting, Dr. Kathy Anderson, president-elect and 2015 convention program chair, has worked diligently to develop a program that blends practical “take home” information as well as cutting-edge scientific abstracts. The Educational Programs Committee, led by Chair Dr. Eric Mueller and Vice Chair Dr. Phoebe Smith, has provided a tremendous amount of support. All are to be commended for their outstanding efforts on behalf of the membership. Las Vegas will prove to be one of the best AAEP conventions ever.

The AAEP Convention would not be possible without the support of our Educational Partners. Their generosity ensures AAEP is able to offer a high quality educational experience in a fun environment to help our members towards their goal of improving the health and welfare of the horse. During your visit to the trade show, please take a moment to thank all our Educational Partners for their efforts on behalf of AAEP and its members.

As your 2015 President, I was proud to represent the AAEP as I met veterinary colleagues in the United States and abroad. As I traveled on behalf of the AAEP, I continually saw the positive impact of AAEP members upon the veterinary and equine communities. I have also been fortunate to have been supported by an outstanding Board of Directors and I thank each of them for ably working so hard to realize the mission of our organization.

It has truly been an honor serving as your President. Thank each of you for the wonderful work you do every day as equine veterinarians.

Have a great convention!

G. Kent Carter, DVM

Raising the Standard in Horse Health
Greetings AAEP Members, Students and Colleagues!

As this year’s Program Chair, I am pleased to welcome you to Las Vegas for AAEP’s 61st Annual Convention. Whatever your interest – in the lecture hall, in the Trade Show or on the entertainment front, there is something for everyone!

Many thanks to all who helped bring you this program, from staff members to member volunteers, with special kudos to EPC Chair Dr. Eric Mueller, Vice Chair Dr. Phoebe Smith and Mrs. Carey Ross for their tireless commitment to providing an excellent educational program. Of course our program also hinges on the quality and expertise of the speakers and moderators – thank you all!

Some highlights of the meeting included in the 100 hours of CE options are:

- **Keynote Speaker** – Dr. Daniel Siegel will address health, lifestyle and wellness issues amongst veterinarians with a focus on skills to improve our lives. These skills will be further explored in the Sunday afternoon session “Mastering Resilience in Everyday Life” – a must for all juggling the demands of practice and daily life.

- **Kester New Hour** – Drs. Carol Clark, Terry Blanchard and Liz Santschi will present “breaking news” in the areas of medicine, reproduction and surgery.

- **Milne State of the Art Lecture** – Dr. Tom Divers presents “The Equine Liver in Health and Disease.”

- **In-Depth Sessions** – Pre-Purchase Exam and Sales, Neurology, Reproduction, AAEP Touch, Trauma/Wound Management – designed to be interactive and clinically useful information with an emphasis on case presentations.

- **“How To” Sessions** – Clinical Pathology, Field Surgery and Field Imaging give you practical information for integrating practice building techniques and services.

- **Equitarian Session** – a medley of experiences showcasing working equids worldwide and the opportunities for both new and seasoned practitioners to assist them.

- **Business Program** – draws on a Las Vegas phenomenon, the “Zappos” team, for a focus on culture and customer experience, AVMA Economic Report with Michael Dicks, and a strong cast of speakers on strategies for success in practice.

- **Table Topics** – Some back by popular demand and some to address emerging topics – discussion with opportunities to brainstorm and network.

**Foundation Celebration:** There will be a live and silent auction, buffet dinner, and 2-hour open bar from 6-8 pm. Sam Riddle will be performing; he is known for his high energy showmanship and his ability as an accomplished pianist.

**Trade Show** – Nearly 350 vendors will be available to demonstrate the latest technologies as well as answer any questions you have on products and programs. Please be sure to thank our educational partners for their part in the success of this meeting.

Thank you all again for joining us in Las Vegas – I hope you hit the jackpot and this AAEP Annual Convention gives you a great payoff to take home with you!

With best wishes,

Kathleen M. Anderson, DVM
2015 AAEP Board of Directors

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2015 AAEP Awards

**Distinguished Life Member** – Dr. Nat A. White II
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 Educator Award (Academia)** – Dr. Virginia Reef
Awarded to an individual educator who by his or her actions and commitment has demonstrated a significant impact on the development and training of equine practitioners.

**Distinguished Educator Award (Mentor)** – Dr. John W. Lee, Jr.
Awarded to an individual who by his or her actions and commitment has demonstrated a significant impact on the development and training of equine practitioners through mentoring.

**Distinguished Service Award** – Mr. Brad Mitchell
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.
General Instructions for Authors
62nd AAEP Convention
Orlando, FL
December 3-7, 2016

To submit a paper, go to https://aaep2016.abstractcentral.com/

ALL papers must be submitted online by March 15, 2016, 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 (see Scoring Criteria section) 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 non-acceptance. 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.

Format:
- 12 point font size
- Double-spaced
- 1” margins
- Times New Roman font

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. Acknowledgments
   i. Declaration of Ethics
   ii. Conflicts of Interest
7. 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.
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.

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.

Declaration of Ethics:
A Declaration of Ethics statement should be included in the paper under the Acknowledgments section. Authors must declare if they have adhered to the Principles of Veterinary Medical Ethics of the AVMA (https://www.avma.org/KD/Policies/Pages/Principles-of-Veterinary-Medical-Ethics-of-the-AVMA.aspx)

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.

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.

Conflicts of Interest:
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.) A Conflict of Interest statement should be included in the paper under the Acknowledgments section whether a conflict exists or not.

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.

All authors are required 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 appropriately influence, or be perceived to influence, their work.

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. 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.

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

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.

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

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


**Book:**


**Chapter in a book:**


**Proceedings:**


**Footnotes:**

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


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


**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 (city, state, and 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\textsuperscript{a} (10-20 mg/kg IV) and butorphanol tartrate\textsuperscript{b} (0.01-0.02 mg/kg IV).

\textsuperscript{a} Dormosedan® Orion Corporation, Espoo, Finland.

\textsuperscript{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.

**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.

**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. For a copy of this list, e-mail Carey Ross at cross@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 15, 2016, 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 binding guidelines below.

Blinding Guidelines:
• The title page and/or front matter 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.

To request a copy of the AAEP Scoring Criteria, e-mail Carey Ross at 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 has a list of members in various areas of expertise that have agreed to volunteer their time to mentor an author who needs guidance. To see this list, email Carey Ross at cross@aaep.org.

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 presenter before approving AAEP’s program for CEU. These criteria are below.

Evidence of this qualification must be provided to the committee by way of:

1. A biography with credential information (i.e. degrees, diplomas, board certification, advanced degrees, current employment, affiliation and any experience related to the subject matter)
2. Other evidence of special knowledge in the subject area being presented
3. Contact information
4. Letters of reference based on the presenter requirements of the RACE Category will be required by the RACE committee for presenters who do not have board certification, advanced degrees or evidence of special knowledge in the subject area being presented.

Scientific Papers: Guidelines for Authors
62nd AAEP Convention
Orlando, FL
December 3–7, 2016

To submit a paper, go to https://aaep2016.abstractcentral.com/

ALL papers must be submitted online by March 15, 2016, 3:00 p.m. E.T.

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.

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**The “How to” Paper: Guidelines for Authors**

**62nd AAEP Convention**

**Orlando, FL**

**December 3–7, 2016**

To submit a paper, go to [https://aaep2016.abstractcentral.com/](https://aaep2016.abstractcentral.com/)

ALL papers must be submitted online by March 15, 2016, 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.

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.

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

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**Review Paper: Guidelines for Authors**

**62nd AAEP Convention**

**Orlando, FL**

**December 3–7, 2016**

To submit a paper, go to [https://aaep2016.abstractcentral.com/](https://aaep2016.abstractcentral.com/)

ALL papers must be submitted online by March 15, 2016, 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.

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.

Review papers should be formatted as described in the General Instructions for Authors. Review papers should be no fewer than 600 words. No upper word limit.
Abstracts ≤ 250 Words: Guidelines for Authors

For those who intend to publish in a refereed journal

62nd AAEP Convention
Orlando, FL
December 3–7, 2016

To submit a paper, go to https://aaep2016.abstractcentral.com/

ALL papers must be submitted online by March 15, 2016, 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. 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

62nd AAEP Convention
Orlando, FL
December 3–7, 2016

To submit a paper, go to https://aaep2016.abstractcentral.com/

ALL papers must be submitted online by March 15, 2016, 3:00 p.m. ET.

The general theme for the 2016 Business of Practice Sessions is “Transitions.” The intent of this theme is to bring forward information to help equine practitioners successfully navigate the many transitions of equine practice. Several potential topics are listed below, and practitioners with expertise or experience in these areas are encouraged to submit papers to be considered for presentation. Please keep in mind that all submissions must follow the guidelines as outlined below and that accepted “How to” papers are allotted a total speaking time of 20 minutes (15 minutes presentation time + 5 minutes questions). Other papers are allotted 25 minutes total. The following topic suggestions are intended to spark ideas that relate to the “Transitions” theme. We also welcome paper submissions on any topic pertaining to the Business of Practice.

Potential Transitions Topics:

- Navigating career transitions such as student to doctor/intern/resident, trainee to associate, associate to owner/partner, practitioner to retirement
- Succession planning, including grooming successor(s)
- Financing the transition to ownership/partnership
- Transitioning from a solo to multi-doctor practice
- Starting a new ambulatory practice or adding a hospital to an ambulatory practice
- When/how to hire new personnel, first technician, office manager, specialist(s)
- When/how to appropriately add new services to a practice
- Transitioning into new technology and the disruptions that can result
- Transitioning to parenthood - pregnancy, childbirth, nursing, practicing as a new parent, re-entry after maternity/paternity leave
- Transitioning within the industry - clinical practice to industry, writing, management, alternative careers
- Planning for the unexpected (death, injury, etc)

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Guidelines:

Failure to adhere to the following format will result in non-acceptance. 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 this document for 'How to’ paper guidelines).
2. Introduction
3. Solution
4. Results
5. Discussion
6. Acknowledgments
   i. Declaration of Ethics
   ii. Conflicts of Interest
7. 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.

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.

Declaration of Ethics:
A Declaration of Ethics statement should be included in the paper under the Acknowledgements section. Authors must declare if they have adhered to the Principles of Veterinary Medical Ethics of the AVMA (https://www.avma.org/KB/Policies/Pages/Principles-of-Veterinary-Medical-Ethics-of-the-AVMA.aspx)

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. 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.

Conflicts of Interest:
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.) A Conflict of Interest statement should be included in the paper under the Acknowledgements section whether a conflict exists or not.

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.

All authors are required 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.

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. 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.

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
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.

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Deadline:
ALL papers must be submitted online by March 15, 2016, 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 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.

Blinding Guidelines:
- The title page and/or front matter 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. To request a copy of the AAEP Scoring Criteria, e-mail Carey Ross at cross@aaep.org.

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:
“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 Las Vegas 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 200 papers were submitted for the 67 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 more than 50 members of the EPC to create the best possible program for the AAEP membership. Several thousand volunteer hours were spent putting together the Las Vegas program, so please thank them for all their hard work creating this program for you.
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Jim Schumacher, DVM, MS, DACVS

Skin grafting should be considered not only for wounds so large that they can heal by no other means but also to speed healing of wounds that might otherwise heal by second intention. Wounds can be grafted using various easily performed techniques that do not require expensive equipment. Author’s address: Department of Large Animal Clinical Sciences, University of Tennessee, Knoxville, TN 37996; e-mail: jschumac@utk.edu. © 2015 AAEP.

1. Introduction

Skin grafting wounds of horses is performed most commonly to speed healing of wounds so large they cannot heal by second intention, but any healthy open wound can be grafted. Grafted wounds heal more rapidly than do wounds that heal by second intention, and more rapid healing may make grafting more economical than a protracted period of bandaging.

The two basic types of skin grafts are the pedicle graft and the free graft. A pedicle graft is created adjacent to the wound and when transferred to the wound still remains connected to the donor site by a vascular pedicle. A wound healed with a pedicle graft has a good cosmetic appearance because the graft is comprised of all components of skin, but pedicle grafting is not used commonly to cover wounds of horses because mobilizing skin of horses to advance a pedicle graft is difficult. Grafts applied to wounds of horses are usually free grafts.

A free skin graft is a section of skin that has been detached completely from its donor site. An autograft, or isograft, is a free graft transferred from one site to another on the same individual and is, by far, the most common type of free graft applied to wounds of horses. An allograft, or homograft, is a free graft transferred between two members of the same species, and a xenograft, or heterograft, is a free graft transferred from a member of one species to a member of another species. The most commonly used xenograft is the porcine xenografts. Allografts and xenografts provoke an immune response and, therefore, are eventually rejected. They are sometimes applied to a wound as a biological dressing (see Using Grafts as Biological Bandages below).

Free skin grafts are also classified according to whether they include the entire dermis or just a portion of it. Full-thickness grafts contain the epidermis and the entire dermis, whereas split-thickness grafts contain the epidermis but only a portion of the dermis. Full- or split-thickness skin grafts can be applied as sheets (i.e., sheet grafts) or implanted within the wound (i.e., island grafts). Each technique of free skin grafting has benefits and drawbacks, and the technique selected, therefore, depends on the circumstances, such as the size or location of the wound, the necessity for cosmesis,
available instrumentation, experience of the surgeon, and the owner’s finances.

The free graft attaches to the wound by fibrin within minutes after it is applied and is nourished initially by imbibing plasma into the graft’s vessels, a process referred to as plasmatic imbibition. Within 2 to 3 days, capillaries and fibroblasts from the wound invade the fibrin. The new capillaries invade other vessels within the graft, establish new vascular channels into its dermis, and anastomose with capillaries in the graft. The graft is revascularized by day 5 and is attached firmly to the wound by day 10. Wounds of horses most commonly grafted are granulating, but fresh tissue is more likely to accept a graft than is granulation tissue because fresh tissue is more vascular. Grafting a wound while the wound is fresh is better than waiting to graft until the wound has filled with granulation tissue. Immature granulation tissue accepts a graft more readily than mature granulation tissue for the same reason. Chronic granulation tissue, therefore, should be trimmed to below the wound’s edge, and grafting postponed until the wound has filled with fresh granulation tissue. The wound should be free of signs of inflammation before it is grafted, and removing the upper portion of the granulation bed not only allows the wound to fill with new, highly vascular granulation tissue, it also removes bacteria and polymorphonuclear cells that contribute to inflammation.

The most common cause of failure of a skin graft to be accepted by a wound is infection, but other causes are accumulation of fluid beneath the graft, shearing forces between the wound and the graft, excessive inflammation of the wound, and poor vascularity of the wound. The wound becomes infected when its concentration of bacteria exceeds its ability to rid itself of bacteria, but the concentration necessary to infect a wound is much less for some bacteria, particularly β-hemolytic streptococci and pseudomonads, than for others. Granulating wounds on the distal portion of limbs of horses are inherently more inflamed than are wounds located elsewhere on the body. Grafts applied to wounds of the distal portion of the limbs of horses, therefore, may be more prone to failure than are grafts applied to wounds proximal to the hock and carpus. An accumulation of exudate, blood, or serum beneath a graft prevents fibrin from attaching the graft to the wound and obstructs revascularization of the graft. Shearing forces between the wound and the graft, caused by movement of the bandage, disrupt fibrin and impair revascularization.

A wound should be assumed to be infected if it shows signs of inflammation. To resolve infection of a granulating wound, the appropriate antimicrobial drug is best administered topically because antimicrobial drugs administered systemically are likely to fail to reach a therapeutic concentration within the surface of granulation tissue. Streptococcae are nearly always susceptible to a β-lactam antibiotic, whereas pseudomonads are usually susceptible to an aminoglycoside antibiotic. A topically applied β-lactam antibiotic may become ineffective in resolving a streptococcal infection if other bacteria in the wound are not susceptible to the β-lactam antibiotic secrete β-lactamase, an enzyme that inactivates β-lactam antibiotics. The efficacy of the β-lactam antibiotic can be preserved, however, by concurrently administering clavulanic acid, a potent inhibitor of β-lactamase. Most bacteria and the inflammatory cells they attract can be removed from an infected, granulating wound simply by excising the superficial layer of the granulation tissue, the site where the bacteria reside.

2. Preparing the Wound for Grafting

Chronic granulation tissue should be trimmed to below the wound’s edge, and grafting postponed until the wound has filled with fresh granulation tissue. The wound should be free of signs of inflammation before it is grafted, and removing the upper portion of the granulation bed not only allows the wound to fill with new, highly vascular granulation tissue, it also removes bacteria and polymorphonuclear cells that contribute to inflammation. Hair surrounding the wound should be clipped, and the wound should be cleansed with an antiseptic solution or with 0.9% saline solution. Skin surrounding the wound should be cleansed with an antiseptic soap, but application of soap to the wound should be avoided because soap may inflame the wound and drive bacteria deep into the granulation tissue.

3. Island Grafting

The simplest method of skin grafting is island grafting, a technique in which small discs, plugs, or strips of full-thickness or partial-thickness skin are implanted into a granulating wound for the purpose of increasing the area of skin from which epithelial cells can migrate to cover the wound. Types of island grafts applied to wounds of horses include punch grafts, pinch grafts, and tunnel grafts.

Punch grafts are full-thickness plugs of skin harvested and implanted into granulation tissue using skin biopsy punches. The grafts can be harvested directly from an inconspicuous site on the horse, such as the portion of the neck that lies beneath the mane or the perineal region, by using a 6- to 8-mm diameter skin biopsy punch. The small wounds created by the punch are usually left unsutured to heal by second intention.

Subcutaneous fascia must be excised from each graft to expose dermal vasculature to permit plasmatic imbibition and revascularization. To excise subcutaneous fascia from a punch graft, one clinician stretches the subcutaneous fascia by applying tension to a hemostat attached to the subcutaneous fascia while grasping the plug with a thumb forceps in the other hand (Fig. 1). Another clinician slices the tensed subcutaneous fascia from the graft.
Pinch grafts are small discs of skin, 3 mm or less in diameter, that are implanted into shallow pockets created in granulation tissue. They are harvested from an inconspicuous site, such as the perineum, neck, ventrolateral aspect of the abdomen, or ventral aspect of the pectoral region because each site of harvest heals with a small scar. Hair is removed from the donor site, and the site is cleaned and desensitized by injecting local anesthetic solution subcutaneously. Skin is most easily excised for excision by using a 20-gauge, hypodermic needle with a bent point, and the tented skin is most easily excised with a No. 11 scalpel blade. The graft is placed onto the wound and a pocket into which the disc is to be implanted is created in the granulation tissue distal to the graft by inserting a No. 15 scalpel blade. The grafts are inserted into the pockets with the graft's epidermis oriented toward the surface of the wound. No consideration need be given to the direction of the hair in the graft because, as with punch grafting, orienting the hair in a direction to match that of the hair surrounding the wound is tedious and has little or no effect on the cosmetic outcome. Implantation should begin distally.

The thin layer of granulation tissue covering each pinch graft usually sloughs within 2 weeks. The superficial, pigmented portion of pinch and punch grafts usually sloughs at approximately this same time exposing the nonpigmented portion of the graft, which may be difficult to discern from surrounding granulation tissue, creating the false impression that the pinch or punch graft has failed to be accepted. Advancing epithelium, which appears as a red ring surrounding each pinch or punch graft, is observed by approximately 3 weeks, and these rings of epithelium expand until they converge to cover the entire wound.

Tunnel grafts are strips of split- or full-thickness skin implanted beneath the surface of granulation tissue. Tunnel grafts can be harvested using a variety of techniques and can be implanted with the horse anesthetized or sedated. Common sites for harvesting tunnel grafts include the ventral aspect of the flank, the neck, and the cranial pectoral region. Tunnel grafts can be harvested by creating linear wheals, several centimeters wide and slightly longer than the width of the recipient bed, by subcutaneously injecting isotonic saline solution (if the grafts are harvested with the horse anesthetized) or local anesthetic solution (if the grafts are harvested with the horse sedated). A straight intestinal forceps is applied to the wheal, and skin protruding between the jaws of the forceps is excised with a scalpel blade. The thickness and width of the graft is determined by the amount of skin protruding between the jaws. If the strip of skin is full thickness,
the donor site is sutured or stapled, and subcutaneous fascia must be excised from the graft to expose the dermis for plasmatic imbibition and revascularization. If the strip of skin is split thickness, the partial-thickness wound at the donor site is left unsutured.

Full-thickness strips of skin can also be harvested from a 2- to 3-cm wide, full-thickness, elliptical section of skin excised from the cranial pectoral region or ventrolateral aspect of the flank. The section of skin is divided into 2- to 3-mm-wide strips after sharply excising subcutaneous fascia to expose the dermal vasculature to permit plasmatic imbibition and revascularization. The donor site is closed in one or two layers. Split-thickness tunnel grafts can be cut from a split-thickness section of skin harvested from the thorax or the abdomen (see Split-thickness Sheet Grafting below).

A tunnel graft is implanted by pushing the shaft of a long, thin alligator forceps through the granulation tissue in the wound, perpendicular to the long axis of the limb, at a depth of 2 to 4 mm until the end of the forceps emerges at the far side of the wound. An end of the graft is grasped by the forceps, and the forceps, with the graft in tow and epidermis oriented toward the surface of the wound, is pulled back through the wound (Fig. 2). The protruding ends of the graft are anchored to the skin with a suture, staple, or cyanoacrylate glue.\textsuperscript{16–18} Grafts are implanted approximately 2 cm apart. A wound too convex to be spanned by the alligator forceps is implanted in two steps. In this circumstance, the end of the forceps is inserted into the granulating wound at the wound’s margin and exited at the center of the wound. One end of the graft is grasped by the forceps and pulled through the tunnel. The end of the forceps is then inserted into the granulation tissue at the margin of the opposite side of the wound and exited close to or at the site of entry of the graft in the center of the wound. The end of the graft is grasped by the forceps and pulled back through the wound. The granulation tissue overlying the graft is likely to slough within a week exposing the revascularized graft, provided that the graft was implanted shallowly (i.e., at ≤ 2 mm) (Fig. 3). If the graft was implanted deeply (e.g., at 4–5 mm), granulation tissue overlying the grafts must be excised a week or more after implantation.\textsuperscript{16–18}

Island grafting requires little expertise and no expensive equipment and can nearly always be performed without anesthetizing the horse. Island grafts are often accepted by wounds incapable of
accepting a sheet graft, such as wounds in areas of high motion. Because island grafting is tedious, it is usually reserved for small wounds. Island grafting should only be used for situations where epithelial scarring is irrelevant to the owner. Because island grafts must be implanted in the wound, they can be applied only to granulating wounds.

4. Full-Thickness Sheet Grafting

A full-thickness graft, which is composed of epidermis and dermis, is usually harvested from the pectoral region, where the skin is relatively mobile. The graft can be harvested with the horse sedated after desensitizing the donor site by depositing local anesthetic solution subcutaneously. The donor skin is incised with a scalpel and then excised from underlying subcutaneous tissue using scissors. The donor site is sutured in one or two layers and a stent bandage is sutured to the sutured wound. Subcutaneous fascia is sharply excised to expose the dermis. The recipient site must be desensitized with local or regional anesthesia when the graft is attached with staples or sutures (Fig. 4). The graft can be attached to the margin of the wound using cyanoacrylate glue without using local or regional analgesia, but to attach the graft using glue, the graft must be larger than the wound. The graft should be attached to the recipient site with slight tension to open the small dermal vessels for plasmatic imbibition and revascularization.\(^\text{19}\)

Although full-thickness grafts can be accepted by granulating wounds, full-thickness grafting is usually reserved for fresh, clean wounds. Full-thickness grafts require more nourishment than do split-thickness grafts and have fewer exposed blood vessels for plasmatic imbibition and revascularization.\(^\text{9,20}\) Full-thickness sheet grafts can be harvested and applied without using expensive instruments and without anesthetizing the horse. Wounds healed with a full-thickness skin graft are more cosmetic than are wounds healed with any other type of graft. Full-thickness grafts, however, can be used to cover only small wounds because the largest graft that can be harvested from the cranial pectoral area, while allowing the donor site to be easily closed primarily, is an ellipse usually not wider than 8 cm. The owner should be cautioned that the sutured donor site might dehisce but reassured that an open wound in the pectoral region heals rapidly and cosmetically.

5. Split-Thickness Sheet Grafting

A split-thickness sheet graft is composed of epidermis and a portion of dermis and is harvested by dividing the dermis with a power-driven dermatome, a drum dermatome, or a free-hand knife. Power-driven dermatomes and drum dermatomes require little experience to operate and allow harvesting of grafts of precise width and thickness. They are, however, expensive, require skilled maintenance, and may malfunction. Drum dermatomes are seldom used to harvest split-thickness sheet grafts from horses because the maximum length of the graft that can be harvested is limited to the length of the circumference of the drum.

Split-thickness sections of skin large enough to graft extensive wounds of horses can be harvested economically with any one of a variety of free-hand dermatomes manufactured for harvesting skin of human beings. A free-hand knife is much less expensive than a power-driven dermatome or a drum dermatome, requires no maintenance, does not malfunction, and can be transported easily. A split-thickness sheet graft can be harvested from the ventral aspect of the abdomen, the least conspicuous donor site, with a free-hand knife, whereas harvesting split-thickness skin from this area with most power-driven dermatomes is difficult to impossible.

The most easily used free-hand knives have an adjustable roller to control the depth of the cut and use a disposable blade. An example of such a knife is the Watson skin grafting knife\(^a\) (Fig. 5). The position of the adjustable roller is controlled by a calibrated knob at one end of the roller. The roller is locked in position by tightening a knob at the other end. The depth at which the skin is cut is governed by the distance between the adjustable

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\(^{a}\) The Watson skin grafting knife is an example of a free-hand knife used for harvesting skin.
The horse must be anesthetized to harvest a split-thickness sheet graft. The donor site of a split-thickness graft should be at an inconspicuous location because the donor site heals leaving a large epithelial scar. The ventral aspect of the abdomen is the least conspicuous donor site, but harvesting from this site using a power-driven dermatome is extremely difficult. To harvest a split-thickness skin graft from the ventral aspect of the abdomen, a free-hand knife is generally required. For this procedure, the horse is positioned in lateral recumbency, hair is clipped from the ventral aspect of the abdomen, and the ventral aspect of the abdomen is scrubbed with an antiseptic soap. The ventral aspect of the abdomen should protrude over the edge of the table so that the table does not restrict up and down movement of the knife during harvest. Draping is not necessary. Harvesting usually begins at the umbilicus and proceeds cranially, and so, if the surgeon is right handed, the graft is more conveniently harvested with the horse positioned in right lateral recumbency. After lubricating the knife and donor site with isotonic saline solution, the knife is pressed into the abdomen with moderate pressure at an angle of 5 to 10°, and the graft is harvested by moving the blade up and down and forward (Fig. 5). The surgeon should pause to examine the graft and donor site after harvesting several centimeters to determine whether the dermis is being cut at the desired thickness. The depth of cut can be changed by repositioning the adjustable roller, by changing pressure applied to the knife, or by changing the angle of incidence at which the knife is held. When a sufficient length of graft has been harvested, the knife is tilted upward to separate the graft from the abdomen. The graft is fixed to the wound’s margin with sutures, staples, or cyanoacrylate glue. Applying the graft after the horse recovers from anesthesia removes the risk of damage to the graft that might occur during recovery. The wound’s margin must be desensitized by using local or regional anesthesia if the graft is applied with sutures or staples after the horse recovers from anesthesia. A graft applied to a large wound or a wound in a highly mobile region, such as the dorsum of a fetlock or hock, can be further secured to the wound with simple-interrupted sutures placed through the graft into the wound. These sutures can be inserted with the horse standing without using local or regional anesthesia if the tissue underlying the graft is granulation tissue because granulation tissue has no sensory innervation.

Split-thickness sheet grafts are accepted more readily than are full-thickness grafts because split-thickness grafts require less nourishment and have more open vessels in the dermis for plasmatic imbibition and revascularization. They can be harvested in sheets large enough to cover wounds too large to be covered by a full-thickness graft or island grafts. Although the appearance of a wound healed with a split-thickness skin graft is superior to that of a wound healed with island grafts, it is inferior to that of a wound healed with a full-thickness skin graft. Harvesting a split-thickness graft is more expensive and less convenient than harvesting a full-thickness graft or island grafts because to harvest a split-thickness graft, the horse must be anesthetized. Piliation at the donor site of a split-thickness skin graft is often poor, and epithelial scarring may be extensive.

6. **Meshing Sheet Grafts**

Although a split- or full-thickness sheet graft can be applied to a wound as a solid or meshed sheet, most are meshed before being applied to a horse, usually to expand the graft so that the graft is able to cover a wound larger than the graft itself (Fig. 6). Meshing a sheet graft also allows escape of exudate or blood interposed between the graft and the wound and enables a topically applied antimicrobial drug to contact a large portion wound. Because fibrin fills the fenestrations, a meshed graft is more stable than a nonmeshed graft, and because a meshed graft can expand, it is better able to tolerate motion. A graft can be meshed tediously with a scalpel blade or easily and rapidly with a meshgraft dermatome. A relatively inexpensive meshgraft dermatome is the Padgett mechanical skin mesher, which consists of staggered, parallel rows of blades housed in an aluminum frame (Fig. 7). The graft is pressed into the blades with a rolling pin. The staggered pattern in which the graft is cut allows the graft to be expanded to three times the graft’s original area. Other meshgraft dermatomes are...
available that allow the graft to be expanded from one and one-half to nine times the original area of the graft. Full-thickness sheet grafts of horses are difficult to mesh on commercial mesh graft dermatomes and so usually must be meshed manually. To manually fenestrate a sheet graft, the graft is attached to a sterile piece of cardboard or Styrofoam with hypodermic needles, and, using a scalpel blade, staggered, parallel rows of incisions are created in the graft. The longer and more numerous the incisions, the greater the expansion. Applying a meshed and expanded graft exposes small portions of the wound within the fenestrations (Fig. 6), and these exposed portions heal by contraction and epithelialization leaving a pattern of diamond-shaped epithelial scars.

7. Aftercare

The grafted wound is covered with a sterile, nonadhering dressing, to which an appropriate antimicrobial agent has been applied. The dressing is secured to the wound with conforming rolled gauze, and a heavy bandage is applied to the region. The bandage is usually not changed for 4 to 5 days after grafting to avoid disrupting the graft, but if nosocomial infection with virulent bacteria is a common problem, the bandage should be changed daily. An antimicrobial drug effective against both β-hemolytic Streptococcus and Pseudomonas spp., such as ticarcillin with clavulanic acid, should be applied topically to the dressing when each bandage is changed. The graft can be considered accepted when it has revascularized and this usually occurs by approximately day 5. The superficial portion of the graft, especially that of a full-thickness graft, sometimes fails to vascularize and becomes desiccated, making it difficult to determine whether the graft has been accepted. The desiccated tissue eventually sloughs revealing vascularized dermis (Fig. 8). The exposed dermis may closely resemble granulation tissue but has a paler color.

8. Storing Split-Thickness Sheet Grafts

Skin grafts placed in sterile isotonic 0.9% saline solution or lactated Ringer’s solution can be stored in a refrigerator for at least week. By using a culture medium, such as McCoy’s 5A medium, to which a small volume of serum has been added, the viability of the stored graft can be extended to at least 3 weeks. The concentration of serum in the medium should be between 10 and 33 percent. To avoid an antigenic reaction to the serum, a commercially available, antibody-free equine serum or the horse’s own serum or should be added. Stored skin can be used to regraft the wound if the primary graft is partially or completely lost. A graft harvested when a horse is anesthetized for treatment for a wound can be stored until the wound is healthy enough to permit grafting. A wound accepts a stored graft more readily than it does a fresh graft because grafts stored for 24 hours or more undergo anaerobic metabolism, which causes release of metabo-
lites that encourage angiogenesis and rapid vascularization of the graft.27

Acknowledgments

Declaration of Ethics

The Author declares that he has adhered to the Principles of Veterinary Medical Ethics of the AVMA.

Conflict of Interest

The Author declares no conflicts of interest.

References and Footnotes


What You Should and Should Not Put In or On a Wound

Dean A. Hendrickson, DVM, MS, DACVS

There are many different dressings and potions marketed for treating equine wounds. Not all of them have evidence of safety or efficacy. It is prudent to understand what effect the dressing will have on the wound before using it. Author's address: Colorado State University, Fort Collins, CO 80523; e-mail: dean.hendrickson@colostate.edu. © 2015 AAEP.

1. Introduction

The main concept in wound cleaning is to remove the necrotic tissue and other debris from the wound, while also reducing the bacterial load. The reduction in necrotic debris and bacteria will help the wound to heal more effectively, both functionally and cosmetically. Consequently, it is important to choose a technique that does not cause more necrosis and subsequent bacterial growth. Every cleaning agent and technique will cause some trauma to the wound. It is therefore important to weigh the cost to benefit of the technique prior to starting. In essence, the benefit of a clean wound must be weighed against the trauma that the agent will cause.

Cleaning agents will generally cause some type of chemical trauma leading to cellular toxicity. The most biocompatible cleaning agent should always be chosen to limit the toxicity to the wound bed. Various cleaning agents will be discussed further in this section. Human chronic wound healing groups have a saying: you should not put something in a wound that you would not be willing to put in your eye. It is a great way to consider limiting the trauma associated with most of our wound-cleaning agents. I think this is especially true in the field of veterinary medicine where we are often entrenched in historical use of cleaning agents and techniques.

Cleaning techniques also have the potential to cause trauma to the wound bed. Most techniques will cause some type of mechanical trauma to the wound. This is especially true when mechanical forces such as scrubbing or high-pressure lavage are used. The trauma to the tissue left behind must be considered when choosing a cleaning technique. The hope is that the veterinarian will choose wisely when selecting wound-cleaning agents and techniques so that wounds will heal quickly and effectively.

2. Saline (Isotonic and Hypertonic)

Isotonic saline has been shown to be as effective as 1% povidone-iodine (PI) in reducing infection rates in the human emergency room. Although the isotonic nature is gentle to the wound bed and unlikely to cause necrosis of the surrounding tissue, isotonic saline is somewhat acidic and it may be better to use a polyionic replacement fluid. Hypertonic saline (20%) is very effective in reducing bacterial numbers in the wound. However, it can be traumatic to nor-
mal cells as well. Hypertonic saline should be used only in wounds that are obviously infected.

3. PI
PI has been used extensively in equine wound care. However, the bulk of research shows that PI is very limited in reducing bacterial numbers in the wound. In one study, isotonic saline reduced bacterial numbers better than PI,\(^2\) whereas in another study there was no difference between PI and isotonic saline in bacterial reduction.\(^1\) In a third study, PI was not an effective substitute for wound debridement.\(^3\) Consequently, PI should only be used around the wound over intact skin, and never in the wound itself.

4. Chlorhexidine
Chlorhexidine has been used since 1946. It has shown a lot of promise in reducing the bioburden of bacteria in intact skin. However, there is limited evidence showing that it can effectively reduce bacterial numbers in wounds without causing further trauma to the wound bed. In a study evaluating the antimicrobial effect of chlorhexidine and saline for irrigating a contaminated open fracture, there was no benefit to using chlorhexidine.\(^4\) Similar to PI, the toxicity to the wound bed most likely leads to tissue necrosis.

5. Hydrogen Peroxide
Hydrogen peroxide (HP) is popular for its effervescent effects. However, HP has not been shown to have antibacterial effects.\(^5\) There may be some limited benefit with regard to wound debridement but probably not enough to warrant the use of HP.

6. Acetic Acid
Common distilled vinegar has been shown to be beneficial in reducing pseudomonas infections in human patients. It is thought that the low pH of the acetic acid creates an environment that cannot be tolerated by select bacteria.\(^6,7\) It is generally used as a 0.25 or 0.5% solution with a 15-minute/day soak or compress. The wound should be rinsed with saline after therapy with acetic acid.

7. Surfactant-Based Cleansers
Surfactant-based cleaners are generally isotonic and are considered minimally toxic and irritating to the wound bed. They generally contain some type of surfactant such as polysorbate-20 or pluronic F-68. In 2005 Wilson et al\(^8\) showed the surfactant-based cleaners were the least toxic of all wound-cleaning agents studied. The most toxic formulations were HP, modified Dakin’s solution (sodium hypochlorite and boric acid), and PI. The surfactant-based cleaners are very effective on minimally contaminated wounds and should be applied, allowed to sit for 1 to 2 minutes, rinsed off, and reapplied as necessary.

8. Topical Antibiotic Silver
Silver is a very effective antimicrobial agent. It has been used in many forms in veterinary medicine, most commonly as silver sulfadiazine cream (SSD). Newer formulated silver dressings also show great promise in reducing bacterial numbers in wounds. In a study comparing the use of SSD and nonadherent wound dressings, the SSD group had less exuberant granulation tissue.\(^9\) In a human burn study, the silver-impregnated dressings lead to significantly reduced length of hospital stay, analgesic use, wound infection, and inflammation compared with SSD.\(^10\)

9. Topical Antibiotic Nitrofurazone
Nitrofurazone was first approved for the use in animals in 1979 and has been applied to wounds in staggering volumes since that time. Yet, research from as early as 1979 has shown that nitrofurazone retards the healing rate by as much as 24%.\(^11\) Nitrofurazone should not be used in open wounds.

10. Topical Antibiotic Triple Antibiotic Ointment
Triple antibiotic ointment has been used since the 1950s. Research has shown the combination of polymixin B, bacitracin, and neomycin to have synergistic effects on bacterial reduction in wounds.\(^12\) A very interesting perspective on triple antibiotic ointment is that the bacterial susceptibility to it has basically remained unchanged since its discovery. Along with silver, it is one of the best topical agents available to use in a wound.\(^13\)

11. Wound Dressings
There are probably as many different wound dressings as there are wound cleaning agents. This lecture will focus on the agents the presenter has the most experience with.

Wound dressings should be chosen based upon the wound’s stage of healing. The author is not aware of any single dressing that provides benefits throughout all stages of the wound healing process. Consequently, the appropriate dressing will vary through the wound treatment. In general, the stages of wound healing can be divided into the following: debridement, wound moistening, granulation tissue development and wound contraction, and epithelialization. The wound should be kept moist in all of these stages as moist wounds will generally heal in half the time as wounds left exposed to air; as long as the appropriate dressing is chosen.

12. Debridement Dressings
Debridement dressings are designed to remove bacteria and necrotic tissue from the wound. Debridement dressings should often be combined with some type of sharp debridement where the bulk of the
necrotic tissue is removed from the wound prior to dressing application.

Hypertonic Saline

Hypertonic saline dressings are woven gauze dressings impregnated with 20% saline. They provide an aggressive, nonselective debridement. They work by drawing the fluid out of bacteria and diseased cells, reducing their attachment to the wound bed, and then lifting them out of the wound when the dressings are changed. The author uses the most effective debridement dressings commercially available. You can make your own hypertonic saline by dissolving 200 g salt in 1 L boiling water. Lower concentrations of hypertonic saline do not seem to be as effective. This debridement dressing should be discontinued when the wound no longer seems infected.

Antimicrobial Dressings

One manufacturer’s antimicrobial dressing is a loosely woven gauze impregnated with polyhexamethylene biguanide (PHMB). PHMB is an antimicrobial that disrupts the cell walls of microorganisms. There is no developing resistance known to PHMB. The dressing was originally developed to apply over a wound to stop bacterial penetration (it is also used in baby wipes and contact lens cleaning solutions). It is now accepted that the dressing will kill bacteria when placed into a wound as well. It comes as a dry dressing and should be moistened with saline prior to use in a wound. Similar to hypertonic saline, this dressing should not be used as a primary dressing after the wound has been effectively debrided and the bacterial numbers have been appropriately reduced. It can, however, be used as a secondary dressing to limit bacterial penetration to the wound bed.

Honey

Honey has been used to improve wound healing for centuries. Some types of honey, such as honey derived from specific plants like the Manuka bush, seem to have even more antimicrobial effect than would be seen with the natural hyperosmolality present in all honey. Not all honey is created equal in this effect, so only honey that is known to have antimicrobial benefits (Manuka honey) should be used in wound care.

13. Moistening Dressings

Although most of the wounds presented to the veterinary practitioner are necrotic and infected, some wounds are dry, often from inappropriate wound care. In these wounds, a gel dressing should be applied to “donate” moisture to the wound and improve the wound healing process. Gel dressings commonly contain water, glycerin, and a polymer. Some gel dressings incorporate a gauze that helps them maintain their normal shape. Either the amorphous or the formed dressings can be used to add moisture to a dry wound. As soon as the wound is moist, another dressing should be used.

14. Granulation Tissue Development and Wound Contraction Dressings

Calcium Alginate (Alginate)

In the past, exuberant granulation tissue has been the bane of the equine practitioner, and to think that you might choose a dressing specifically to encourage granulation tissue would have been frowned upon. However, one of the complications of equine wound healing is the lack of inflammatory response that is formed by the horse after wounding. Calcium alginate dressings will lead to an effective inflammatory process that will help wound healing proceed in an effective order. Another valuable benefit of the alginate dressings is that they contain a lot of calcium that is “donated” to the wound to encourage wound contraction. These dressings can also be placed directly on exposed bone that has been curetted to minimize bone sequestrum formation. As soon as granulation tissue fills the wound, these dressings should be discontinued.

15. Epithelialization Dressings

Semiocclusive foam dressings help to finish off the wound healing process. The foam dressings will increase the surface temperature of the wound by 1–2°F, which will preferentially select for epithelialization. They are a relatively closed-cell design so that the granulation tissue does not grow into the foam. The added benefit of the AMD foam is that it contains the same PHMB as described above in the debridement dressings to limit bacterial growth on the surface of the wound. It is not recommended to add any other agent to the wound during the epithelialization process.

In summary, there are many options for wound-cleaning agents and wound dressings that have either negative effects or unknown effects on the wounds. It is the veterinarian’s job to make sure to select a wound cleaning agent or a dressing that will encourage the most functional and cosmetic end result. If you are not sure what the dressing does, you should make sure to find out before using it. Many of these materials have quite detrimental effects on the wound.

Acknowledgments

Declaration of Ethics

The Author declares that he has adhered to the Principles of Veterinary Medical Ethics of the AVMA.

Conflict of Interest

The Author has written a book on equine wound care but has no conflict with the companies that manufacture the dressings and cleaning agents described in this article.
References and Footnotes


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Get the Home Field Advantage in Managing the Traumatized and Infected Wound

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Successful management involves considering the wound’s entire ecology, including the source and extent of any contamination, the presence of bacterial refugia (foreign bodies, surgical implants, devitalized tissue, inflammatory/necrotic debris, bacterial biofilms), the patient’s immunocompetence, and tissue perfusion, in addition to identifying the wound pathogens and determining their antibiotic sensitivities. Author’s address: New Bolton Center, University of Pennsylvania, School of Veterinary Medicine, 382 West Street Road, Kennett Square, PA 19348; e-mail: orsini@vet.upenn.edu. © 2015 AAEP.

1. Introduction

Wound infections are common in equine practice, but most respond to routine antibiotic therapy and wound care, so they’re not often considered a hot topic for discussion. However, wound infections are becoming more of a challenge as multiple antibiotic resistance becomes more common among bacterial pathogens in humans and animals. Multidrug-resistant strains are still relatively uncommon in equine practice, but we have seen a dramatic increase in their prevalence and diversity in the past 20 years, particularly in hospitalized patients (i.e., nosocomial infections) but also on-farm (i.e., community-acquired infections). It’s time we readjust our expectations and start anticipating that at least some wound infections are and will be caused by multidrug-resistant pathogens against which we have few good antibiotic options.

In other cases, antibiotic sensitivity is not the issue; rather, tissue perfusion or the patient’s immunocompetence is the limiting factor. We may not think of these host factors as primary determinants in the development and persistence of wound infection until the patient has failed to respond to antibiotic therapy, by which time the infection has metaphorically run away with the ball.

Getting the home-field advantage with serious wound infections basically means making the best use of the local conditions and resources. We know this field and we know the players; we simply must make the best use of our knowledge. In particular, we must get into the habit of looking beyond our customary reliance on antibiotics primarily or exclusively, and address the wound ecology globally. Targeted antibiotic therapy that is directed by the results of bacterial culture and antibiotic sensitivity testing remains a cornerstone of effective management for serious wound infections. However, other factors beyond in vitro antibiotic testing are crucial to a successful outcome in severely traumatized and infected wounds.

2. Extensive Contamination

Some wounds in horses are complicated by extensive contamination, which results in overwhelming numbers of bacteria in the wound. Examples include:
Treatment Approach

For wounds that remain contaminated with fecal matter or other material with a large bacterial load, the following dictum is useful: “The solution to pollution is dilution.” High-volume lavage is indicated for these wounds. Many of these wounds also require surgical debridement and establishment of good drainage.

Lavage

Thoroughly irrigate the wound with sterile isotonic fluid until all visible contamination and purulent discharge have been removed:

- Use sterile 0.9% saline, Lactated Ringer’s, or Hartmann’s solution.
- Use copious quantities, as much as necessary to visibly and thoroughly cleanse the wound.
- Use moderate delivery pressure, in the range of 10–15 pounds per square inch (psi); this range can be achieved by forcefully depressing the plunger on a 20- or 30-mL syringe through an attached 18-gauge needle.
- Direct the fluid into every part of the wound.
- Repeat lavage as necessary.

Avoid using profoundly hypotonic fluids, such as plain water because of the difference in osmolality and the associated tissue injury; use plain water only as a last resort, and only to remove gross contamination. Also avoid delivery pressures much greater than 15 psi, because high pressures may cause cell damage and/or drive bacteria deeper into the tissues. Conversely, delivery pressures much lower than 10 psi are ineffective.

Adding an antiseptic such as povidone-iodine or chlorhexidine to the lavage fluid is unnecessary and unlikely to provide additional benefit to high-volume, pressurized lavage. Not only is the contact time too brief, but some antiseptics (eg, povidone-iodine) are inactivated by organic matter.

Debridement

Surgical debridement is another important component of management for wounds that contain heavily contaminated and compromised tissue:

- Debride all tissue that looks devitalized or irreparably damaged (e.g., necrotic skin, fascial tags, frayed tendon or ligament, soft and discolored bone).
- Open the wound as much as necessary to allow thorough lavage and debridement; there is no value in preserving the healing/healed skin incision if it encompasses infected tissue.
- Repeat lavage to remove any residual debrided tissue.
- Repeat debridement as necessary.

Maggot debridement therapy may be worth considering as an alternative or adjunct when complete surgical debridement is difficult or impossible without causing damage to viable tissue. Maggot therapy may be particularly useful in contaminated wounds with deep or multiple tracts, such as penetrating injuries to the foot and fistulous withers.¹ ²

Not only does debridement reduce bacterial numbers by removing heavily contaminated material from the wound, it also enhances the effectiveness of wound lavage by removing physical impediments to fluid flow. In addition, debridement aids subsequent drainage of inflammatory debris and infected material from the wound.

Drainage

Severely infected wounds tend to be effusive, often with copious accumulation or discharge of purulent material. This bacteria-laden, inflammatory exudate must have an egress for the immune system to effectively control the infection and orchestrate wound repair. Thus, drainage is a key component of wound management, not just for wounds involving extensive contamination, but for every infected wound.

Encourage gravity-assisted (“dependent”) drainage from the most distal or ventral parts of the wound, taking advantage of the location, orientation, and shape of the wound where possible. If necessary, surgically create a drainage portal in the distal/ventral portion of the wound.

In so doing, avoid opening the wound any more than is necessary to allow thorough lavage, debridement, and continued drainage, as it is important to avoid exposing the interior of the wound to further contamination. Along the same lines, take care when using Penrose drains and setons because these
strategies may suck or wick bacteria into the wound. For deep bone infections, thorough surgical debridement under general anesthesia may best be followed by use of an antibiotic-impregnated implant such as polymethylmethacrylate beads, collagen sponges, or sterile plaster of Paris, and the wound closed primarily, leaving only a small opening for further drainage.

The exception to the rule is when there is gas accumulation in the deeper tissues. For these wounds, the larger the egress, the better. Wounds that act as one-way valves, such as deep wounds to the axilla or inguinal area, suck and trap air within the tissues, sometimes causing spectacular subcutaneous emphysema that may take several days to dissipate. Clostridial myonecrosis is often accompanied by extensive subcutaneous and intramuscular gas accumulation that may extend along fascial planes. These wounds are best left open, and enlarged if necessary, to prevent air trapping in the first instance and facilitate treatment of the anaerobic infection in the second.

Manual and Movement Therapy

Wound drainage may be further encouraged with controlled movement. Specific techniques depend on the nature of the wound, the stage of healing, and the potential for harm with excessive or inappropriate movement. Massage and/or passive range-of-motion exercises may be appropriate for horses restricted to a stall. To avoid complications, manual therapy is best directed by a physical therapist or veterinary rehabilitation therapist when treating wound infections (see American Association of Rehabilitation Veterinarians: www.rehabvets.org).

Hand-grazing or hand-walking, and even limited turnout in a pen or small pasture, may be appropriate for more ambulatory patients. Avoid unattended turnout when wound dressings include bandages or leg wraps that may loosen and either leave the wound exposed or cause injury during unrestricted activity.

Movement may have additional benefits by helping to optimize blood flow to, from, and within the wound; limit or prevent restrictive fibrosis; prevent or resolve digestive and musculoskeletal problems associated with inactivity; relieve the stress, social isolation, and/or boredom of confinement; and otherwise improve the patient’s physical and psychological health and wellbeing. These aspects of nursing care are particularly important when the nature of the wound necessitates stall confinement for weeks or months.

Negative-Pressure Wound Therapy

Drainage may also be achieved with negative-pressure wound therapy (NPWT), also known as vacuum-assisted closure. Subatmospheric pressure of between 75 and 125 mm Hg is applied to the wound using a small vacuum pump and occlusive dressing. This technique speeds wound closure, evacuates inflammatory fluid and debris (including bacteria) from the wound, and improves microcirculation within the wound bed. Originally developed for use in human medicine, NPWT is increasingly used in small-animal medicine for grossly contaminated or otherwise-complicated wounds. Developments in dressing design and in pump size and portability make NPWT a viable option for use in horses as well.

Avoiding Transmission

Not only do infected wounds in animals carry the potential for human infection (zoonosis), but humans can transmit pathogenic bacteria such as Staphylococcus aureus, S. epidermidis, and enterobacteria to the animal patient (reverse zoonosis or “humanosis”). To avoid transmission in either direction, communicate well with the nursing staff and encourage the practice of good hygiene: wear gloves or wash hands with soap and warm water before and after handling the wound or dressings, and promptly dispose of all dressings and cleaning materials in the garbage or a biohazard container.

3. Bacterial Refugia

In this context, a refugium is any substance or circumstance that protects bacteria within the wound from the host’s defenses and from effective concentrations of antibiotic drugs, thus enabling the infection to persist and potentially to progress. Refugia commonly associated with wound infections in horses include:

- Foreign bodies—most often pieces of metal, wood, or other plant material (thorns, awns, etc.).
- Surgical implants—metal plates, screws, pins, wire; surgical mesh; prostheses; embedded suture, etc.
- Devitalized tissue—bone, muscle, tendon, ligament, fascia, or skin.
- Inflammatory/necrotic debris—pockets or pools of free purulent material, abscessation.
- Mucoid biofilms produced by the bacteria themselves.

We are already in the habit of considering the presence of foreign bodies and most of these other items in persistent wound infections, but bacterial biofilms have not received as much attention in veterinary medicine as they have in human medicine, where they commonly contribute to catheter-related infections and chronic wounds. Treatment of wounds complicated by foreign bodies or other inanimate refugia is fairly straightforward, requiring surgical removal, debridement, and/or drainage. However, bacterial biofilms require a somewhat different approach.

Bacterial Biofilms

Bacterial biofilms are not a new phenomenon, but their importance in the persistence—and resis-
Bacterial biofilms are gel-like substances composed of an extracellular matrix of polysaccharides, proteins, glycolipids, bacterial DNA, water, the bacteria that secreted the matrix, and often other microbes that are taking advantage of the protected environment. 14,15

Although most biofilms in wounds are microscopic, a well-established biofilm may be visible to the naked eye as a shiny coating or sheen on the surface of the affected tissue or implant, and it may feel slippery or slimy. 15 Some bacteria in biofilm mode produce pigments (e.g., Pseudomonas aeruginosa produces a greenish pigment). 15 Discoloration of the wound surface or dressing is not a reliable indicator of a specific pathogen, nor of the presence of a biofilm and does not replace a properly sampled and handled culture. Unlike serious exudate, purulent discharge, and sloughed cells, a bacterial biofilm is not easily washed away.

The following dynamics are described 15 for bacterial biofilms under experimental conditions that are similar to those found with naturally occurring wounds:

- Within minutes, bacteria attach to an available surface.
- Within 2–4 hours, strongly attached microcolonies have formed.
- Within 6–12 hours, an initial biofilm has formed, which becomes increasingly well organized and tolerant of antiseptics and antibiotics.
- Within 2–4 days, the biofilm is mature; it is now highly resistant to adverse host and environmental influences, including antiseptics and antibiotics, and it sheds bacteria singly and as microcolonies within fragments of biofilm that can colonize other areas.
- Within 24 hours of mechanical disruption, the mature biofilm has reformed.

The biofilm provides a secure attachment for the bacteria and a moist, stable, and nutrient-rich environment that protects against desiccation, deluge, and other adverse physical conditions. 14,15 It also protects the bacteria from the host’s defenses and from potentially lethal concentrations of antibiotic drugs. 15,16

Depending on the antibiotic, diffusion through the matrix may be impeded and/or the drug may be inactivated by chemical constituents of the matrix. In the presence of a mature biofilm, the minimum inhibitory concentration of an antibiotic or antiseptic to which the wound pathogens are susceptible in vitro may be increased by as little as 2-fold and as much as 200-fold. 15–19 Furthermore, bacteria within biofilms alter their metabolism and gene expression in ways that promote survival, including the formation of subpopulations that are metabolically inactive (quiescent), and thus relatively invulnerable to antibiotics at any concentration. 15,16,20

Even more problematic is the sharing of antibiotic resistance among bacteria within the biofilm. In a polymicrobial biofilm, antibiotic-binding or -degrading substances secreted by bacteria that are resistant to a particular drug may also protect bacteria within the biofilm that are susceptible to that drug. In addition, there is potential for antibiotic-resistance genes to be transferred from one bacterial species to another within the protected environment of the biofilm. 15,16

Polymicrobial biofilms are documented to occur in equine wounds of various types (traumatic, surgical, acute, chronic). 21,22 Under experimental conditions, many different bacteria isolated from wounds or normal skin in horses are capable of producing biofilms. 20,22 However, the conditions under which clinically relevant biofilms form in equine wounds require further study. In humans, problematic biofilms are most common in wounds with poor perfusion, in malnourished patients, and in those with comorbidities that impair immune function (e.g., in diabetic ulcers and severe burns). 14–16

This aspect of wound care is paramount when dealing with a biofilm-infected wound; even though many different wound pathogens are capable of producing a clinically relevant biofilm, most do not because tissue perfusion and bactericidal host defenses are adequate to prevent its establishment.

Treatment Approach

Bacterial biofilms present a particular therapeutic challenge. Not only do they protect the enclosed bacteria from host defenses and from antibiotics, but they are highly resistant to chemical damage. A mature biofilm is relatively impervious to common antiseptics, alcohols, acids, bleach, hydrogen peroxide, and other generators of oxygen radicals (e.g., ozone) unless concentrations are used that are toxic to the host cells. 16,17

Plaque on dental enamel is a good example, and a handy reminder, of how quickly bacterial biofilms can form in a suitable environment and what is required to remove them. The biofilm must be physically degraded to expose the bacteria within to antibacterial substances, which then slow the reformation of the biofilm. Mouthwashes are of limited and short-term benefit if not preceded by thorough tooth brushing and flossing. Another essential element is frequent removal (i.e., regular tooth brushing).

Biofilm-infected wounds require the same three-step approach: 15–17,23

1. **Physically Degrade the Biofilm**

   Vigorously debride the wound to remove purulent discharge, necrotic tissue, and any discernible biofilm, even if it causes minor bleeding; in fact, fresh blood contains antibacterial components that facilitate Step 2. The goal is to remove as much of the biofilm and associated bacteria as possible and expose the remaining bacteria to biocides (Step 2).
Depending on the wound, options include the following:

- Drag a gauze swab (i.e., mildly abrasive material) across the surface.
- Use pulsed lavage (e.g., waterpik) at moderate pressure to dislodge and flush away the biofilm (this option is better than swabbing for very uneven surfaces).
- Use low-frequency ultrasonic debridement.
- Draw the sharp or blunt edge of a scalpel blade across the surface.

Sharp debridement and low-frequency ultrasonic debridement are most studied in human wound care.\textsuperscript{15–17,23} Surfactants such as polyhexamethylene biguanide (polyhexanide [PHMB]) may be useful adjuncts because they reduce the surface tension of the biofilm, which facilitates its degradation and removal.\textsuperscript{15}

**AXIOM I**

Wear gloves to protect against zoonotic and reverse-zoonotic infections; and when using a waterpik, also wear a surgical mask and protective eyewear, particularly if the wound infection is known or suspected to involve methicillin-resistant *S. aureus* (MRSA) or other multidrug-resistant bacteria.

This step may be uncomfortable for the patient, so use sedation and local or regional anesthesia. For deep or extensive wounds, and for very uncooperative patients, general anesthesia may be needed for the first debridement and periodically thereafter until the wound infection is under control.

2. **Prevent or Delay Reconstitution of the Biofilm**

Once the protective matrix has been removed or significantly degraded, most of the bacteria within the biofilm resume the antibacterial sensitivities they would have outside of a biofilm.\textsuperscript{15–17} If current culture and sensitivity results are available, use the most appropriate antibiotic(s) both topically and systemically.\textsuperscript{16} The efficacy of regional antibiotic delivery is also likely to be greatest immediately after debridement of the biofilm.

If sensitivity results are still pending, apply a topical broad-spectrum antibacterial cream,\textsuperscript{16} such as povidone-iodine, chlorhexidine, 1% silver sulfadiazine,\textsuperscript{23,24} or 1% hydrogen peroxide.\textsuperscript{24} Raw honey is another option.\textsuperscript{16,25} The biofilm bacteria are susceptible to a wide variety of biocides immediately after debridement, including ionic silver solutions\textsuperscript{16,26} and ozone,\textsuperscript{27} but the best choices may be those that have a residual effect by virtue of their adherent properties (gels, creams, honey). Antibiotic-containing dressings, such as silver-impregnated wound dressings,\textsuperscript{26,28–30} inhibit biofilm formation and re-formation, but their efficacy decreases after the first 24 hours,\textsuperscript{29} so these dressings must be changed frequently. An added advantage to topical silver and other antibacterial agents is that they increase the susceptibility of biofilm bacteria to antibiotic drugs.\textsuperscript{16,29}

Plasma (natural or hyperimmune) may also be useful by inhibiting bacterial adhesion and replication.\textsuperscript{31–33} Platelet-rich plasma products may aid in wound healing, but the plasma component seems to have the principal bacteriostatic effect,\textsuperscript{33} so it may not be necessary to take the extra steps of concentrating the platelet portion of the plasma for this purpose. Whether antigen-specific equine hyperimmune plasma (see Section 4. Immunocompromise, below) is superior to plain plasma for biofilm inhibition remains to be determined, but it is plausible that antigen-specific hyperimmune plasma would be of greater benefit when the specific pathogen has been cultured from the wound.

Other antibiofilm agents under investigation include lactoferrin, xylitol, ethylenediaminetetra-acetic acid (EDTA), RNA III inhibiting peptide, dispersin B (a bacterial glycoside hydrolase), galium, acetylalicylic acid, various phytochemicals, bacteriophages (antibacterial viruses), glucose oxidase, various proteases, UV light, and low-voltage pulsed electric fields.\textsuperscript{16,34,35}

Regardless of which methods are selected, the aim is to target the biofilm bacteria both from above (topically) and below (systemically and/or regionally).\textsuperscript{15–17}

3. **Repeat Frequently for as Long as Needed**

Mature biofilms can reform in as little as 24 hours after debridement, so there is a narrow window of opportunity (≤ 72 h) in which any bactericidal substances will have a significant effect on the biofilm population.\textsuperscript{15–17,23} Repeat steps 1 and 2 daily or every other day until the infection is well under control, as evidenced by a reduction in the signs of infection and the resumption of wound healing. Most biofilms are microscopic, and bacterial culture of biofilm-protected inhabitants may be unrewarding or incomplete.\textsuperscript{15,21,22} So until a practical, stall-side biofilm identification test is available, clinical signs are the best guide. Also, reassess frequently and add or change to a different method of debridement (Step 1) or inhibition (Step 2) as needed.

Each step is equally important. Physically degrading the overlying biofilm restores the susceptibility of the bacteria to antibacterial agents; however, with a well-established biofilm in a severely infected wound, one round of treatment is not sufficient. Hence, the need for daily treatment, or treatment at least every other day, using a combination approach until the infection is resolved or well on the way.\textsuperscript{15–17,23}

Admittedly, this intensity of treatment would disrupt the wound bed and interfere with wound repair in an uncomplicated wound. However, biofilm-infected wounds are not simple or straightforward wounds, and many are chronic.\textsuperscript{14} Wound repair cannot proceed normally in the presence of a bacte-
rial biofilm, so the priority is to address the biofilm
and thereby resolve the infection. Thereafter, the
intensity and invasiveness of treatment can be re-
duced, and tailored to the rate and quality of wound
repair.

The higher initial costs of this approach are coun-
terbalanced by lower overall costs. In human
patients with chronic, biofilm-infected wounds, fre-
fquent debridement as a component of a multifaceted
approach shortens the duration of treatment and de-
creases the overall costs of wound care.17

**Biofilms on Surgical Implants**

Biofilms on unstable surgical implants are best ad-
dressed by implant removal. Biofilms on stable
surgical implants are more of a challenge because
there may be no other indication for implant re-
moval, and good reason to leave the implant in place
if it is still performing the function for which it was
inserted (e.g., a bone plate stabilizing a fracture).
In such cases, it may be best to surgically expose the
implant (or infected portion) and perform Steps 1
and 2 under general anesthesia in a sterile surgical
suite, then pack the wound with an antibiotic-im-
pregnated material (polymethylmethacrylate beads,
collagen sponges, or plaster of Paris)36 in place of
Step 3.

**AXIOM II**

Biofilms in wounds are most likely to form and per-
sist in the presence of immunocompromise and/or
poor perfusion, so these conditions must also be
identified and addressed for effective treatment.

**4. Immunocompromise**

We often overlook the fact that antibiotic therapy
alone is inadequate in the face of an incompetent
immune system. Antibiotics are only ever adjunc-
tive therapy, albeit a very important component of
the management for serious wound infections.
The patient’s immunocompetence is of primary im-
portance in the prevention and treatment of wound
infections.

A number of conditions or circumstances may
compromise the patient’s ability to prevent or res-
olve bacterial infection, including:

- Age—especially neonatal foals (< 2 wk) and
  very old horses.
- Antibody deficiency—failure of passive trans-
  fer of maternal antibodies (neonatal foals);
  congenital deficiencies of Ig production; pro-
  tein-losing enteropathy, nephropathy, or der-
  matopathies (i.e., extensive third-degree burns,
  large open wounds).
- Malnutrition—of macronutrients (i.e., protein-
  calorie malnutrition) or micronutrients (i.e.,
  specific trace mineral, essential fatty acid, or
  amino acid deficiencies).
- Chronic physical or psychological stress—int-
  ense athletic training, long-distance trans-
  port, competitive events, social isolation, sus-
  tained hypothermia (i.e., cold stress), hos-
  pitalization, etc.
- Diseases involving the pituitary-adrenal
  axis—notably, pituitary pars intermedia dys-
  function (PPID) or equine Cushing’s disease,
  which causes hypercortisolemia ± hyperglycemia.
- Corticosteroid administration.
- Sepsis (causes early hyperinflammation then
  severe immunosuppression); other concurrent
  systemic infection or debilitating systemic
disease.

Hyperglycemia is a risk factor for wound infec-
tions and biofilm formation in human patients,16 but
there is little evidence that wound infections are
more likely or more problematic in horses with hy-
perglycemia unrelated to PPID, possibly because hy-
perglycemia is an inconsistent feature of equine
metabolic syndrome and diabetes mellitus is rela-
tively rare in horses.

**Treatment Approach**

In most cases, patients with serious wound infec-
tions have already undergone initial systemic as-
essment and treatment. Even so, it is wise to
review or further investigate the patient’s signal-
ment, history—including recent medications, physi-
ical examination findings, and blood work—and
repeat the physical examination and/or blood work
as needed.

If possible, treat any condition identified that may
be compromising immune function. For example, a
severely infected wound in an older horse with PPID
is unlikely to respond well to wound treatment until
the PPID is controlled with pergolide.

When no specific treatment is available, and
even as adjunctive therapy to complement specific
treatment, consider administering supplemental
plasma.31,32 Fresh plasma from a compatible do-
nor living on the same premises (and thus exposed
to the same pathogens) is suitable. Antigen-spe-
cific hyperimmune plasma may be of particular
value when the wound pathogen is known. Com-
mercial hyperimmune plasma31 is available in the
United States for Escherichia coli, Salmonella
spp., Streptococcus equi, Rhodococcus equi, and
various Clostridium spp. Plasma may be applied
topically and/or administered intravenously.

Also take care to avoid immunosuppressive ther-
apies such as corticosteroids and cryotherapy (ther-
apeutic hypothermia).37 Although both are highly
effective anti-inflammatory therapies, they also in-
crease the risk for infection. If corticosteroids are
considered necessary, such as for initial treatment of
systemic inflammatory response syndrome, then use
a short-acting drug (e.g., dexamethasone) and a
short course of treatment (≤ 48 h).
General Supportive Care

Good nursing care and nutritional management are also important, particularly when specific treatment for the immunocompromising state is not possible:

- Maintain clean, dry bedding in a well-ventilated stall that is large enough for the horse to lie down and rise without risk of injury.
- Provide compatible company to prevent the stress of social isolation; even if the infection dictates that the horse be physically isolated from others, isolation stalls or barns should not cause social stress or sensory deprivation.
- Keep the area quiet and dark at night to encourage rest.
- Feed a well-formulated diet that provides adequate protein, calories, and micronutrients, yet is low in simple carbohydrates (starch and sugars)\(^3^8\); base the diet on high-quality forages, and when extra calories are needed, use supplemental fat (e.g., vegetable oils, rice bran).

5. Poor Perfusion

An effective immune response to bacterial invasion relies not just on local tissue resources but also on the delivery of leukocytes and molecules (complement, antibodies, oxygen, nutrients, etc.) to the site of infection via the systemic circulation. Thus, any circumstance that limits optimal blood flow to, or at, the site of infection inevitably limits the host’s immune response, as well as its wound repair capabilities, which renders the wound vulnerable to re-infection. Examples include the following:

- Sustained hypovolemia or hypotension (e.g., endotoxemia).
- Thrombotic states (e.g., disseminated intravascular coagulation, vasculitis).
- Extensive tissue trauma, particularly crushing, tearing, or strangulating injuries that result in local ischemia.
- Fibrosis at or proximal to the site of injury.
- Location of a foreign body, surgical implant, or devitalized tissue that impedes blood flow.
- Pressure from an improperly applied bandage or cast, or from postural necessity (e.g., recumbency).
- Severe local or regional edema (inflammatory or dependent).

Edema is so common with infected wounds that it is easily overlooked as a potentially problematic factor, other than its adverse effect on patient comfort and client perception of treatment efficacy. However, severe edema, whether inflammatory or merely dependent, may adversely affect blood flow at the site of infection by creating local interstitial pressures that exceed the perfusion pressure of the small vessels and capillaries, thereby decreasing tissue perfusion within the wound bed and interfering with wound healing.\(^3^9\)

Treatment Approach

Blood flow is tightly regulated, so there is a limit to how much blood flow can be increased in any tissue. Nevertheless, serious wound infections that are complicated by poor perfusion are difficult to manage unless any impediments to optimal blood flow are identified and addressed:

- Ensure good hydration, provide specific treatment for systemic disorders as needed.
- Remove any impediments to blood flow within the wound; in particular, debride traumatized tissue that has been ischemic for longer than a few hours and is becoming, or already, necrotic.
- Use nonsteroidal anti-inflammatory drugs to address any vasculitis and to control inflammatory edema, but avoid corticosteroids and cryotherapy if possible, as they are also immunosuppressive.
- Control dependent edema using manual and movement therapy (see Section 2. Extensive Contamination: Drainage, above) and compression bandages where appropriate; apply the bandage from distal to proximal, and take care to avoid excessive or focal pressure.
- Consider using NPWT if available (see Section 2. Extensive Contamination: Drainage, above).

Oxygen Therapy

When blood flow cannot be improved further, one of the oxygen therapies may be useful. Hyperbaric oxygen therapy increases the oxygen tension in the tissues, which facilitates all aerobic processes (both defensive and reparative) and is potentially lethal to obligate anaerobes.\(^4^0,4^1\) It may also stimulate the release of stem cells from the bone marrow into the peripheral blood.\(^4^2\)

Ozone therapy and 1% hydrogen peroxide cream are other options. Oxygen and hydrogen peroxide are oxygen donors, but their primary value in wound care may be as biocides,\(^2^4,2^7,4^3–4^6\) as they increase the concentration of oxygen-derived free radicals within the wound, which supplements the bacterial oxidative burst by neutrophils. Medical ozone may be delivered topically or intravenously (dissolved in sterile 0.9% saline solution); topical ozone may be delivered in gaseous form (ozonized air or medical-grade oxygen), in solution (dissolved in water or isotonic saline), or as ozone-infused oil (e.g., vegetable oil).\(^2^7,4^4,4^5\)

AXIOM III

Oxygen radicals also cause oxidative damage to the patient’s cell membranes, so overuse of these therapies may be counterproductive.\(^4^5\)
Antibiotic selection and delivery. Following are some recommendations for optimal wound management, particularly in wounds containing biofilms. Even when conventional culture techniques are now known to identify only some of the bacterial genera and species that may be found in wounds, particularly in wounds containing biofilms. Inherent antibiotic resistance—i.e., inappropriate drug choice for the pathogen(s) involved. Acquired antibiotic resistance—e.g., methicillin resistance in *S. aureus*; unlike inherent resistance, acquired resistance is unpredictable. Inappropriate drug dosage, route, or duration of treatment—each may result in subtherapeutic antibiotic concentrations at the site of infection, even when the pathogen is susceptible to the drug. Poor perfusion—it, too, may result in subtherapeutic antibiotic concentrations at the site of infection, even with an appropriate drug choice and dosage. Protection from inhibitory or lethal antibiotic concentrations by refugia, particularly purulent material, necrotic tissue, and bacterial biofilms.

The last two points are covered in earlier sections. Following are some recommendations for optimal antibiotic selection and delivery.

**Treatment Approach**

Effective antibiotic therapy involves these three elements:

1. Identify the principal wound pathogens.
2. Determine or confirm their antibiotic sensitivities.
3. Achieve effective concentrations of the antibiotic(s) at the site of infection.

It is essential to perform bacterial culture and antibiotic sensitivity testing with serious wound infections, and repeat it if the wound has not responded to therapy as expected. Time spent on providing empiric antibiotic therapy is time wasted if culture and sensitivity testing is not already underway. It is crucial to determine antibiotic sensitivity—and resistance—for the particular wound pathogen(s) as soon as possible.

Conventional culture techniques are now known to identify only some of the bacterial genera and species that may be found in wounds, particularly in wounds containing biofilms. Even when concerted efforts are made to sample the biofilm, bacteria in biofilm mode, especially the metabolically inactive subpopulation, can be difficult to grow in culture. For this reason, rapid molecular methods of bacterial identification are increasingly being used to guide antibiotic therapy in human patients with chronic wounds. But although molecular identification methods are becoming more widely available in commercial microbiology laboratories, we are still left with the current culture-based methods of determining antibiotic sensitivities in each case. Thus, routine bacterial culture and antibiotic sensitivity testing remains an essential component of management for serious wound infections.

**Ensuring Reliable Culture Results**

In addition to aseptic sample collection and prompt submission in appropriate transport media, it is important to sample the surfaces and materials where the wound pathogens are most likely to reside, factoring in the various types of refugia that may complicate infected wounds in horses. Be sure to collect samples from the deepest recesses of the wound, and sample multiple sites if the wound has multiple pockets, fissures, or layers. If a biofilm is suspected, draw the swab across the surface with enough pressure to collect a sample of the biofilm, but not enough pressure to cause bleeding given that blood contains antibacterial elements. Superficial culture of a wound with a biofilm is likely to yield an incomplete and potentially misleading sample.

Whenever possible, include tissue samples from the wound (e.g., debride and submit some of the debrided material). Wound exudate is easier and less invasive to collect, but tissue samples from the site of infection are more likely to yield reliable results. For wounds involving or suspected to involve a synovial structure (joint, tendon sheath, or bursa), aseptically collect a sample of synovial fluid using percutaneous aspiration at a site distant from the wound. Use the same techniques and precautions required when tapping a joint for other diagnostic or therapeutic purposes.

Also submit any other material removed from the wound, such as a foreign body, surgical implant, or sequestrum. In fact, submit all representative samples. It is false economy to submit only one sample when several different types of material (e.g., exudate, implant, tissue, biofilm) have been collected. Confirming that the bacteria cultured from a foreign body or infected implant are the same as those cultured from the wound tissues is valuable; finding that different bacteria were cultured from the object is more valuable still, particularly if the wound is complicated by a biofilm.

**Concurrent Antibiotic Use**

Collect all samples before starting or changing antibiotic therapy. If the horse is already receiving antibiotics, either suspend therapy or aim to collect the culture samples immediately before the next scheduled dose. Ideally, suspend therapy for a period that is at least 8 hours longer than the dosing interval; for example, for antibiotics administered every 12 hours, suspend therapy for at least 20 hours before collecting samples for culture. This extended interval allows the residual bacteria to
re-enter a log phase of active growth, making positive culture more likely. However, when time is of the essence, proceed with the culture immediately, and notify the laboratory staff of the horse’s antibiotic regimen and timing of the most recent dose in relation to sample collection.

The goal is not simply to identify the pathogens that may be insensitive to the current antibiotic regimen, but to form as complete a picture as possible of the microbial ecology of the wound to formulate an antibiotic treatment plan that addresses each pathogen and its sensitivities.

Interim Antibiotic Selection

With serious wound infections, interim systemic therapy with an experience-guided (empiric) antibiotic selection can be critical to a successful outcome while awaiting culture and sensitivity results. Table 1 lists the common bacteria isolated from various types of wounds in horses as a basis for empiric antibiotic choices that are most likely to be useful.

Unless a specific pathogen with a well-established sensitivity pattern is strongly suspected (e.g., penicillin for clostridial myonecrosis), broad-spectrum antibiotic therapy generally is the best approach for interim therapy with serious wound infections. A combination of penicillin G (crystalline or procaine penicillin) and gentamicin is widely used in horses, but for some pathogens penicillin G is best replaced with a synthetic penicillin (e.g., ampicillin) or a cephalosporin (e.g., ceftiofur, cefazolin, cephalothin), and amikacin is sometimes a better choice than gentamicin.

It is also wise to collect a separate swab or tissue sample from the wound and perform a Gram stain in-house. This simple procedure provides immediate information that can be very useful in directing interim antibiotic therapy. Table 2 lists some key cytologic features of common equine wound pathogens, and Table 3 lists likely antibiotic sensitivities for each pathogen based on published studies of various equine populations in North America, Europe, Australia, and New Zealand during the past 20 years.

AXIOM IV

Antibiotic sensitivity patterns are quite variable and are always evolving. Sensitivity patterns vary among geographic regions and are heavily influenced by the extent of antibiotic use in the particular population of animals and their human caretakers. Pathogens isolated from hospitalized horses or those on farms where antibiotics are frequently used are more likely to show antibiotic resistance than are pathogens isolated from horses at premises on which antibiotic use is less common. Thus, these published antibiotic sensitivity patterns should be used as a general guide only, to aid in making rational choices for interim therapy, and never as a substitute for patient-specific, current bacterial culture and antibiotic sensitivity results.

Options for Multidrug-Resistant Bacteria

At the present time, two antibiotic drugs remain in the arsenal for multidrug-resistant bacteria: vancomycin and imipenem. Note, however, that bacterial resistance is now documented for both drugs, so they are by no means “silver bullets,” and they will not remain viable options in veterinary or human medicine if they are used indiscriminately. Their use should therefore be reserved for situations in which culture and sensitivity results clearly indicate sensitivity to the particular drug and absence of reasonable alternatives.

Vancomycin

Vancomycin is active against many Gram-positive bacteria, both aerobes and anaerobes, including MRSA and Clostridium difficile. Vancomycin, either alone or in combination with an aminoglycoside, is therefore an option for serious infections caused by Staphylococcus sp. or Enterococcus sp. that are resistant to all other available options.

The recommended systemic dosage in horses is 7.5 mg/kg every 8 hours, administered in saline by slow IV infusion over 30 minutes. For infections confined to the distal limb, vancomycin may be administered by regional perfusion, either IV or intraosseous delivery. A vancomycin dosage of 300 mg in 60-mL sterile isotonic saline seems well tolerated and achieves therapeutic concentrations in the synovial fluid and medullary sinusoidal plasma of the perfused area. These regional modes of delivery help optimize the treatment of vancomycin-susceptible infections in the distal limb while minimizing the cost of treatment, potential for toxicity, and development of bacterial resistance.

Imipenem

Imipenem-cilastatin is active against a wide range of Gram-positive and Gram-negative organisms, both aerobes and anaerobes, including multidrug-resistant enteric pathogens (E. coli, Klebsiella sp., Salmonella sp., etc.), and most strains of P. aeruginosa and Bacteroides fragilis. However, it is not effective against MRSA, and resistance is documented for some Enterococcus sp.

A recommended dosage in horses is 10–20 mg/kg by slow IV infusion every 6 to 8 hours. Intravenous administration is described in case studies, the dose ranging from 10 to 250 mg. Imipenem may also be administered by IV regional perfusion. A dose of 500 mg imipenem in 100-mL sterile isotonic saline, delivered into the cephalic or saphenous vein, sustained imipenem concentrations in the metacarpal/tarsal/halsal joint well above the minimum inhibitory concentration of most susceptible pathogens for approximately 6 hours. Smaller perfusion volumes are also described for IV regional perfusion, but the safety of higher imipenem concentrations (solutions > 5 mg/mL) have not been established.
### Table 1. Common Bacterial Isolates From Various Wounds in Horses, and Recommendations for Interim Systemic Antibiotic Therapy

<table>
<thead>
<tr>
<th>Tissue Involved</th>
<th>Common Genera</th>
<th>Therapeutic Considerations</th>
</tr>
</thead>
</table>
| Skin (normal microflora) | Predominant: *Staphylococcus, Enterococcus*, *Bacillus Micrococcus*  
Lesser: *Streptococcus, Streptomyces*, other nonenteric *Corynebacterium pseudotuberculosis* (western US) | Environmental contamination, particularly with fecal matter, and human contact can alter the normal skin flora. These contaminants often play an important role both in traumatic and surgical wound infections. genera; **Base empiric antibiotic selection on the deeper structures involved**, as detailed below. |
| Wounds (in general) | Acute: *Staphylococcus, Aerococcus*, *Micrococcus, Alcaligenes, Bacillus, Propionibacterium, Acinetobacter, Clostridium*  
Chronic: *Pseudomonas (aeruginosa)*, *Staphylococcus (epidermidis, aureus)*, *Serratia, Enterococcus, Enterobacter, Proteus* | Causal organisms are often resistant to penicillin, so use a cephalosporin + an aminoglycoside (e.g., ceftiofur + gentamicin/amikacin)  
**Enrofloxacin alone is a reasonable alternative in adult horses.**  
For ulcerative lymphangitis in *C. pseudotuberculosis* endemic areas, consider a penicillin or a macrolide |
| Subcutis (cellulitis/ ulcerative lymphangitis) | Most common: *Staphylococcus*  
Less common: *Streptococcus*, Gram-negative aerobes, anaerobes; *C. pseudotuberculosis* (western U.S.) | **Enrofloxacin alone is a reasonable alternative in adult horses**  
Add metronidazole for wounds on the distal limb or otherwise likely to have fecal contamination (i.e., likely presence of obligate anaerobes)  
As for synovial structures |
| Synovial structure (joint, tendon sheath, bursa) | Traumatic: Gram-negative enteric genera, *Streptococcus, Staphylococcus*, anaerobes; polymicrobial infection is common  
Surgical: *Staphylococcus, Streptococcus*, *Enterobacter*, other enteric genera, *Pseudomonas* | **Enrofloxacin alone is a reasonable alternative in adult horses** |
| Bone or physeal cartilage | Traumatic: *Enterobacter, Streptococcus, Staphylococcus*; in young foals, Gram-negative enteric genera  
Surgical: *Streptococcus zoopneumonia, S. aureus*, other *Streptococcus* sp. | A cephalosporin (or penicillin) + an aminoglycoside + metronidazole (many *Bacteroides* sp. are resistant to penicillin and cephalosporins)  
**A penicillin (penicillin G or ampicillin) + gentamicin. Ceftiofur can be substituted for penicillin, and amikacin for gentamicin. Add metronidazole if there is extensive tissue necrosis or dead space** |
| Distal limb or foot | Polymicrobial infection that typically includes Gram-negative enteric genera and, with the foot, anaerobes such as *Bacteroides* | As for synovial structures |
| Respiratory tract (e.g., penetrating head, neck, or chest wounds) | Combinations of Gram-positive aerobes (especially *S. zoopneumoniae*), Gram-negative aerobes (e.g., *Actinobacillus, Pasteurella*, enterobacteria), and anaerobes (e.g., *Bacteroides, Fusobacterium, Peptostreptococcus*) | A penicillin (penicillin G or ampicillin) + gentamicin. Ceftiofur can be substituted for penicillin, and amikacin for gentamicin. Add metronidazole if there is extensive tissue necrosis or dead space |
| Intestinal tract (e.g., penetrating abdominal or perineal wounds, open drainage for septic peritonitis) | If the bowel wall is penetrated: Gram-negative enteric genera and anaerobes | Penicillin (penicillin G or ampicillin) + gentamicin + metronidazole. Ceftiofur can be substituted for penicillin, and amikacin for gentamicin |
| Muscle | Common: skin microflora  
Gaseous myonecrosis: *Clostridium*  
Deep IM abscess: *C. pseudotuberculosis* (western U.S.) | Penicillin (penicillin G or ampicillin)  
Add metronidazole for clostridial myonecrosis  
Add rifampin for *C. pseudotuberculosis* |

**Interim antibiotic therapy is an empiric drug selection, instituted while awaiting results of bacterial culture and antibiotic sensitivity testing. For all serious wound infections, rational antibiotic therapy must be guided by current culture and sensitivity results.**  
**Note:** Enrofloxacin and other quinolones are not recommended for use in foals.

**Bacteriostats**  
Bacteriostatic antibiotics include tetracyclines, sulfonamides (including trimethoprim-sulfonamide combinations), macrolides (erythromycin, azithromycin, etc.), and chloramphenicol. In general, severe wound infections are best treated with bactericidal drugs (penicillins, cephalosporins, aminoglycosides, quinolones [in adult horses],...
metronidazole, rifampin) rather than bacteriostatic drugs. However, these distinctions are somewhat artificial and do not always reflect the drug’s antibacterial activity in the patient. When laboratory results indicate sensitivity to a bacteriostat and resistance to most or all of the available bactericidal drugs, use of the bacteriostat should at least be considered. In combination with the physical measures discussed in this paper that render the wound environment less hospitable to bacteria, inhibition of bacterial replication may be enough to tip the scales in favor of the host and thus resolution of the infection, particularly when systemic administration is accompanied by local and/or regional antibiotic delivery.

These decisions must be made on a case-by-case basis. If persistence or progression of the wound infection would have life-threatening or career-ending consequences, then an appropriate bactericidal drug should be used, even if it is one of the reserved drugs (vancomycin or imipenem).

Local and Regional Antibiotic Delivery
In most cases of serious wound infection, systemic parenteral antibiotic therapy is indicated, at least initially, particularly if there is evidence of a systemic inflammatory response (fever, malaise, inappetance, neutrophilia/neutropenia, hyperglobulinemia, elevated plasma fibrinogen or other acute phase proteins). However, for sequestered infections, wounds involving surgical implants that must remain in place, infections involving poorly perfused tissue (whether anatomical or pathological), and biofilm-infected wounds, local and/or regional delivery of the antibiotic(s) may also be needed, and may in fact be pivotal to a successful outcome. In some cases, these more targeted modes of antibiotic delivery may obviate the need for ongoing systemic antibiotic therapy.

Local and regional routes of antibiotic delivery include topical application, intrathecal infusion (for wounds involving a synovial structure),72,74 regional IV or intraosseous perfusion (for infections at or below the carpus/tarsus)36,75–85 and antibiotic-impregnated implants.36,86–92 These alternate modes of drug delivery are well covered elsewhere.36,75,86 Suitable antibiotics, recommended dosages, and some clinical notes are summarized in Table 4.

Duration of Treatment
A final component of effective antibiotic therapy is duration of treatment, which is primarily dictated by the patient’s response to therapy:
Reduction and ultimately resolution of inflammation and purulent discharge.

Resolution of any systemic signs of inflammation (fever, malaise, inappetance, neutrophilia, etc.); note: absence of fever is not a reliable indicator in horses on nonsteroidal anti-inflammatory drugs.

Continued improvement in comfort and function.

Negative culture result.

Normal rate of wound healing.

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<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Likely Sensitivity</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gram-positive</strong></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
| *Clostridium sp.*| Penicillins, metronidazole | 1. Exposure to air or other oxygen source (hydrogen peroxide, ozone, hyperbaric oxygen therapy) facilitates antibiotic therapy, and may be sufficient against this obligate anaerobe.  
2. Some clostridial isolates are resistant to metronidazole.  
3. Vancomycin is likely to be effective, but this drug should be reserved for documented multidrug-resistant, vancomycin-sensitive pathogens. |
| *Corynebacterium sp.* | Penicillins, macrolides | 1. Drainage is important for a successful outcome in cases with deep IM abscessation, as antibiotic penetration of the dense abscess wall is poor.  
2. Gentamicin is likely to be effective against many† isolates, but amikacin is unlikely to be effective.  
3. Tetracyclines are likely to be effective against many† isolates.  
4. Vancomycin and imipenem are each likely to be effective, but these drugs should be reserved for documented multidrug-resistant, vancomycin- or imipenem-sensitive pathogens. Resistance to each of these drugs is documented for *Enterococcus sp.* |
| *Enterococcus sp.* | Penicillins, chloramphenicol | 1. Ampicillin is more likely than penicillin G to be effective; cephalosporins are effective against few isolates.  
2. Gentamicin is likely to be effective against many† isolates, but amikacin is unlikely to be effective.  
3. Tetracyclines are likely to be effective against many† isolates.  
4. Vancomycin and imipenem are each likely to be effective, but these drugs should be reserved for documented multidrug-resistant, vancomycin- or imipenem-sensitive pathogens. Resistance to each of these drugs is documented for *Enterococcus sp.* |
| *Staphylococcus sp.* | Cephalosporins, enrofloxacin, aminoglycosides, chloramphenicol, rifampin | 1. Amikacin is more likely than gentamicin to be effective, but susceptibility to amikacin in equine MRSA isolates is variable.  
2. Penicillins, macrolides, tetracyclines, and TMPS are each likely to be effective against many isolates.  
3. Vancomycin is appropriate for documented cases of MRSA when other drugs are not suitable. |
| *Streptococcus sp.* | Penicillins, cephalexins, chloramphenicol | 1. Gentamicin is likely to be effective against many† isolates; amikacin less so.  
2. Enrofloxacin, macrolides, tetracyclines, TMPS, and rifampin are each likely to be effective against many† isolates. |
| **Gram-negative** |                   |                                                                                                                                                                                                                                                                  |
| *Actinobacillus sp.* | Cephalosporins, enrofloxacin, tetracyclines, TMPS, chloramphenicol | 1. Penicillins are inconsistently effective; ampicillin may be more likely than penicillin G to be effective.  
2. Aminoglycosides and macrolides are each likely to be effective against many† isolates. |
| *Enterobacter sp.* | Ceftiofur, enrofloxacin, aminoglycosides | 1. Amikacin is more likely than gentamicin to be effective.  
2. Tetracyclines and TMPS may each be effective against many† isolates. |
| *E. coli, other enterobacteria* | Enrofloxacin, aminoglycosides | 1. Amikacin is more likely than gentamicin to be effective.  
2. Ceftiofur, ampicillin, tetracyclines, TMPS, and chloramphenicol may each be effective against many† isolates.  
3. Resistance to quinolones is documented for *Klebsiella pneumoniae*. |
| *Pasteurella sp.* | Penicillins, cephalexins, enrofloxacin, aminoglycosides, tetracyclines, TMPS, chloramphenicol | 1. Ampicillin may be more likely than penicillin G to be effective.  
2. Gentamicin may be more likely than amikacin to be effective. |
| *P. aeruginosa* | Amikacin | 1. *P. aeruginosa* is resistant to most commonly used antibiotics in equine practice.  
2. Gentamicin and enrofloxacin are each likely to be effective against some isolates, as are carbenicillin, ticarcillin, and piperacillin.  
3. Imipenem use is appropriate for documented multidrug-resistant, imipenem-sensitive infections. |

Abbreviations: IM, intramuscular; MRSA, methicillin-resistant *Staphylococcus aureus*; TMPS, trimethoprim-sulfonamide combinations.

Note: Enrofloxacin and other quinolones are not recommended for use in foals.

*Sensitivity shown for at least 80% of clinical equine isolates.
†Sensitivity shown for 50 to 79% of clinical equine isolates."
<table>
<thead>
<tr>
<th>Route</th>
<th>Drugs and Doses (Adult Horses)</th>
<th>Clinical Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Topical</td>
<td>Silver (e.g., 1% silver sulfadiazine cream, silver-impregnated dressings), raw honey, 1% hydrogen peroxide cream, other antibacterial ointments and dressings ozone therapy</td>
<td>1. Topical antibacterial therapy is particularly important in the treatment of biofilm-infected wounds, along with frequent debridement of the biofilm (see text).</td>
</tr>
</tbody>
</table>
| Intrathecal infusion (joint space, tendon sheath, bursa) | Amikacin: 500–1000 mg  
Gentamicin: 150–500 mg  
Ceftiofur: 150 mg  
Cefazolin: 250–500 mg  
Na/K penicillin: 2–5 million units | 1. Although injecting an antibiotic, use a strict aseptic technique to avoid further contamination.                                                                                                           |
| Regional limb perfusion (IV or IO perfusion of carpus/tarsus or distal limb) | Amikacin: > 250 mg, up to 2.5 g  
Gentamicin: 100–300 mg  
Na/K penicillin: 10–20 million units  
Ampicillin: 10–20 million units  
Ceftiofur: 2 g  
Enrofloxacin: 700 mg (1.5 mg/kg)  
Marbofloxacin: 300 mg (0.67 mg/kg) | 1. Amikacin doses > 250 mg are required to exceed MIC in perfused tissues; the dose used is dictated by the size of the perfused region: 500–1000 mg for smaller areas (e.g., the digit via a palmar/plantar digital vein; the isolated carpus/tarsus); 2–2.5 g when perfusing the distal limb via the cephalic/saphenous vein. |
| 2. The size of the perfused region also dictates the perfusion volume; typically, 20–30 mL for IV perfusion of the digit via a digital vein, 60 mL for larger areas and for IO perfusion, up to 100 mL for IV perfusion of the distal limb via the cephalic/saphenous vein. Higher drug doses in lower perfusion volumes are proving to be well tolerated and effective for some antibiotics (e.g., 500 mg gentamicin q8 10 mL sterile saline for perfusion of the distal limb via a palmar digital vein). |
| 3. Enrofloxacin may cause vasculitis even at the therapeutic dosage, so reserve for documented enrofloxacin-sensitive infections with no other reasonable options. Marbofloxacin appears to be well tolerated. |
| Antibiotic-impregnated PMMA beads         | Suitable drugs: aminoglycosides (gentamicin, amikacin, tobramycin, streptomycin)  
Cephalosporins (cefazolin, ceftiofur) penicillins, metronidazole | 1. When using more than one antibiotic, make a separate batch of PMMA beads for each drug.                                                                                                              |
| 2. Therapeutic concentrations may be sustained in the surrounding tissue for days or weeks, but bead removal may be required after treatment. |
| 3. Metronidazole may also be mixed with hoof acrylic for polymicrobial infections of the foot. |
| Gentamicin-impregnated collagen sponges    | Commercial product containing gentamicin, 130 mg/sponge | 1. Collatamp G2  
2. Gentamicin concentrations in wounds are higher in the first few days after implantation than with PMMA beads.  
3. These implants biodegrade in <60 days, so removal may not be needed.  
4. May be used intrathecally (e.g., septic arthritis or tenosynovitis). |

Abbreviations: IO, intraosseous; IV, intravenous; MIC, minimum inhibitory concentration; PMMA, polymethylmethacrylate.
Treatment response is dependent on many different factors, so it can vary greatly among individual patients. Table 5 summarizes some general guidelines for duration of antibiotic therapy with different types of wounds.

**Monitoring Response to Therapy**

It is important to monitor the horse frequently (at least once a day initially) until the infection is resolved; a positive response should be seen within a few days. Continue antibiotic therapy until signs of infection have resolved completely and wound healing is proceeding as expected.

If there is no improvement within 3 or 4 days of starting or changing treatment, review the case; check that antibiotics are being administered as directed, repeat the physical examination and routine blood work, and further explore and debride the wound as needed.

Repeat bacterial culture and antibiotic sensitivity testing if, at any point, recovery is not proceeding as expected. A single sampling may not be enough, particularly in wounds with polymicrobial infections involving multidrug-resistant organisms.

Re-culture the wound:

- If there is a poor response to treatment.
- If signs of infection recur during therapy or after therapy has concluded.
- During prolonged antibiotic therapy (weeks or months).
- Within a week of discontinuing antibiotic therapy for polymicrobial infections or infections involving multidrug-resistant organisms.

At every turn, adjust the treatment plan accordingly.

Also watch for signs of laminitis with injury or infection that causes a nonweight-bearing lameness in one limb or when infection is accompanied by marked systemic inflammation.

And Then . . .

Once the infection is resolved, methods for enhancing wound healing can be considered. Depending on availability and on the nature and location of the wound, they include delayed closure with suture material, negative-pressure wound therapy, topical application of growth factors or extracellular matrix material, and skin grafting.93

### Table 5. Guidelines for Duration of Antibiotic Therapy in Specific Types of Wounds

<table>
<thead>
<tr>
<th>Tissue Involved</th>
<th>Guidelines for Antibiotic Therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wounds (general)</td>
<td>Staphylococcal infections tend to be insidious in onset and incite a chronic inflammatory response, so therapy is typically required for weeks; base discontinuation of therapy on the health of affected tissues and negative culture. Streptococcal infections typically are more antibiotic sensitive, so treatment generally is shorter (10–14 d).</td>
</tr>
<tr>
<td>Synovial structure</td>
<td>Usually require treatment for weeks using a combination of methods: Systemic, parenteral initially; depending on sensitivity results, may be switched to oral once significant improvement is seen Intrathecal lavage and antibiotic infusion; repeated if needed Regional perfusion (IV or IO) for wounds at or below the carpus/tarsus; repeated if needed Antibiotic-impregnated PMMA beads or collagen sponges; particularly important when complete debridement is not possible or when a surgical implant must remain Repeated surgical debridement may also be needed Specific biofilm treatment as needed (see text)</td>
</tr>
<tr>
<td>Bone or physeal cartilage</td>
<td>Usually require treatment for weeks or months using a combination of methods: Systemic, parenteral initially; depending on sensitivity results, may be switched to oral once significant improvement is seen Regional perfusion (IV or IO) for wounds at or below the carpus/tarsus; repeated if needed Antibiotic-impregnated PMMA beads or collagen sponges; particularly important when complete debridement is not possible or when a surgical implant must remain Repeated surgical debridement may also be needed</td>
</tr>
<tr>
<td>Respiratory tract</td>
<td>Often respond quickly, needing only a short course of treatment (5–10 d) When chest wounds are complicated by septic pleuritis, treatment may be required for months and must include pleural lavage and drainage</td>
</tr>
<tr>
<td>Intestinal tract</td>
<td>Systemic antibiotic therapy is needed for weeks in most cases; parenteral initially. Peritoneal lavage and drainage is also important with septic peritonitis</td>
</tr>
<tr>
<td>Muscle</td>
<td>Typically respond very well, needing only a short course of treatment (5–10 d) Open drainage is easy at most sites and speeds resolution, particularly with IM abcessation Clostridial myonecrosis can rapidly cause severe systemic illness, but with aggressive surgical debridement and aeration/oxygen therapy, local and systemic antibiotic therapy usually is successful within days</td>
</tr>
</tbody>
</table>

Abbreviations: IM, intramuscular; IV, intravenous; IO, intraosseous; PMMA, polymethylmethacrylate.
Acknowledgments

Declaration of Ethics
The Author declares that he has adhered to the Principles of Veterinary Medical Ethics of the AVMA.

Conflict of Interest
The Author declares no conflicts of interest.

References and Footnotes

17. Wolcott RD, Kennedy JP, Dowd SE. Regular debridement is the main tool for maintaining a healthy wound bed in most chronic wounds. J Wound Care 2009;18:54–56.

IN-DEPTH: TRAUMA/WOUND MANAGEMENT


aSilvadene, Pfizer, New York, NY 10017.
bLake Immunogenics, Ontario, NY 14519.
cEquilox, Equilox International, Pine Island, MN 55963.
dCollatamp G, Schering Corporation, United Kingdom.
Triglyceride Concentrations in Healthy, Suckling Foals

Emily H. Berryhill, DVM*; K. Gary Magdesian, DVM, DACVIM, DACVECC, DACVCP; and Judy M. Edman, SRA

Healthy, suckling neonatal foals have higher serum triglyceride concentrations than adult horses. These were highest at 1–2 days of age, but still higher than adults at 10–12 days of age. Authors’ addresses: William R. Pritchard Veterinary Medical Teaching Hospital, (Berryhill); and Department of Medicine and Epidemiology; School of Veterinary Medicine, (Magdesian, Edman) University of California, Davis, CA 95616; email: ehberryhill@gmail.com. *Corresponding and presenting author. © 2015 AAEP.

1. Introduction
Anecdotal reports exist of neonates with transient hypolipidemia; however, little research is available to substantiate this and evaluate effects of age on serial serum triglyceride concentrations. The hypotheses of this study were: 1) healthy foals 1–2 days old would have higher serum triglycerides compared with foals immediately post-parturition, foals 10–12 days old, and postpartum mares; and 2) serial measurement of triglycerides would demonstrate wide variability.

2. Materials and Methods
This was a prospective study in which serial serum samples were obtained from seven foals and mares post-parturition at 1–2 days of age and at 10–12 days of age. Triglyceride concentrations were measured by enzymatic colorimetric assay and compared among groups.

3. Results
Serum triglyceride concentrations in foals immediately postpartum were not different than those in mares (median 28 and 20 mg/dL, respectively). Foals 1–2 and 10–12 days of age had higher serum triglycerides compared with the immediate postpartum period and their dams (P < .001). Foals 1–2 days of age had higher concentrations than foals 10–12 days (median, 89 and 60 mg/dL, respectively; P < .001). Highest concentrations occurred at 1–2 days of age.

4. Discussion
Foals can be hyperlipidemic relative to adult horses and have triglyceride variability within the neonatal period. A milk diet may contribute to hyperlipidemia given that neonatal triglyceride concentrations were similar to their dams just after parturition and increased progressively as foals nursed.

Acknowledgments
This study was supported by the Center for Equine Health, University of California, Davis, with funds from the Oak Tree Racing Association, the State of California pari-mutuel wagering fund and contributions from private donors and the Roberta A. and Carla Henry Endowed Chair in Emergency Medicine and Critical Care.

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Conflict of Interest
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Research Abstract—for more information, contact the corresponding author

NOTES
Correction of the Area Under the 24-Hour L-Lactate Time Curve for Age in Sick Neonatal Foals

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The area under the l-lactate concentration versus time curve (LAC Area) is different between surviving and nonsurviving sick foals. Correcting LAC Area for the known age-dependent decrease in normal l-lactate over the first week of age should further improve discrimination between surviving and nonsurviving critically ill foals. Authors’ addresses: Department of Veterinary Clinical Medicine, College of Veterinary Medicine, University of Illinois, Urbana, IL 61801 (Sheahan, Wilkins, Vander Werf); Department of Veterinary Medical Sciences, University of Bologna, Bologna, Italy 40126 (Castagnetti); Department of Large Animal Clinical Sciences, College of Veterinary Medicine and Biomedical Sciences, Texas A&M, College Station, TX 77845 (Hardy); Vetsuisse Faculty, University of Zurich, Zurich, Switzerland CH-8057 (Schoster); and Department of Clinical Studies, New Bolton Center, University of Pennsylvania, Kennett Square, PA 19348 (Boston); e-mail: *Corresponding author, pawilkin@illinois.com; †presenting author, bjsheahandvm@gmail.com. © 2015 AAEP.

1. Introduction

L-Lactate (LAC) is a normal metabolite present in the blood of neonatal foals. Abnormally increased L-lactate concentration ([LAC]) is recognized as a fairly reliable biomarker of disease severity and outcome in general populations of critically ill foals, correctly classifying survival to hospital discharge in approximately 75% to 80% of cases.1–5 Increased [LAC] at admission ([LAC]Admit) captures the severity or degree of initial hyperlactatemia, whereas the change in [LAC] with time ([LAC]Δ) captures the duration or persistence of initial hyperlactatemia in the face of treatment. Both, when abnormal, are associated with decreased survival to hospital discharge in ill neonatal foals.1–5 Whereas initial hyperlactatemia, determined from [LAC]Admit, commonly results from poor oxygen delivery to tissues, persistent hyperlactatemia, indicated by minimal [LAC]Δ when [LAC]Admit is increased, has been associated with dysmetabolism and poor oxygen utilization by tissues in the face of adequate oxygen delivery.1,2

This report expands the investigation of the area under the 24-hour [LAC] versus time curve (LAC Area), a unique measure of [LAC] exposure that encompasses both the severity and duration of hyperlactatemia by correcting LAC Area for known age-specific differences.6,7 To our knowledge, there are no studies that have evaluated the relationship between age-corrected LAC Area and outcome in ill neonatal foals.

We hypothesized that age-corrected LAC Area would improve discrimination between foals that survive to hospital discharge and those that do not. In addition, we wanted to compare the performance of the area under the [LAC] versus time curve using
Materials and Methods

Animals

Neonatal foals aged less than 30 days that presented to 1 of 4 university or private referral hospitals in 2014 for the treatment of conditions that require intensive or critical care were studied after having received client-informed consent. The study protocol was approved by the Institutional Animal Care and Use Committee.

Sampling Protocol

Details of the sampling protocol have been previously published.7 Briefly, whole blood (0.5–1 mL) was obtained by either direct venipuncture of a jugular vein using a preheparinized syringe or preplaced sterile intravenous catheter upon admission. Additional samples were similarly obtained at 6, 12, 18, and 24 hours after the initial sample.

L-Lactate Measurement, Calculation of LACArea and Determination of Outcome to Hospital Discharge

[LAC] was determined using the [LAC]-measuring techniques currently in use at each participating location. LACArea was determined using samples obtained during the first 24 hours of hospitalization in 2 ways: first by using only the admission and 24-hour values and second by summing individual areas calculated at 6-hour intervals by the trapezoidal method for numerical integration.8

The 24-hour LACArea calculated by summation was then age-corrected using 24-hour LACArea values determined from normal foals and the age at presentation of each individual foal (Tables 1 and 2; Fig. 1). Short-term outcome was defined as survival or nonsurvival to hospital discharge and recorded by the participating clinician at each hospital. Only foals that survived the entire initial 24-hour hospitalization period were included in the study. In the example shown in Fig. 1 and Table 3, the foal is 36 hours old at the time of initial evaluation. The timeline shown in Fig. 1 outlines when l-lactate measurements were taken. It is important that the same measuring device is used at each sample time to reduce variability. A total of 5 l-lactate measurements were obtained at 6-hour intervals between the initial evaluation and 24 hours after the initial evaluation. The timeline is 24 hours; each arrow in Fig. 1 represents a sample time. From these l-lactate measurements, as shown in Table 3, the LACArea can be calculated using the method demonstrated in Table 4. As shown in Table 4, the 6-hour interval LACArea is calculated by adding the 2 values flanking the interval (time interval), dividing that sum by 2, and then multiplying that number by 6. This is the formula for calculating the area of a trapezoid (see Fig. 2). These 6-hour interval areas are then summed to get the 24-hour total LACArea of 114.6 mmol/L·h. The appropriate age correction is then applied (in this case 37.0 mmol/L·h for 36 hours of age at presentation).

Table 1. Mean ± SD and Median (25th to 75th Percentile Range) for L-Lactate Area (LACArea) Determined From Normal Foals From Birth to 24 Hours, Day 3 (48–72 Hours), and Day 7 of Life (144–168 Hours)

<table>
<thead>
<tr>
<th>Age (h)</th>
<th>LACArea (mmol/L·h): Mean ± SD</th>
<th>LACArea (mmol/L·h): Median</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–6</td>
<td>16.8 ± 11.3</td>
<td>13.2 (11.1–16.1)</td>
</tr>
<tr>
<td>6–12</td>
<td>13.8 ± 4.5</td>
<td>13.1 (11.1–14.2)</td>
</tr>
<tr>
<td>12–18</td>
<td>12.0 ± 3.9</td>
<td>11.3 (9.2–13.1)</td>
</tr>
<tr>
<td>18–24</td>
<td>10.7 ± 3.0</td>
<td>9.00 (8.78–12.8)</td>
</tr>
<tr>
<td>0–24</td>
<td>53.3 ± 17.8</td>
<td>42.1 (40.1–54.2)</td>
</tr>
<tr>
<td>48–54</td>
<td>8.8 ± 2.0</td>
<td>8.4 (7.5–9.2)</td>
</tr>
<tr>
<td>54–60</td>
<td>8.2 ± 1.9</td>
<td>7.8 (7.5–9.0)</td>
</tr>
<tr>
<td>60–66</td>
<td>8.1 ± 1.4</td>
<td>7.5 (6.9–9.3)</td>
</tr>
<tr>
<td>66–72</td>
<td>8.2 ± 1.6</td>
<td>7.8 (8.7–12.3)</td>
</tr>
<tr>
<td>48–72</td>
<td>33.5 ± 4.2</td>
<td>32.4 (30.0–36.0)</td>
</tr>
<tr>
<td>144–150</td>
<td>6.2 ± 1.0</td>
<td>6.0 (5.7–6.9)</td>
</tr>
<tr>
<td>150–156</td>
<td>6.6 ± 0.6</td>
<td>6.9 (6.00–7.1)</td>
</tr>
<tr>
<td>156–162</td>
<td>7.2 ± 1.3</td>
<td>6.9 (6.3–8.4)</td>
</tr>
<tr>
<td>162–168</td>
<td>6.7 ± 1.2</td>
<td>6.9 (5.6–7.2)</td>
</tr>
<tr>
<td>144–168</td>
<td>26.8 ± 3.1</td>
<td>26.4 (24.8–27.9)</td>
</tr>
</tbody>
</table>

Table 2. Values Used for Age Correction of 24-Hour L-Lactate Area (LACArea) in Sick Neonatal Foals

<table>
<thead>
<tr>
<th>Age at Presentation (h)</th>
<th>LACArea: Normal Foals (mmol/L·h)</th>
<th>Correction (mmol/L·h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–24</td>
<td>42.1</td>
<td>42</td>
</tr>
<tr>
<td>24–48</td>
<td>ND</td>
<td>37</td>
</tr>
<tr>
<td>48–72</td>
<td>32.4</td>
<td>32</td>
</tr>
<tr>
<td>72–96</td>
<td>ND</td>
<td>29</td>
</tr>
<tr>
<td>96–144</td>
<td>ND</td>
<td>27.5</td>
</tr>
<tr>
<td>144+</td>
<td>26.4</td>
<td>26</td>
</tr>
</tbody>
</table>

ND = not determined.
to reach the final corrected 24-hour total LAC\text{Area} of 77.6 mmol/L-h.

Statistical Analysis
Data were tested for normality using the Shapiro–Wilk method. Differences between surviving and nonsurviving foals were tested using Kruskal–Wallis ($P < 0.05$, see Fig. 3).

3. Results
A total of 49 foals were enrolled for this report, 42 with complete datasets were used. A more complete description of this dataset has been recently published.\(^7\) There were 5 nonsurviving foals (5/42; 11.9%). Age at admission was not normally distributed. The median (25th to 75th percentile) was 18.5 hours (8.00–45.75) and was not different between survivors and nonsurvivors ($P = 0.312$). Admission [LAC] was 3.25 mmol/L (1.90–4.75) and also was not different between survivors and nonsurvivors ($P = 0.923$).

The area under the 24-hour $l$-lactate time curve (LAC\text{Area}) was 58.5 mmol/L-h (44.7–118.4) and clearly distinguished between survivors and nonsurvivors (54.0 vs. 122.4, $P = 0.03$; Fig. 2). The most commonly applied age correction was 42 mmol/L-h (23/42; 55%); 37 mmol/L-h was the second most commonly applied correction (9/42; 21%). No differences were found between age correction values for survivors or nonsurvivors ($P = 0.700$). Age-corrected LAC\text{Area} was 19.2 mmol/L-h (8.8–76.4) and seemed to further clarify differences between survivors and nonsurvivors (17.1 vs. 80.4, $P = 0.034$; Fig. 2). The 24-hour lactate area calculated using only admission and 24-hour [LAC] values was 65.4 mmol/L-h (46.8–104.1) and failed to distinguish survivors from nonsurvivors (62.4 vs. 103.2, $P = 0.135$; Fig. 2).

4. Discussion
The use of the area under the [LAC] time curve (LAC\text{Area}) as a tool for improving prognosis generation gained attention after it was used in septic pediatric human patients, where LAC\text{Area} was found to be superior to both [LAC]\text{Admit} and [LAC]Δ.\(^9\) Large prospective studies of general populations of sick neonatal foals have demonstrated that both [LAC]\text{Admit} and [LAC]Δ can be useful as biomarkers of disease severity and prognosis but, at best, correctly classify only 75% to 80% of studied foals in regard to survival to hospital discharge.\(^1\text{-}^3\text{,}^5\) There is evidence from both prospective and retrospective studies that both $l$-lactate parameters perform better as prognostic indicators in foals with certain specific diagnoses, such as sepsis and diarrhea, and are essentially irrelevant in others, such as meconium impaction and isolated failure of passive transfer of maternal immunity.\(^1\text{-}^2\text{,}^10\)

It is generally recognized that [LAC] decreases rapidly after birth in foals. Normal foals have [LAC] larger than that of adult horses at and after birth, with 1 recent study demonstrating a significant progressive decrease in [LAC] and LAC\text{Area} over the first week of life, both being indistinguishable from adult values by day 7.\(^6\) Fetal energy metabolism from mid-gestation to just prior to foaling includes a reliance on $l$-lactate.\(^1\) The foaling process also contributes to increased [LAC] at birth as the fetus transitions to neonatal life.\(^12\) For these reasons, the normal range of both [LAC] and LAC\text{Area} in neonatal foals will be higher than the normal range of [LAC] in adult horses over the first several days of life. Most sick neonatal foals are presented for treatment within 96 hours after birth; the median age in this small study was 18.5 hours. This makes considering age-related adjustments in $l$-lactate parameters important when determining prognosis, particularly over the first 3 days of life when normal $l$-lactate concentrations are largest and potentially confounding.

The results of this preliminary investigation suggest that age correction of LAC\text{Area} might improve its performance as a prognostic measure. LAC\text{Area}, corrected or uncorrected for age, certainly outperformed the calculation of area under the $l$-lactate concentration time curve using only the 2 points of admission and 24 hours, which could not distinguish between survivors and nonsurvivors in this report (Fig. 3). Because more than half the foals received the same age correction value, it is possible that the low median age at presentation for the foals in this study (18.5 hours) masked some of the influences of age correction.

### Table 3. Example $l$-lactate Values From a Sick Neonate Presented at 36 Hours of Age

<table>
<thead>
<tr>
<th>Sample Time</th>
<th>Lactate Concentration (mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial</td>
<td>8.9</td>
</tr>
<tr>
<td>6 hours</td>
<td>6.2</td>
</tr>
<tr>
<td>12 hours</td>
<td>4.0</td>
</tr>
<tr>
<td>18 hours</td>
<td>3.2</td>
</tr>
<tr>
<td>24 hours</td>
<td>2.5</td>
</tr>
</tbody>
</table>

### Table 4. Demonstration of LAC\text{Area} Calculation Using the Values Shown in Table 3

<table>
<thead>
<tr>
<th>Time Interval</th>
<th>(Sample a + Sample b)/2 * 6</th>
<th>LAC\text{Area} (mmol/L^2hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial-6 hours</td>
<td>(8.9 + 6.2)/2 * 6</td>
<td>45.3</td>
</tr>
<tr>
<td>6–12 hours</td>
<td>(6.2 + 4.0)/2 * 6</td>
<td>30.6</td>
</tr>
<tr>
<td>12–18 hours</td>
<td>(4.0 + 3.2)/2 * 6</td>
<td>21.6</td>
</tr>
<tr>
<td>18–24 hours</td>
<td>(3.2 + 2.5)/2 * 6</td>
<td>17.1</td>
</tr>
<tr>
<td>24 hour TOTAL</td>
<td>N/A</td>
<td>114.6</td>
</tr>
<tr>
<td>Age correction</td>
<td>N/A</td>
<td>−37.0</td>
</tr>
<tr>
<td>Corrected 24 hour TOTAL</td>
<td>N/A</td>
<td>77.6</td>
</tr>
</tbody>
</table>

The 6-hour interval LAC\text{Area} are calculated first, then summed together to achieve the 24-hour total LAC\text{Area} value. The age correction factor is then removed from the total 24-hour LAC\text{Area} to obtain the final corrected 24-hour LAC\text{Area} value.
There are several limitations to this small preliminary study. Although prospective and multicenter in nature, the sample size was small, and there were very few nonsurvivors, limiting our ability to more fully investigate the role of LACArea as a prognostic tool. There was also an insufficient number of foals on which to perform valid statistical evaluations by major diagnostic category, for example, or to perform logistic regression to determine the odds of survival based on LACArea—either corrected or uncorrected. One further limitation is that not all sites used the same L-lactate measuring device, introducing uncontrolled variability in the reported values. However, the results are sufficiently
encouraging such that a multicenter prospective study should be undertaken, preferably one that uses similar L-lactate measurement devices at each participating hospital.

Questions of practicality always arise when new techniques are introduced, particularly when mathematical formulas are involved, potentially explaining the failure of some complex predictors of outcome, or sepsis, in sick foals in gaining widespread popularity and the persistent use of the sepsis score tally sheet over time despite evidence that its performance is somewhat location-dependent and has changed over time. Should the application of LAC Area prove useful for estimating the prognosis of a critically ill foal, rest assured that the calculation is simple and can be done by hand or with a calculator (and is potentially quite adaptable to a score sheet). Calculation requires 5 measurements of [LAC] from admission to 24 hours of hospitalization at 6-hour intervals using a single measuring device, commonly performed as part of routine monitoring of sick foals that require intensive care. The area of each 6-hour interval is calculated as the average of the 2 measurements flanking that 6-hour interval. That mean value is then multiplied by 6 hours to determine the 6-hour area of interest. The 24-hour LAC Area is simply the sum of those 4 6-hour areas. Once standards for age correction are established and found to be useful, correction can be applied, and the result can be compared to probability estimates for survival, stratified by diagnosis, developed from large multicenter studies. The goal, of course, is to develop a relatively simple and accurate tool for assisting sick neonatal foals and improving the accuracy of prognoses.

Acknowledgments

The Authors would like to acknowledge funding provided by the Hatch Funds project ILLU-888–384 for the project L-Lactate Area (LAC Area) in Neonatal Foals that provided data for normal foals used in the current study.

Declaration of Ethics

The Authors declare that they have adhered to the Principles of Veterinary Medical Ethics of the AVMA.

Conflict of Interest

The Authors declare no conflicts of interest.

References


Rhodococcus equi–Specific Hyperimmune Plasma Decreased Rhodococcal Pneumonia Severity in Newborn Foals After Experimental Infection

Macarena G. Sanz, DVM, MS, DACVIM, PhD*; Alan Loynachan, DVM, DACVP, PhD; and David W. Horohov, PhD

Whereas infection after challenge was not prevented by R. equi–specific hyperimmune plasma (HIP) administration, severity of clinical pneumonia decreased. Authors’ addresses: Maxwell H. Gluck Equine Research Center (Sanz, Horohov), and Veterinary Diagnostic Laboratory (Loynachan), Department of Veterinary Science, University of Kentucky, Lexington, KY 40546-0099; e-mail: macarena.sanz@uky.edu. *Corresponding and presenting author. © 2015 AAEP.

1. Introduction
Because a vaccine against R. equi is not available, HIP is commonly used, although its efficacy remains controversial. The objective of this study was to evaluate the ability of a commercially available HIP to prevent clinical rhodococcal pneumonia in neonatal foals after experimental challenge.

2. Materials and Methods
Nine foals were given intravenous HIP after birth while nine remained as controls. Foals were challenged the first week of life (10³ cfu/foal R. equi) and were monitored for 8 weeks. Virulence associated protein (VapA) specific IgG and IgG subclasses in serum and in bronchoalveolar lavage fluid (BALF) were evaluated using ELISA.

3. Results
One foal in the HIP group and four in the control group developed clinical pneumonia; however, the power of the study was too low to detect a statistical significant effect of treatment. HIP foals had significantly lower weekly ultrasonographic scores (P < .05), lower white blood cell counts (P = .03), platelet counts (P = .01), and fibrinogen concentration (P = .01) than controls. Serum VapA–specific IgG, IgGa, and IgGb were significantly higher in HIP foals, and IgGa and IgG(T) significantly increased (P < .001) over time only in control foals. VapA-specific IgG (P = .02) and IgGb (P = .04) were significantly higher in BALF of HIP foals.

4. Discussion
HIP administration decreased severity of pneumonia, which reduced the need for antimicrobial treatment. Antibodies present in HIP transferred to BALF of foals shortly after HIP administration. VapA-specific IgG(T), which increases with R. equi infection, was only elevated in control foals.
Acknowledgments

Declaration of Ethics

The Authors declare that they have adhered to the principles of Veterinary Medical Ethics of the AVMA.

Conflict of Interest

Dr. Sanz had a scholarship from Zoetis. Funding from this project was provided by the Jes E. and Clementine Schlaikjer endowment.
Transabdominal Ultrasonography for Monitoring Ascarid Burdens in Foals

Martin K. Nielsen, DVM, PhD, DEVPC, DACVM*; John M. Donecker, VMD; and Clara K. Fenger, DVM, PhD, DACVM

Large *Parascaris* spp. burdens can cause small intestinal impactions that are associated with a guarded to poor prognosis for survival. A transabdominal ultrasound technique was developed and found useful for determining ascarid burdens above ten worms. Authors' addresses: M.H. Gluck Equine Research Center, University of Kentucky, Lexington, KY 40546 (Nielsen); Zoetis Outcomes Research, Reidsville, NC 27320 (Donecker); and Equine Integrated Medicine, PLC, Georgetown, KY 40511 (Fenger); e-mail: martin.nielsen@uky.edu. *Corresponding and presenting author. © 2015 AAEP.

1. Introduction
Ascarid parasites pose a significant threat for small intestinal impaction and rupture. The post-surgical long-term survival is in the range of 9 to 60%. Anthelmintic treatment has been identified as a significant risk factor for small intestinal impaction. Ascarid egg counts suffer from low negative predictive value and do not correlate with the worm burden.

2. Materials and Methods
Ten foals underwent repeated transabdominal ultrasonographic examination. A scoring system was developed with a scale of 1 through 4, and one score was assigned for each foal on each examination day. Foals were euthanized and full worm counts performed. Fifteen foals were then randomly allocated to three treatment groups: ivermectin, oxibendazole, and no treatment. Blinded ultrasound examinations were performed daily for 5 consecutive days following treatment. Foals were both ultrasound twice by the same investigator, and by two different investigators.

3. Results and Discussion
Two consecutive examinations were found to reliably detect worm burdens larger than ten ascarids. Ascarid scores declined in response to both anthelmintic treatments, although differences were not statistically significant. Kappa values indicated fair to moderate intra- and inter-observer agreements. The ultrasonographic screening techniques can be a useful tool for monitoring ascarid burdens in foals.

Acknowledgments
Declaration of Ethics
The Authors declare that they have adhered to the Principles of Veterinary Medical Ethics of the AVMA.

Conflict of Interest
This study was conducted with support from the 2013 Zoetis HorseCall Grant.
How to Perform Cardiopulmonary Resuscitation on Newborn Foals in the Field

SallyAnne L. Ness, DVM, DACVIM*; Gillian A. Perkins, DVM, DACVIM; Kevin T.T. Corley, BVM&S, PhD, MRCVS, DACVIM, DACVECC; and Daniel J. Fletcher, PhD, DVM, DACVECC

1. Introduction

Birth is a high-risk event in which a myriad of both maternal and fetal factors may result in failure of a foal to properly transition from fetal to neonatal physiology. In the field setting, this failure to transition often presents as a foal that is born alive (i.e., with heartbeat and pulse), but fails to spontaneously breathe. Without intervention, hypoxemia-induced bradycardia ensues, followed shortly thereafter by asystole and death. In this situation, a well-prepared plan and the ability to quickly assess the foal and perform effective cardiopulmonary resuscitation (CPR) can mean the difference between perinatal death and a live, and healthy foal.

Clearly, a foal that fails to spontaneously breathe at birth represents an emergency not amendable to retroactive planning, equipment retrieval, or travel to the farm. In this situation, preparedness is key, and a positive outcome depends entirely on the presence of someone possessing the knowledge, skill, and equipment to perform effective resuscitation. Ideally, a veterinarian would be present at high-risk foalings; however, this is not always possible or predictable. Depending on experience and expertise, breeding farm personnel can be trained to effectively perform the basic life support techniques described here to improve a foal’s chance of survival in the absence of a veterinarian.

2. Materials and Methods

Equipment

Supplies can be organized together in a crash kit that allows for easy transport from clinic or truck to the foaling stall (Fig. 1). Breeding farms may similarly assemble a kit that can be placed outside of foaling stalls for easy access during and after parturition. A portable oxygen tank and flow meter can be rented or purchased from local human medical suppliers (Fig. 2). Medications should be well labeled and checked annually for expiration. A dosage chart may be printed and kept in the crash kit to minimize the need for time-consuming calculations and help prevent dosage mistakes in an emergency (Fig. 3).
Supplies
The following supplies should be available in the crash kit:

- Clean towels
- Nasotracheal tubes: 8–10-mm internal diameter × 55 cm long
- Syringe (5 mL) for inflating nasotracheal tube cuff
- Self-inflating Ambu bag
- Mask that fits over muzzle and both nostrils
- Syringes (3 mL)
- 20 g × 1-inch needles
- White tape
- Bulb syringe
- Umbilical clamps
- Light source (small flash light)
- Laryngoscope (optional)
- 14 g or 16 g intravenous catheters appropriate for use in foals
- Portable oxygen tank and flow meter
- Injectable Medications
  - Epinephrine (1 mg/mL)
  - Vasopressin (20 U/mL)

3. Recommendations for Performing CPR
The recently published Reassessment Campaign on Veterinary Resuscitation (RECOVER) Initiative was created to provide veterinarians with evidence-based guidelines for performing CPR and improving outcomes in small-animal patients.1 Although not specifically aimed at equine patients, the principles set forth by the RECOVER Initiative apply across species and have been adapted into the recommendations below.

Initial Assessment of the Newborn Foal
Normal foals should exhibit spontaneous respiration and movement within seconds of birth. In these cases, intervention is unnecessary and may negatively interfere with maternal-neonatal bonding. For the foal that fails to move and breathe upon delivery, however, rapid assessment and immediate intervention is warranted. Foals should be placed in sternal recumbency to optimize ventilatory efforts and membranes manually removed from the nostrils. A bulb syringe can be used to clear the nasal passages and nasopharynx of excessive fluid and, if present, meconium. Bleeding umbilical stumps should be quickly clamped to prevent excessive hemorrhage. A stethoscope on the chest wall can be used to auscult heart rate and rhythm while simultaneously palpating femoral pulses on the upper medial thigh. Normal foals will have heart rates greater than 60 bpm (and usually closer to 80–100 bpm). Sinus arrhythmia may be noted during the first hour of life. Bradycardia (< 60 bpm) is reason for concern and should alert the clinician that intervention may soon be needed.2–4

Tactile stimulation of the head, neck, and body by vigorous drying and rubbing with a towel is often effective at initiating respiration and resolving mild bradycardia. Stimulating the inner ears or nasal cavities with a finger or piece of bedding straw may also be helpful. Initial assessment and the decision to initiate further resuscitative therapies should all occur within the first 2 minutes of life, and ideally, within the first 30 seconds.2–4

Fig. 1. Neonatal emergency crash kit.

Fig. 2. Portable oxygen tank and flow meter.
If the Foal Fails to Breathe and/or is Bradycardic

Foals that remain apneic or display an irregular or gasping respiratory pattern should receive immediate ventilatory support. Additionally, foals with heart rates less than 50 bpm should also receive respiratory intervention, given that neonatal bradycardia is often hypoxemia-mediated and will resolve with adequate ventilation.2–4

Several options exist for providing ventilatory support to foals in the field. The preferred method is intubation through either the nose or the mouth. For intubation, a foal should be placed in lateral or sternal recumbency with its head extended as much as possible to help guide the tube into the trachea. For nasal intubation, the tube is passed ventromedially through the ventral meatus into the nasopharynx (Fig. 4). For oral intubation, the tongue is gently pulled forward to stabilize the larynx and the tube is passed over the base of the tongue. For either technique, gently rotating the tube can help guide it through the glottis and into the trachea.

![Fig. 3. Neonatal emergency drug dosage chart.](image)

### CPR Emergency Drugs and Doses: NEONATE

<table>
<thead>
<tr>
<th>DRUG</th>
<th>DOSE</th>
<th>Weight (kg)</th>
<th>2.5</th>
<th>5</th>
<th>10</th>
<th>20</th>
<th>30</th>
<th>40</th>
<th>50</th>
<th>60</th>
<th>70</th>
<th>80</th>
<th>90</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Weight (lb)</td>
<td>5</td>
<td>10</td>
<td>20</td>
<td>40</td>
<td>60</td>
<td>80</td>
<td>100</td>
<td>120</td>
<td>140</td>
<td>160</td>
<td>180</td>
</tr>
<tr>
<td>Epi Low (1:1000; 1mg/ml) every other BLS cycle x3</td>
<td>0.01 mg/kg</td>
<td>0.03</td>
<td>0.05</td>
<td>0.1</td>
<td>0.2</td>
<td>0.3</td>
<td>0.4</td>
<td>0.5</td>
<td>0.6</td>
<td>0.7</td>
<td>0.8</td>
<td>0.9</td>
<td></td>
</tr>
<tr>
<td>Epi High (1:1000; 1 mg/ml) for prolonged CPR</td>
<td>0.1 mg/kg</td>
<td>0.25</td>
<td>0.5</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>7</td>
<td>8</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>Vasopressin (20 U/ml)</td>
<td>0.6 U/kg</td>
<td>0.08</td>
<td>0.15</td>
<td>0.3</td>
<td>0.6</td>
<td>0.9</td>
<td>1.2</td>
<td>1.5</td>
<td>1.8</td>
<td>2.1</td>
<td>2.4</td>
<td>2.7</td>
<td></td>
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<td>Atropine (0.54 mg/ml)</td>
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<td>0.18</td>
<td>0.4</td>
<td>0.8</td>
<td>1.1</td>
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<td>Flumazenil (0.1 mg/ml)</td>
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<td>0.25</td>
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### Defibrillation

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Fig. 3. Neonatal emergency drug dosage chart.

Fig. 4. Nasotracheal intubation in a foal. (© Veterinary Advances, Ltd., 2012)
Tubes should be placed as far down the trachea as possible to minimize dead space. Once in the trachea, the cuff is inflated, the Ambu bag is connected, and the tube is secured in place by wrapping white tape around the tube and muzzle. Administering a breath should result in visible excursion of the chest wall and auscultable bronchovesicular sounds over the lung fields. Care should be taken to ensure the tube has not been placed in the esophagus, which results in a palpable tube-like structure dorsal to the trachea, and abdominal enlargement rather than chest wall excursion in response to breath administration. Intubation should be attempted for no longer than 20 seconds before considering alternative methods of respiratory support.2–4

If nasal or oral intubation is not possible, ventilation via a mask or mouth-to-nose resuscitation may be attempted. A commercially available self-inflating resuscitation pump may be attached to a tightly fitting mask and used to administer breaths (Fig. 5).3 To perform mouth-to-nose resuscitation, the foal is placed in lateral recumbency and the down nostril is occluded with one hand while the other hand is used to gently occlude the esophagus and prevent aerophagia (Fig. 6).3,4

Once intubated, the first delivered breath should have a prolonged inspiratory phase lasting approximately 5 seconds to help inflate the lungs and displace fluid across alveolar membranes into the interstitium.4 Additional breaths should be delivered at a rate of 10 breaths per minute.1,2 Breaths should be quick and last only long enough to expand the lungs, as prolonged inspiration times raise intrathoracic pressure and impair venous return to the heart.1 The foal should be reassessed 30–60 seconds after starting ventilation. Worsening bradycardia, cardiac arrest, or heart rates less than 40 bpm warrant immediate initiation of thoracic compressions (see next section).2,3,4

The use of oxygen during CPR is controversial, with studies suggesting that inducing hyperoxia is deleterious to patient outcome,1 and the 2010 International Consensus on Cardiopulmonary Resuscitation recommended that human infants be resuscitated using only room air after several studies failed to show any benefit of oxygen supplementation.4 With that said, in the field setting, the use of free-flow 100% oxygen either intranasally or attached to the Ambu bag in the intubated foal is unlikely to cause harm when used for the relatively short time periods required for birth resuscitation, and increasing inspired oxygen levels may help bolster arterial oxygenation when cardiac output is poor. Until further studies are performed in equine neonates, the use of supplemental oxygen remains controversial and clinician dependent.

If the Foal has a Questionable or Absent Heartbeat and Fails to Breathe

A foal that is truly stillborn or has been dead for any length of time is unlikely to respond to CPR. However, a foal that is experiencing acute cardiopulmonary arrest at the time of delivery may very well present as a stillborn but respond favorably to immediate initiation of resuscitative techniques. In either case, the clinician has nothing to lose by attempting to resuscitate the “dead” foal, and if nothing else, efforts made to revive a stillborn foal will likely be viewed more positively by owners and observers than doing nothing.

To perform thoracic compressions, gently but quickly palpate the foal’s ribcage for rib fractures and place the foal in lateral recumbency on hard ground. If rib fractures are present, they should be placed on the down side. If bilateral rib fractures are identified, the side with the most cranial fractures should be placed down.2,4 The RECOVER initiative recommends a “push hard, push fast” ap-

Fig. 5. Ventilatory support using a mask and self-inflating resuscitation pump. (© Veterinary Advances, Ltd., 2012)

Fig. 6. Mouth-to-nose resuscitation in a foal. (© Kevin Corley and Jane Axon, 2004)
proach to thoracic compressions. The rescuer should kneel behind the foal’s back and place one hand over the other (Fig. 7). Arms should be kept as straight as possible with elbows locked to allow the core and back muscles to perform most of the physical work and prevent rescuer exhaustion. Compressions should center over the heart and should aim for a compression depth of one third to one half the width of the chest at a rate of 100 to 120 compressions per minute.

If the foal is experiencing cardiac arrest, thoracic compressions should be initiated immediately. Time should not be taken to first intubate the foal given that delivering oxygenated air to the lungs will have no effect on arterial oxygenation in the absence of circulation. Furthermore, studies in humans have demonstrated that maintaining circulation of relatively deoxygenated blood via uninterrupted thoracic compressions results in better survival rates than pausing compressions for extended periods of time to give breaths. With that said, most cardiac arrests in foals occur secondary to respiratory arrests, and therefore, intubation should be attempted as soon as possible (or simultaneously by a second person), or mouth-to-nose ventilation administered in the case of single-rescuer CPR.

If the foal is intubated and more than one person is available, CPR should be performed continuously at a rate of 100 to 120 chest compressions per minute and 10 breaths per minute. In the intubated foal, chest compressions should not be stopped during delivery of breaths. Rescuers can perform CPR in 2-minute cycles; rotating every 2 minutes to prevent fatigue and allow for brief patient assessment before resuming compressions. If only one rescuer is present, and/or if the foal is not intubated, simultaneous compressions and ventilation is not possible. In this case it is recommended to perform CPR with a 30:2 ratio; delivering 30 chest compressions immediately followed by two quick breaths. This cycle is repeated continuously and the foal is reassessed every 2 minutes.

Drug Therapy
In foals suffering from cardiac arrest or progressive bradycardia, vasopressor and vagolytic therapies may be used to increase heart rate, increase contractility, and induce peripheral vasoconstriction to redistribute blood to the heart, brain, and lungs. Epinephrine is a nonspecific catecholamine that acts on all adrenergic receptors. Whereas its β1 and β2 actions have inotropic, chronotropic, and bronchodilatory effects, the vasoconstrictive effects of its α1 actions have been shown to be of most benefit during CPR. In fact, increased myocardial workload and oxygen consumption as a result of epinephrine’s beta actions may, in fact, be deleterious to patient survival. Epinephrine is administered at a so-called low dose of 0.01 mg/kg IV every other CPR cycle (i.e., every 4 min during CPR). The epinephrine high dose of 0.1 mg/kg IV can result in prolonged peripheral vasoconstriction, renal failure, and ileus, and should be reserved for use only as a last resort. If venous access is not possible, epinephrine may be administered intratracheally via a red-rubber tube at a dosage of 0.1 to 0.2 mg/kg; however, efficacy and absorption from this route is highly variable. Vasopressin is a vasopressor that exerts its potent vasoconstrictive effects via peripheral V1 receptors. It has no cardiac effects. Vasopressin can be administered in place of epinephrine at a dose of 0.6 U/kg IV every other CPR cycle (i.e., every 4 min during CPR). If enough people are available, an IV catheter may be placed during CPR to facilitate drug administration. In the absence of extra hands, the author recommends placing a 20 g needle in the jugular vein and leaving it in place for venous access and drug administration until a catheter can be placed.

When to Stop CPR
In the hospital setting, end-tidal CO2 (capnography) and electrocardiogram monitoring provide rapid means of confirming a return to spontaneous circulation and restoration of normal sinus rhythm, respectively. In the field setting, however, a clinician must rely on physical examination and observation to signal respiration and return to spontaneous circulation. Ventilatory support can be discontinued when spontaneous respiration at a rate of greater than 16 breaths per minute is observed; however, the foal should be closely monitored for recurrence of apnea and assisted ventilation reinitiated if needed.

Thoracic compressions should continue until a regular heart rate of greater than 60 bpm is present. Pauses in CPR to assess for return of circulation should last no longer than 10 seconds before resuming compressions. Foals that fail to show any signs of life after 15 minutes of continuous CPR are unlikely to survive.

Post-Resuscitation Monitoring
Foals that arrest during or after birth should be considered high-risk patients throughout the neonatal period. Any maternal or fetal risk factors lead-
ing to cardiopulmonary arrest may also be associated with systemic sequelae for the foal, in addition to global hypoxic insult incurred as a consequence of the arrest itself. If possible, foals resuscitated in the field should be transported immediately to a referral hospital for further observation and treatment, or at the very least, closely monitored on the farm for repeat arrest.

4. Discussion
The described techniques provide practical guidelines for performing CPR in foals that without intervention would undoubtedly succumb to respiratory and cardiac arrest. Success rates will vary by case and depend on a combination of patient and clinician factors including the underlying cause of arrest, time to initiation of CPR, and effectiveness of CPR technique. Fortunately, the long-term neurologic sequelae frequently noted in humans following cardiopulmonary arrest seems to be rare in foals, reported in only 6% of large-animal neonates undergoing successful CPR in a recent retrospective. With preparation and practice, basic CPR can be performed in the field with relative ease, clinicians are encouraged to prepare themselves to put these techniques into action when the appropriate emergency situation arises.

5. Additional Resources
Additional information and step-by-step instruction on performing CPR on foals is available via the free online application, Foal CPR by Veterinary Advances, Ltd., at https://appsto.re/ie/lZ0iG.i.

Acknowledgments

Declaration of Ethics
The Authors declare that they have adhered to the Principles of Veterinary Medical Ethics of the AVMA.

Conflict of Interest
Dr. Corley is a director of and author for Veterinary Advances Ltd, that produces Apps for veterinarians including free Apps on CPR.

References
Gestation Length and Racing Performance in 115 Thoroughbred Foals With Incomplete Ossification

Lillian M.B. Haywood, VMD*; Bonnie S. Barr, VMD, DACVIM; Deborah L. Spike-Pierce, DVM; Dimitria Mathys, MsPH, VMD; and Dixie Mollenkopf, MS

Thoroughbreds with more profound incomplete ossification have a shorter gestational length, are less likely to race, and earn less than their maternal siblings. Authors' addresses: Rood and Riddle Equine Hospital, PO Box 12070, Lexington KY 40580-2070 (Haywood, Barr, Spike-Pierce); and College of Veterinary Medicine, Department of Veterinary Preventive Medicine, The Ohio State University, Columbus, OH 43210 (Mathys, Mollenkopf); e-mail: lhaywood@roodandriddle.com. *Corresponding and presenting author. © 2015 AAEP.

1. Introduction
Incomplete ossification of the tarsal and carpal cuboidal bones is a problem in equine neonates with potentially life-long consequences.

2. Materials and Methods
Hospital records were searched for Thoroughbred foals less than 90 days of age who had tarsal radiographs performed. The radiographs were examined and graded on a scale of 1 to 4 based on the Adams Skeletal Ossification Index.1 Gestational length was calculated from farm records and Jockey Club birth dates. Race records were obtained from Equibase.com, and affected foals’ records were compared with maternal siblings.

3. Results
Grade 1 and 2 foals were premature (gestation length ≤ 325 days); Grade 3 and 4 foals were not. Foals with Grades 1, 2, and 3 were significantly less likely to race than their maternal siblings, and foals with Grades 1, 2, 3, and 4 earned less money. Foals with incomplete ossification raced more frequently as 3-year-olds than as 2-year-olds.

4. Discussion
Incomplete ossification, especially Grades 1 and 2, is associated with a shorter gestation length, which is expected given that ossification of the cuboidal bones occurs during the end of gestation. Foals with Grades 1 through 3 of incomplete ossification were less likely to race and earned less than their unaffected siblings, and therefore should be considered poor racehorse candidates.

Acknowledgments

Declaration of Ethics
The Authors declare that they have adhered to the Principles of Veterinary Medical Ethics of the AVMA.

Conflict of Interest
The Authors declare no conflicts of interest.

Reference
Novel Continuous Positive Airway Pressure System for Respiratory Support of Foals

Rosemary McKean, BVetBio/BVSc (Hons.1)*; Sharanne L. Raidal, BVSc, MVSt, PhD, GradDipEd, FANZCVSc; Sharon G. Nielsen, BAppSc, Dip. Ed., Grad Cert Biom, MPhil; and Christopher Quinn, BSc(Vet), BVSc, MACVSc, DipACVAA, DipECVAA

A human sleep apnoea continuous positive airway pressure (CPAP) unit was adapted to deliver safe, portable, efficacious, and cost-effective respiratory support to foals, and with refinement may prove suitable for treatment of respiratory distress in foals. Authors’ addresses: Moorong Veterinary Clinic, Wagga Wagga 2650, Australia (McKean); School of Animal and Veterinary Sciences (Raidal, Quinn), and Quantitative Consultancy Unit, Research Office (Nielsen), Charles Sturt University, Wagga Wagga 2650, Australia; e-mail: rose.mckean@bigpond.com. © 2015 AAEP.

1. Introduction
Respiratory insufficiency in foals is routinely managed by nasal insufflation or, more rarely, ventilator support. In humans, especially neonates, CPAP optimizes both ventilatory mechanics and respiratory efficiency. The current project aimed to critically evaluate a practical foal CPAP system suited to clinical equine practice.

2. Materials and Methods
A commercial device was adapted to provide CPAP to five foals (age 6–10 wk) following pharmacological induction of respiratory insufficiency. Respiratory support derived from CPAP (10 cmH₂O plus 5 L.min⁻¹ O₂) was compared with non-pressurized mask O₂ (5 L.min⁻¹) by randomized crossover design, with foals receiving both treatments on two occasions in random order, followed by nasal O₂ insufflation. Respiratory function was assessed by analysis of arterial blood gases, oxygen saturation, tracheal gas composition, spirometry (flow/volume), respiration rate, and blood pressure.

3. Results
CPAP and mask O₂ normalized pre-treatment hypoxemia. Oxygen extraction and CO₂ elimination were highest with CPAP at a lower respiratory rate than mask O₂ (P < .05). Nasal O₂ caused supra-physiological arterial oxygen pressures, decreased tidal volume and increased respiratory rate compared with CPAP (P < .05). All treatments caused modest hypercarbia with no adverse cardiovascular effects.

4. Discussion
The CPAP system provided an improved method of clinical respiratory support for foals without ventilator-assisted respiratory therapy. Additional
studies are required to optimize delivery and characterize efficacy in compromised neonates.

Acknowledgments

Declaration of Ethics
The Authors declare that they have adhered to the principles of Veterinary Medical Ethics of the AVMA.

Conflict of Interest
The Authors declare no conflicts of interest.
Primary Hypothyroidism in Mares From a Herd Affected With Congenital Hypothyroidism

Mariana Diel de Amorim, DVM, DVSc, DACT*; Nat Messer, DVM, DABVP; and Claire Card, DVM, PhD, DACT

Primary hypothyroidism was present in mares from a herd affected by congenital hypothyroidism dysmaturity syndrome (CHDS). Authors’ addresses: Department of Large Animal Clinical Sciences, The Western College of Veterinary Medicine, Saskatoon, SK S7N 5B4, Canada (Diel de Amorim, Card); and Department of Veterinary Medicine and Surgery, University of Missouri-Columbia, Columbia, MO 65211 (Messer); e-mail: mariana.dieldeamorim@usask.ca. *Corresponding and presenting author. © 2015 AAEP.

1. Introduction
Primary hypothyroidism, in which the thyroid gland fails to respond to thyrotropin-releasing hormone (TRH) is rare in horses.

2. Materials and Methods
A CHDS-affected herd was investigated. CHDS was confirmed by three foal necropsies, of which 6/16 foals met the case definition. Mares (n = 16) were blood sampled at time 0. Two weeks later a TRH stimulation test (1.0 mg TRH, IV) was performed (n = 7) at pre- and 2 hours post-treatment for measurement of T₃ and T₄ (competitive chemoluminescent enzyme immunoassay).a

3. Results and Discussion
At time 0, 8/16 and 11/16 mares had deficient (<0.461 and <12.87 nmol/L) baseline T₃ and T₄ levels, respectively, and 6/16 mares had T₃ levels below detection. TRH stimulation showed pre levels deficient in 2/7 mares for T₃ and T₄, respectively, and post TRH results included 4/7 and 7/7 failing to double in T₃ and T₄, respectively.

Acknowledgments
We thank Rebecca Johnston, Brad McKell, and Kayla Nielsen for their assistance. Financial support was provided by Saskatchewan Agriculture Development Fund.

Declaration of Ethics
The Authors declare that they have adhered to the Principles of Veterinary Medical Ethics of the AVMA.

Conflict of Interest
The Authors declare no conflicts of interest.

Note
aThe compounded product used in this study was not used as a therapy but rather as a diagnostic pharmaceutical product. There are no U.S. Food and Drug Administration–approved TRH products for veterinary use.
Review of Prognostic Studies of Neonatal Foals: Making Sense of the Noise

Peter R. Morresey, BVSc, MACVSc, DACT, DACVIM (Large Animal)

Estimating the prognosis of the sick neonate has profound clinical, economic, and client-relationship implications. A plethora of information is available; however, inconsistency between study location and methodology has led to disparate results, and research center diagnostic capabilities are largely unavailable in field settings. However, in some study populations readily observable parameters have been found to be good indicators of prognosis, reinforcing the utility of a comprehensive clinical examination. Author’s address: Rood and Riddle Equine Hospital, PO Box 12070, Lexington, KY 40580–2070; e-mail: pmorresey@roodandriddle.com. © 2015 AAEP.

1. Introduction

Central to the viability of the equine breeding industry is live foal production. This is consistent between various locations at approximately 80% of mares bred. Economic modeling has determined that to maintain financial viability for the individual broodmare, six foals must be produced over a 7-year period and remain viable so as to be sold at auction. Survival of each foal delivered is therefore critical to the health of the overall equine industry.

A number of studies have investigated the prognostic value of clinical and laboratory findings in neonatal foals at time of admission to intensive care units. From these values mathematical models have been created to assist decision making and prognosticate on survival. However, repeatability between different locations or between different groups of foals from the same location where the model was created but in different years was found to be poor. Mathematical model accuracy was lessened for conditions that developed after the initial assessment. It has been suggested that sepsis scoring should not be used to define sepsis in clinical study situations unless the score has been previously validated for the particular study center. However, when compared with a regression model derived from objective clinical data, the modified sepsis score performed favorably.

In field clinical settings utility of such prognostic models is complicated as parameters available in academic settings are likely not measurable in the field. Also, if important values are measurable, results are typically not available in a reasonable timeframe that would allow meaningful application of the information. Even with readily available information, prolonged delay in hospital referral may alter both clinical and laboratory findings sufficient to affect prognosis.

2. History and Initial Evaluation

Readiness for birth of the neonatal foal is intuitively likely to have a bearing on prognosis, yet in one study prematurity or dysmaturity of the neonate (as indicated by gestational age) did not differ between survivors and nonsurvivors. Likewise, age of the
neonate at admission did not affect survival. However, duration of clinical signs prior to admission was found to significantly negatively affect outcome. In one study a 5.8-fold increase in the risk of death for each 24-hour period of delay was demonstrated.

Among physical parameters determined at the time of admission, a heart rate ≥ 70 beats per minute, a respiratory rate ≥ 60 breaths per minute, a rectal temperature greater than 99°F, a normal appearance of mucous membranes, and the ability to stand at admission positively affected survival in one study. In contradiction to these findings, in foals presented for respiratory disease the majority of clinical examination variables at initial evaluation were unassociated with case outcome. With respect to septic foals, fever was found to be an inconsistent finding in one study.

Maternal disease (both systemic and placental) was found to be higher among nonsurviving than surviving foals. Events at parturition, particularly dystocia, have been suggested to be associated with negative outcomes.

3. Signs of Sepsis
Signs suggestive of sepsis have been a negative prognostic indicator in many neonatal studies. Sepsis was identified as the most common problem in both survivors and nonsurvivors in one study. Diagnosis was assisted by sepsis scoring, this being considered more reliable that any individual parameter. Bacteremia in one study was most consistently due to Escherichia coli, although mixed infections were also found. Of 423 bacteremic foals in this study 254 survived, this being affected by a number of historical and clinical findings. A positive blood culture is associated with nonsurvival.

4. Hematology and Clinical Chemistry
Hematological and clinical chemistry values have been integral in many neonatal foal prognostic models. A low segmented neutrophil count, low total erythrocyte count, and elevated fibrinogen concentration were significant indicators of mortality in a number of studies. Immunoglobulin G (IgG) concentration also factored in a number of studies involving survival and prevention of infection. A recent study found odds of nonsurvival increasing proportionally with lower IgG concentrations, with higher total protein associated with a decreased likelihood of low IgG, and increased albumin associated with an increased likelihood of low IgG.

Blood glucose has been shown in other species to be indicative of survival. Hyperglycemia, both degree and duration, has been associated with nonsurvival in human medicine. Hypoglycemia also is problematic, and although critical glucose levels have not been determined, prolonged human neonatal hypoglycemia is associated with negative outcomes, both neurological and survival. In neonatal foals, hypoglycemia has been associated variably with survival, being associated with nonsurvival in septic foals, but not overall foal admissions. Hypoglycemia at admission has been associated with sepsis, bacteremia, and the systemic inflammatory response syndrome, with blood glucose outside the range of 50 to 180 mg/dL associated with decreased survival. In another study blood glucose > 120 mg/dL was associated with increased likelihood of survival. It remains uncertain whether derangements in blood glucose are the cause of, or merely reflective of, the pathophysiological processes decreasing survival.

5. Lactate
Lactate is widely reported in the assessment of health and prognosis. Lactate is considered a marker of circulatory competence, with the rate of clearance predicting survival in some studies. In human sepsis cases, serial blood lactate is considered to predict development of multiple organ failure. In sick human neonates blood lactate elevations are associated with increased mortality, with increasing levels preceding clinical indicators of deterioration.

In neonatal foals, admission arterial blood lactate has shown to be associated with survival and occurrence of systemic inflammatory response syndrome. Venous blood lactate elevation, and persistent hyperlactatemia, has been shown associated with survival and severity of illness. The highest admission lactate concentrations were present in hemorrhagic shock, sepsis, and complicated perinatal asphyxia in one study, but in younger, premature, or encephalopathic foals in another. Regardless of initial diagnosis, lactate is significantly increased in nonsurvivors vs survivors.

6. Metabolic and Endocrinological Pathways
Academic studies regarding the prognosis for neonatal survival have advanced to involve variables difficult to measure at point of care in routine clinical practice. Septic nonsurvivors have been shown to have elevated arginine vasopressin and adrenocorticotropic hormone, with a subcategory of neonates having concurrent normal to decreased cortisol implying a relative adrenal insufficiency. Hypoperfusion of critically ill foals, with the attendant clinically evident signs of poor peripheral perfusion, has been associated with elevated levels of both arginine vasopressin and aldosterone. Anion gap elevation and decreased venous PO2 were also associated significantly with nonsurvival. Associated with sepsis in humans, plasma adrenomedullin has been shown elevated in critically ill foals but lacks the specific association with sepsis seen in other species. As more is revealed about the complex endocrinological pathways of the neonatal foal during the transition to extrauterine life, additional
Table 1. Summary of Clinical or Clinicopathological Variables Affecting Outcome Versus Author (in Chronological Order)

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Table 1 (continued). Summary of Clinical or Clinicopathological Variables Affecting Outcome Versus Author (in Chronological Order)
markers of sepsis and dysfunction will no doubt be uncovered, some of which may be applicable to field assessment settings.

7. Discussion—How Much More Do We Know, and How Can it Help Us?

The 1980s are looked upon as the birth of equine neonatology. During this time, prognostication of the severity of illness and likely outcome was based upon clinical parameters derived from experience, and routine laboratory values available at the time. Attempts to formulate prognostic mathematical equations followed with varying success. These equations were based upon weighting factors derived from clinical data collected at various research sites, but unfortunately uniformity and transference between sites was difficult to achieve. Through the 1990s and early 2000s aspects of the presenting physical examination and laboratory values were integrated to provide a basis for prognosis, yet this also suffered from an inability to be transferred between hospitals and sometimes within the same hospital over time. Metabolic status became more closely studied with the widespread validation and application of lactate levels. Largely from the 2010s, research has turned toward homing in on endocrinological processes present in the neonatal foal and their disturbances due to in utero deprivation and periparturient events (Table 1). A number of studies suggest appropriate basic physical examination parameters, blood work that does not suggest the likelihood of a septic process, and a sufficient Ig concentration to be associated with survival. This information is readily assessed in field situations. In recent studies, some degree of linkage between endocrinological dysfunction and clinically evident comprises has been made.37

Throughout this period, the importance of the clinical examination has not been diminished, and in fact has been enhanced as in multiple studies involving many different populations of foals in various centers, physical parameters and observations have been retained in many prognostic models. Therefore, whereas the search for an objective, consistent, and transferrable marker of prognosis continues, veterinarians should rededicate themselves to conducting searching and comprehensive clinical examinations of at-risk neonates.

Acknowledgments

Declaration of Ethics
The Author declares that he has adhered to the Principles of Veterinary Medical Ethics of the AVMA.

Conflict of Interest
The Author declares no conflicts of interest.

References
10. Rohrbach BW, Buchanan BR, Drake JM et al. Use of a multivariable model to estimate the probability of discharge in hospitalized foals that are 7 days of age or less. J Am Vet Med Assoc 2006;228:1748–1756.
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Leveraging Free Resources and Strategic Planning Strategies That Work

Gregory A. Twedt

This article presents how you can take advantage of the free mentoring and counseling services that are available to small business owners, managers and entrepreneurs from SCORE. You also will get an introduction to a proven strategic planning process that you can use to grow your business. Author’s address: 300 South 4th Street, Suite 400, Las Vegas, NV 89101; e-mail: gtwedt@scorelv.org. © 2015 AAEP.

1. Introduction
Starting, running, and growing a small business can be a challenge for anyone, especially for those who are trained in a technical field but lack education and experience in basic business disciplines, including planning, marketing, finance, and human resources (HR) just to name a few. How do you fill these gaps when your financial resources are likely stretched and your time is limited? Whether you are already in business or looking to start your practice, there are resources available to help you. Of course, there are certified public accountants, lawyers, business consultants, and specialists available as well if you have the financial resources to hire them, but if you don’t or if you’re not sure, free resources are available. The US Small Business Administration (SBA) has resource partners that exist to assist the small business community. These partners include SCORE, small business development centers, and the Women’s Resource Center. This article will focus first on how SCORE can help you succeed and second on a process you can use to develop a strategic plan to move your business ahead.

2. What Is SCORE?
SCORE is America’s premier source of free and confidential small business mentoring and advice. The SCORE organization consists of experienced business people who volunteer their time to help small business owners, managers, and entrepreneurs succeed. As a national organization, SCORE has more than 10,000 members distributed across 320+ chapters. Chances are very good that there is a chapter near you, but if not, there are still ways to tap into SCORE’s resources. SCORE’s volunteers understand the needs and challenges of managing successful businesses because they have been there. They have owned and operated their own businesses or have served in management positions in various types of businesses. The basic service provided by SCORE is mentoring and advice delivered through face-to-face sessions, telephone calls, e-mails, or, in some cases, video conferencing. Clients seeking advice on a specific topic are generally paired with a mentor most closely matching their needs. One goal of SCORE volunteers is to establish an ongoing mentoring relationship with their clients. The primary mentor may often route clients to other men-
tors who have the experience or expertise in the specific area of concern. In addition to one-on-one mentoring, SCORE can assemble a team of mentors to visit the client site to analyze the situation—a SWAT team approach, so to speak, to assisting the client. In addition to mentoring, SCORE also helps to educate the small business community by holding local workshops conducted by the chapters as well as online seminars and workshops available through the national organization.

3. Who Does SCORE Serve?
SCORE serves entrepreneurs starting their business/practices, owners managing and growing their business, and owners facing times of trouble/opportunity. Let’s take a look at how SCORE mentors can help you in each of these situations.

Entrepreneurs Starting a Business
Chasing your dream is an exciting endeavor; however, before acquiring your business license, buying equipment, and opening your doors, there is a process that you should follow to get off to a good start. The author suggests that you first visit a SCORE mentor to discuss your idea and to map out a plan to get you there. Generally, you should perform a feasibility analysis to test your idea and answer basic questions such as the following:

- What is your value proposition to the customer?
- Who is your target audience?
- How big is the market?
- What is the competitive situation?
- What is your competitive advantage?
- What will your startup costs be?
- How will you operate the business?
- What are your preliminary financial projections (revenue and costs)?

After performing this preliminary analysis and reviewing it with your SCORE mentor, you should be ready to move on. This could include digging deeper into the previous questions, determining the organizational entity (LLC, S corporation, partnership, etc.) and licensing requirements of your jurisdiction, refining your financial projections, and calculating your funding requirements. If outside funding is required, all of the above can be assembled into a formal business plan for submission to funding sources. Your SCORE mentor can then review your final plan, help coach you on how to present it, and direct you to possible funding sources.

Owners Managing and Growing Their Business
All right—so you’ve opened your doors and are in the early phases of operating your business. Now a new set of issues have presented themselves, such as how to attract more business, what to do if you run out of cash, how to grow your business, etc. SCORE can help answer these and similar questions through both mentoring and education. As when you started your business, you should have a plan for growing your business. Developing an annual strategic plan is a key element in managing and growing your business (more on this later). Your SCORE mentor can guide you through this planning process and help to hold you accountable for executing the plan. In addition to planning, you can come to SCORE for advice on the following specific issues:

- Marketing and sales
- Product pricing
- Business processes
- Hiring and firing of employees
- General management
- Financial control
- Cash flow
- Funding

Your SCORE mentor or chapter has the resources to assist you. Additionally, you may want to consider adding your mentor to your advisory board if you have one. Your SCORE mentor wants to have an ongoing relationship with you and wants at a minimum for you to keep them abreast of how your business is progressing.

Owners Facing Times of Trouble/Opportunity
Although we don’t like to think about it, there can be times when serious problems arise that may threaten the life of the business. Then again, there could be tremendous opportunities that arise that demand special attention. Regardless, SCORE mentors can assist you. SCORE can assemble a team to help you analyze the situation and chart a course of action (provided you haven’t waited too long).

Sometimes a problem can be so severe, however, that there is no recovery path. Don’t let this happen to you. Work with your SCORE mentor to develop a set of key results indicators (KRI s) that can serve as an early warning mechanism to head off any problems before they become too severe. In addition, after developing a strategic plan and reviewing the plan’s progress with your mentor, you will have an additional set of objective eyes looking at your business for potential problems and/or opportunities.

4. Take Advantage of SCORE and Other Free Resources
SCORE and other free resource organizations can provide valuable advice for helping you grow your business. Whether you are just starting out, dealing with issues of managing and growing your business, or simply need someone to talk to, SCORE can help. Visit http://www.score.org to learn more. Here you can get information on many topics, view webinars on timely subjects, and most importantly locate a SCORE chapter nearest you and request a mentoring session. You can also start an e-mail mentoring session if you are not close to a SCORE
office or just need an answer to a question. Additional valuable information can also be found at the SBA website at http://www.sba.gov.

5. Strategic Planning: A Key to Growth
As mentioned previously, a strategic plan is a vital tool in managing and growing your business. This section outlines a process for developing a strategic plan for your business. Although it may seem like a daunting task, it doesn’t have to be. It does, however, require that you get away from the daily tasks of running the business and spend some quality time thinking about what you want to accomplish and how to do it.

Your plan should specify your goals and objectives along with the strategies and tactics necessary to accomplish them. It will help you to be proactive rather than reactive, keep you focused on what is important, and provide you with a yardstick to measure performance. The plan should be clearly and concisely written down—it doesn’t have to be a work of literary art. To get you started, you first need to establish your process (i.e., what steps you will follow) and then put together a schedule. An example of a workable process is as follows:

- Establish your planning team
- Review or set mission, vision, and values
- Set your destination
- Build your marketing plan
- Build your operational plan
- Build your financial plan

If you are new to planning, you may want to just focus on the next year. As you refine your process and become more comfortable, you can expand the planning horizon out to 3 to 5 years.

Choosing your planning team is an important element in developing a good plan. You do not want to try and go it alone. Having people who can and will contribute to the thinking process and provide critical feedback is imperative. You want people that know and care about you and your business. They could be knowledgeable/key employees or outside resources such as your accountant or lawyer, business associates, friends, and SCORE mentor.

Your mission, vision, and values set the parameters for your planning. If you have them, that’s great. If not, it’s worth spending some time writing down your thoughts, but be careful not to let this task drag on too long. Your mission is essentially your long-term direction. Your vision is what the business will look like in the future. Your core values specify how you want your business to behave. Expressions such as “customer first,” “integrity,” “quality,” “innovation,” or “green” are examples of core values. These core values will generally reflect those of the owner. They are useful in hiring (and selecting partners) because you want people who share your values.

Setting your destination specifies where you want the business to go. This step actually has two components. The first is to honestly assess where you are today; the second is to define where you want to go. To assess where you are today, document your target market, products and services, competition, customers, capabilities, and financial performance. You should also take note of any trends (such as those on social media) or external factors (such as government regulations, the economy, and technology) that may affect the business. Finally, you should perform a SWOT analysis on your current business to exploit strengths and opportunities and to address weaknesses and threats.

After examining where you are today you can now establish where you want to be at the end of the planning horizon. Note here that if you are looking out 3 to 5 years you’ll want to set goals, objectives, and strategies that establish vectors for your business. After the long-term direction is set, you can then bring your planning down to the next year. Here you set goals and objectives that are attainable in the next year and consistent with the long-term direction. Your goals and objectives can address several categories:

- Revenue and profitability
- Products/services/markets/locations
- Capabilities/resources
- Operational improvements
- Customer relations
- Systems and procedures

Regardless of the category, your objectives should be specific, measurable, achievable, relevant, and timely. One note of caution: you may set more objectives than you can reasonably accomplish with the resources you have available; therefore, you should also establish priorities. As you further develop the plan into action items and budgeting, it may become apparent that you do not have the resources necessary, and the priority can be used to adjust the plan.

Your marketing plan is the first step in defining how you will accomplish your goals and objectives. Here you may need to do some market research to gain further insights into your existing market or new markets you want to attack. For new markets, research the market’s size, unique characteristics, what its needs and problems are, any special buying factors, pricing, etc. Preferably, you have done this research before you set an objective to enter a new market. If you do not have a good handle on your existing market, a similar analysis should be performed. You also should analyze your competitors and understand their strengths and weaknesses, products and services, pricing, marketing messages, and positioning. Study their website for insights and solicit feedback from your customers.
You can define the 4 Ps for your business as follows:

- **Product:** What are your products/services?
- **Price:** What is your pricing/bundling strategy?
- **Place:** Where will you deliver the product/service?
- **Promotion:** How will you promote the business?

Next, for each step in the customer cycle, develop a strategy to address acquiring and retaining customers. The customer cycle, definition, and possible strategies include the following:

**Suspects:** Suspects are those who could potentially use your service. Strategies to reach suspects include creating a website, optimizing search engines, direct mailing, and advertising.

**Prospects:** Prospects have a need/interest for your service. Strategies to entice prospects include offering incentives, coupons, and white papers.

**Customers:** Customers have purchased your service. Strategies to “wow” customers include providing a quality customer experience, excellent service, and post-service follow-up.

**Repeat customers:** Repeat customers are those who have purchased goods or services from your business multiple times. Strategies for retaining repeat customers include offering perks, sending out newsletters, and following up.

**Raving fan:** A raving fan is someone who loves your company. Strategies for rewarding raving fans include offering perks and reference bonuses.

The idea is to develop and implement strategies that move customers from suspects to raving fans.

Finally, develop a clearly articulated value proposition and marketing message that you will integrate into every marketing program and vehicle (website, brochures, advertising, e-mail blasts, social media, etc.). Note that you could have a different marketing message depending on the target market (e.g., 1 for the horse owner and 1 for the breeder). The message should communicate the benefits of your service and correspond to the needs of your audience.

**Step 2 in defining how you will accomplish your goals and objectives is your operational plan.** This step determines the strategies and tactics bundled together as programs that your business will execute to achieve your objectives. You can establish programs in areas such as the following:

- Sales and revenue
- Product development/product line expansion
- Infrastructure—website, automation, equipment
- Systems and procedures
- Hiring/training/HR

The programs should support specific goals and objectives. Within each program you should define the strategies you will use and the tactics/action plans that will be required to execute the strategy.

This is best explained by the following example:

**Objective:** Increase sales by 50%.

**Strategy 1:** Expand the sales force.

**Tactics:** Develop a quick-start sales training program in Q1 and add new salesperson in Q2 and Q3.

**Strategy 2:** Expand the marketing program to increase lead generation (prospects).

**Tactics:** Update website with a new look and feel and better search engine optimization in Q1 and implement an e-mail marketing and social media strategy in Q2 and Q3.

Please note that each tactic has a delivery timeframe (by quarter in this example) and should include a responsibility assignment. As the year proceeds, you will tighten up these program schedules by developing project plans with tasks, timelines, milestones, and task responsibility. To repeat an earlier warning, choose your objectives wisely. Don’t have too many, and focus on those that are most important. Keep your plan in harmony with your ability to deliver.

The last step in the planning process is creating a financial plan to support your goals and objectives, strategies, and tactics. The financial plan includes the following:

- Revenue projections
- Costs/expense budget
- Scenarios
- KRI

Revenue projections are your forecast of how much and when you will generate revenue. The projection should be built from the ground up for each product group you market. It is often not feasible to forecast every product you sell, so forming groups of similar products with similar margins makes the projection process more manageable. For example, estimating how many customers you will have for the product each month and multiplying that by the average revenue per customer will generate a monthly revenue figure. In this example, both the number of customers and average revenue per customer can be tracked as you move through the month as one of your KRI (more on that later).

Your cost and expense budget lays out the cost of running the business or, in other words, where the money goes. Some costs can be expensed, whereas others may need to be capitalized. Typical categories of costs that are expensed include the following:

- **Fixed:** costs incurred every month regardless of revenue (rent, utilities).
- **Variable:** costs associated directly with revenue (cost of goods sold, commissions).
- **Step function:** costs that increase when revenue reaches certain levels (hire an additional employee for sales, lease more space).
- **Discretionary:** costs associated with imple-
menting your programs (more flexibility in timing these programs if business conditions do not follow the plan).

As with revenues, costs should be slotted by month, thus giving you the components for your projected monthly income statement. This is another point at which you can test your plan for feasibility.

Other factors to consider in building your financial plan include allowance for bad debt, average collection period for receivables, payment period for payables, inventory levels, repayment of debt, and capital expenditures. These factors can affect your cash flow and need to be managed. You can be profitable and still run out of cash.

This part of your plan will help you to identify any financing needs you may have for working capital and capital expenditures. To prepare you for the possibility that things won’t go exactly as planned, you should run some variations, or scenarios, on your projections and budget. With a good spreadsheet you can see what happens if revenues fall short or exceed your projections. Try using a best-/worse-case adjustment of ±25% to 30%. The same can be done with costs you don’t control, although it probably won’t be as dramatic a swing as revenues. This exercise will get you thinking about what you would do if reality doesn’t follow the plan.

Finally, you can establish key results indicators (KRI) to serve as metrics you use to track your business performance before you see it reflected on your financial statements. These indicators vary by type of business but could include the following:

- Month-to-date sales/daily sales
- Order backlog
- Pipeline (prospects becoming customers)
- Cash balance
- Accounts receivable
- Inventory
- Billable hours/utilization percent

These KRIs can be reported daily or weekly as you move through the month so you’re not in for any surprises when the financial reports arrive on your desk. If necessary, you can take early action to head off potential problems.

6. Your Plan Is Done... Now What?

Your strategic plan now becomes a tool to help you manage your business. If you have employees, share some or all of it with them to get everyone on the same page. Align their goals and performance measurements with your plan. Use your plan to manage the business. On a daily or weekly basis, review your KRIs in the form of a flash report. Examine your financial statements once a month and compare actual results to what was planned. Then take time to understand any significant variances from your plan. You may also want frequent updates on operational programs that are in progress. Finally, review the overall status of your progress against your plan each quarter—preferably with your planning team—and make any necessary adjustments.

If you “plan your work and work your plan” you will have a good chance of reaching your goals and objectives.

One final note. This may seem like a lot of work and a distraction from day-to-day operations, but remember as the owner and manager of the business one of your key responsibilities is to plan. Also, it doesn’t have to take too long. A series of 5 to 6 team meetings preceded by individual thinking time can result in a good plan in 2 to 4 weeks. Get started. You won’t regret it.

Acknowledgments

Declaration of Ethics
The Author declares that he has adhered to the Principles of Veterinary Medical Ethics of the AVMA.

Conflict of Interest
The Author declares no conflicts of interest.
Global Expansion of Your Practice as a Strategy

Joop B.A. Loomans, DVM, PhD

Many sport horses now travel the world, sometimes to countries that lack the infrastructure for professional care of equine athletes. Thus, global travel may potentially jeopardize the wellbeing of these sport horses, but at the same time provides opportunities for the equine veterinary profession. Author's addresses: Oculus-Insights B.V., Appelgaarde 95, 3992 JD Houten, The Netherlands; and Heilan International Equestrian Club, Xinqiao Town, Jiangsu Province, China; e-mail: jloomans@oculus-insights.com. © 2015 AAEP.

1. Introduction

In most of the Western countries, recreational use of horses evolved from an agricultural, transportation, and military necessity to a professional sport, racing, and leisure industry.1 Our careers as equine veterinarians rely heavily on this industry where the bond between the horse and the rider/owner can be very strong both emotionally as well as financially. We have created an infrastructure with academic education for (equine) veterinarians, further specialization opportunities, created (inter)national organizations of equine veterinarians and specialists, built clinics, referral centers, centers of excellence, etc. In “our world,” our customers take this for granted and see it as an easily accessible commodity.2

Recently, new countries have entered the world of equestrian sport, which is good for the sport as a whole and the Olympic status of the sport. Expanding their global network is the strategy of the two leading governing bodies of equestrian sport, the Fédération Equestre Internationale (FEI) and the International Federation of Horseracing Authorities (IFHA), who, in 2013, joined forces and created the International Horse Sports Confederation (IHSC). Because globalizing equestrian sport involves movement of the equine athletes around the world, making the global transportation of the horses easier is a priority for the IHSC.3 As a result, the organization has intensified their cooperation with the Animal World Health Organization (OIE).

According to the 2013 Annual Report of the IFHA3: “The IHSC’s primary mission will be the exchange of information and technical knowledge, and collaboration on issues that represent the collective interests of both our organizations, including the international movement of horses with the Animal World Health Organization (OIE), horse welfare, anti-doping, coordinated communications and fair play.”

“Under the session titled ‘Developing and Promoting International Competition’, the affiliation between the IFHA and the OIE was discussed. The presentation made by Bernard Vallat, Director General of the OIE, groups detailed the facilitation of the international movement of racehorses, including a concept known as High Health, High Performance (HHP) which would allow for a streamlined movement of sport horses and race-
horses that met criteria as being low risk for disease transmission."

The aim of the HHP horses status is to allow for these horses to be transported more easily because their health status is monitored more frequently than that of other (local) horses and the risk of disease transmission is minimized. Transporting the horses to new emerging countries for competition is only one of the challenges; keeping them healthy during their stay is yet another. Many of these emerging economies/countries lack the infrastructure to provide for the complex needs of these horses once they arrive. Professional equine veterinary care, amongst other needs, is often completely unavailable. Challenges and opportunities exist for equine veterinarians and equine veterinary practices that consider expanding their activities abroad as part of their strategic plan.

2. The Global Equine Industry

The FEI is the global regulatory body of most equestrian disciplines and is located in Lausanne, Switzerland. They have registered approximately 90,000 athletes (riders and horses) and regulate 3500 events per year.3 The organization is also a good source of information for what is taking place in equestrian sports worldwide given that they provide annual reports with statistics of their events. Between 2007 and 2013, the number of events organized under the FEI umbrella increased by 56% despite the economic recession in many parts of the world. Fig. 1 shows the differences in percentage per discipline with 2007 as the starting point. In all disciplines with the exception of driving, the number of events increased from 2007 to 2014.

Table 1 provides information about the total number of equestrian competitions organized under FEI rules per country in 2013 as an indication of the importance of these countries in the equestrian industry. France, United States, Germany, Italy, Austria, Spain, United Kingdom, Australia, The Netherlands, and Belgium are the ten countries that provide greater than 100 competitions annually. Table 2 shows which countries made the biggest growth in percentage of competitions between 2009 and 2013; i.e., the most active newcomers in equestrian sports in these recent years. Here Estonia, Costa Rica, Tunisia, Oman, China, India, Venezuela, Jordan, and Slovakia are at the top with more than 200% growth in their competitions. Many of these countries have the ambition to become important players on the world stage, and given recent results at the Olympics, World Equestrian Games, and other international competitions the equestrian sport is globalizing rapidly.

The IFHA also provides information on their website regarding starts, breeding, prize money, betting, etc., and this provides a good insight into what is happening globally in the racing and Thoroughbred breeding industries. Table 3 shows the 30 countries with the most horse races (both flat and jump) in the world in 2013 and the prize money involved. The United States, Australia, and Japan are shown to be the key players in this industry. Table 4 provides information about the number of breeding horses (foals, mares, and stallions) per country and the percentage of growth between 2011 and 2013. Globally, the number of Thoroughbreds used for breeding decreased 11.1% during this period, but there are large differences between countries; some of the most active horse racing countries in the
world are shrinking substantially in number of racehorses produced. This industry contracture would be expected to have a negative effect on the workload and income of equine veterinarians working in this sector in these countries.

3. Opportunities

Many equine veterinarians who work with breeding farms, stallions, and sport horses already have contacts abroad. Horses are examined for export, laboratory samples are taken, and pre-purchase examinations are performed. Semen and embryos are exported, and sport horse services are provided to international sport and racehorses. Additional touch-points are the team veterinarians of national teams who travel with horses to competitions, FEI official vets or treating vets at international competitions and races, and advisors of international operating equine industries. Veterinary practices can utilize these contacts and develop an international presence as part of their strategy. Even without providing services in foreign venues, veterinarians can provide an important service to their clients by being a resource and advisor. Did you ever wonder where the horses that you are preparing for export end up, what calibre of veterinary care is available abroad, and whether you should provide advice to the buyer and/or seller on these matters?

An excellent way to learn more about the equine veterinary industry abroad is through foreign interns, externs, or associates who work at your clinic, or in your region. There are also trade missions of specific sectors to different countries where you can team up with breeders, horse dealers, horse feed industry, pharmaceutical industry, etc. Very often these kinds of missions are organized by local embassies or consulates of your country and they are also very helpful and equipped to provide local information about our industry. It is necessary to actively research these different recourses, connect with local equestrian federations, and create your own network.

4. Challenges

Working in other countries and other cultures can be challenging, especially in emerging countries where our profession is not well understood, or the specific knowledge and clinical skills of working with sport and racehorses does not exist locally. If a strategic decision as a practice or as an individual is made to investigate these new business opportu-

### Table 2. The 30 Countries With the Biggest Growth of FEI Competitions in Percent, 2009–2013

<table>
<thead>
<tr>
<th>Growth, %</th>
<th>Country</th>
<th>Events in 2013, n</th>
</tr>
</thead>
<tbody>
<tr>
<td>1300</td>
<td>Estonia</td>
<td>14</td>
</tr>
<tr>
<td>1000</td>
<td>Costa Rica</td>
<td>22</td>
</tr>
<tr>
<td>1000</td>
<td>Tunisia</td>
<td>11</td>
</tr>
<tr>
<td>800</td>
<td>Oman</td>
<td>8</td>
</tr>
<tr>
<td>700</td>
<td>China</td>
<td>8</td>
</tr>
<tr>
<td>600</td>
<td>India</td>
<td>6</td>
</tr>
<tr>
<td>500</td>
<td>Venezuela</td>
<td>6</td>
</tr>
<tr>
<td>300</td>
<td>Jordan</td>
<td>8</td>
</tr>
<tr>
<td>225</td>
<td>Slovakia</td>
<td>26</td>
</tr>
<tr>
<td>200</td>
<td>Lithuania</td>
<td>12</td>
</tr>
<tr>
<td>200</td>
<td>Romania</td>
<td>6</td>
</tr>
<tr>
<td>200</td>
<td>Indonesia</td>
<td>2</td>
</tr>
<tr>
<td>200</td>
<td>Paraguay</td>
<td>2</td>
</tr>
<tr>
<td>167</td>
<td>Colombia</td>
<td>24</td>
</tr>
<tr>
<td>150</td>
<td>Slovenia</td>
<td>20</td>
</tr>
<tr>
<td>133</td>
<td>Ecuador</td>
<td>7</td>
</tr>
<tr>
<td>125</td>
<td>Turkey</td>
<td>9</td>
</tr>
<tr>
<td>119</td>
<td>Switzerland</td>
<td>46</td>
</tr>
<tr>
<td>100</td>
<td>Kazakhstan</td>
<td>8</td>
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<td>100</td>
<td>Azerbaijan</td>
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<tr>
<td>100</td>
<td>Georgia</td>
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<tr>
<td>100</td>
<td>Latvia</td>
<td>2</td>
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<tr>
<td>100</td>
<td>Puerto Rico</td>
<td>2</td>
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<tr>
<td>100</td>
<td>El Salvador</td>
<td>1</td>
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<tr>
<td>100</td>
<td>Hong Kong</td>
<td>1</td>
</tr>
<tr>
<td>100</td>
<td>Singapore</td>
<td>1</td>
</tr>
<tr>
<td>83</td>
<td>Thailand</td>
<td>11</td>
</tr>
<tr>
<td>79</td>
<td>Chile</td>
<td>50</td>
</tr>
<tr>
<td>71</td>
<td>Austria</td>
<td>166</td>
</tr>
<tr>
<td>67</td>
<td>Bulgaria</td>
<td>10</td>
</tr>
</tbody>
</table>

From the 2013 FEI Annual Report.

### Table 3. The 30 Countries Hosting the Most Horse Races and Their Prize Money in 2013

<table>
<thead>
<tr>
<th>Races, n</th>
<th>Prize Money, €</th>
<th>Country</th>
</tr>
</thead>
<tbody>
<tr>
<td>43,135</td>
<td>682,811,690</td>
<td>United States</td>
</tr>
<tr>
<td>19,626</td>
<td>381,707,190</td>
<td>Australia</td>
</tr>
<tr>
<td>16,122</td>
<td>572,433,883</td>
<td>Japan</td>
</tr>
<tr>
<td>10,146</td>
<td>137,006,673</td>
<td>Great Britain</td>
</tr>
<tr>
<td>7,146</td>
<td>120,188,545</td>
<td>France</td>
</tr>
<tr>
<td>5,525</td>
<td>44,658,244</td>
<td>Argentina</td>
</tr>
<tr>
<td>4,892</td>
<td>23,992,881</td>
<td>Chile</td>
</tr>
<tr>
<td>3,967</td>
<td>18,810</td>
<td>Brazil</td>
</tr>
<tr>
<td>3,809</td>
<td>22,029,975</td>
<td>South Africa</td>
</tr>
<tr>
<td>3,675</td>
<td>65,379,685</td>
<td>Canada</td>
</tr>
<tr>
<td>3,269</td>
<td>34,966,952</td>
<td>Italy</td>
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<tr>
<td>3,007</td>
<td>29,629,679</td>
<td>New Zealand</td>
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<tr>
<td>2,746</td>
<td>8,441,146</td>
<td>Venezuela</td>
</tr>
<tr>
<td>2,546</td>
<td>50,780,663</td>
<td>Turkey</td>
</tr>
<tr>
<td>2,534</td>
<td>15,120,100</td>
<td>India</td>
</tr>
<tr>
<td>2,530</td>
<td>45,986,000</td>
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<tr>
<td>1,935</td>
<td>6,862,005</td>
<td>Peru</td>
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<td>1,904</td>
<td>134,875,854</td>
<td>Korea</td>
</tr>
<tr>
<td>1,828</td>
<td>3,225,495</td>
<td>Mexico</td>
</tr>
<tr>
<td>1,646</td>
<td>10,532,339</td>
<td>Uruguay</td>
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<tr>
<td>1,332</td>
<td>6,549,264</td>
<td>Panama</td>
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<tr>
<td>1,286</td>
<td>6,377,864</td>
<td>Morocco</td>
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<tr>
<td>1,275</td>
<td>12,884,371</td>
<td>Germany</td>
</tr>
<tr>
<td>994</td>
<td>39,310,284</td>
<td>Singapore</td>
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<tr>
<td>980</td>
<td>6,789,340</td>
<td>Cyprus</td>
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<tr>
<td>794</td>
<td>4,535,653</td>
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<tr>
<td>771</td>
<td>83,201,938</td>
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<tr>
<td>672</td>
<td>7,821,835</td>
<td>Sweden</td>
</tr>
<tr>
<td>608</td>
<td>8,366,832</td>
<td>Saudi Arabia</td>
</tr>
</tbody>
</table>

Adapted from the 2013 IFHA Annual Report.
Table 4. Change in the Number of Registered Thoroughbred Horses for Breeding (Foals, Mares, Stallions) 2011–2013

<table>
<thead>
<tr>
<th>Growth, %</th>
<th>Growth, n</th>
<th>Country</th>
<th>Registered 2013</th>
</tr>
</thead>
<tbody>
<tr>
<td>83</td>
<td>217</td>
<td>Spain</td>
<td>477</td>
</tr>
<tr>
<td>71</td>
<td>−5</td>
<td>Uzbekistan</td>
<td>12</td>
</tr>
<tr>
<td>46</td>
<td>77</td>
<td>Qatar</td>
<td>244</td>
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<tr>
<td>39</td>
<td>62</td>
<td>Tunisia</td>
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</tr>
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<td>38</td>
<td>98</td>
<td>Ecuador</td>
<td>353</td>
</tr>
<tr>
<td>26</td>
<td>24</td>
<td>Norway</td>
<td>118</td>
</tr>
<tr>
<td>19</td>
<td>298</td>
<td>Philip- pines</td>
<td>1907</td>
</tr>
<tr>
<td>16</td>
<td>573</td>
<td>Saudi Arabia</td>
<td>4211</td>
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<tr>
<td>13</td>
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<td>Thailand</td>
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<tr>
<td>11</td>
<td>2,103</td>
<td>Ireland</td>
<td>20,606</td>
</tr>
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<td>11</td>
<td>89</td>
<td>Morocco</td>
<td>933</td>
</tr>
<tr>
<td>9</td>
<td>13</td>
<td>Kazakh- stan</td>
<td>153</td>
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<tr>
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Adapted from the 2013 IFHA Annual Report.
take internationally as well. Most surgical instruments, suture materials, bandage materials, and other needed items can also be transported globally. Stocks can be made readily from local material, as can a twitch and a stomach tube. Constructing surgical facilities and obtaining specialized equipment to safely induce and maintain horses under general anesthesia is more difficult to accomplish. When there are facilities available elsewhere in the country, it is necessary to determine whether there are horseboxes or trucks available and allowed to transport horses to these destinations quickly and safely.

Local Legislation
Even when there are no equine veterinarians available in a particular country, that does not automatically give a foreign veterinarian the right to practice there. It is important to research local legislation, including whether your diploma is acknowledged and whether you are allowed to work in the country. Tax implications, whether you work as an employee or start your own business abroad, are important to investigate both in the new market as well as at home.

Local Health Threats for Horses
When you start from scratch in a new culture in a new country, you want to know as much as possible about the possible threats for the horses. These do not necessarily have to be infectious diseases, parasites, midgets, mosquitoes, etc. Simple things, such as the temperature and humidity in different parts of the year; availability of good roughage, grains, or pellets, drinking water; stable quality; and pasture availability may have a large effect on equines’ health and welfare. The presence of good farrier care should also be investigated. At one’s home country these elements are often taken for granted, but in a new environment they may be impossible to get or of inferior quality.

Personal Safety and Insurance
Working with horses carries significant risk and accidents during work do happen. In an environment that lacks horsemanship and up-to-date infrastructure these risks should be expected to increase. Therefore, investigation of the human healthcare system is essential. It is very important to make sure you are well prepared and know whether and where help is available. Insurances policies for health and disability at home may have to be adjusted to accommodate a change to a work environment abroad. Professional liability insurance also is likely to require modification.

5. Discussion
International sport horse competitions are expanding all around the globe. In emerging economies, the middle class and wealthy have discovered the leisure and competition horse and want to play a role in the international scene. Betting on horses is also very popular in many of these countries. Horses travel easily around the world and sometimes end up in places that have no proper infrastructure and are not equipped to provide professional care up to our standards. Due to these circumstances the welfare of these horses can be compromised. Both the FEI and IFHA are working with the OIE to make it easier for sport horses to travel the world. This provides opportunities in the equine industry. Equine veterinarians who are professionally involved with the export of horses are particularly well positioned to embrace this strategy. Providing education to owners, grooms, riders, and veterinarians in these regions can be quite a challenge but is often rewarding. The most important factor for success internationally is the engagement of the emerging countries’ riders and horse owners (individuals and companies) and fostering their willingness to invest in (veterinary) care. The equine veterinary profession in these regions is empowered through the help of equine practices from other parts of the world. This does not have to be an altruistic enterprise but can be part of a business strategy to expand practice activities overseas, providing not only veterinary services but also equine welfare, veterinary education, and horsemanship. In a shrinking local economy, a global presence can be an opportunity for growth and a strategy for expansion and survival of your business.

Acknowledgments
Declaration of Ethics
The Author declares that he has adhered to the Principles of Veterinary Medical Ethics of the AVMA.

Conflict of Interest
The Author declares no conflicts of interest.

References
The Equine Liver in Health and Disease

Thomas J. Divers, DVM, DACVIM, DACVECC

This article is dedicated in memory of Dr. Doug Byars, a wonderful friend, colleague, teacher, and equine clinician. Author’s address: Department of Clinical Sciences, College of Veterinary Medicine, Cornell University, Ithaca, NY 14853; e-mail: tjd8@cornell.edu. © 2015 AAEP.

Anatomy and Histology

The liver is the second largest single organ in the horse (skin is first), accounting for approximately 1.5% of body weight. In the adult horse, the liver is positioned obliquely in the cranial abdomen, with the left lobes located ventrally near the 7th to 10th intercostal spaces and the right lobes positioned more dorsally and opposite the 9th to 16th intercostal space. The smaller ventral quadrate and right dorsal caudate lobes are located mid-abdomen.

The liver on the left encompasses approximately one third of the liver mass and is often adjacent to the more caudally located spleen; this provides an opportunity, when visualized with ultrasound, to compare the relative echogenicity of each organ. The liver should be hypoechoic compared with the spleen (Fig. 1), and with hepatic fibrosis the liver and spleen appear more similar in echogenicity. An unusual anatomical variant in horses is atrophy of the right lobe of the liver that occurs in some middle-age-to-older horses. In this condition, there is a loss of hepatocytes with condensation of the hepatic stroma and a thick, wrinkled hepatic capsule develops. This atrophy may occasionally prevent ultrasound visualization of the right liver. The cause of this atrophy is somewhat unique to the horse, and is not believed to be a result of divergence of arterial or venous blood flow. Instead, it is believed to result from compression of this portion of the liver by the right dorsal colon and cecum. In the fetus and newborn foal, the liver is relatively much larger and on fetal ultrasound the liver is the easiest organ to identify.

At the light microscopic level, the liver can be divided into structural units called hepatic lobules. The classic hepatic lobule is a polygonal histologic unit composed of numerous plates of liver cells (hepatocytes), radiating toward a central vein. Situated around the perimeter, at each “corner” of the lobule, are branches of the hepatic artery, hepatic portal vein, and bile duct (together called the hepatic triad), plus lymphatics. Blood from hepatic arterioles and portal venules mixes together in hepatic sinusoids, which surround the plates of hepatocytes, thus bathing each hepatocyte in a mix of arterial blood plus venous blood that has come mainly from the gut and spleen via the hepatic portal system. Sinusoidal blood then travels along the plates of hepatocytes toward the central vein, eventually entering larger hepatic veins that converge near the diaphragm to enter the caudal vena cava. Between each hepatocyte are microscopic bile canaliculi into which bile produced by each hepatocyte is secreted.

Bile flow within each hepatic lobule is opposite that of blood, with bile heading toward bile duct tributaries at the periphery of each lobule. The ducts from each lobe join and eventually form the...
left and right hepatic ducts. These unite to form the common hepatic ducts leading to the common bile duct. Both the bile duct and pancreatic duct empty into the duodenum at the major duodenal papilla, located on a slightly raised mucosal ridge. This ridge can be seen on endoscopic examination of the duodenum, just prior to the cranial flexure of the duodenum at the beginning of the sigmoid loop.

Seventy percent of blood flow to the liver is deoxygenated blood from the hepatic portal vein. The hepatic portal vein receives blood from the stomach, spleen, pancreas, small intestines, cecum, and large colon, making the liver the most important organ for assimilation and metabolism of nutrients, orally administered drugs, and gut-derived toxins or bacteria. The portal vein does not receive blood from the most distal colon and rectum, kidneys, reproductive organs, or from the mammary gland. The other 30% of blood to the liver is oxygenated, provided by the accompanying hepatic artery. Approximately 10% of the total blood volume at any one time is found in the liver.

1. Pathophysiology and Pathology

Insults to the liver and secondary diseases are common due to hepatic filtration of enteric-derived toxins, both bacterial and chemical. Fortunately, the liver is an amazingly regenerative organ and most diseases do not progress to failure. Although hepatocytes are fully differentiated cells they are capable of active proliferation by self duplication in response to acute external stimulation and up-regulation of humoral factors, all without any contribution from hepatic stem cell progenitor cells. However, in the chronically injured liver, hepatocytes do not proliferate well and biliary cells commonly proliferate and form ductules around the portal veins. The biliary cell proliferation associated with chronic injury may contain progenitor cells, which can give rise to both hepatocyte and biliary cells in an attempt to repair the liver.

The liver is a vital organ, one that performs many essential functions such as production of most plasma proteins (including albumin), production of almost all coagulation factors, and production of acute phase proteins (e.g., fibrinogen, amyloid A, hepcidin). The liver also stores glucose as glycogen and performs glycogenolysis when required. It performs gluconeogenesis from glycerol, lactate, volatile fatty acids such as propionate, some amino acids, and in general, the liver is the most important organ in the intermediate metabolism of proteins, carbohydrates, and lipids. It is an important storage organ for fat-soluble vitamins (A, D, E, and K) and many elemental nutrients (iron, copper, selenium, and molybdenum). The liver is also the detoxifying organ of the body for many xenobiotics (a chemical compound; as a drug, pesticide, or carcinogen that is foreign to a living organism) and is the prime organ for first-passage metabolism of many drugs and for excretion of some.

Liver pathology can, simply from a clinical perspective, be divided into acute vs. chronic, and when possible, into predominately hepatocellular vs. biliary injury. Laboratory biochemical testing can be helpful in determining predominantly hepatocellular vs. predominantly biliary types of disease when the relative increases between hepatocellular and biliary enzymes and between direct and indirect biliuribin are compared. Distinguishing between acute vs. chronic liver disease is best accomplished by evaluation of history, clinical findings, ultrasound and/or biopsy evidence of fibrosis (evidence of fibrosis in particular is an important determinant of long-term prognosis). Resolution of acute hepatic injury often results in a rapid and complete recovery whereas chronic injury is more likely to cause fibrosis. Differentiating whether biliary or hepatocellular disease predominates can be important in narrowing the differential diagnosis list and, when possible, allows early, focused therapy. In many cases, it may not be possible to make clear-cut distinctions between the two. In the horse, the rate at which fibrosis can occur is surprising. When investigating ferrous fumarate toxicity and hepatic failure in newborn foals in 1983, 3- and 4-day-old affected foals commonly had evidence of both fibrosis and regeneration.

Following injury, hepatic fibrosis is believed to be promoted predominantly by hepatic stellate cells (also known as perisinusoidal or Ito cells). Hepatic stellate cells are located in the perisinusoidal space (space of Disse), in close proximity to hepatocytes, just beneath sinusoidal endothelial cells and adjacent to Kupffer cells. These stellate-shaped cells are normally quiescent and are present in relatively small numbers; they contain intracellular...
lipids that serve to store vitamin A as retinal ester. When the liver is damaged, Kupffer cells produce tumor necrosis factor alpha, causing the stellate cells to contract individually but proliferate in numbers. These activated cells become chemotactic, decrease their vitamin A stores, secrete collagen (thus causing fibrosis) and eventually become senescent.\ref{8,14}

Kupffer cells are specialized macrophages that are part of the reticuloendothelial system. Kupffer cells are responsible for destroying gut-derived bacteria, endotoxins, and other foreign substances. They also help in recycling iron from senescent or injured red blood cells and, as a result, Kupffer cells accumulate hemosiderin and this can be pronounced even in disease-free horses.\ref{15}

2. Biochemical Testing for Liver Disease and Failure

Biochemical testing is imperative when attempting to diagnose liver disease or liver failure. From a clinical perspective, biochemical results can be helpful in narrowing the differentials for the liver injury and, when evaluated over time, can help predict prognosis. Biochemical testing can also be used to identify subclinical hepatotoxin exposure, such as is seen with pyrrolizidine alkaloid toxicity or drug-induced liver disease.\ref{16} Liver-specific enzymes include sorbitol dehydrogenase (SDH), glutamate dehydrogenase (GLDH), and gamma glutamyltransferase (GGT), which respectively reflect predominantly hepatocellular (SDH, GLDH) and biliary injury (GGT).\ref{11} Aspartate aminotransferase (AST) and alkaline phosphatase (ALP) also, respectively, reflect hepatocellular and biliary injury, but AST and ALP are not liver specific. Increased values of serum SDH, GLDH, and AST are expected with even mild hepatocyte injury. SDH is released from the cytosol of the hepatocyte and has a short half-life (hours) and repeated SDH measurements can be helpful in determining resolution or progression of hepatic insult.\ref{17} GLDH is localized in hepatic mitochondria and is markedly increased in the serum of most horses with acute liver disease resulting from or with necrosis of hepatocytes; sensitivity for GLDH elevations in detection of hepatic necrosis and hepatic lipidosis was 78 and 86%, respectively.\ref{18} GLDH is thought to be more stable and have a slightly longer $T_{1/2}$ than does SDH, although SDH is stable for at least 24 hours when the sample is refrigerated.\ref{19}

GGT is an excellent screening test for hepatic disease in the horse.\ref{20} In my experience it is rare that a horse with severe liver disease will not have elevated plasma/serum GGT. GGT often continues to elevate for several days after a hepatic insult is no longer present, presumably due to biliary hyperplasia. Although the greatest elevations in GGT are seen with biliary disease, there is some GGT release into plasma with hepatocellular injury.\ref{21} On rare occasion, and with severe chronic fibrosis, SDH and GLDH may return to normal range despite a fatal outcome.

The magnitude of elevation of hepatic-derived enzymes may not correspond to functional abnormalities and should, therefore, be considered tests that measure disease and not tests that measure function (the magnitude of increases in hepatic enzymes [especially GGT] should not routinely be used by itself to determine prognosis). During a 2-year farm investigation of a forage-associated hepatopathy in Europe, more than 70 weanlings, yearlings, and adults had elevated GGT values, some as high as 1000 IU/l (mean, 180 IU/l) and GLDH values as high 1200 IU/l, yet not a single horse has demonstrated signs of hepatic failure nor any obvious clinical signs (author, personal observation). The prognosis for horses with hepatic failure is best determined by function test abnormalities, etiology, and presence or absence of hepatic encephalopathy.\ref{22} Somewhat unique to the horse, hematocrit, serum iron, and percentage iron saturation are sometimes high in horses with severe liver disease and remain after rehydration. Racehorses occasionally have mild-to-moderate increases in GGT (50–140 IU/l) without any other biochemical evidence of liver disease. This phenomenon has been observed for at least 30 years and the elevation in GGT has often been temporally linked to poor performance.\ref{23} In some situations, multiple horses stabilized together may have elevations in GGT. Possible causes include drug administration causing induction of GGT, toxin exposure and disease specific to the biliary tract, viral infections causing only increases in GGT at the time of sampling, and overtraining with depletion of hepatic glycogen.\ref{24} GGT activity between 70 and 100 U/L has previously been associated with poor health, oxidative stress, and overtraining the horse, and further, those horses with high GGT values showed a markedly reduced incidence of increased GGT following reduction in training intensity in subsequent years.\ref{23} Furthermore, GGT levels in these horses almost always return to normal within a few weeks with rest. Recent studies demonstrated that serum GGT activity was correlated to cumulative training load and racing frequency\ref{25} and maladaptation to training.\ref{26}

It should be noted that hepatocellular liver enzymes may be elevated with many systemic inflammatory disorders. This likely reflects inflammatory, vascular, hypoxic, and toxic insults to the liver secondary to the primary disorder. Bile acids can be elevated in horses with intestinal disorders such as colic and enteritis and in equine dysautonomia where the elevations in bile acids may be due to both liver pathology and ileus. The severity of these laboratory abnormalities in horses with colic, especially moderate-to-markedly-elevated bile acids, has been associated with prognosis.\ref{27}

Liver function tests may only become abnormally elevated when approximately 70% or more of liver function is lost.\ref{28} These tests include conjugated (direct) and unconjugated (indirect) bilirubin, blood ammonia, bile acids, and prothrombin (PT)/partial
thromboplastin times (PTT). An increase in conjugated bilirubin of more than 0.2 mg/dL above normal range has, in my experience, been a sensitive marker of liver failure. When the abnormally high conjugated bilirubin comprises 25% or more of the total bilirubin, this is often suggestive of a pronounced biliary and obstructive disease. Septic neonatal foals and horses with intestinal ileus sometimes have serum-conjugated bilirubin elevations with no additional evidence of hepatic dysfunction. Treatment in such cases should focus on the sepsis and intestinal ileus. Conjugated bilirubin is water soluble and when increased in serum, bilirubinuria may result, which can be detected by green-colored bubbles after shaking urine in a tube or by using urine test strips that detect bilirubin (Fig. 2). On very rare occasions a healthy horse will have a positive bilirubin reading on the urine test strips, although I do not know why. Unconjugated bilirubin is not water soluble and does not normally pass in the urine. Increased unconjugated bilirubin is also a sensitive test of liver failure but lacks specificity given that elevations may also occur with anorexia, hemolysis or, on rare occasion, marked elevations may be seen in an otherwise-healthy horse (presumed congenital deficiency in glucuronyl transferase). Although it is common knowledge that horses that are off feed can have unconjugated bilirubin concentrations that are slightly outside normal laboratory ranges, this latter group of healthy horses likely reflects some variation in uridine-diphosphate glucuronyltransferase activity.

Serum or plasma bile acid levels may be early predictors of liver failure when values increase above 30 μmol/L. Mild elevations in bile acids (as high as 20 μmol/L) may be seen after 2 to 3 days of anorexia. In some healthy neonatal foals, serum bile acid concentrations may be well above normal adult horse values and should therefore not be used alone as disease or function tests in this age group. In a state of negative energy balance, serum triglycerides are increased in equines but hepatic lipidosis resulting in liver failure is rarely found in these patients except in those with lipemia. In foals with hepatic failure hypoglycemia is often present, whereas in adult horses blood glucose is more commonly normal or increased but may be low in some cases. Plasma lactate is frequently high and bicarbonate is usually low in horses with fulminant hepatic failure. Serum albumin is inconsistently and only mildly low in horses with either chronic (18%) or acute (6%) severe liver disease/failure. Conversely, serum globulins are increased in 64% of horses with liver failure from multiple causes. These findings, reported by Dr. Gary Carlson, who had a long-time interest in diseases of the equine liver, are somewhat unique to the horse. The absence of hypoalbuminemia in many horses with liver failure is partially explained by a longer half-life (19 days) of albumin in horses than in other domestic species. Volume contraction was not believed to be the cause of the elevated total protein in most cases. The increase in globulins is explained by either an increased exposure or antigenic response to enteric-derived antigens that would normally be cleared by the healthy liver and its Kupffer cells, or to an immune response to the diseased liver.

There may be a decrease in blood urea nitrogen (BUN) and increase in PT and PTT clotting times in horses with liver failure. The low BUN is presumed to be associated with failure of the hepatic urea cycle and the increased clotting times are due to insufficient lack of synthesis of factors II, V, VII, IX, X, XI, and XII (VIII is produced mainly in endothelium). Coagulation abnormalities may not always occur with obstructive biliary diseases causing failure despite the importance of normal enteroic bile acids in the absorption of vitamin K and its importance in activating factors II, VII, IX, and X. Although coagulation abnormalities exist in many horses with liver failure, clinical bleeding is uncommon and liver biopsies can be safely performed in the great majority of cases. One explanation for this is that platelet counts generally remain normal in horses with liver failure. Fibrinogen, an acute phase protein made in the liver, is usually in normal or low range in most cases of equine liver failure, except with cholangiohepatitis where it may be high.

3. Liver Biopsy

Liver biopsy can be performed safely in the horse following ultrasound examination and identification of an appropriate site. For many years, liver biopsy was safely and generally successfully performed on the patient’s right side using anatomical landmarks although the biopsy report would occasionally return with the reading, “normal lung” or “normal diaphragm.” A recent study reported that using the traditional landmarks as biopsy sites may result
in unsuccessful sampling of the liver in many horses and could puncture the lung or intestine. The study concluded that the practice of blind percutaneous liver biopsy in horses is not recommended because of the potential risk of serious complications. Although biopsy is safe after identifying the liver by ultrasound, biopsies may not be needed in many cases. If historical, clinical and laboratory information clearly suggest a diagnosis such as Theiler’s disease or hepatic lipidosis then biopsy is unlikely to be of additional help in managing the case. Hepatic biopsy is best used, in my opinion, when the cause of the diagnosis is unknown, or any time when a biopsy sample might help determine clinical treatments or prognosis. Sampling should be considered in cases of chronic liver diseases of unknown etiology (and there are many) or sampling for bacterial culture. Although samples should be obtained, I must admit that biopsies of chronic hepatitis/hepatopathy cases rarely provide a specific etiology or etiopathogenesis (toxic or infectious or immunologic), and even in bacterial cholangiohepatitis case approximately 50% of samples are culture negative. Regardless of these limitations, the biopsy is still the most direct way of evaluating the liver when clinical information and biochemical testing leaves us searching for a diagnosis, treatment plan, or prognosis. Prognosis should not be solely based upon microscopic assessment of the amount of fibrosis but must include clinical findings, biochemical tests, assessment of other microscopic findings from the biopsy and ultrasound examination.

An experienced equine hepatic sonographer is often able to accurately predict the severity and extent of hepatic fibrosis (Figs. 3A and B). Assessing PT and PTT prior to biopsy may not be necessary if platelet count is normal because, as noted previously, severe hemorrhage following liver biopsy is extremely rare. If the PT and PTT are known to be prolonged this should not prevent sampling if the biopsy results might prove important to case management.

On rare occasions the liver cannot be visualized on the right but can be visualized and biopsied on the left. This is not my preference as the few colics I have witnessed following hepatic biopsy have mostly occurred when the left liver was sampled. There are spring-loaded and ultrasound-guided biopsy techniques but I still prefer: 1) visualization of the liver with ultrasound; 2) selection of the anatomic site and the proper needle angle needed to allow for penetration of the maximal liver thickness; 3) routine preparation of the site for biopsy; and 4) using the old fashioned “Tru-Cut” needles, which seem to me to provide better sample size.

Causes of Liver Failure

1. Cholestatic Causes of Liver Disease

Obstructive or cholestatic disorders of the liver include any diseases that have, as the primary dysfunction, obstruction of bile flow. Liver failure with biliary obstruction as the predominant dysfunction is characterized clinically by colic and icterus. Hepatic encephalopathy is uncommon in these cases. The expected biochemical abnormalities with cholestatic liver disease include marked elevations in GGT, abnormally high conjugated bilirubin with 20% or more of the total bilirubin often being in the conjugated form, and markedly elevated serum bile acid concentration. The causes of cholestatic liver disease and failure include suppurative cholangiohepatitis, cholelithiasis, displacement of the left colon to the right with obstruction of the common bile duct, and duodenal ulcerations in foals that occur either at or just caudal to the opening of the bile duct. Primary cholangiohepatitis (CH) and cholelithiasis (CL) are two of the most frequent causes of obstructive hepatic disease in horses. Inflammation of the biliary tract is called cholangitis but in adult horses with clinical disease there is usually a concurrent inflammation of hepatocytes hence, cholan-
giolhepatitis. Studies have identified CH and CL as diseases of middle-age-to-older horses with no obvious sex or breed predilection. Although the etiopathogenesis of CH and CL in horses is uncertain, retrograde bacterial infection from the small intestine is considered probable and this hypothesis is supported by the studies by Johnston et al., Reef et al., and Peek. The isolation of enteric, predominantly Gram-negative organisms from clinical cases of cholangiohepatitis and cholelithiasis is consistent with this hypothesis. Plant material has occasionally been found inside the stones, further supporting retrograde invasion of bacteria and even ingesta from the duodenum as a predisposing cause of the disease. As bacteria gain entrance to the biliary system from the duodenum (bile is normally sterile), it would be expected that an inflammatory response would occur with tissue swelling thus potentially increasing the pressure within the common bile duct and forcing infection into the biliary canaliculi. The ascending infection is thought to initiate a cholangitis, which may then extend into the periportal region of the liver causing cholangiohepatitis. The occurrence of enteritis or inflammatory bowel disease prior to the onset of CH in some cases may also support the ascending enteric bacterial infection hypothesis, although most cases of CH do not have a clear history of either prior enteritis or inflammatory bowel disease. The most common organisms cultured from the affected horses are E. coli, Actinobacillus equuli, Streptococcus spp., Klebsiella sp., Enterococcus spp., Clostridium spp., and Bacteroides sp. CH may predispose to hepatolith or cholelith formation. Equine choleliths are predominantly composed of calcium bilirubinate and to a lesser extent calcium phosphate. Ascending enteric bacterial infection is thought to be critical in the development of brown pigment stones (calcium bilirubinate) in man. Deconjugation of bilirubin digluconide by bacterial beta glucuronidase such as Clostridium spp., Bacteroides spp., Escherichia coli, and Peptococcus spp. occurs with subsequent precipitation of calcium bilirubinate, and cementation by anionic glycoproteins is thought to result in the formation of these calculi. It is clinically important to realize that calcium bilirubinate crystals may form initially, followed by sludge formation and then stone formation. If that hypothesis is true, then each stage of the process may signal further decreases in the efficacy of medical treatment. In the majority of clinical cases of equine cholelithiasis, stones are found in more than one area of the biliary tree and can include intrahepatic and extrahepatic choleliths, as well as choleddoliths (stones of the common bile duct). Multiple stones are common, with one extreme case reported as having 1,120 stones. Stones in the common bile duct would almost always be expected to produce signs characteristic of obstructive failure whereas stones in other areas of the biliary tree may be silent.

Clinical signs associated with these conditions include pyrexia, colic, icterus, and in chronic cases, significant weight loss. In a small percentage of cases, both neurologic signs caused by hepatic encephalopathy (HE) and photosensitization may be seen. Unless the disease is chronic and extensive fibrosis has occurred, hepatocellular function is not severely compromised, thus accounting for the absence of HE in many cases. Although nonspecific, colic and marked icterus are the most common clinical findings in horses with obstructive CH and CL. Fever would be expected but is not always evident on a single exam. Some degree of depression may be present. Affected horses have markedly elevated serum concentrations of the hepatobiliary enzymes GGT and ALP and bilirubin. A proportionate increase in the conjugated bilirubin fraction to greater than 25% of the total bilirubin is common but in approximately one third of cases the percentage of conjugated/total bilirubin is lower than 25%. Affected horses almost always have an increase in hepatocellular enzymes SDH, GLDH, and AST (commonly 2–4 times normal), although the proportionate increase above normal values is usually less than that seen with serum GGT elevations (commonly 7–20 times normal). Hyperfibrinogenemia was common in nine of 10 horses diagnosed with CH in our initial retrospective study of CH at the University of Pennsylvania but that was not the case in our second study of nine horses at Cornell. Other notable serum biochemical abnormalities with CH include increased serum bile acids and hyperglobulinemia. If one is trying to diagnose CH and discovers that globulins are not elevated then this could be a red flag for either a wrong diagnosis or some complicating factor; I learned that from a case that Dr. Sandy Tasse discussed with me in 2012. That case had all of the classic clinical and laboratory findings associated with CH but the total protein was only 5.6 g/DL and albumin was normal. The horse did have liver failure due to infectious CH but did not respond to routine and extended therapy for CH on the farm. After consulting with Dr. Julia Felippe at Cornell, it was found that the horse also had combined variable immunodeficiency necessitating euthanasia. I felt badly that I had not diagnosed it earlier.

Clotting function tests (PT and PTT) were consistently normal in our second study, despite biochemical evidence of advanced hepatobiliary disease but there are reports of severe CH and CL causing prolongation of both PT and PTT times.

Transabdominal ultrasound examination of the liver with a 5 ± megahertz (MHz) sector scanner is required for diagnostic imaging purposes and for monitoring treatment of equine liver disease. The liver can almost always be visualized on the right in horses with cholelithiasis, even in older horses that commonly have atrophy of the right lobe, because hepatomegaly is present in all cases except for those with chronic and diffuse fibrosis. The ultrasono-
graphic findings in horses with CH include subjective evidence of liver enlargement and distended, thickened bile ducts with variable liver echogenicity. In horses with CL, increased hepatic echogenicity, hepatomegaly, distended bile ducts, and occasional calculi are the salient features of the condition. Stones can often best be visualized in the 7th to 10th intercostal spaces on the right side (Fig. 4), just ventral to the lung border, and are seen in approximately 50 to 75% of horses with CL; stones are uncommonly seen on the left. The biliary tree is usually not detectable ultrasonographically in normal horses but dilated bile ducts can be seen as enlarged channels running parallel to the portal veins in approximately 40% of horses with obstructive CL and this finding should be considered suspicious for either intra- or extrahepatic biliary obstruction even if no stones are seen. Hypercholecholithiasis with acoustic shadowing can occur, whereas other less mineralized calculi or sludge may have only faint or no acoustic shadowing. If several bile ducts are severely distended that suggests a likely obstruction (cholelith) in the common bile duct. If no stones can be imaged this may suggest a favorable prognosis for CL with surgery (assuming absence of hepatic parenchyma hyperechogenicity suggesting fibrosis) because there may be only a single obstructing stone. In contrast, if stones can be seen in the image field with classic findings of hepatic failure due to obstruction, one can assume there is at least one other stone obstructing the common bile duct and the presence of multiple stones may lower the prognosis. Stones obstructing the common bile duct cannot be imaged on transcutaneous abdominal ultrasound but when endoscopic exam of the duodenum is performed, with luck the stone or a “bulge” may be noted at the bile duct opening (Fig. 5).

Histopathology of biopsy material can provide valuable information as to the severity of inflammatory CH and the extent of accompanying hepatic fibrosis. Historically, marked periportal and bridging fibrosis have been interpreted as poor prognostic indicators in horses with suspected or known CH or CL. Case reviews suggest that extended survival times (months or years) may be possible in some horses despite histological evidence of chronic hepatoportal damage (severe periportal and bridging fibrosis). An estimate of the degree of fibrosis, based upon hepatic echogenicity, can also be determined by ultrasound exam and may be nearly as prognostic as a liver biopsy. There is some suggestion that horses with biliary obstruction have a characteristic circumferential biliary hyperplasia (K. Butterworth, personal communication, 2013) on microscopic examination of a liver biopsy and this can be helpful in the diagnosis and prognosis when an obstructing stone cannot be seen on ultrasound or duodenoscopy and surgery is being contemplated. This finding does not have 100% sensitivity so if all other clinical and laboratory findings are characteristic of CL then surgery (if an option and appropriate) should not be ruled out based on the absence of this microscopic finding. Another indication for biopsy is to provide a sample for bacterial culture, in hopes of determining the etiologic agents such that specific antimicrobial therapy can be chosen. Unfortunately, as noted previously, cultures obtained from biopsy samples are negative in approximately 50% of cases.

Based upon previous studies it would seem that long-term administration of systemic antibiotics is critical for the successful treatment of CH and in some cases of CL in horses. Median treatment period in one study, where seven of nine horses had liver failure due to CH but survived, was 51 days (range, 17–124 days). Antibiotic selection should

Fig. 4. Sonogram of the right liver of an 8 year old Thoroughbred gelding with chronic colic, icterus and fever. A distended bile duct is visible containing two hyperechoic foci (sludge or stones causing mild shadowing). Acoustic enhancement, characteristic of bile can be seen beyond the distended duct.

Fig. 5. Endoscopic photograph of the proximal duodenum at the level of the major duodenal papilla. A bulge seen on the right side of the image was determined to be an obstructing cholelith. Cholecotolithotomy was successful in this horse and he is currently healthy, 4 years after discharge.
ideally be based upon culture and sensitivity results from hepatic biopsy material but clinicians are frequently required to select an initial antimicrobial agent empirically or when the culture results are negative. The isolation of predominantly Gram-negative enteric and mixed anaerobic organisms from cases of CL suggests that antibiotics with good Gram-negative activity (enrofloxacin, third-generation cephalosporins, aminoglycosides, and possibly trimethoprim sulfamethoxazole) in addition to antimicrobials effective against enteric anaerobes (penicillin, metronidazole, chloramphenicol) would be reasonable choices. Although the liver metabolizes nearly 50% of metronidazole, its use in CH is unlikely to be a problem or require a dose adjustment unless there is marked liver fibrosis. In the absence of positive culture results, clinicians may not only have to select an agent empirically but should also be prepared to change antibiotics according to clinical response. In one study, in all horses that improved, clinical improvement was seen days to several weeks before normalization of serum biochemical indices of hepatobiliary disease/function occurred (GGT, alkaline phosphatase [AP], bilirubin, and bile acids). Furthermore in that study, serum GGT actually increased during the initial period of clinical improvement in all horses. I do not believe that there is a level of GGT that accurately predicts treatment failure in CH, as long as the horse is clinically stable, the ultrasound examination does not reveal numerous obstructing stones or diffuse fibrosis, fever abates, and laboratory tests improve within 7–10 days after medical therapy commences. The highest GGT in an affected horse that I have successfully treated with medical therapy was 2,270 IU/L but I consulted on a case in Virginia where the horse recovered despite a GGT of 3,200 IU/L. Continuing antimicrobial therapy until both clinical and biochemical recovery has occurred may be important in achieving a successful outcome and I suggest monitoring serum GGT activity as a biochemical marker of recovery. Supportive medical care using intravenous fluids and anti-inflammatory drugs was a critical part of the treatment of several cases in our reports.

Therapeutic agents, such as the bile salts chenodeoxycholic acid and ursodeoxycholic acid (ursodiol), that are specifically used to encourage dissolution of cholesterol-rich stones in man, have no effect on calcium bilirubinate calculi but, as anti-inflammatory and choleretic agents that increase bile production, their use may make bile more liquid and easier to excrete. These bile salts were initially predicted to be potentially toxic in horses due to their ability to be metabolized to the hepatotoxic compound lithocholic acid by intestinal bacteria in another hindgut fermenter, the rabbit. During the past 4 years we have now used ursodiol in eight horses with CH when standard medical treatments as listed above were not providing a favorable response. Five of these horses had somewhat surprising recoveries, including one that clearly had an obstructing stone and others with “sludge” visualized on ultrasound examination. There has been no recognizable evidence of toxic effects in any case but additional studies on safety and efficacy are needed. Administration of ursodiol may increase serum bile acid concentrations. There is direct experimental support for the use of dimethyl sulfoxide (DMSO) in the treatment of brown pigment stones in man because it is a direct solubilizer of calcium bilirubinate and I still recommend its use for CH and CL.

Previous reports have described the surgical treatment of cholelithiasis. Unfortunately, intrahepatic calculi are largely inaccessible to the surgeon and the fact that many cases of equine cholelithiasis possess multiple intrahepatic and extrahaepatic choleliths means that recurrent biliary obstruction would be likely even if an obstructing stone is removed. The best surgical outcome has been when there is thought to be a single stone obstructing the bile duct and when hepatic fibrosis is not severe. Surgery should be considered for horses that have intermittent or persistent abdominal pain, ultrasonographic evidence of obstruction to biliary outflow, moderate-to-no hepatic fibrosis, few or no stones visible on ultrasound, and an unsuccessful response to medical therapy. Recommending surgery when you are uncertain whether it is a single obstructing stone causing the clinical disease can be difficult so one must use all of the information above to make that decision. In addition, consultation with a voice of experience such as the one Dr. Jim Becht and I received on a case in 1985 is always helpful. In that case we were struggling to make a diagnosis as to the reason for persistent colic and liver failure when Dr. Whitlock suggested, “if I were you boys I would consider cholelithiasis in that mare and explore her,” the mare did well following a choledocholithotomy and went on in later years to deliver a foal by Seattle Slew. Thanks to Dr. Whitlock for the “suggestion.”

Right dorsal displacement of the large colon (RDDLC) can cause nonstrangulating obstructions, preventing the normal flow of ingesta through the bowel lumen without resulting in intestinal vascular compromise. It may be difficult to make a definitive diagnosis of a RDDLC because of the vague clinical findings (often mild colic, questionable abnormalities on rectal exam, etc.) although the diagnosis is easier in horses that have the highly specific but not highly sensitive ultrasound examination finding of horizontally flowing mesenteric blood vessels in the right cranial to mid-abdomen. In the late 1980s we initially noticed that some horses with RDDLC had intense icterus and marked elevations in the serum activity of GGT, often accompanied by an increase in concentration of conjugated bilirubin. The elevations in GGT returned to normal after surgical correction of the displacement and liver biopsies showed minimal pathology, suggesting that the elevations are due to some feature of the colic...
episode rather than being caused by primary hepatic disease. In 2005, Dr. Rachel Gardner reported on the surgical and postmortem examinations of horses with RDDLC that demonstrated how positioning of the colon in horses with RDDLC sometimes results in compression of the bile duct causing extrahepatic bile duct obstruction and a subsequent elevation in serum GGT activity and direct bilirubin. The use of GGT as a diagnostic test for RDDLC would result in many false negatives. However, the presence of high GGT activity in a horse with examination findings consistent with a colon displacement is supportive of RDDLC, and in those circumstances was found to have a specificity of 98%. Horses not responding to medical therapy and exhibiting continual abdominal pain with examination findings suggestive of a RDDLC should not have surgery delayed because of elevations in serum GGT or bilirubin concentration unless historic or ultrasonographic evidence are suggestive of a primary hepatic disease.

Gastric outflow obstruction in suckling foals is caused by duodenal and pyloric ulcer disease and commonly follows an episode of diarrhea 1–4 weeks earlier. CH can occur in these foals if the obstruction is at or distal to the opening of the bile duct; this is reported to occur in 28% of cases. Surgical correction by gastrojejunostomy or gastroduodenostomy and often an additional jejunojejunostomy will resolve both intestinal and bile obstruction in most cases. Antimicrobial therapy is required in these cases because feed material and bacteria can be found in the bile duct. Initial reports suggested that having CH decreased prognosis in these cases but a recent report did not confirm that. If the obstruction is at the opening of the common bile duct, a hepaticojejunostomy as first described by Orsini and Donawick will be needed in addition to the intestinal bypass surgery. Even with successful corrections of obstructions just distal to the hepato-pancreatic ampulla, continued bile reflux into the stomach may cause chronic gastric ulcers and less-favorable growth in some affected foals. It may be difficult to determine the extent of CH in the foals prior to surgery because GGT is often only modestly increased. With hepatic duct obstruction, GGT and direct bilirubin are more markedly elevated. Barium studies may show barium entering the biliary tree if the obstruction is distal to the opening.

An additional and recently reported cause of cholangiohepatitis occurred in a suckling foal associated with Bartonella henselae infection of the liver. This 3½-month-old foal was treated with minocycline and seemed to recover.

2. Infectious Causes of Nonobstructive Liver Disease and Failure

Tyzzer’s Disease

There are several infectious diseases of foals that may result in liver failure, Tyzzer’s disease (Clostridium piliforme) being the most reported and best described. Listeria monocytogenes has been reported as causing similar findings to Tyzzer’s disease and, on very rare occasions, Actinobacillus spp., Equine herpesvirus-1, Streptococcus zooepidemicus, or Leptospira spp. may cause liver failure in young foals. All of these have a concurrent bacteremia/septicemia. In adult horses, other than previously discussed bacterial cholangitis, infectious causes of severe liver disease or failure include viral hepatitis and infectious necrotic hepatitis (Black disease or Clostridium novyi).

Tyzzer’s disease, a cause of acute and generally fatal hepatitis and bacteraemia in 5–36-day-old foals is caused by Clostridium piliforme. Clostridium piliforme is thought to be common in the environment but given that it is difficult to culture, little is known regarding the epidemiology and pathogenesis of the disease. Dr. Swerczek, at the University of Kentucky who first reported the disease in the United States in foals in 1973, has recently authored an excellent retrospective study on 148 cases of the disease. The average age of foals at death in that study was 20 days and there was not believed to be a breed or sex predisposition. Relative risk of disease seems to be greater in the later months of the foaling season and one could speculate that, similar to Rhodococcus equi, shedding by both mares and healthy foals increase environmental contamination as the foaling season progresses. Foals normally consume freshly deposited feces from their dam from day 2 to 35 and the mare’s feces may serve as the source of infection for the foal. Affected foals are generally well fleshed at the time of illness, and an increase in protein and carbohydrate diets fed to the mare and foal has been suggested as possibly affecting the intestinal microbiome, permitting overgrowth of the C. piliforme bacteria and predisposing the foal to clinical disease. The disease is generally sporadic both on and between farms and of low overall incidence, likely less than 0.1% of foals per year. Multiple cases have been reported on some farms and increased environmental contamination and bacterial colonization of the mares leading to increased fecal-oral transmission is possible. One of the more interesting Tyzzer’s case scenarios was described to me by Dr. Tennant: A draft mare with twins had one foal that died from confirmed Tyzzer’s disease after being admitted to the hospital and while the mare and other live foal were waiting to go home, the second foal developed fever and hepatitis and was treated successfully with antibiotics for suspected Tyzzer’s disease. Affected foals, especially younger ones, are often found dead in the pasture without prior clinical signs being observed. Clinical signs are acute in onset and include signs of both septic shock and liver failure, including fever, weakness, tachycardia, icterus, recumbency, seizure, and coma. Laboratory findings are also suggestive of septic shock and liver failure, including leukopenia, toxic changes in neutrophils, hypoglyce-
mia, hyperammonemia, severe lactic acidosis, elevated creatinine, markedly elevated hepatocellular liver enzymes, increased PT and PTT, and abnormal elevations in both unconjugated and conjugated bilirubin, with unconjugated usually comprising the greatest increase. Rarely is treatment successful.

The first published case report of successful treatment of suspected Tyzzer’s disease, using fluid therapy, penicillin, and trimethoprim sulfad was by Drs. Peek and Byars. The only confirmed case of Tyzzer’s disease (polymerase chain reaction [PCR] positive on liver biopsy) successfully treated occurred in a foal treated at University of California Davis with ampicillin, gentamicin, fluids, and nutritional support.

The diagnosis of Tyzzer’s is usually made by clinical findings and autopsy observation of widespread multifocal hepatic necrosis with the intracellular filamentous bacteria. Enteritis and carditis may also occur with Tyzzer’s disease as the organism can commonly be found in those tissues at autopsy.

Theiler’s Disease

Theiler’s disease has been the most baffling equine liver disease of the last century. In November 1914, a condition of “acute liver atrophy” was recognized and reported for the first time in horses in South Africa. Affected horses were being used for African horse sickness immunization experiments performed by Dr. Arnold Theiler. Immunization consisted of simultaneously inoculating horses both with serum from recovered horses and live African horse sickness virus. One thousand one hundred forty eight horses were passively immunized against African horse sickness; the total number of cases of acute liver failure was 27, including five recoveries. The disease was observed in four horses that never received any inoculations, which is the earliest scientific suggestion that the disease we now know as Theiler’s disease could be both infectious and contagious.

Theiler also provided a summary of the results of immunization of horses belonging to farmers in eight different South African districts between October 1916 and January 1917. Of 1154 horses inoculated, 210 were reported to have died of acute liver failure but, again, seven cases were believed to have occurred in nonimmunized horses. The principal pathologic changes that Theiler described are classic for our current pathologic description of Theiler’s disease, also known as acute hepatitis or serum hepatitis. The liver is reduced in size, the edges are sharp and flattened, and its consistency is usually firm. On microscopic examination, the liver cells were seen to have undergone necrosis. Emigration of round cells and regeneration of bile ducts are additional commonly identified features. In rare cases, the blood of dying horses showed distinct hemolysis and hemoglobinuria. This is likely the earliest report of hemolysis occurring in horses with liver failure, a finding that Dr. Tennant in 1972 also reported on in a U.S. horse with hepatic failure.

Theiler described the clinical course of the disease as a very rapid one, usually approximately 24 hours. Sometimes it lasted less than 6 hours whereas other fatal cases lasted up to 3 days. Recovery was observed to occur in a minimum of 4 days. There was no fever present. The onset of clinical signs was peracute and characterized by violent neurologic signs in most instances. If the horse was noted to be semi-conscious, it was often standing in the stable with its head hanging in a corner and refusing food. The gait was slightly swaying or staggering. In the more violent cases the symptoms increased rapidly in intensity or were present from the very onset. Horses were noted to push their heads violently forward against a wall and in so doing assumed all possible positions, resulting in bruising and laceration of the skin of the head and point of the shoulders. These violent symptoms were followed by partial or complete coma. In the somnolent or semi-conscious stage, the eyes were staring, the horse apathetic toward its surroundings, with twitching of the lips, chomping of the teeth, opisthotonus, kicking at the abdomen, and yawning noted. Despite Dr. Arnold Theiler’s brilliant work, no determination as to the cause of the serum inoculation-related acute hepatic necrosis could be made.

Since Theiler’s observations, clinically similar illnesses have been described in many parts of the world. Most reported cases have been observed 1 to 3 months after horses have been treated with serum or plasma products produced in other horses. The first reported cases of acute equine hepatitis associated with equine biologic administration in the United States occurred during the pandemic of western equine encephalomyelitis that first was observed in 1930 in California and then spread rapidly throughout the western states. Madsen and Marsh described a second wave of disease (hepatitis) that occurred 2 to 3 months following initial outbreaks of western equine encephalomyelitis and use of the prophylactic serum treatment. Since these outbreak reports, hepatitis following the administration of equine plasma or serum has been described in horses from around the world and cases undoubtedly occur in the United States every year. Acute hepatitis has been reported following treatment with a variety of equine serum products including tetanus antitoxin (TAT), commercial equine plasma; anthrax antitoxin; *Streptococcus equi* antiserum; antisera against Clostridium perfringens, equine influenza antisera; antisera against Salmonella enteritis and Salmonella abortus-equis; and pregnant mare’s serum. Epidemics with identical clinical, clinical pathologic laboratory findings, and pathologic disease have also been reported in which equine biologicals were not administered. Regardless, most reported cases of Theiler’s disease have been associated with the prophylactic or therapeutic use of serum or plasma.
products of equine origin and it would seem that products containing pooled serum/plasma from multiple donors may have a higher risk of causing disease than products produced from a single donor. The overall risk of Theiler’s disease and acute hepatic failure in association with equine blood product inoculation is not known given that many cases likely go unreported. We know of at least six confirmed cases that occurred in 2014, all associated with the administration of tetanus antitoxin. It is also likely that some horses may have less fulminant and even subclinical liver disease following administration of certain blood products or specific lot numbers of those products. An etiology similar to that of viral hepatitis B or C in humans has been suspected to be the cause of Theiler’s disease because of the similarities between low incidence of clinical disease in inoculated humans and horses and the long incubation period following inoculation of blood products. The long period (generally 45–60 days) between inoculation of blood and onset of clinical disease in humans is known to be associated with the slow increase in serum antibody, which is followed by a rather sudden attempt to clear virus from infected hepatocytes.

To summarize information available on equine acute hepatitis/Thorller’s disease, there seem to be three separate epidemiologic diseases with identical clinical, clinical pathologic, and autopsy findings: 1) acute, often fulminant hepatitis in adult horses that have received equine origin blood products approximately 4–10 weeks earlier and less fulminant or subclinical disease in some other horses receiving the same product; 2) acute, sometimes fulminant, hepatitis occurring in an adult horse that was in contact with a blood product-inoculated Thorller’s diseased horse but did not itself receive the equine origin blood product; and 3) acute fulminant hepatitis in adult horses, often broodmares on pasture, with no known blood product administration. This third syndrome is sometimes associated with farm outbreaks involving a few horses over a 2–6-week period and is most common in the autumn season. Two farms that Dr. Tennant and I have investigated had more than one outbreak with several years in between outbreaks. The classic Thorller’s disease, with a history of blood product administration 4–10 weeks prior to acute liver failure, might be more common in the spring but that might be associated with foals and mares receiving tetanus antitoxin following foaling. In a review of our last 14 cases of Thorller’s disease at Cornell, only four cases were confirmed to be associated with TAT administration and one was associated with commercial plasma. Although the overall incidence of Thorller’s disease in adult horses receiving TAT is low, tetanus antitoxin has for the past 40 years been the most common blood product associated with the disease in the United States. Thorller’s disease may be more commonly associated with TAT administration than equine plasma because of either the more frequent administration of TAT or because TAT is produced as a pooled product while commercial units of normal plasma are most often collected from a single donor. A small number of cases have occurred following intravenous commercial plasma administration and one case that I know of occurred following intrauterine plasma administration. From personal research experience, Thorller’s disease was produced in a failed Lyme disease project of ours in 1986 when Borrelia burgdorferi was mixed with 4–8 mL of commercially purchased serum, which was then administered subcutaneously to adult ponies and then 6 and 7 weeks later two ponies developed Thorller’s disease and died; there was no indication that Borrelia itself caused the disease.

The clinical disease (acute hepatitis, Thorller’s disease, serum hepatitis) is characterized by fulminant hepatic failure and encephalopathy in adult horses. Clinical signs are jaundice, discolored urine, ataxia, and signs of acute encephalopathy including cortical blindness. As previously mentioned, neurologic signs may range from mild to severe depression or coma to severe maniacal behavior and seizure. The discolored urine (bilirubinuria) that is typically seen in horses with hepatic failure will have green-colored bubbles when shaken and this could serve as a point of care test to separate liver failure and HE from other causes of encephalopathy and from encephalitides. In some severe cases of Thorller’s disease and in liver failure of other causes, hemolysis and hemoglobinuria may be found and is a poor prognostic finding in my experience. The cause of the hemolysis is unknown but may be due to physical damage to the red blood cells passing through the sinusoids of the diseased liver, oxidative damage resulting from liver failure, or release of large amounts of copper or iron from the diseased liver. Some horses may have photosensitivity and abdominal pain. Laboratory findings in Thorller’s disease are typical of acute severe liver disease predominantly affecting hepatocellular function and include marked elevations in serum hepatocellular enzymes in all clinically affected horses with Thorller’s disease. The aspartate aminotransferase (AST) often is nearly 2000 IU/L; greater than 4000 IU/L seems to be associated with a poor prognosis. In the last 14 Thorller’s cases diagnosed at our hospital the mean AST was 2545 (1116–5267). There are also marked elevations in SDH and GLDH and a marked decline in those enzymes after 2 or 3 days of treatment may suggest a favorable prognosis if there is improvement in clinical signs as well. There is a moderate increase in biliary-derived enzymes with GGT usually increased to 100 and 300 IU/L (mean, 197). Horses that are recovering from the disease (improved clinical signs and decreases in SDH and GLDH values) may still have some further mild increase GGT. This should not be interpreted as a bad prognostic finding as the continued elevation in GGT is likely related to bile duct hyperplasia and
the liver trying to regenerate/repair itself. Both unconjugated (mean, 12.5; range, 9.3–17.2 mg/dL) and conjugated bilirubins (mean; 2.7; range, 0.9–4.7 mg/dL) are increased, with conjugated often comprising less than 20% of the total. Prolongation of PT and PTT are almost always present, and a small number of the horses have thrombocytopenia, likely indicating disseminated intravascular coagulation. Bile acids, anion gap, and lactate are almost always noticeably increased. Blood glucose is frequently in the 140–170-mg/dL range and no horse in our last 14 cases was hypoglycemic. Ammonia concentration in our last 14 cases has ranged from 91 to 495 mmol/L (normal, < 90 mmol/L).

Signalment and history are important in the diagnosis of Theiler's disease: Any adult horse with clinical signs of acute fulminant hepatic failure that has received serum (especially TAT) or plasma approximately 4–10 weeks earlier would likely have Theiler's disease as the top differential. If the horse has appropriate signs and laboratory findings of Theiler's disease but no history of recent blood product administration and there is no suspicious toxic cause for the hepatic failure then nonbiologic origin acute hepatitis should be considered. Ultrasound examination of the liver in horses with Theiler's disease may seem unremarkable other than the liver is sometimes hard to image due to its small size; if the liver is seen it may seem hypoechoic. I generally do not perform a liver biopsy on a horse with “classic” Theiler's disease as the utility of a liver biopsy is marginal because histologic data will not change therapy. Unfortunately, in many cases a diagnosis is often made at necropsy where the liver is almost always smaller than normal (Fig. 6). Measurement of liver weight in comparison with body weight (BW) in our last 13 cases of Theiler's disease found that the liver in Theiler's disease horses was 1.0% of BW and normal controls were 1.6% of BW, suggesting a 40% decrease in hepatic size following disease. Affected livers show severe centrilobular or massive liver necrosis/apoptosis,77,84–86 with portal areas often less severely affected but with a mononuclear cell infiltration and slight-to-moderate bile duct proliferation, sometimes with fibrosis. Vasculitis has been occasionally reported in horses with Theiler's disease and was found in three of 12 of our most recent necropsy cases at Cornell (Sean McDonough, personal communication). Alzheimer type II astrocytes are present in the brain in virtually all the cases.

Treatment focuses on treating HE when present or preventing it from occurring (HE treatment, page 92), along with supportive care including fluids, nutritional support (page 95), antioxidant treatments, anti-inflammatory and antibiotic treatments. Pentoxifylline, 7.5 mg/kg by mouth (PO) or IV (compounded), two or three times daily, may be helpful in decreasing systemic inflammation, which undoubtedly occurs with Theiler's disease. A bactericidal antibiotic (e.g., ceftiofur) should be administered in all cases of acute liver failure to inhibit bacterial translocation (gut to blood). Bacterial translocation is of increased risk in acute fulminant liver disease because of loss of Kupffer cell numbers and function, in addition to the potential for increased intestinal permeability resulting from ischemia and congestion of the intestine. Acetylcysteine has been proposed as an antioxidant treatment for acute fulminant liver failure in humans. Acetylcysteine is best known as the specific treatment for acetaminophen toxicity but as a potential glutathione donor and antioxidant, it could have value in treating other acute fulminant liver diseases. I have administered the sterile acetylcysteine solution marketed for nebulization by slow intravenous administration (up to 100 mg/kg) by mixing in 5% dextrose. A preparation labeled for intravenous use in humans is not available in the United States and the sterile nebulization product is used in human hospitals.

Horses with Theiler's disease have a guarded to poor prognosis if there is fulminant HE. Horses that continue to eat for 3 days and have supportive treatments may then make a quick recovery. My experience is that horses with Theiler's disease are usually either dead or “on their road to recovery” within 3–5 days. There are no proven long-term consequences in horses that recover.

Newly Discovered Equine Hepatitis Viruses
Recently, three distinct equine hepatitis viruses have been discovered in horses: nonprimate hepato-

Fig. 6. Livers from two horses of similar body weight, normal liver on left and shrunken Theiler's disease liver on the right.
civirus (NPHV; also called hepacivirus), Theiler’s disease–associated virus (TDAV) and equine pegivirus (EPgV), which are all close Flaviviridae family relatives of human hepatitis C viruses (HCV). The horse seems to be unique among our domestic animal for harboring these so-called “hepatitis viruses.”

The recent discovery of these equine hepatitis viruses is directly related to the use of the newer technology involving nucleic acid deep sequencing of samples. Unknown nucleotides are then reassembled using de novo computerized sequencing to identify new agents. One of the problems with this technology, for us as clinicians, is that finding new and infectious agents may not be clinically important.

NPHV was the first of the three new blood borne viruses to be reported. NPHV was found in pooled equine serum and in the serum of eight of 103 normal horses (7.8%), of which 35% were NPHV-antibody positive; samples tested were those submitted for equine infectious anemia (EIA) testing at Cornell, and it was therefore assumed that horses were healthy. Since that description in the United States, NPHV viremia has been reported in three of 142 horses (2.1%) in Great Britain and in seven of 210 horses (3.3%) from Germany. Multiple NPHV infections have also been reported in Japan and Brazil. Similar incidence of NPHV infection in clinically healthy horses is found in France (Stephane Pronost, personal communication). Currently, accumulated data from Europe, North America, and Asia suggests that nearly 40% of all adult horses are seropositive, prevalence of active infection is 4% and approximately one fifth of those 4% are chronic carriers. No infections from either of these three new viruses (NPHV, TDAV, EPgV) have been found in mules or donkeys. NPHV is a member of the Hepacivirus genus in which human HCV are the prototype members and of the three new viruses found in horses, NPHV is most closely related genetically to HCV. NPHV has also been found commonly in bats, rodents, and Old World moneys and it is the only one of the three newly discovered equine hepatitis viruses known to be hepatotropic in the horse. As regards clinical importance, the testing of a somewhat random assortment of naturally infected horses has suggested that there is little documented association between NPHV infection and clinical hepatic disease in those horses.

In NPHV experimentally infected horses there is mild disease documented by elevations in liver enzymes and lymphocytic portal inflammation and piecemeal hepatocyte necrosis. The elevations seem to occur shortly after development of measureable antibodies, suggesting that antibody-mediated activity related to the virus could cause some disease. When severe combined immunodeficient foals were experimentally infected, they produced no antibody and there was no increase in GGT, further supporting an immune response to the infection and association with disease. The only report of clinical disease associated with NPHV infection is a case report from Europe of Theiler’s disease with the horse having high levels of NPHV viremia and as the viremia diminished the horse recovered. Although our data set is small, horses that are chronically infected with NPHV do not seem to develop clinical disease. We have one horse at Cornell that has retrospectively from frozen and stored samples been infected for 10 years (thanks to Dr. Doug Antczak for storing serum for such a long time). This horse has no elevations in liver enzymes and a liver biopsy obtained after at least 9 years of infection was normal. Most horses infected with NPHV seem to have relatively slow development of NPHV specific antibody (approximately 6–8 weeks) followed by viral clearance in the following months. In 2014, with the collaboration of scientists at both Rockefeller and Columbia Universities, we were able to successfully infect a seronegative horse with infectious clones of NPHV, yielding high RNA titers in the serum and liver of the horse and establishing the molecular components of a functional NPHV genome; cell culture of HCV like viruses is extremely difficult requiring the use of infectious clones for inoculation. Delayed seroconversion, slightly elevated circulating liver enzymes, and mild hepatitis was observed, followed by viral clearance. Hepatotropism was confirmed by level of viremia in the liver and by finding negative-strand RNA in the liver, a hallmark of viral replication.

In August 2011 we were asked to consult on an outbreak of acute hepatitis in horses. Two and a half months earlier four horses from the affected farm had developed botulism caused by ingestion of toxin-contaminated hay. Twenty-two horses eating that hay had been treated, either therapeutically or prophylactically, with Clostridium botulinum antitoxin (equine origin). Seven of 17 horses treated with one particular lot of botulinum antitoxin developed clinical and/or biochemical evidence of hepatitis 6–8 weeks later. Hepatitis was not observed in five horses that were treated with a separate lot of botulinum antitoxin produced from different horses or in 53 farm horses that had not received antitoxin. Using high-throughput pyrosequencing, a new virus belonging to the family Flaviviridae (the family of human HCV) was identified in the serum of two clinical cases of acute hepatitis/Theiler’s disease and in the botulinum antitoxin the horses received 47 and 52 days prior to the onset of illness. The nucleic acid sequences of the viruses in the two hepatitis cases and in the botulinum antitoxin were virtually identical. The virus was named Theiler’s Disease–Associated Virus (TDAV). A reverse transcriptase–polymerase chain reaction (RT-PCR) then was used to test the serum of horses for the newly discovered equine hepatitis–associated virus (TDAV). All 17 of the horses that received the incriminated lot of botulinum antitoxin tested positive.
for the new virus, including the seven horses with acute hepatitis. One of the donor horses that produced the antitoxin was positive. Serum from all other horses was RT-PCR negative for the virus. Follow-up testing 6 months later demonstrated that the donor horse had cleared the virus and the product was again widely used with no other cases of acute hepatitis being reported, further supporting the association between the virus and disease. The original 17 TDAV-positive horses were retested after 1 year by qRT-PCR and TDAV was undetectable in 13 of the previously infected horses. However, in four horses, persistent TDAV viremia was demonstrated.100 A large portion of the horses from the index farm have been retested since the outbreak and no additional horses have become PCR positive, suggesting direct horse-to-horse transmission or transmission by insect vectors may not occur or is not common. This work would not have been possible except for the excellent collaboration of the attending veterinarian, Dr. Anita VanBlaricum, who recognized the disease syndrome and provided appropriate samples for testing. Her keen observations have persisted; she found that three of the clinically affected horses with liver disease went on to have colon displacements weeks after the hepatitis. Is it possible the liver disease affected colon motility or caused a mild anatomical shift predisposing those horses to right displacements?

Follow-up studies since 2012 find that TDAV seems to be a relatively rare virus in the United States horse population and it has not been found in horses in the United Kingdom and France or in any other species so far.103 Interestingly, blood product-associated Théler’s disease has not been reported in France and United Kingdom for four decades. On the four separate premises in North America where we have found the TDAV virus, there are some historical links to Théler’s disease, but no current cases were discovered on three of those premises. Thanks to Drs. Laurie Beard, Phillip Johnson, Kara Lascola, Beatrice Sponseller, and other referring veterinarians, we have been able to test serum and/or livers from six horses with fulminant acute hepatic necrosis (Théler’s Disease) that had 4–10 weeks earlier received tetanus antitoxin and were unable to find TDAV virus. It is, therefore, likely that TDAV might cause a milder yet clinical form of Théler’s disease but another virus that we are investigating may be responsible for the fulminating form of the disease.

The most recent of the new equine blood-borne equine flaviviruses was reported by Kapoor et al.101 and has been named the Equine Pegivirus (EPgV).103 EPgV and TDAV are both members of the Pegivirus genus but are genetically distinct. Pegiviruses, although a common infection in humans and other species, are not known to cause clinical disease in other species. Three of the original 12 EPgV-infected horses from Alabama were reported to have had elevated liver enzymes but were not clinically ill.101 qRT-PCR testing of both lymph node biopsy and liver biopsy tissue from infected horses failed to demonstrate hepatotropism of the virus.101 Two of the horses were shown to be carriers of EPgV for at least 3.5 years. Nine liver samples obtained from random equine necropsies in California were tested for EPgV and one was positive.101 Although there is little data to support an association between NPHV or EPgV infections and clinical equine liver disease at this time, this possibility cannot be ruled out.103 Although all three viruses (NPHV, TDAV, and EPgV) are believed to be transmitted by blood, the high seroprevalence of both NPHV and EPgV suggest there must be modes of transmission other than plasma and antitoxin inoculations. The role of and importance of co-infection in disease is unknown.

Hepatic Abscesses

Hepatic abscesses are rare in horses and, when present, usually cause weight loss, fever, and laboratory signs of inflammation rather than indications of liver disease or failure. Corynebacterium pseudo-tuberculosis and Streptococcus spp. are listed as the most common organisms associated with bacteremic abscess formation in adult horses and Rhodococcus equi and Streptococcus spp. abscesses being most common in growing foals.29 Parasitic causes of liver failure are extremely rare, but parasitic infections may cause focal granulomas.111,112 Heterobilharzia americana causes a remarkable starry sky image of the liver on ultrasound exam, but horses have no signs of liver disease.111 Cystic lesions can be caused by Echinococcus granulosus, but they are generally an incidental finding at necropsy; in the United States they are mostly in imported horses. Parascaris equorum and Strongyle spp. migration through the liver in foals can cause hemorrhagic and focal necrosis and eosinophilic granulomas, but are not expected to cause dysfunction, although secondary abscesses could occur.112 Infectious necrotic hepatitis (Clostridium novyi or Black disease) has been described in horses,58,113 but rather than causing signs and laboratory findings of liver failure, findings are mostly the result of peritonitis and a systemic inflammatory response. Ultrasound of a liver with abscessation or necrosis as occurs with Black disease would likely show an inconsistent appearance in different locations of the liver. Gas echoes may be seen with Black disease or abscesses containing other anaerobic organisms. Parasite migration (flukes) are a well-documented cause of liver necrosis in ruminants providing an anaerobic environment conducive for proliferation of clostridial spores, but in horses this link is not common.

3. Toxic Hepatopathies

Pyrrolizidine Alkaloid Toxicity

Toxic causes of liver disease and liver failure are relatively common in horses and most are associated...
with plant ingestion. There is little doubt that pyrrolizidine alkaloid (PA)–containing plants are the most common plants, worldwide, causing toxic liver failure in horses. There is a long list of PA-containing plants, but Amsinckia intermedia (tarweed), Cynoglossum officinale (hound’s tongue), Heliotropium europaeum (heliotrope), Senecio jacobaea (tansy ragwort), Senecio vulgaris (common groundsel), Senecio riddellii (sand groundsel), Senecio flaccidus (threadleaf groundsel), and Echium plantagineum (Patterson’s curse) are some the more common species reported as causing hepatotoxicity in horses in North America.114 Recent reported outbreaks of Crotalaria species hepatotoxicity in horses have mostly been from Brazil and Australia.115 The incidence of Crotolaria toxicosis in the southeastern, central, and mid-Atlantic states, areas where the plant is found, seems low in comparison with toxicity from other PA-containing plants found in the western United States and parts of Canada. All parts of PA-containing plants, fresh or dried, are toxic but toxin concentrations may vary during the course of the growing season and for the perennial Senecio jacobaea and many other PA-containing plants, toxin concentration is highest during budding and flowering; the onset of flowering heralds the period of greatest risk for toxicity for those plants.116 Threadleaf groundsel is an evergreen and maintains foliage throughout the year. For Crotolaria, the seeds are most toxic.116 Most PA-containing plants have a bitter taste and, therefore, variable palatability. Hepatotoxicity from plant ingestion is usually associated with chronic feeding of contaminated hay but with drought conditions and sparse grasses these same toxic plants will be consumed in the pasture.

The liver is important in both toxic pyrroles formation and in detoxification of PAs. The parent PAs in the plants are relatively unreactive but when metabolized by the horse can produce highly toxic pyrroles.114 Pyrrolizidine alkaloids are absorbed and metabolized in the hepatocytes, especially in the zone 3 region with more acute intoxication, where hepatocytes with the highest level of drug-metabolizing enzymes are located.7 PAs are dehydrogenated to yield pyrroles which cross-link DNA and thus impair hepatic cell division and protein synthesis hence causing megalocytosis.7 Another important toxic metabolite is trans-4-hydroxy-2-hexenal, a reactive aldehyde that causes lipid peroxidation.117 Other than megalocytosis, characteristic pathologic and histopathologic lesions with chronic ingestion include karyomegaly, progressive fibrosis, variable nodular regeneration, bile duct proliferation, and veno-occlusion. With chronic disease, the liver may eventually become small and fibrotic.115 Horses that ingest large amounts of PA plants over a few days, usually in contaminated alfalfa hay, may die from subacute liver disease and hepatic lesions in these cases might have diffuse centrilobular necrosis118 and, unless megalocytes are observed, lesions could look similar to Theiler’s disease pathology. Megalocytes may not be seen in the liver on microscopic exam until 30 days or more after exposure to the PA.119

The effect of PA toxins on the liver is cumulative and it is estimated that horses must ingest approximately 2% or more of their body weight to develop hepatic failure.119 In many instances, by the time clinical signs develop weeks or months later, contaminated hay is often long gone from the property. If confirmation of the diagnosis cannot be made by investigation of the feeds or from histopathology of the liver, chromatography/mass spectrometry testing of frozen liver could be performed to detect pyrrolic metabolites.119 Although the disease is chronic, acute onset of neurologic signs (HE) is a common presentation and onset may be precipitated by a number of stressors. Weight loss, diminished appetite, jaundice, and photosensitization can be early clinical findings, followed by acute onset of neurologic signs due to hepatic encephalopathy. Weight loss and decline in appetite often go undetected in many cases. Laryngeal paralysis and gastric impaction also occur in a small percentage of affected horses.120–122

Horses with PA-induced hepatic failure have a guarded-to-poor prognosis due to the extensive fibrosis that occurs in most cases prior to the development of any clinical signs. Treatments for hepatic encephalopathy (page 92), antioxidant therapy with vitamin E, S-adenosyl methionine (SAMe) and milk thistle extract, anti-inflammatory/anti-fibrosis treatment with pentoxifylline, supportive care with fluid therapy, and nutritional management providing a high-energy and adequate-protein diet (page 95) are, to my knowledge, the most frequent treatments provided to horses with PA toxicity and liver failure. There is minimal proof of efficacy for those treatments in affected horses but most of the treatments seem reasonable based upon pathophysiology of the disease. The addition of dietary supplements of cysteine, butylated hydroxyanisole, 200 micrograms of vitamin B12/kg of feed, and 5 mg of folic acid/kg of feed did not alter toxicity in ponies fed tansy ragwort.123 A recent study in rats found that administration of SAMe and vitamin E administered before and after monocrotoline pyrrole exposure modulated the hepatic oxidative stresses induced by that toxic PA pyrrole.124 Unfortunately, pharmacokinetic and efficacy studies on SAMe are not available in horses and bioavailability of silymarin in horses may be less than 1%.125 When the above treatments are administered relatively early in the course of the disease, and with a dose of luck, a moderate percentage of clinically affected horses (including even a few with signs of HE), will have transient resolution of clinical signs and survive for several months or have complete recovery.126,127 I suspect that earlier detection of disease, increased antioxidant therapies, and better nutritional management including provision of...
mild-to-moderate-protein diet might have improved the prognosis since my internship year in California in the mid-1970s when the outcome of treatment was generally poor. One of the more favorable outcomes I know of was from cases that Dr. Karen Unger managed in 2013. Although some horses died from PA toxicity, four horses with persistent and marked increases in AST, GGT, and bile acids (up to 108 µmol/L) for 3–4 weeks survived and appeared clinically normal 3 years later. Serum bile acid concentrations greater than 50 µmol/L generally indicate a poor prognosis for long-term life.118 When a case of PA toxicity is confirmed or suspected on a farm, measurement of GGT in other horses on the farm is a sensitive, although not 100% sensitive, test to detect subclinical cases.16 GGT testing of horses potentially exposed to plant or environmental hepatotoxins, such as alsike clover, Panicum spp. should be routinely recommended.

Alsike Clover Poisoning

Alsike clover (Trifolium hybridum) poisoning is one of the most common pasture-associated hepatotoxicities seen in some areas of the northeastern United States and in some provinces of Canada (Ontario, Quebec, Alberta); it is also seen in Europe.128–130 Much of the original description of the disease came from outbreaks of hepatic failure in horses in Ontario where the disease was called “big liver disease.”128 The disease most often occurs when horses graze pastures or are fed hay containing large amounts of alsike clover. An increased incidence of poisoning is reported during wet seasons when the clover grows luxuriantly on heavy clay soil. Two presentations of the disease have been described.128 The first is dermatitis-stomatitis, previously referred to as trifoliosis or clover disease. The second presentation of the disease, believed to be associated with more long-term consumption of the plant, is hepatic dysfunction, characterized by icterus, colic, and nervous system signs. It remains unproven whether photosensitivity without signs of liver failure is really a primary photosensitivity or whether it is secondary to biliary disease that permits phylloerythrin accumulation without other signs of liver failure. We have found marked elevations in GGT in exposed, asymptomatic, horses grazing fields of alsike clover that might support the latter suggestion. The liver of horses that die from the disease may be noticeably enlarged with rounded edges, sometimes weighing 5% of body weight or 25 kg in a 500-kg horse. Other cases may have normal or even smaller-than-normal livers if there is extensive fibrosis. Characteristically there is little inflammation in the liver and the parenchyma is mostly intact except where it collapses by expanding fibrosis. Bile duct proliferation and fibrosis, most severe in the portal areas, is found in all cases but these two findings are common to several other toxins including PA toxicity (however, PA toxicity and most other hepatotoxins will cause more severe parenchymal disease). Both biliary and hepatocellular enzymes are increased in horses with alsike clover–associated liver failure, as are serum bile acids and direct and indirect bilirubin. Despite the consistent and often severe portal bile duct proliferation and fibrosis there is little evidence of bile stasis, which helps explain why this, a predominant biliary disease, may not have the typical high percent of conjugated bilirubin in the serum that is expected with an obstructive biliary diseases. Interestingly, we have found that horses exposed to the same alsike clover fields as horses exhibiting liver failure, have moderate-to-marked elevations in GGT with normal SDH, suggesting that the disease begins as a predominant biliary toxicity.

The toxic principle in alsike clover has yet to be identified. It is not known whether the agent is a toxic metabolite from the plant itself or a mycotoxin produced by a fungus (Cymodothea trifolii) living on the plant.131 We have noticed a pattern where no cases will occur for many years followed by several cases occurring in other years, suggesting that the toxicity may be related to a mycotoxin that may grow best on the alsike clover under certain climatic conditions. Treatment would be the same as for PA toxicity except the antifibrotic drug colchicine (0.03 mg/kg PO once daily) could be used and might have some additional benefit in inhibiting fibrosis.

Panicum Toxicity

Three different Panicum sp. (panic grasses) have been found to cause outbreaks of liver failure in horses. The first outbreak132 involved Panicum coloratum (kleingrass), a species introduced to Texas in 1952 from Kimberly, Union of South Africa, and subsequently widely planted in the state. The second involved Panicum virgatum (switchgrass), and occurred in eastern Nebraska.133 In the autumn of 2004, Dr. Melinda Freckleton discovered 14 horses in a single stable in Virginia that were clinically affected with liver disease, some with failure resulting in death, shortly after being fed a new shipment of hay.134 We had, anecdotally, on two other prior occurrences (1 and 3 years earlier), received information on outbreaks of hepatotoxicosis in horses in the mid-Atlantic area and had become suspicious of fall panicum toxicity based upon limited testing of the hay from those prior outbreaks but were unable to acquire a sufficient volume of the presumed toxic hay for a feeding trial. Thanks to Dr. Freckleton’s efforts, we were finally able to obtain hay from the third outbreak, conducted a feeding trial, and confirmed the hepatotoxicity of Panicum dichotomiflorum (fall Panicum). The onset of severe hepatotoxicosis after fall Panicum hay exposure was rapid (1–2 weeks) in both the naturally occurring cases and in the experimental horses. Horses with Panicum coloratum hepatotoxicosis had either prolonged grazing on the kleingrass pasture or were fed hay for 150 days before showing signs of liver failure.132 Hepatic pathology
in the experimental horses fed fall Panicum was similar to that seen in horses with spontaneous disease: individual hepatocyte necrosis, interpreted as apoptosis, with prominently clumped chromatin. Early fibrosis was not apparent. The accumulation of lipofuscin suggested some degree of oxidative stress. Experimental horses also had increases in creatine kinase and cardiac troponin I, suggesting concurrent hepatic, skeletal, and cardiac myocyte toxicity. Similar to alsike clover, the epidemiology and specific toxicity associated with panicum grasses is unclear given that many horses may be grazed or fed hay containing panicum without known toxicosis. Potential plant factors include strain variation, stage of growth during cutting, or variation in saponin concentrations. Environmental variables such as soil composition, weather conditions, or fungal contamination, also may play a role. For the three panicum toxicity outbreaks that we have investigated, all occurred in late fall or early winter when horses were fed fall Panicum hay harvested during the same year. The toxic principle of fall Panicum is unknown. A synergy between plant saponins and other hepatotoxins, particularly mycotoxins, might explain the sporadic incidence of hepatic disease. The absence of fibrosis in the research animals suggests that immediate cessation of feeding toxic fall Panicum hay should allow most animals to recover from acute exposure. Medical treatment and nutritional management would be as for PA toxicity. There are many other plant-induced hepatopathies in other areas of the world that time and space do not permit me to review.

Mycotoxins

Feeding horses grains that are sufficiently contaminated with Fusarium causes the classic disease, leukoencephalomalacia. The mycotoxin can also cause a concurrent hepatopathy with marked elevations in AST, GGT, and bilirubin. Lesions in the liver include hepatocyte vacuolation with centrilobular fatty change, hepatocyte necrosis, mild mononuclear infiltrate, mild bile duct proliferation, and perportal fibrosis. Unlike in swine and other species, liver failure caused by aflatoxins in horses is rare. It has been reported that the difference in aflatoxin toxicity in the horse could be related to differences in enzyme hepatic P450 activity detoxifying aflatoxin. This finding suggested that horses have microsomal-associated glutathione (GHS) S-transferase with the capacity to collapse GSH-conjugation of the reactive and toxic aflatoxin product AFB1-8,9 epoxide. Experimental administration of aflatoxin B1 to ponies has produced variable responses, from no or minimal disease (elevated liver enzymes but no clinical signs), to severe illness and death. Prior exposure to the mycotoxin AFB1, which affects hepatic metabolism upon subsequent exposure, and duration of exposure before studies, may explain the different results. The target organ in horses, as in other animal species, is the liver and horses suffering from aflatoxicosis show signs of inappetence, depression, fever, tremor, ataxia, and cough. Necropsy findings include a yellow-brown liver with centrilobular necrosis, icterus, hemorrhage, tracheal exudates, and brown urine. Iron Toxicity

Iron is an absolute requirement for most forms of life but is a potentially toxic substance. The liver is the major storage site for iron absorbed from the intestinal tract and it is a primary site for heme iron sequestration. When the capacity of the system to store iron is exceeded, hemochromatosis may occur. Hemochromatosis is the excessive deposition of iron into hepatocytes such that toxicity occurs. Hemosiderosis is the term used to describe iron deposits in Kupffer cells and hepatocytes without destruction of liver parenchyma and is a common finding during histologic examination of the normal equine liver, much of which is due to phagocytosis of heme from broken-down red blood cells. Both processes are more commonly seen around the perportal region of the liver. Iron is a known hepatotoxin and horses are often exposed to high-iron diets yet poisoning of adult horses with orally administered iron is rare. One explanation for this is that horses, as in most other species, have the protective ability to decrease iron absorption when body stores of iron are normal or high. For example, in one of our experimental equine motor neuron disease–induction trials, horses were fed a highly soluble ferrous fumarate at 3–6 times the National Research Council (NRC) recommendation for iron for 21–30 months without having abnormal hepatic iron concentrations. Pearson fed 50 mg/kg of ferrous sulfate to ponies for 8 weeks (NRC requirement is 1–2 mg/kg body weight), although hepatic iron, serum iron, and percent saturation of transferrin increased, there were no clinical signs or histopathologic lesions in the liver. He concluded that previous reports of hepatopathies in animals with hemosiderin accumulation might have represented another primary hepatopathy with secondary hemosiderin accumulation, especially if the only source of iron is via oral feed consumption. There are, however, individual case reports of iron overload associated (hemochromatosis) and liver fibrosis in horses. Portal fibrosis and/or excessive iron in hepatocytes were a feature of the iron toxicity in those case reports. Antemortem diagnosis of iron hepatotoxicity is difficult because observing large amounts of iron in the diseased liver is not diagnostic. It is known that large amounts of hemosiderin (hemochromatosis) is a common histologic observation in the liver of horses that may occur independent of liver pathology. In addition, using measurement of serum iron in horses with liver disease as a determinant of cause should not be relied on because horses with a variety of other known causes of liver failure (both acute and chronic) may have very high serum iron concentrations and nearly 100% saturation, without hemo-
chromatosis. The reason for the high serum iron in those horses with liver failure is unknown but one possible theory is that during severe liver disease iron might be released from the iron storage protein and iron detoxicant, ferritin, or from the considerable hemosiderin that seems to normally accumulate in horse liver. In my experience, after evaluating serum iron and saturation values in adult horses for more than 15 years, extremely high serum iron and nearly 100% saturation is most commonly seen in adult horses with liver disease and may be seen in up to 20% of horses with severe liver disease. This does not apply to newborn foals, who normally have very high serum iron for the first few days of life. There is a recent abstract concluding that horses drinking heavily iron-contaminated water (72.5 mg/L, or 150 times acceptable levels) for a prolonged period can result in hemochromatosis and liver failure.

Iron toxicity has been well documented in newborn foals. In the spring of 1983, more than 60 foals died at 2–5 days of age in association with the administration of a probiotic/nutritional supplement paste containing only 64 mg of ferrous fumarate. Foals that died were always administered the product before nursing colostrum. Iron toxicity in these foals was likely related to the direct passage of iron to the liver in foals that had not received the antioxidant benefits of colostrum. Foals were most commonly observed to be ill at 2 days of age with variable degrees of icterus and neurological signs. Hypoglycemia was almost always present; this is common in foals with hepatic failure and may reflect the relatively low glycogen content of foals. Both total and conjugated bilirubins were increased with conjugated bilirubin less than 10% of total. Foals were severely acidic, also characteristic of liver failure in foals. GGT enzymes were increased in all foals and, amazingly, SDH was normal in 3- and 5-day-old foals, likely reflecting massive hepatic necrosis on day 1 and the very short half-life of SDH. Interestingly, a small amount of liver on the periphery seemed normal, allowing some function to occur. This was believed to be a result of dilution of the iron toxin between the central area of the liver and the main entry point of the iron, the portal vein. Three surprising findings were discovered from these unfortunate cases: bile ductule proliferation can be rapid and pronounced in the equine, Alzheimer type II cells associated with hyperammonemia can develop in 48 hours or less, and detectable fibrosis may be seen within 5 days. It was hard to fathom that 64 mg of ferrous fumarate would kill a newborn foal when 50 mg/kg could be given to adult ponies without evidence of liver disease (clinical, biochemical, or microscopic), but it did and we learned. All potential toxins, even in small amounts, administered to foals prior to colostrum consumption could be harmful and should be routinely avoided.

Another less-well-understood hepatic failure possibly associated with iron overload has occurred in foals with neonatal isoyrolythesis that required one or more blood transfusions. Affected foals may have progressive liver disease (increasing liver enzymes) within days after they receive one or more transfusions and these foals may exhibit liver failure as soon as 5 days following transfusion or up to 3 months later. Histologic findings in the liver consist of hepatocellular necrosis with extensive biliary proliferation. A greater number of transfusions and lower packed cell volume (PCV) on hospital admission were risk factors for liver failure. Histologic findings are similar to those seen in the newborn foals that received ferrous fumarate, suggesting that a combination of hepatic hypoxia and iron overload from transfusions may have caused the severe liver disease. It is unlikely that either one alone would have caused the disease in so many foals. Regardless, liver failure is one of the most common complications seen in foals with extremely low PCVs who have been transfused. Regardless of the etiology of liver failure in those foals, treatment with subcutaneous deferoxamine should be considered because this drug increases iron elimination.

The liver is not only the key organ for iron homeostasis and storage but is also the main producer of acute phase proteins. Acute phase proteins include the iron-regulating protein hepcidin, serum amyloid A, and fibrinogen. A decrease in serum iron (a negative phase reactant) is known to occur quickly in many species following an acute inflammatory response and is believed to be an evolved method for decreasing the iron available to invading bacteria. Except for a few bacteria exceptions such as my nemesis Borrelia spp., iron is the most important element required for bacterial growth. Dr. Alexandre Borges, while on sabbatical at Cornell, demonstrated that low serum iron was a better predictor of systemic inflammation than plasma fibrinogen. Heparin is known to be the key regulator of iron metabolism that leads to hypoferremia during inflammation. Dr. Borges was further able to clone and sequence the equine hepcidin gene and perform expression analysis from different equine tissue. That work has shown that equine hepcidin is predominantly expressed in the liver of horses. When horses were challenged with endotoxin, plasma iron concentration was decreased significantly from the pre-infusion level by 8 hours and relative real-time RT-PCR analysis showed that liver hepcidin and IL-6 mRNA expression was up-regulated at 6 hours post lipopolysaccharide (endotoxin) infusion. The totality of Dr. Borges’ work suggests that hepcidin acts as an acute-phase protein in horses, its regulation of iron (hypoferitenemia) is more rapid than most other markers of inflammation and the serum concentration of iron is correlated with the severity or course of inflammation, with a return to normal as the inflammation resolved.
Drug-Induced Hepatotoxicity

Luckily for those of us in equine practice, and for the horse, drug-induced causes of hepatic failure in the horse are rare. There are rare reported cases of steroid hepatopathy in horses.\(^{158,159}\) Imidocarb can cause severe hepatic disease in donkeys\(^{160}\) but does not seem to do so in horses. A severe toxic hepatopathy has been described in nursing foals being treated for *Rhodococcus equi* with both rifampin and doxycycline.\(^{161}\) This combination therapy as a cause of liver disease in other species is apparently nonexistent or at least rare. Regardless, this combination of antimicrobials should be used with caution in the foal. Rifampicin is thought to be one of the most common antibiotics associated with drug-induced liver diseases in humans. Nonsteroidal anti-inflammatory drug (NSAID) hepatopathy has not been reported in horses, which is surprising considering that acetaminophen is by far the most common drug-associated cause of acute hepatic failure in humans.

There is a paucity of literature on the equine cytochrome P450 enzymes and hepatic metabolism of drugs in the horse compared with knowledge in many other species. Hepatic metabolism can play a critical role in determining both the efficacy and the residence time of drugs in the body, as well as in modulating the response to toxic chemicals. Age, species (horse vs. donkey, for example), and other genetic profiles may affect hepatic metabolism and body elimination. Donkeys, and especially miniature donkeys, have a much greater capacity for metabolism of some drugs than other equines.\(^{162}\) Miniature donkeys were also noted to be overrepresented in one retrospective study of hepatic cirrhosis, calling into question whether increased metabolism of hepatotoxins might also occur in donkeys (S. McDonough, personal communication, 2014). Little is known about the effects of aging on the hepatic drug metabolizing capacity of horses, but newborn foals are generally believed to have decreased hepatic metabolism (often along with increases in bioavailability and differences in protein binding) of many drugs.\(^{163}\) Compared with adult horses, young horses may have less ability to metabolize aromatic hydrocarbons and less conjugative ability, and may be at greater risk when exposed to xenobiotics requiring metabolism by these systems.\(^{164}\) This might explain why we have seen more markedly increased hepatic enzymes in foals vs. adult horses exposed to the same toxic pasture containing red fescue (author’s experience). Some thought should be given to the dose of potentially toxic drugs that undergo hepatic metabolism, such as metronidazole and lidocaine, in foals and a reduced dose or prolonged interval may be required.\(^{165}\)

Phosphine gas, associated with feeding a pelleted ration treated with aluminum phosphide, caused hepatic failure in a group of horses.\(^{166}\) Horses and ponies seem to be very resistant to any hepatotoxic effects of high dietary copper and adult horses fed ten times the NRC requirements for copper for 21 months or more had 100 times the amount of hepatic copper as control horses without elevated liver enzymes or hepatic lesions on necropsy.\(^{143}\)

4. Primary Hyperammonemia

A primary or enteric hyperammonemia syndrome has been well described in horses. The first case report was by Dr. Tim Mair in 1995\(^{167}\) and we followed that report with an additional four cases in 1997.\(^{168}\) Since that time there have been numerous case reports of the condition and a large study has been published involving 36 horses.\(^{169}\) It is a relatively common and unique disease of horses and when we first recognized the disorder in the mid-1990s we could find no other animal model for the equine disorder. Even today, the horse seems to be the domestic animal most predisposed to fulminant enteric hyperammonemia. Dr. Skip Hintz was consulted on the cases we had in the 1990s as he had experimentally caused urea poisoning in ponies\(^{170}\) previously, and the ponies clinical and laboratory findings were similar to what we were observing in the horses with primary hyperammonemia. With Dr. Hintz’s help and further evaluation of serum urea (urea poisoning causes a rather marked increase in urea, which was not present in our horses) and epidemiologic investigations, we were able to sufficiently rule out urea poisoning. The characteristic presentation is one of anorexia and mild colic initially, followed by an acute onset of encephalopathy 24–48 hours later. Head pressing, circling, other maniacal behavior and blindness with dilated pupils are the most common neurologic signs (Fig. 7). Infectious causes of anorexia, enteritis, and colitis have also been described as predisposing factors. In fact, hyperammonemia may be the number one cause of death in horses and ponies with coronavirus enteritis.\(^{171}\) We have also, on rare occasion, observed the fulminant hyperammonemic encephalopathy while treating horses with Potomac Horse Fever or salmonellosis. We have not identified any unique risk factors in those horses other than the primary disease. The possibility of more frequent recognition of the disease in horses and ponies with coronavirus infection and the absence of large bowel signs (tympany, etc.) in most of the abdominal pain cases, may point to small intestinal dysfuction or microbiome changes as an initiator for the disease. Many of our cases have not been associated with those aforementioned infectious diseases and instead have been otherwise healthy, nonfebrile horses with mild colic that preceded by 12–24 hours the acute neurologic signs. It may sound easy, but with the history of colic in the preceding 24 hours, it has sometimes taken me an hour or so to figure out that the current signs are not those of abdominal pain. On rare occasions, an affected horse has even undergone abdominal surgery for abdominal pain without finding a lesion at surgery; those horses
have usually not recovered from anesthesia. Although gastrointestinal diseases may predispose to the hyperammonemia condition, there are reports of starved horses developing hyperammonemia after being refed and in a small number of cases there is no evidence of gastrointestinal disease. Dr. Linda Mittel and I recently reviewed 14 documented cases of primary/enteric hyperammonemia evaluated at Cornell; epidemiologic data did not reveal any breed or sex predisposition and the average age was 10 years (range, 2–17 years). Young foals, especially those with meconium impaction, have been reported with hyperammonemia but their syndromes are not as fulminant as what we routinely see in the adult horses. In our review, it was more common for affected horses to be on pasture but a few were stabled horses. The disease occurred as a single case on a farm with little or no predisposing risk factor in 12 of the 14 cases. On one farm in March 1997, we had two horses affected at the same time and those were febrile horses so I tend to think they were likely coronavirus-infected horses, before we even knew about coronavirus infections in adult horses.

Affected horses almost uniformly have no evidence of liver disease (increased hepatic enzymes) or abnormal liver function to explain the hyperammonemia. Characteristic laboratory findings have been hyperammonemia (often > 200 μmol/L), marked hyperglycemia, erythrocytosis, and hyperlactatemia (l-lactate). Horses with concurrent enteritis/colicitis may have neutropenia and other biochemical abnormalities (e.g., azotemia, hyponatremia, and hypoalbuminemia) characteristic of those disorders. The measurement of blood ammonia is affected by temperature, time and air exposure. Samples should be collected in vacuum blood tubes, kept on ice and measured in 30 minutes. If the horse has died just prior to arrival and you suspect hyperammonemia, aqueous fluid can be collected, stored, and tested as described above with values less than 100 umol/L suggesting that the horse did not have hyperammonemia. Ammonia will progressively increase in the aqueous humor following death, making high levels somewhat hard to interpret (Linda Mittel, personal communication).

Although the cause of the hyperammonemia is unproven it is likely a combination of increased enteral ammonia production and increases in intestinal permeability that overwhelm the normal liver's ability to metabolize the ammonia causing the metabolic and neurologic disturbances. Overgrowth of urease-producing bacteria has been discussed but until the microbiome is evaluated in affected and control horses, the cause will remain speculative. Treatment for the condition is treatment of the primary disease, if known, and treatment of HE. Studies in some species suggest that the largest amount of enteric ammonia is generated by Gram-negative anaerobes, clostridia, enterobacteria, and Bacillus spp. Lactobacilli formed very little ammonia. If this is true in the horse, a combination of metronidazole and neomycin could be used, in addition to lactulose and probiotics given separately from the antibiotics. Treatments specific for hyperammonemia of more than 1 or 2 days are usually not required. For cases with mild colic followed by idiopathic hyperammonemia I often tell the owners that the outcome will be known within 2 days as approximately half the cases quickly deteriorate to coma and death with the other half showing improvement following HE treatment within 24 hours and often a complete recovery within 72 hours (except when associated with traumatic injuries). If you have not seen this disease, I can almost guarantee that you will eventually see one or more cases if you are routinely examining horses presenting with intestinal signs. When I have shown movies of affected horses and described the clinical syndrome at veterinary meetings, it is common for at least one practitioner in the group to remark, “I just saw a case of that.”
Hepatic lipidosis with concurrent hyperlipemia is the most common metabolic disorder causing equine liver failure. Hyperlipemia with hepatic lipidosis is a metabolic condition most frequently seen in fat ponies, donkeys, miniature equines, and occasionally in horses with Cushing’s disease. In some practices, hepatic lipidosis may be the most commonly diagnosed cause of equine liver failure. Hyperlipemia is defined as serum triglycerides greater than 500 mg/dL, grossly discolored plasma or serum (lipemia), and concurrent fatty infiltration of the liver (hepatic lipidosis). Hyperlipidemia is an elevation in serum triglycerides without lipemia.

Lipid metabolism is mostly determined by dietary intake of digestible nutrients, energy requirements, and insulin activity. Decreases in feed intake, increased energy requirements such as pregnancy or lactation, and increased stress hormone release are all known to increase lipolysis of fat stores. Lipolysis associated with negative energy balance is stimulated by decreased production of the antilipolytic hormone insulin. Increases in stress hormones (e.g., cortisol and beta 3 receptor–activated catecholamines) enhance lipolysis by either decreasing insulin sensitivity or by up-regulation of hormone-sensitive lipase and adipose triglyceride lipase.

Lipolysis of triglycerides stored in peripheral fat results in release of free fatty acids (FFAs), also known as non-esterified free fatty acids (NEFFAs), and glycerol into circulation. Circulating FFAs are taken up by the liver, where they may be oxidized in the Krebs cycle for energy, stored as triglycerides, or tryglyceride released back into circulation as lipoproteins. In the equine liver, incomplete oxidation of free fatty acids seems to be minimal, as marked ketonemia rarely occurs. With hypertriglyceridermia, there is an increased activity of endothelial lipoprotein lipase (LPL), an enzyme needed to hydrolyze triglycerides into FFAs so they can be used as energy substrate or stored as triglycerides in the peripheral tissue (fat deposits). There is an increased activity of LPL activity in ponies with hyperlipemia, suggesting that equine hyperlipemia is mostly a result of exaggerated lipolysis and increased hepatic secretion of triglyceride-rich very low density lipoproteins and is not commonly a primary problem with decreased peripheral clearance of triglycerides. In azotemic equines, clearance of triglycerides is thought to be decreased because of uremic inhibition of LPL activity.

Pre-renal or existing renal diseases and azotemia may be compounded by renal steatosis that also occurs with hyperlipemia. Lipolysis and hypertriglyceridemia are commonly seen in all equines that have either negative energy balance or decreased insulin activity but lipemia only develops in ponies, donkeys, miniature equines, and occasionally in horses with Cushing’s disease. On rare occasions, lipemia will be noted in horses with neoplasia, and I have assumed this may in part be due to increased tumor necrosis factor and its effect on enhanced lipolysis and decrease in LPL activity.

Adult ponies, especially Shetland or fat ponies, donkeys and miniature equines of all ages experiencing enhanced lipolysis can quickly develop lipemia and hepatic lipidosis and without prompt treatment they often die from metabolic abnormalities, liver failure, or occasionally rupture of the liver and hemorrhagic shock (Fig. 8). Interestingly, horses and ponies with Cushing’s disease often have some hepatic lipidosis and are, on rare occasion, hyperlipemic but they do not seem to have the same metabolic/hepatic failure crisis that occurs in other ponies, donkeys, and miniature equines.

The most common predisposing factors initiating the negative energy balance and marked lipolysis include intestinal diseases, esophageal choke, pregnancy, dental disorders, transportation, decrease in available feed or appetite, parasitism, lactation, laminitis, respiratory diseases, and Cushing’s disease. Donkeys afflicted with social reordering and donkeys that pine for missing mates are affected as well. Females are most commonly affected because late pregnancy and lactation seem to be significant risk factors for the disease. The disorder seems to be common in older donkeys, which could be a reflection of concurrent Cushing’s disease or dental problems in those animals. There are cases of hyperlipemia and hepatic lipidosis in fat adult ponies that do not have any additional obvious risk factors, suggesting that insulin resistance is the basic mechanism for the lipemia in those ponies although Freestone in an earlier publication did not find that to be the case.

Clinical findings in equines predisposed to hyperlipemia and hepatic lipidosis include signs associated with the predisposing disease, as well as signs caused by the hepatic lipidosis. Clinical signs that...
can be directly attributed to the hyperlipemia and hepatic lipidosis include depression, anorexia, jaundice, signs of HE, discolored urine, dysphagia, and ventral edema. Tachycardia is present in most cases. Depression is observed in almost all affected equines with hyperlipemia and some will progress to fulminant encephalopathy (HE) with blindness. Ventral edema occurs in approximately 30% of the cases and nearly 50% of hyperlipemic animals look obese. Although laminitis can trigger hyperlipemic/hepatic lipidosis, laminitis does not in my experience seem to be commonly caused by hyperlipemia itself, unless serum insulin concentrations remain very high for a prolonged time.

Laboratory findings include lipemia and hypertriglyceridemia, but the degree of lipemia does not always correlate with the triglyceride concentrations in the plasma. Some ponies may have gross lipemia with triglyceride levels of only 400–700 mg/dL. Free fatty acids are increased (normal, < 0.4 mEq/L), as are total cholesterol and some liver enzymes. Handheld, point-of-care devices could be used for measuring triglycerides and FFAs. The pattern of elevation in hepatic enzymes seems inconsistent with nearly normal GGT in many cases, but marked elevation (several hundred IU/L) does occur in a few patients. Hepatocellular enzymes are often moderately elevated in the serum but are variable. This may reflect a significant variation in amount of inflammation of hepatocytes and very variable biliary obstruction between cases.

Bile acids are generally increased and are often in the 80–120 μmol/L range, PT and PTT prolonged, direct bilirubin increased, and blood ammonia is frequently high, all suggesting significant decrease in liver function (failure). Blood glucose is variable with many cases having low glucose, especially young miniature equines, yet glucose is high in others, likely related to insulin resistance. Concurrent pancreatitis can occur, possibly associated with the excessive hydrolysis of circulating triglycerides in the pancreas, which might cause inflammation due to calcium/FFA deposits. Hyperlipemia is known to be associated with primary pancreatitis in foals, dogs, and some humans. Although the equine pancreas is one of the organs that contains the GGT enzyme, pancreatitis in equines does not seem to cause elevations in GGT. This is supported by two case reports of acute severe pancreatitis in a donkey and in two acute, fatal cases of pancreatitis in foals.

Management of hyperlipemia should target treating any underlying disease, reversing the negative energy balance, normalizing plasma lipid concentrations and treating hepatic lipidosis/failure. Treatment for all of these should begin immediately and concurrently due to the sometimes rapid progression of disease. Treatment of predisposing disorders will vary but maintaining hydration, decreasing systemic inflammation, and decreasing any pain with drug therapy would seem appropriate in most cases; endotoxin, systemic inflammatory mediators and azotemia all may inhibit LPL activity. Broad-spectrum antibiotics may be needed for the primary disease or to combat bacterial translocation from the intestinal tract. Pergolide should be administered to lipemic horses or donkeys with Cushing’s disease and in some cases is the only treatment needed.

Nutritional support for miniature horses, donkeys, and ponies with hyperlipemia and hepatic lipidosis is the most important indication for nutritional intervention in equine liver disease. Providing calories in the form of mostly carbohydrates and protein in the meal or forced enteral feeding is often successful in quickly correcting this life-threatening disorder, assuming the predisposing cause can be promptly corrected. The provision of adequate calories will inhibit mobilization of FFAs from adipose sites, and supplemental protein has in some species been shown to be an important treatment. Enteral support could consist of simply feeding the lipemic animal at a higher carbohydrate level and feeding more palatable feed. Syringe feeding 30 mL of corn syrup two to six times a day has been recommended as a simple method of increasing caloric intake. Unfortunately, most lipemic equines have a depressed appetite and interventional nutrition will likely be required. There are reports of fat-free diet formulas that were successful when given via nasogastric tube in treating ponies with hyperlipemia. These generally include glucose as the carbohydrate supply and are mixed with casein and alfalfa meal as protein sources; the combination of carbohydrate and protein intake is likely ideal. A common formula used in our clinic for ponies and miniature horses with hepatic lipidosis/ hyperlipemia is 100–200 g glucose (depending upon blood glucose levels), 400 g of whey, and 50–100 g of alfalfa meal mixed in two liters of water and administered every 8 hours via nasogastric feeding tube. Five to ten grams of potassium chloride and multiple B vitamins should also be provided in the slurry. The mixture can be given via a standard stomach tube of proper size or through a commercially available enteral feeding tube. The gruel must be kept sufficiently thin so that it will flow through the tube without clogging. There are also numerous commercial enteral products that could be used but many of the products marketed for humans contain fats. When commercially available enteral nutrition products are used, a low-fat or medium-chain-triglyceride fat supplement is preferred for forced enteral feeding.

Parenteral nutrition using 5 to 20% dextrose with amino acids (1 g/kg body weight) can be used in the equine patient with hyperlipemia and hepatic lipidosis where enteral diets cannot be administered because of esophageal or intestinal disorders. Parenteral nutrition with a moderate dextrose con-
successful and prompt correction of the predisposing 50% but the prognosis is variable, depending on the lipidosis reported in one case series is approximately heparin to a pony with hyperlipemia as the pony think I made that mistake once of giving too much and intravenous fluids with dextrose, the horse had ing 2 days of enteral feeding, selenium treatment proportion (2 g twice daily) had no effect on plasma FFAs have used it in treatment of hyperlipemia) as a energy metabolism and it would be of interest (and I administration of B-vitamins daily. Although l-carnitine supplementation is known to affect β-oxidation and energy metabolism and it would be of interest (and I have used it in treatment of hyperlipemia) as a potential treatment for hyperlipemia/hepatic lipodosis, the feeding of dietary l-carnitine supplementation (2 g twice daily) had no effect on plasma FFAs and triglyceride concentrations in healthy ponies. Additional studies on l-carnitine in ponies with hyperlipemia would be of interest.

Heparin therapy is generally not recommended given that LPL may already be maximally activated in equine hyperlipemia184 and, with severe fatty liver, PT and PTT are often prolonged. I think I made that mistake once of giving too much heparin to a pony with hyperlipemia as the pony developed a severe hemorrhagic crisis.

The mortality rate from hyperlipemia and hepatic lipodosis reported in one case series is approximately 50% but the prognosis is variable, depending on the successful and prompt correction of the predisposing cause(s) and on the ability to supply adequate nutritional support (enteral or parenteral or both).183 Prognosis should not be based upon the level of triglycerides in the serum or upon liver enzyme elevations. For example, one miniature horse mare with masseter myopathy (and no detectable selenium in her whole blood) had lipemia and a plasma triglyceride concentration of 1926 mg/dL. Following 2 days of enteral feeding, selenium treatment and intravenous fluids with dextrose, the horse had a triglyceride concentration of 120 mg/dL, with marked improvement in liver function tests and clinical signs. The horse was able to eat on her own on day 4. Hyperlipemic horses with Cushing’s disease that are receiving pergolide treatment in addition to attention to general health care generally have a good prognosis.

The prevention of hepatic lipidosis revolves around identifying at-risk equines, especially pregnant or lactating pony mares, obese or geriatric ponies and donkeys, and miniature equines of all ages with diarrhea and other predisposing disorders. Donkeys that have any change in their social status, such as loss of a companion, should be considered at high risk for disease. Providing adequate caloric intake is important in late-pregnant and early-lactation mares. As a preventive, fat could be fed to increase caloric density and feeding fat to healthy ponies increases lipoprotein lipase and decreases circulating triglycerides.19 This would not be recommended in at-risk equines that already have hyperlipidemia. Performing routine body condition scoring and periodic sampling of at-risk equines for triglycerides and free fatty acids could be helpful in identifying metabolic abnormalities before the onset of clinical signs. Proper maintenance health care and husbandry are of utmost importance in at-risk equines. Testing for Cushing’s disease should be instituted in all older at-risk animals.

6. Vascular, Genetic and Miscellaneous Causes of Liver Failure

Hepatic disorders of vascular or genetic origin that result in severe central nervous system signs (hepatic encephalopathy or HE) include congenital portosystemic shunts (PSS), a suspected ammonia metabolism defect in Morgan weanlings198–200 and portal vein thrombosis. Additional miscellaneous causes of liver disease or failure include umbilical vein abscesses, liver lobe torsion, a congenital deficiency in bilirubin uridine diphosphate glucuronyltransferase activity, and primary and secondary hepatic neoplasias.

Portosystemic Shunts

PSSs are not common in foals and, when present, are often not detected until the foal is 6 to 12 weeks of age.198,201–203 This relatively late clinical detection could be because hindgut development in young foals is necessary to produce enough enteric ammonia to cause sufficient elevations in blood ammonia. We have also noticed that young calves with PSS do not generally exhibit clinical signs until they are ruminants.198,204 The diagnosis of PSS is made after looking at the patient’s age, clinical signs of HE with marked elevation in blood ammonia and bile acids and normal serum hepatic enzymes. The signs of HE in foals with PSS are variable and include lethargy, disorientation, compulsive circling, ptyalism, seizures, central blindness, walking through fences and so on. Blood ammonia can be
very high (up to 300 umol/L) in some PSS foals without evidence of encephalopathic signs. The chronicity of the hyperammonemia and, in some cases, its gradual development may somehow allow the brain to adapt to the elevated ammonia levels. PSS can be confirmed by liver ultrasonography, by ultrasound-guided, percutaneous trans-splenic injection of 10 mL agitated saline into the spleen with simultaneous echocardiography of the right heart (“bubblegram”) or by transrectal hepatic scintigraphy. The bubblegram may have higher sensitivity because air bubbles will appear in the right heart almost immediately after splenic injection if PSS is present. Theoretically, if portal circulation is normal the liver will filter the bubbles and the bubbles will not reach the heart. If the majority of technetium is detected first in the liver then PSS can be ruled out. Conversely, if most of the radioactivity appears in the heart first then the foal either has a PSS or the technetium was not administered “high” enough in the distal bowel (substances absorbed from the distal rectum enter the caudal vena cava, not the portal circulation). If a liver biopsy is performed (usually unnecessary), lobular atrophy characterized by decreased distance between the central veins and portal areas and arteriolar proliferation in the portal tract with mild proliferation of interlobular arteries are usually seen. The number and size of portal veins may be subjectively less than normal. These findings may vary depending upon location of the shunt (extra- vs. intrahepatic), but all of the changes develop as a result of decreased oxygen supply to the liver caused by the PSS (recall that the majority of oxygen to the liver is delivered via the portal vein, not the hepatic arteries). Positive-contrast portography following anesthesia and catheterization of a mesenteric (usually jejunal) vein or computed tomographic angiography (Fig. 9) with contrast and ultrasonography may all be required to determine the shunt location prior to considering surgical repair. Shunts may occur as either intrahepatic or extrahepatic and be single or multiple shunts. Shunts may occur due to aberrant connections between the systemic circulation (often the caudal vena cava or azygous vein) and virtually any gastric, splenic or mesenteric vessel. Surgical repair has been successful using suture ligation, cellophane bands, or casein–stainless steel rings (ameroid rings) placed around the shunt. Immediate, total surgical ligation of the shunt vessel can cause life-threatening portal hypertension with increased mesenteric venous pressures and congestion of the bowel. As opposed to suture ligation, cellophane bands or casein rings can be fitted around the shunt vessel, modestly occluding blood flow initially; over the following weeks gradual attenuation of the shunt occurs due to either vascular constriction associated with the inflammation caused by cellophane banding or because of gradual casein swelling in the ameroid rings. Placement of transvenous coils or umbrellas into shunts could also result in slow embolization and progressive closure of the shunt, but experience has shown that the umbrellas made for children are too small to cause a sufficient thrombus in the vessel in neonatal large animals. While preparing for diagnostics and surgery, treatments for foals with HE secondary to PSS are similar to HE treatment caused by intrinsic liver disease and failure (page 92). Enemas are often used on foals with HE if the manure becomes abnormally firm. One foal with a PSS and a blood ammonia as high as 300 umol/L was maintained on lactulose and minocycline per os, in addition to enemas, until surgery could be attempted.

Hyperammonemia Syndrome in Morgan Foals

An acute onset of clinical signs such as coma, blindness, and seizures has been associated with the hyperammonemia syndrome seen in Morgan foals, usually 4–7 months of age. The onset of clinical signs often occurs soon after weaning. Liver enzymes may be mildly elevated and variable degrees of liver pathology can be seen on microscopic examination but the degree of microscopic disease is modest and other liver function tests are not abnormal in affected foals. The cause of the disease is unknown but it seems to be a disorder of metabolism of ammonia and possibly other amino acids. The blood ammonia is extremely high in affected foals (300–500 umol/L), indicating that they have likely developed some compensatory or protective mechanism against the persistent elevation in ammonia to
survive to several months of age. The author has pedigree information on 12 affected foals and, interestingly, there is a common grandsire on the paternal side in 11 of the foals and in the other foal the stallion is a grandsire on the maternal side. One mare was bred to the same stallion in consecutive years with both foals dying from the disorder. The disease is fatal and may end with a terminal hemolytic crisis. Some foals may survive the first crisis and live for days or even weeks before dying with a second HE crisis. I have not seen or heard of a case in 3 years so perhaps it has been “bred out” of the breed.

Portal Vein Thrombosis
Portal vein thrombosis (PVT) occurs rarely in foals following enteritis or in association with Rhodococcus equi infection. Portal vein thrombosis has been reported in other species as a sequella to sepsis, hypercoagulability, and inflammation. In older animals, hepatic neoplasia may predispose to thrombosis. Although rare, horses with cirrhosis may occasionally develop portal vein thrombosis.

Clinical signs in foals attributed to complete portal vein thrombosis include diarrhea and HE signs. Diarrhea may occur from increased mesenteric venous pressure and intestinal wall edema if the thrombosis is acute and completely obstructs the portal vein. Diarrhea may be a predisposing cause of or a result of the PVT. The neurologic signs are secondary to hyperammonemia, caused by the collateral flow of the portal blood into the vena cava and elsewhere, thus bypassing the liver. If the obstruction is not complete or portoportal collateral compensatory circulation develops, clinical signs may be absent. One foal with both abdominal lymphadenitis, septic tarsitis, and hepatitis caused by Rhodococcus equi had a large but incomplete obstruction of the portal vein and with long-term antimicrobial therapy the patient recovered. An aged horse developed an acute onset of HE from portal vein thrombosis secondary to invasion of a metastatic gastric adenocarcinoma. Treatment to remove PVT in horses to my knowledge has not been attempted.

Umbilical Vein Abscess
Foals with umbilical vein abscesses extending into the liver are well documented. Although the infection in the umbilical vein (which normally becomes the round ligament of the liver) may extend into the liver parenchyma, inflammation of the liver and increase in hepatic enzymes are uncommon. The infected and enlarged vein can be easily imaged during ultrasound examination as it courses into the liver and, in some cases, can be palpated through the body wall. Affected foals are febrile, depressed, and may concurrently have infected joints and lameness. Because of the extent of the infection, surgical removal is generally recommended. If the infection extends into the liver such that it cannot be completely removed, then a stump of the vein extending into the liver can be marsupialized to the ventral body wall.

Liver Lobe Torsion
Liver lobe torsion is an uncommon disorder in horses. Based upon the small number of cases reported, it would seem that the left medial or accessory lobe is most commonly affected, although the entire left lobe was believed to be involved in two horses. Predisposing causes were rarely, if ever, identified. Of the eight cases reported, only three were presented for colic. Clinical signs are most commonly anorexia of 1 or more day’s duration, tachycardia, and clinical and laboratory signs associated with systemic inflammation resulting from necrosis of the twisted liver lobe and peritonitis. Peritonitis seems to be present in all cases and peritoneal fluid is often serosanguineous. Serum levels of hepatic-derived enzymes are usually unremarkable although SDH and GLDH may be increased in association with the systemic inflammatory response. Ultrasound examination is generally unremarkable as the affected lobe may be obscured from imaging by the gastrointestinal gas. If the entire left lobe is “torsed,” then the affected portion may appear as a mixed echogenic mass. Surgical repair is via stapled resection of the affected lobe and this has been successful in five cases.

Persistent Hyperbilirubinemia
In otherwise-healthy horses, persistent bilirubinemia is believed to be a result of congenital deficiency in bilirubin uridine diphosphate-glucuronyl transferase activity. A deficiency of this enzyme results in a decreased rate of conjugation of bilirubin within the hepatocyte endoplasmic reticulum. We have examined or have knowledge of several cases of persistent hyperbilirubinemia in healthy adult horses with total bilirubin values between 9 and 16 mg/dL on repeated testing. Virtually all of the increase in the bilirubin is unconjugated (indirect). Affected horses have normal appetite, no anemia, normal liver enzymes, and normal serum bile acids. The normal serum bile acids concentration likely rules out a hepatic anion uptake abnormality given that unconjugated bilirubin, bile acids, and free-fatty acids are all organic anions that somewhat competitively enter the hepatocytes at the sinusoidal hepatic surface with the help of cytosolic transport proteins. The majority of the cases have been healthy Thoroughbred horses and most were first noted to be icteric upon reading their tattoo while completing health papers. Illness in these horses has not been recognized, even when monitored for 3 or more years.

Hepatic Tumors
Primary hepatic tumors are rare in horses with hepatoblastoma, hepatocellular carcinoma, lymphoma, hemangiosarcoma, and cholangiocarcinoma
Hepatoblastoma is the most common of the primary hepatic tumors, usually diagnosed in stillborn fetuses, neonates, and juvenile horses less than 3 years of age. The clinical signs in horses with hepatoblastomas are generally nonspecific but include anorexia, lethargy, abdominal enlargement, weight loss, mucous membrane congestion, and fever of unknown origin. Signs directly associated with liver failure have not been reported. The tumor can be a single mass or multiple masses likely metastasizing within the liver from a primary site. The left lobe of the liver may be more commonly affected. Affected individuals may have increases in serum hepatic enzymes caused by hepatocellular injury, biliary hyperplasia, and cholestasis. Erythrocytosis is a common finding (hematocrit [HCT] of 50–60% is common) and believed to be associated with increased erythropoietin and from extramedullary hematopoiesis. Ultrasonographically, the liver may be markedly enlarged and a mass of mixed echogenicity may be seen. No treatment has been reported. Hepatocellular carcinomas are rare in the horse but have been reported in a young horse and, similar to horses, with hepatoblastoma, polycythemia, and increases in serum alpha fetal protein may occur. Hepatocellular carcinomas are better defined by immunohistochemistry than are hepatoblastomas. Hypoglycemia may also be seen with hepatocellular carcinomas or other hepatic tumors.

Cholangiocellular carcinoma is also rare but may occur in aged horses. The onset of the disease is usually insidious, as a consequence of slow and progressive neoplastic infiltration from the bile duct epithelium throughout the entire parenchyma. Clinical signs, including intermittent episodes of colic, diarrhea, and fever are most likely related to obstructive cholangitis although bacterial cholangitis may also be present. On rare occasion lymphoma may diffusely infiltrate the liver and cause hepatic failure; hypoglycemia would be likely.

7. Chronic Fibrosing Liver Disease

Except for PA toxicity, evidence-based information on chronic fibrosing liver disease in horses is rare. In Jubb and Kennedy pathology text, there is limited discussion (other than on PA toxicity) on chronic fibrosis, cirrhosis, or chronic active hepatitis in the horse. Causes of chronic hepatitis that may result in progressive fibrosis include chronic biliary infection and/or obstruction from hepatocholeliths, plant toxicities, rarely iron toxicity, chronic viral infection, chronic hypoxia, and immunologic reaction directed against self antigens or foreign antigens in the liver. Some degree of fibrosis may occur in any liver disease if hepatic stellate cells are activated and collagen is deposited. In most diseases the fibrosis is reversible if the primary insult is removed, PA toxicity being an exception. In contrast, if the toxic, obstructive, infectious, or immunologic insult continues, then progressive fibrosis, often with concurrent biliary hyperplasia and nodular areas of regeneration, will occur. Eventually, fibrosis results in sufficiently reduced functional hepatic mass and dysfunction occurs (cirrhosis).

The clinical presentation of chronic progressive hepatitis in the horse, sometimes referred to as chronic active hepatitis, may include weight loss, chronic colic, intermittent fever, photosensitization and other skin conditions, decreased appetite, and eventually neurologic signs associated with HE. Weight loss is likely to be the predominant clinical finding. Fever may occur with either chronic infections or immunologic reactions. In addition to the skin lesions characteristic of photosensitization observed in white-haired areas or on mucous membranes, a variety of other more diffuse dermatologic signs including alopecia and seborrhea and coronary band lesions may be found with chronic hepatitis. Although unproven, these skin changes may occur from increased circulating bile acids, decrease in absorption of fat and fat-soluble vitamins, or mineral abnormalities associated with hepatic fibrosis. A multisystemic, eosinophilic, epitheliotrophic disease can severely affect liver, intestine and other organs concurrently; the etiology is unknown and the condition carries a poor prognosis.

Other inflammatory or infiltrative bowel diseases may cause secondary liver disease but rarely, if ever, as severe as that seen with multisystemic, eosinophilic, epitheliotrophic disease. Hepatic amyloidosis has occurred most commonly in horses used for plasma production. Rupture of the liver subsequent to amyloid deposition may cause acute hemoperitoneum.

The most common biochemical abnormalities with chronic progressive liver disease are marked elevations in either GGT or AST or both. GLDH and SDH seem to be more variable, likely related to their short half-life and the slow progression of disease in many horses with cirrhosis. If there is sufficient liver disease to cause dysfunction, then serum bile acids and bilirubins will be increased. BUN and albumin will generally be low and globulins high regardless of the cause of cirrhosis. Ultrasound examination of the liver may reveal increased echogenicity due to fibrosis.

Horses with chronic liver disease should have a liver biopsy performed. In my opinion, chronic liver disease may be the best indication for liver biopsy in the horse. Although PT and PTT may be prolonged with liver failure, biopsy rarely causes complications if performed properly and if the patient’s platelet count is normal. Periportal fibrosis with inflammation and bile duct hyperplasia are perhaps the most frequent findings on biopsy of horses with chronic liver disease, although a few toxins, drugs, and hypoxia cause more severe disease in the centrilobular area (zone 3). Although histopathologic examination of liver samples...
obtained by biopsy rarely provides a specific etiology for the disease, it may provide information that guides treatment recommendations. If neutrophils predominate in perportal inflammation, a course of antimicrobial therapy in addition to pentoxifylline and vitamin E would be reasonable. If there is no clinical and serum biochemical improvement in 2 weeks and there is evidence of biliary obstruction (>25% of bilirubin is conjugated bilirubin), then urso-diol (10 mg/kg PO every 24 hours) treatment could be added. If fibrosis is judged to be moderate to severe on ultrasound or biopsy, colchicine (0.03 mg/kg PO every 24 hours) could be used to inhibit or even diminish fibrosis. Colchicine is an alkaloid derived from meadow saffron (Colchicum spp.). Colchicine’s beneficial effect in treating hepatic fibrosis is inhibition of collagen synthesis along with anti-inflammatory and immune-modulatory effects.\(^\text{227}\) Colchicine should not be used if PA toxicity is suspected or if megalohepatocytes are present, as it is known to inhibit mitosis, as do PAs. There is one report of fatal bone marrow suppression in a horse being treated for chronic active hepatitis with colchicine.\(^\text{226}\)

Silymarin, a standardized extract of the milk thistle seeds (Silybum marianum) is commonly used in the treatment of chronic liver diseases in several species including the horse.\(^\text{229,230}\) Flavonolignans are the most common class of compounds present in milk thistle extract, and of the group, silybin (silibinin) is considered to have the most hepatoprotective properties.\(^\text{229,230}\) The percentage of silybin was found to be quite variable among commercial milk thistle extract products. Experimental studies have demonstrated antifibrotic, antioxidant, and metabolic effects of silybin but human studies have been insufficient in confirming the clinical efficacy in chronic liver disease.\(^\text{229}\) In normal horses, a standard bioavailability study of orally administered silibinin found less than 1% available.\(^\text{125}\) SAMe is believed to stabilize cell membranes, promote secretion of bile, and has anti-inflammatory properties. Despite these potentially favorable benefits and information to suggest that SAMe synthesis is depressed in chronic liver disease, high-quality randomized studies to demonstrate clinical efficacy were not found.\(^\text{232}\) There are no studies on bioavailability or efficacy in the horse.

If the inflammatory reaction in the diseased liver is more lymphocytic than neutrophilic then immunomodulatory therapy with corticosteroids or azathioprine can be used. In some horses with chronic liver disease and a mononuclear inflammatory response, treatment with 0.1 mg/kg dexamethasone for 5–7 days followed by a tapering dose of dexamethasone or switching to prednisolone has resulted in clinical and laboratory improvement for several months in several cases that I have treated, and a few cases have had a full recovery. Nutritional support for chronic liver disease is extremely important and some guidelines are found on page 95. One nutritional recommendation for chronic liver disease that has recently changed is that protein intake in patients with chronic liver disease should not be reduced unless HE is present. In years past, the traditional recommendation for nutritional support in cases of chronic liver disease was to feed a low-protein diet.\(^\text{233}\)

Prognosis for chronic, active liver disease in horses can often be determined by clinical and serum biochemical response to therapy.\(^\text{22,147,234–236}\) Quantitative assessment of hepatic function using 99mTc-mebrofenin could be performed but studies in horses with liver disease are not available.\(^\text{227}\) Prior to therapy, the degree of fibrosis and level of serum bile acids can be used as prognostic indicators.\(^\text{22}\)

8. Hepatic Encephalopathy

HE is a neurologic disorder associated with liver failure. HE is common in horses with either acute or chronic liver failure, and in foals with portosystemic shunts. Primary hyperammonemia syndromes in horses also have clinical and laboratory findings (increased ammonia) similar to HE. The pathophysiology of HE is complex, and likely involves several gut-derived neurotoxins, cerebral and systemic inflammation, cerebral vascular dysfunction, and neuroendocrine abnormalities.\(^\text{238,239}\)

High concentrations of blood and cerebrospinal fluid (CSF) ammonia have been most commonly incriminated as causing the pathophysiologic events of HE.\(^\text{240}\) Ammonia (NH\(_3\), unionized and a weak base) is toxic at high concentrations and believed to easily pass the blood-brain barrier (BBB) by diffusion while the relatively nontoxic ionized ammonia (NH\(_4^+\)) is limited to a transeellular route for translocation. Ammonia is produced mainly within the gut during protein digestion and amino acid deamination and the concentration of ammonia in the blood is regulated by the urea cycle in the healthy liver. Therefore, liver failure often leads to hyperammonemia in both blood and CSF.\(^\text{240}\)Astrocytes normally use ammonia when synthesizing glutamate from glutamine, but excessive ammonia presented to astrocytes can disrupt the equilibrium.\(^\text{241}\) Ammonia is believed to play a key role in the development of HE, with increased glutamate, effects of varying amounts of glutamate and neurosteroids on N-methyl-D-aspartate receptor activity, all playing a central role in ammonia metabolism and HE.\(^\text{241}\)

Increased ammonia metabolism by brain astrocytes results in accumulation of glutamine, which is known to disrupt water balance in the brain leading to cytotoxic edema.\(^\text{238,241,242}\) Increased ammonia concentra-
tions in the brain may have direct effects on pH, membrane potential, and neurotransmission in addition to cellular metabolism and cerebral water balance. Although undoubtedly the most important single neurotoxin involved in HE, ammonia should not be considered the sole cause. In fact, a moderate number of horses with HE do not have blood ammonia values above the reported normal range. Other neurotransmitters are frequently discussed as playing a role in HE but their association has not been well documented; these neurotransmitters include endogenous benzodiazepine-like compounds, mercaptans, monoamines, and aromatic amino acids.

Increased blood brain barrier (BBB) permeability for gut-derived neurotoxins may also play an integral role in HE. The BBB is the physical and metabolic barrier separating the peripheral circulation from the central nervous system and regulates exchanges between the two. Changes in BBB permeability may be due to both structural alterations in tight junctions and inflammatory vascular effects, as well as functional changes. Increases in blood ammonia, metalloproteinase activity, and endotoxin may increase permeability of the BBB in liver failure, permitting transport of other neurotoxins to the brain.

Systemic inflammation and sepsis from endotoxin or bacterial translocation are often part of the syndrome of HE. Breakdown products of injured hepatocytes as well as increases in systemic inflammatory cytokine production, free radicals, and metalloproteinases in liver failure have severe systemic effects in addition to increasing permeability of the BBB. The toxic effect of these events on the brain with HE is believed to result in both cytotoxic (intracellular swelling without increased permeability of BBB) and vasogenic (increased permeability of BBB allowing net gain of fluid) brain edema. The clinical signs of HE in horses may be mild with only depression, anorexia, and frequent yawning. In other cases the signs can be fulminant and include head pressing, blindness, circling, and coma. Ataxia can be noticeable in some cases and absent in others. Rarely, ataxia may precede HE signs or icterus in acute liver failure. Clinical examination may reveal decreased muscle tone of the lower lips, delayed or absent response to touching the inner nares, and cortical blindness often accompanied by mydriasis. These signs are all a result of the cortical disease that occurs with HE. Laryngeal paralysis or dysphagia (presumably due to HE and functional disturbance in the nucleus ambiguous) and gastric impaction/rupture (presumably due to autonomic nerve dysfunction) are additional findings that may be associated with HE in horses.

Laboratory findings that are directly associated with HE include hyperammonemia, occasionally hypoglycemia, and metabolic acidosis. Increased blood ammonia is common, but certainly not always above the normal range in all horses with HE. The normal range of blood ammonia in the horse varies between laboratories but is generally less than 90 μmol/L. Many cases of HE have concentrations in the 100–200 μmol/L range, a few cases are greater than 200 μmol/L, and the highest percentage of cases seem to be in the upper-normal range, 70–90 μmol/L. Horses with HE as a result of liver failure generally do not have ammonia levels as high as those seen with primary enteric hyperammonemia, portosystemic shunts, or Morgan foal hyperammonemia syndrome, which often have blood ammonia concentrations greater than 300 μmol/L. Metabolic acidosis, mostly due to lactic acidosis, is common in horses with HE. The increase in L-lactic acid is likely a result of decreased hepatic metabolism of lactate, poor perfusion and cellular hypoxia, and enhanced hepatic and extra-hepatic aerobic glycolytic activity and lactate production. Hypoglycemia is rather uncommon in adult horses with HE but common in young foals with HE. In one report of 50 horses with liver failure none had hypoglycemia, but we have occasionally examined a horse or pony with liver failure and hypoglycemia. Dr. Bud Tennant reminded me of a HE case that was hypoglycemic and comatose and following glucose treatment quickly stood up and eventually recovered.

Pathologic findings in the central nervous system of horses and other animals with HE or other hyperammonemia syndromes consist of reactive astrogliosis in gray matter, Alzheimer type II cells, and cerebral edema. Alzheimer type II cells (enlarged astrocytes with basophilic nucleoli that seem to be metabolically hyperactive) are closely associated with increased blood and CSF ammonia; these cells are also routinely found in the brain of horses dying from primary intestinal hyperammonemia and we know from foal studies with acute hepatic failure that Alzheimer cells can be found in the brain within 2 days of the hepatic failure.

Treatments for HE revolve around decreasing entero-derived neurotoxins (primarily ammonia), decreasing cerebral edema, correcting glucose, electrolyte, and acid-base abnormalities, and maintaining perfusion and oxygenation to the brain and other vital organs. Providing specific treatment for the liver disease per se, such as hepatic lipodosis or bacterial cholangiohepatitis, is imperative but in many cases of liver failure there are no specific treatments for the hepatic disease and only supportive care, as outlined below, can be provided. Additional general supportive treatments that inhibit both systemic and neuronal inflammation, reduce oxidative stress, and prevent multiple organ dysfunction are recommended.

Correction of intravascular volume deficits should be the initial focus for fluid therapy in most horses with liver failure (LF). Expansion of the vascular volume can improve perfusion to the diseased liver and other organs that may be secondarily in-
Horses with liver failure may have intravascular volume deficits for several reasons including lack of fluid intake, decreased vascular tone and/or endothelial dysfunction, and increased urinary loss due to low urea and its negative effect on renal tubular water absorption. Hypertonic saline (7.5%, 4 mL/kg) can be administered in adult horses with HE if there is clinical or measurable evidence of severe hypotension and abnormally low cardiac preload. An alternative to the administration of hypertonic saline as a resuscitation fluid would be to administer in the first hours of therapy 20-30 mL/kg of a balanced crystalloid with 50 g of dextrose and 20 mEq of KCl added to each liter. Supplemental potassium is generally recommended given that horses with HE are anorexic and would most likely be deficient in total body potassium. Hypokalemia is known to increase proximal tubular ammoniogenesis with the increased ammonia being returned to circulation and worsening the symptoms of HE. Ideally, a crystalloid with an acetate rather than lactate buffer should be used, but if lactate fluid is all that is available then use what you have because fluid volume is likely more important in the treatment than the buffer composition. The liver is the primary organ responsible for glucose production and glucose support should be considered in the fluid therapy plan for all patients with LF. This is especially important in foals with LF because they are much more likely to be hypoglycemic than are adult horses. Adult horses with LF are rarely hypoglycemic but still might benefit from glucose treatments to decrease the need for hepatic glucogenesis and glycogenolysis (the little that may remain). Normal perfusion to the kidney and muscle are very important given that they are the only other organs that can help with ammonia and glutamine metabolism or elimination. Most horses with HE have a metabolic acidosis but still might benefit from bicarbonate administration to increase the ammonia to ammonium ratio, and decrease urinary elimination of ammonia.

Ideally, all sedatives should be avoided in HE but horses with HE can have propulsive cortical signs that may require sedation to properly attend to the horse and to prevent injury to both the horse and humans. A low dose of detomidine (5–10 µg/kg IV) may suffice and is what I usually use to control maniacal behavior in horses with HE. It is important to not overly sedate a horse with HE because that might cause excessive lowering of the head in the standing horse and promote cerebral edema in addition to the potential negative effects on the brain, liver, and other organ perfusion. Propofol (2 mg/kg IV), another short-acting sedative available to veterinarians, could be used in foals with HE to control seizure or maniacal behavior, or for sedation prior to general anesthesia, but the volume needed for adult horses would likely not be practical. Propofol does not require a change in dose in patients with liver failure and does not worsen HE when used as a sedative in humans with cirrhosis. Respiratory depression may occur with the use of either propofol or detomidine, and the use of supplemental intranasal oxygen would be ideal if available. Diazepam use in HE is controversial as it can induce astrocyte swelling and worsen HE. In humans with HE, haloperidol is one of the preferred drugs for controlling abnormal behavior. I have not personally used haloperidol for HE in horses and the form that has been used in horses for behavior issues is haloperidol deconate, which has a delayed but long-acting effect and would likely not be of use in most horses with HE. If more long-term sedation is required, pregabalin (3 mg/kg every 12 hours PO) or gabapentin (5–12 mg/kg every 12 hours PO) could be used given that these drugs undergo minimal metabolism and are eliminated by the kidney and could provide sedation in HE if needed. Conversely, horses with profound coma could be treated with sarmazenil (0.04 mg/kg IV) or flumazenil (0.1 mg/kg IV), which could have a positive effect in reversing those neurologic disturbances of HE via inhibition of benzodiazepine receptor ligands, which may play a role in the pathogenesis of HE by increasing GABAergic tone. Controlled clinical trials showed that flumazenil had a transient beneficial effect in only a subpopulation of HE patients. One of the most important goals in the treatment of HE is to reduce the blood and CSF ammonia concentration. The primary means for reduction of blood/CSF ammonia is to reduce the production or absorption of ammonia from the gut. Therapeutic options include neomycin 10–20 mg/kg PO every 8 hours or another poorly absorbed antibiotic. Neomycin decreases ammonia production via its effect on microbial population and decreases in ammonia producing bacteria. Neomycin was used successfully for decades in human medicine as a first-line per os treatment for HE but has fallen out of favor because of risk of ototoxicity and nephrotoxicity, which would be very unlikely to occur in horses treated orally for only a few days. Rifaximin, a poorly absorbed and expensive rifamycin derivative antibiotic, is currently the antibiotic of choice in humans with HE but studies to demonstrate efficacy of this antibiotic over neomycin were not found. Orally administered antibiotics should not be prolonged beyond 1–3 days if possible to lower the risk of antibiotic-associated diarrhea. Lactulose, a poorly absorbed carbohydrate, has been used (0.3–0.5 mL/kg every 8 hours) in horses to decrease intestinal ammonia production but no efficacy studies are available. Lactulose decreases ammonia when its metabolism in the large bowel results in increased H+ production and conversion of some ammonia ions (NH3) to poorly absorbed NH4+ (ammonium) salts. Lactulose also can be a helpful cathartic given that constipation must be avoided in animals with HE. It may, therefore, be preferred to...
combine neomycin with lactulose. It would be ideal if the treatments softened the stool (a cathartic effect) without causing diarrhea. Ideally, all oral medication should be given by dose syringe because the nasal bleeding resulting from passage of a stomach tube (probably never happens to you) will result in ingestion of considerable amounts of blood protein, which would be likely to increase blood ammonia production.251

Other oral treatments that have been used in humans in hopes of lowering enteric ammonia production include probiotics and prebiotics, which may have some efficacy in treating HE by increasing non-urease producing bacteria.263 Treatments to decrease neuroinflammation and disruption of the BBB are mostly unproven, but include N-acetylcyesteine, minocycline, neurosteroids such as progesterone or allopregnanolone, and hypothermia.238,242,245 Treatments to support energy metabolism and antioxidant activity to the brain include B vitamins, vitamin C, and DMSO, although all are unproven. Treatment of cerebral edema with mannitol or hypertonic saline may have some temporary effect in decreasing cerebral edema.

There are no highly effective, safe, and specific parenteral treatments for decreasing ammonia that is present in the blood and CSF of patients with HE. Glycerol phenylbutyrate has been used in human clinical studies, sometimes combined with branch chain amino acids (BCAAs), to lower ammonia by providing an alternative to the ammonia-to-urea pathway with excretion of phenylacetylglutamine in the urine.264,265 L-ornithine-l-aspartate (LOLA) reduces ammonia levels by increasing hepatic ammonia disposal and its peripheral metabolism. It is available in Europe in both intravenous formulations and oral formulations and some clinical trials have found it to be effective in treating hepatic encephalopathy.266,267

Another commonly recommended administered treatment for HE is oral supplementation of BCAA valine, leucine, and isoleucine. Their role in treating HE is controversial with some reports demonstrating beneficial effects possibly via improving glucose metabolism, decreasing protein and muscle catabolism, and as an alternative pathway (in muscle) for ammonia detoxification.268 They may, therefore, be of particular benefit in treatment of HE caused by hepatic lipodosis, but other reports point out concerns of BCAA administration increasing glutamine synthesis and worsening of HE.269

**Nutritional Management of Acute and Chronic Liver Failure**

Nutritional management specific for horses with liver disease, other than removing potential hepatotoxins and feeding an adequate diet, is generally not necessary unless hepatic dysfunction has occurred. Nutritional support for miniature horses, donkeys, and ponies with hyperlipemia and hepatic lipodosis is the most important indication for nutritional intervention in equine liver disease.263 Although there are few, if any, evidence-based guidelines for nutritional management of horses with severe liver disease, some recommendations may be made intuitively based upon an understanding of the function of the healthy liver and its repair following disease. Provision of adequate enteric glucose or glucose precursors to the equine with severe liver disease may help maintain normal plasma glucose concentrations, decrease the workload of the failing liver, and inhibit lipolysis and muscle protein breakdown. When the liver is sufficiently diseased, liver glycogen stores and hepatic glucose production via glycogenolysis and gluconeogenesis are likely diminished. Depletion of glycogen reserves in addition to insulin resistance, which may occur with some causes of liver failure, increase reliance on gluconeogenesis from protein and this, often coupled with inappetence, tends to lead to an increased reliance on body protein for energy. Despite this metabolic dysfunction, most adult horses with liver failure have normal or even elevated blood glucose values, possibly related to insulin resistance.20,29 In a small percentage of cases, hypoglycemia may occur, which could worsen the neurologic signs associated with liver failure (HE) and cause generalized neuromuscular weakness. This is especially pronounced in foals with hepatic failure because foals consistently and quickly develop hypoglycemia with the onset of liver failure.36 With an enhanced reliance on protein and fat catabolism for energy, there is both a rapid muscle breakdown and increased ammonia production in addition to increased serum triglycerides, the latter of which can be life threatening to miniature equine, ponies, and donkeys.374,180 Provision of adequate enteric glucose or glucose precursors in horses may decrease the workload of the failing liver, inhibit lipolysis and muscle protein breakdown and in those foals or horses prone to hypoglycemia, maintain plasma glucose concentrations. It may, therefore, be imperative in normoglycemic or hypoglycemic horses with severe liver disease that are still eating to provide an appropriate supply of glucose or easily digestible glucose precursors to help supply daily energy requirements, inhibit muscle and fat catabolism, and decrease the need for the liver to perform gluconeogenesis or glycogenolysis. Compared with other products of carbohydrate hindgut fermentation (e.g., acetate, propionate, lactate) that can be used for energy, glucose provides 2–3-fold more ATP.270 The frequent (every 4–6 hours) feeding of small amounts of sugars, easily hydrolyzable carbohydrates, and nonresistant starches that are readily digested to glucose and absorbed in the small intestine should be beneficial in maintaining a constant supply of enteric derived glucose.271 Feeds with high amounts of sugars or hydrolyzable carbohydrates include most sweet feeds (especially those containing ingredients such as oats, cooked corn/barley, and molasses), whole oats, or soaked beet
pulp with molasses. Pre-cecal digestibility of processed oats is higher than unprocessed corn and barley.\textsuperscript{272,273} Palatability of the feeds must be considered, given that it is important to keep the horse eating something (even if it is not the ideal feed). Only small amounts of the high-glycemic feeds (≤ 1.0 g/kg) should be fed at a time to improve small intestinal starch digestibility as well as prevent hyperglycemia, extreme insulin surges, and small intestinal overload, which could result in excessive starch passing undigested into the cecum.\textsuperscript{271,274,275} The amount fed may need to be modified based upon appetite of the horse, blood glucose concentrations, and consideration of individual metabolic conditions, e.g., presence of any insulin dysregulation. Structural carbohydrates that are converted to volatile free fatty acids (VFAs) in the large bowel are an important source of energy for the horse and should be fed to supplement the energy potential obtained from sugars and hydrolysable starches digested in the small intestine. Some of the VFAs (i.e., acetate) may be used directly for energy,\textsuperscript{276} whereas others such as propionate and lactate that undergo hepatic gluconeogenesis\textsuperscript{277} may be of less value with a severely diseased liver. A highly palatable grass or grass hay (legumes may be too high in protein for horses with liver failure) should be fed at approximately 1.5% of body weight dry matter basis for hay if the horse will eat this amount, in addition to the small feedings of a grain-based feed as described above depending upon blood glucose levels. Palatability of fresh grass may be higher than hay or even some concentrates and horses ill with severe liver disease may only be willing to ingest grass. If the horse with severe liver disease has markedly diminished appetite for grain and hay, grazing a nonlegume grass should be offered as long as the grazing is monitored (gorging while grazing/eating too much too quickly would likely be harmful) and grazing should be performed at night or in shaded pastures to prevent photosensitization in predisposed horses. Horses with severe liver disease and concurrent insulin dysregulation or those prone to laminitis should not have uncontrolled grazing on potentially high water-soluble carbohydrate (fructan and simple sugars) pastures unless appetite is poor. Assuming the horse with liver disease is still eating, the above outlined combination of grains and forages might be able to maintain glucose within a normal range, decrease hepatic work necessary for glycogenolysis and gluconeogenesis, and hopefully allow the horse to maintain body condition. Horses with liver failure that are anorexic may need to be supplemented by forced enteral feeding (especially ponies, miniature horses, and donkeys with hepatic lipidosis) or by continual intravenous administration of 5% glucose with potassium chloride, B vitamins, and supplemental protein.\textsuperscript{178}

There are no evidence-based guidelines available for dietary protein feeding in horses with liver failure. In horses with liver failure and neurologic signs of HE, it can be assumed that the amount of protein in the diet should initially be restricted. The old concept of providing a low protein diet to all animals with chronic liver failure with no evidence of HE is no longer believed to be standard of care in other species and should be considered in horses as well. With HE, it can be assumed that the amount of protein in the diet should initially be restricted in hopes of preventing or decreasing hyperammonemia. Feeding a low-protein feed to horses with hyperammonemia may decrease enteric production of ammonia and concentration of proteins that may be metabolized to ammonia or other neurotoxins, which are believed to contribute to HE. It is, however, important to maintain adequate energy supply by feeding carbohydrates (as discussed above) and fat (except with hyperlipidemic conditions) to horses with liver failure, or there could be sufficient breakdown of muscle protein to cause an increase in blood ammonia. It is also very important that horses with liver disease have adequate fluid volume (either self-drinking or interventional fluids) to maintain hydration and sufficient dietary potassium which may decrease resorption of ammonia from the kidneys.\textsuperscript{255} The gut, liver, muscle, and kidney are all involved in regulating blood ammonia concentrations!

There is some evidence that feeding BCAAs (valine, leucine, and isoleucine) and increasing the BCAA-to-aromatic-amino-acid ratio (AAA) can be helpful with the treatment of liver disease, liver failure, and HE in humans.\textsuperscript{278} The rationale for recommending BCAA in the treatment of liver failure is based on their unique metabolic and pharmacologic properties; muscle metabolism for energy and stimulatory effect on ammonia detoxification to glutamine by muscle rather than hepatic metabolism.\textsuperscript{269} In horses with PA toxicity or in foals with toxic hepatopathy there was an increase in concentration of AAA without increases in BCAA.\textsuperscript{150,279} Although BCAA treatment for severe liver disease is often recommended, there is no published data to confirm their benefit in treating horses with liver disease and in humans with HE the evidence is relatively low.\textsuperscript{269} A proper dose of BCAA in horses with liver disease is also not known and excessive amounts could actually enhance ammonia production from excessive glutamine breakdown in the intestine and the kidneys, worsening HE.\textsuperscript{269} I have, therefore, generally taken a conservative approach with BCAA supplementation, recommending 10 g/adult horse every 12 hours. The BCAA can be given as a commercial paste product or if the horse is still eating, sorghum and soaked beet pulp can be fed as these concentrate sources have a relatively high BCAA:AAA ratio. Corn also has a high BCAA:AAA ratio but if unprocessed has a decreased prececal carbohydrate digestibility compared with the other two products. Sugar supplemented beet pulp can be offered to diminish total protein intake, yet provide a high BCAA:AAA ratio and a digestible energy equal to oats, but with less fluctuation in
urea. Beet pulp should be adequately soaked pre-feeding to decrease chances of esophageal choke and to provide critically important fluids. BCAA feeds or products may be of greatest benefit in horses with clinical signs of HE. If the horse has chronic liver disease but no signs of HE then a normal-protein diet with sufficient quality of comprehensive and balanced amino acids should be fed. As previously mentioned, providing low-protein diet to all animals with chronic liver failure is no longer believed to be standard of care in other species. One beneficial effect of long-term intake of protein and specifically BCAA in humans with liver cirrhosis is likely related to improved muscle mass and nutritional status rather than to the ammonia-lowering effect of BCAAs themselves.

Although fat is the most energy-rich food available, the feeding of a high-fat diet to horses with liver disease may not be beneficial. The absorption of fat and fat-soluble vitamins may be hindered by decreased bile acid secretion into the small intestine in horses with severe liver disease. Some amount of dietary fat is required to facilitate the absorption of fat-soluble vitamins (A, D, E, and K) that are stored in the liver. Some modest amount of supplemental fat in the diet of horses with nonobstructive liver disease would likely be appropriate and the extra energy will, at least in healthy horses, decrease triglyceride concentrations and increase lipoprotein lipase activity. Adding oil to the diet of horses, especially those with severe liver disease, should be gradual and accompanied by additional vitamin E supplementation. Although similar to findings in horses, feeding fat to healthy Shetland ponies decreases plasma triglycerides (likely by enhanced lipoprotein lipase activity) but feeding fat to equines that have hyperlipemia/hepatic lipidosis disorder is not recommended because there will be no further increase in lipoprotein lipase activity and fat feeding may enhance glucose intolerance and insulin resistance.

Nutritional supplements that may have some benefit in horses with severe liver disease are mostly those that are normally stored in high concentration in the liver (vitamins E and K, selenium and zinc), supplements that may decrease oxidative injury or inflammatory/fibrotic progression or ones that may improve metabolism. If the horse with chronic liver disease is still ingesting green forage, supplementation with vitamin A is not needed and excessive amounts may activate hepatic stellate cells and promote fibrosis. If the affected horse is not eating green forage then supplementation with betacarotene and vitamin E are recommended. Vitamin E should be supplemented with a soluble product at 5 IU/kg body weight daily or more if supplemental fat is being fed; B vitamins can be administered orally. Additional supplements that have been suggested for chronic liver disease in other species include SAM-e and n-3 polyunsaturated fatty acids in hopes of decreasing cellular oxidation associated with free radicals. There is no proof of efficacy for these supplemental therapies in treating liver disease in the horse. Milk thistle supplements contain silymarin, which are believed to have antioxidant and other liver-protecting actions and are commonly used in treating horses with chronic liver disease. The bioavailability of one preparation was found to be low in healthy horses and only minor alterations in antioxidant capacity were found. Iron and copper supplements should not be given to horses with liver disease as they may promote the Fenton reaction and trigger free radical damage to the liver.

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Declaration of Ethics
The Author declares that he has adhered to the Principles of Veterinary Medical Ethics of the AVMA.

Conflict of Interest
The Author declares no conflicts of interest.

References and Footnotes


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Pre-Purchase Examination in a Practice Focused on Horses Offered at Public Auction

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For veterinarians who focus on providing services to those who buy and sell Thoroughbreds both in training and juveniles, there are many issues to consider. In addition to the obvious technical veterinary issues, there are other considerations, including client selection and communication and ethical, legal, and reporting issues.

First, the job description for veterinarians employed by clients who wish to buy at a public auction has some features that are obvious and some that are not so obvious. The actual physical examination can be quite variable in terms of thoroughness and depends upon the wishes of the buying client. The standard examination would include, at the very least, an assessment of the horse's eyes, heart, external genitalia, and its overall body condition as well as a visual and manual examination of each of its four limbs. Part of the routine examination process would be watching the horse in motion to detect any subtle lameness or neurologic deficits. This part of the examination will vary depending upon the age of the horse. At a weanling or yearling sale it is customary to watch the horse walk and turn; at a sale of 2-year-olds in training or older horses in training it is also customary to observe the horse at the trot at the end of a shank. (Note: One aspect of the examination that is unique to a “Horses of Racing Age” sale is the access to previous racing performances and public workouts in the sales catalog. Veterinarians should become familiar with how to read and interpret this information to have an idea of the level of competition the horse has been in and whether the horse has been competitive at that level, whether the horse's performance has been increasing or decreasing, and how that historical information might affect the level of risk involved with the purchase of that horse.)

In the process of completing a thorough physical examination, endoscopic examination of the upper airway, and radiographic examination of a standard set of images of the distal limbs, a list of findings is created. That part is obvious. However, the mere presence of findings does not necessarily disqualify a given horse from consideration for a particular client. Many veterinarians will discover findings that they may consider significant and automatically “fail” the horse and remove it from consideration for purchase. This is essentially deleting an important step in the communication process, as if the client is totally averse to risk. The most effective outcome is reached when the findings, once discovered, have a level of risk attached to them by the veterinarian and a complete discussion about the findings and the risk they pose is had with the client. This part of
the examination are just that—findings—and should be communicated to the buyer and seller in an identical manner and expressed with the same degree of significance. This becomes easier when findings are placed in risk categories and when that level of risk is clearly explained to both parties.

Another issue is that of radiographic technique. This has both technical and ethical aspects. Whether the purchaser’s veterinarian is actually taking the radiographs during the course of the examination or reading the radiographs taken by another veterinarian, a proper evaluation of the standard views of the skeletal system cannot be done if the images are inadequate. Poor positioning, motion, inadequate technique, and missing views make it impossible to visualize the areas of interest, i.e., areas where pathology would commonly occur. This is a common problem at public auctions, where radiographs housed in a radiographic repository may vary widely in quality. In cases where there are missing or inadequate views, a veterinarian reviewing the images must either obtain retakes of these poor-quality views if time permits or report to the client that the images cannot be used to evaluate the horse adequately. The ethical aspect to this issue is that there are abundant educational materials available for veterinarians to learn how to take a full set of good radiographs (see resources provided by the American Association of Equine Practitioners [AAEP] at http://www.aaep.org) so there is no excuse for lack of acceptable quality.

There has been much discussion about radiographic reports generated by sellers’ veterinarians for the radiographic repository, what they mean, and consequently how reliable they are. There are two schools of thought on the part of the veterinarians who generate these reports. One group records the findings seen in areas where pathology would commonly occur, a sort of cataloguing of findings irrespective of perceived significance. Others record only those findings they consider significant and basically list all other joints as NSF (no significant findings). The latter method fails to address the fact that each buying client has his or her own level of risk tolerance and intended purpose for the horses that he or she buys, so an omission of information may render the report less than useful. The former method creates a need for a higher level of communication between veterinarian and buyer in which the veterinarian, based on his or her understanding of the client’s unique risk tolerance, can guide the client in the purchase of any individual horse.

This is precisely why the use of the radiographic reports as a marketing tool in the back walking ring at public auctions is inappropriate and puts both consignor and veterinarian at risk of increased legal exposure. Regardless of how they are written, radiographic reports are essentially subjective in nature. They are not like a Carfax report, and no matter how diligent a veterinarian is in accurately describing all findings, there will always be an element of opinion in each report. Legal counsel ad-
vises that these reports be a complete and accurate report of all findings to minimize legal exposure. There are some aspects to legal exposure regarding radiographic reports at public auction that are counterintuitive and will be discussed by attorneys Casey and Meuser.

There are currently no recognized guidelines for generating these reports. If veterinarians are to be held to a standard of reporting all findings, it is incumbent upon the sales companies to make that standard known so that the vets can comply. Creating a standard for reporting findings would potentially require a group of qualified individuals to study the issue and compile a list of reportable findings for the knees, hocks, stifles, front and hind fetlocks of sales horses. Joints absent of those findings on the list could be notated as “No Findings” (thus eliminating the word “significant” which is problematic for legal reasons.) The cataloguing of reportable findings would be presumably based upon available veterinary scientific literature.

The potential use of videoendoscopy of the upper airway at public auctions has been discussed by veterinarians and consignors over the last few years. Although not currently in usage at sales in the United States, throat videos have become common at European sales, particularly in France. The topic has resurfaced recently within consignor groups and is currently under consideration for inclusion in repository settings. Several years ago, an AAEP task force set forth criteria for videoendoscopic examination of the upper airway, and presumably these criteria will be applied if and when throat videos are utilized at sales. Videoendoscopy of the upper airway, whether used in public or private sales, can be useful from the standpoint of archiving the form and function of the throat at the time of purchase examination. Given the possibility of changes in throat function over time, the saved dynamic images may be helpful from both a diagnostic and legal standpoint.

In conclusion, it is incumbent upon veterinarians working in a public auction setting to become familiar with not only the technical aspects of performing a thorough pre-purchase examination within a very limited time frame but also the communication, ethical, and legal issues surrounding that environment.

Acknowledgments

Declaration of Ethics
The Author declares that he has adhered to the Principles of Veterinary Medical Ethics of the AVMA.

Conflict of Interest
The Author declares no conflicts of interest.
Pre-Purchase Examinations in a Sport Horse Practice

Christopher (Kit) Miller, DVM

For sport horse practitioners, pre-purchase examinations are a particularly important service in which veterinarians play the role of clinician and advisor in the decision to purchase a particular horse. These transactions are frequently both financially and emotionally significant for the buyer, and the decisions are, in large measure, contingent on the veterinarian’s clinical expertise and understanding of the specific discipline or industry for which the horse is intended.

Pre-purchase examinations are an opportunity for clinicians to provide highly valued information that, in time, can help to develop and nurture a strong doctor–client relationship. Conversely, pre-purchase examinations have the potential to make that bond more tenuous should the outcome, for whatever reason, be perceived as unsatisfactory. From a business perspective, a thorough pre-purchase examination can be a significant source of revenue provided the veterinarian is appropriately compensated for his or her time in performing the clinical examination, acquiring and interpreting the images, and communicating with clients.

It is particularly important when performing pre-purchase examinations to have a complete understanding of the client’s objectives in purchasing a horse. Horses may be purchased for pleasure, athletic endeavors, and investment purposes, and the client’s goals in owning a particular horse should guide the nature and extent of the examination. In sport horse practice, the majority of the pre-purchase examinations are performed on horses intended to compete at some level of athletic activity. In addition, many of the clients have a professional trainer. In my opinion, it is the responsibility of the trainer—not the veterinarian—to advise the purchaser on price and on the physical ability or suitability of the horse for its prospective owner and rider. These variables should have been discussed and agreed upon before the veterinarian becomes involved in the transaction, and the pre-purchase exam should be performed in an effort to establish whether the horse is physically capable of performing at the intended level of athletic activity.

In my practice, we frequently perform pre-purchase examinations on horses that have had extensive athletic careers and have, as a consequence, acquired some degree of wear and tear. An important and often difficult feature of pre-purchase examinations is the ability of the veterinarian to form and convey an opinion as to whether the nature and extent of specific exam findings are likely to prevent a horse from continuing to perform at the intended level for an acceptable period of time. Being able to formulate such an opinion requires clinical experi-
ence, a thorough understanding of the discipline in which the horse will compete, and a complete understanding of the buyer’s expectations. For example, a client may be willing to purchase an experienced equitation horse knowing full well that horse may have a prior injury or some degree of degenerative joint disease, because the horse is known to be safe and reliable and will be competitive at specific events. For such buyers, a horse’s reliability and safety may be acceptable tradeoffs for durability, and it is important for veterinarians to have that understanding to offer effective and appropriate advice.

There are a multitude of ways of performing the actual clinical examination. It is important for practitioners to develop their own routine that is thorough, repeatable, and appropriate for the horse’s intended discipline or use. The same is true for ancillary testing such as lab work and imaging. The extent of the radiographic evaluation should be appropriate for the horse’s intended use. Additional modalities, such as survey ultrasounds and upper airway endoscopy, should be made available and performed based upon the results of the clinical examination and the clinician’s opinion as to the relevance of the information to be acquired. Because imaging is frequently one of the most costly line items in pre-purchase examinations, it is often useful to discuss options and cost estimates with buyers in advance. As with the rest of the examination, it is important to understand and meet the clients’ expectations.

Invariably, information arises that will be subject to the clinician’s interpretation. Scars, radiographic abnormalities, and positive flexion tests are all clinical findings that veterinarians will be asked to interpret to best advise their clients. There are very few absolutes, and the veterinarian’s opinion will likely be shaped over time by clinical experiences. If the veterinarian has uncertainty about specific findings, it is valuable to have resources to consult. In my opinion, clinicians who perform pre-purchase examinations should not hesitate to avail him or herself to additional opinions when needed. These are often “mentor” veterinarians who are either employers or colleagues in the industry and are available for additional expertise or simple counsel. It is important to acknowledge that the summaries of most pre-purchase examinations will be the veterinarian’s best estimate and that there is often some degree of uncertainty about specific findings. In these instances, it is helpful for practitioners to provide information about the relative risks associated with specific findings and the available remedies in the event of an unwanted or unanticipated outcome.

Pre-purchase examinations are challenging because they require practitioners to perform a thorough clinical examination and to interpret the findings in a concise and meaningful way. It is an opportunity to provide a valuable service to clients, both professional and amateur, provided the clinician has a thorough understanding of the specific athletic discipline and of the client’s objectives. In addition, it is a valuable learning tool because the clinical exam and image findings can be referred to as an individual horse’s career progresses.

**Acknowledgments**

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How to Estimate White Blood Cell Mass and Differential From a Peripheral Blood Smear

Kelsey A. Hart, DVM, PhD, DACVIM (LAIM)*; and Melinda S. Camus, DVM, DACVP

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1. Introduction

Knowledge of the white blood cell (WBC) count and differential distribution of the leukogram is integral to correctly diagnosing and treating disease in any ill animal. In ambulatory equine practice, obtaining this information typically requires submitting a complete blood count (CBC) to a diagnostic laboratory, and is often associated with a delay of a day or more until the results are available. This can delay appropriate diagnosis and initiation of therapy and ultimately can affect the patient's prognosis.

Although board-certified veterinary clinical pathologists are the most qualified to perform detailed microscopic analysis of blood and other samples, general practitioners can use simple techniques and inexpensive tools to make a peripheral blood smear from the CBC tube and use this to rapidly estimate the WBC mass and differential. This information can help practitioners make a tentative diagnosis and institute appropriate therapy without delay while awaiting confirmatory results from the laboratory. The same tools can also be used for other cytologic applications in practice, ultimately improving patient care and client satisfaction due to decreased delays in treatment while providing additional income through billable services.

Even in a referral institution with an in-house clinical pathology service during routine hours, evaluation of blood smears and cytologic samples from out-of-hours cases can be very useful to expediently confirm presumptive diagnoses and initiate appropriate treatment. The goals of this session are to demonstrate how to make and interpret a peripheral blood smear to estimate the WBC mass and differential in equine samples, and to illustrate common leukocyte abnormalities encountered in equine practice. The accuracy of bench-top CBC analyzers at providing a differential cell count varies substantially among models: some impedance counters are only able to provide a total WBC count, whereas flow cytometer models can provide an extremely accurate differential. However, even with top-of-the-line equipment with excellent cell type differentiation, a manual differential and peripheral blood smear interpretation is considered standard of care in veterinary clinical pathology laboratories to ensure that important cytologic findings such as nucleated eryth-
rocytes, band neutrophils, toxic changes in leukocytes, or neoplastic cells are not missed.

## 2. Materials and Methods

There are a number of different ways to prepare and stain a peripheral blood smear, and as long as the chosen method produces a “feathered edge” and a region where the cells are spread in a monolayer (Fig. 1), the particular method used is really a matter of personal preference. The method described below has been compiled from best practices used by clinical pathology and large-animal internal medicine personnel in our hospital who routinely prepare and evaluate peripheral blood smears in routine and emergent cases.

### What you will need:

- A properly filled ethylenediaminetetraacetic acid (EDTA) anti-coagulated blood sample (purple top tube) from the patient
- Plain glass microhematocrit tubes (no heparin) or two wooden-handled Q-tips
- Two glass microscope slides and coverslips
- Commercial hematology stain set (any stain in the Romanowsky family of stains is acceptable.)
- A microscope, minimally with a 10× objective and a 40× objective, and ideally with a 100× (oil immersion) objective. There are a number of reasonably priced ($400–800), easy-to-use microscopes suitable for general use in clinical practice.

### Preparation of the Blood Smear

1. Blood smears should be made within 2 hours of collection to prevent artifacts that alter cell morphology. Store the sample in a refrigerator if you cannot make the smear immediately. Make sure the sample is brought back to room temperature prior to making slides and that the slides themselves are never refrigerated.

2. Mix the sample by repeated inversion for 30–60 seconds if recently collected, or for 3–5 minutes if it has been refrigerated.

3. Place one microscope slide on the table with a long side facing you.

4. Partially fill the microhematocrit tube with blood OR dip a wooden-handled applicator stick into the well-mixed blood tube.

5. Place a small drop of blood on the slide approximately 0.5 cm from the short end of the slide closest to your dominant hand.

6. Take a second slide (pusher slide), hold it on the long sides fairly close to one short end, and gently touch it at an approximately 30° angle onto the first slide, adjacent to the blood drop.

7. Slowly back the pusher slide into the blood drop, wait while the blood spreads to the edge of the slide, then gently and smoothly push the pusher slide across the first slide in one smooth motion to the end of the slide.
   a. Do not push down: the weight of the slide alone will spread the blood.
   b. If you must make additional smears, you may reuse the pusher slide as long as you do not reuse the same edge that was used to push the blood on the first attempt.

8. Dry the slide immediately by waving it in the air to prevent crenation of the cells.

### Staining of the Blood Smear

There are a number of different ways to stain blood smears and cytologic preparations, but for general applications a three-step Romanowsky-type stain such as Wright’s stain is simplest and most useful. This generally will stain proteins pink, DNA/nuclei purple, and bacteria and fungi blue. Follow the manufacturer’s directions for specific stains. If you plan to stain and analyze “dirty” samples (e.g., fecals, transtracheal washes, abscess aspirates, etc.), it is helpful to keep two sets of stain: one for clean samples such as blood smears, and one for the likely contaminated samples listed above. In addition, make sure to store and change out your stain properly according to the manufacturers’ protocols, to avoid contamination with bacteria, fungus, or stain precipitate that can impair your ability to analyze your stained samples.

In our laboratory, we use a stain with the following basic protocol:

1. Once slide is air dried, hold it by one short end away from the sample and dip it in the fixative (clear or light blue) for 10–20 dips (~15 s),
then blot the short end opposite to the one you are holding on a paper towel.
2. Dip the slide in the stain (pink), for 10–20 dips (~15 s) and blot the short end again.
3. Dip the slide in the counterstain (purple) for 10–20 dips (~15 s), and blot the short end, then rinse gently with tap water.
4. Air dry again by waving in the air before viewing under the microscope, and label it with the patient name or number on one edge away from the smear.

Examination of the Blood Smear

1. Visually examine the slide to determine the best area for microscopic examination (Fig. 1).
2. Place the slide on the microscope stage, and focus on it using the lowest power objective (4× or 10×). Find an edge of the smear near the butt end and then quickly scan the whole smear from butt end to feathered edge to note cell clumps and thin areas.
3. Using the 10× objective, examine the feathered edge. Big things such as platelet clumps and large cells get dragged out to the feathered edge. Equine platelets frequently clump in EDTA anticoagulant, which can cause the automated platelet count to be falsely low. If you see platelet clumping, do not panic over a low platelet count on a CBC.
4. Now move three to four 10× fields back into the smear, to the monolayer, assuming that the patient is not anemic. This is where cellular morphology is best evaluated and where the differential WBC count is best performed. You can tell you are at the right part of the slide when erythrocytes are close together but not touching (Fig. 2). If you cannot find an area like this on the slide, make another smear and try again.
5. Place a coverslip on the slide and change to the 40× objective. The coverslip is important because it is required for the 40× objective to focus properly. Scan back and forth across the short axis of the slide, noting the following:
   a. Do you see platelets? Are they clumped? You should see 15–20 platelets per field at this magnification unless they are severely clumped.
   b. Examine leukocyte morphology.
      i. Get a general idea: are they all neutrophils or all lymphocytes? Horses should have more neutrophils than lymphocytes (Based on equine reference ranges established in our laboratory, this is usually at least an approximately 2:1 to 3:1 neutrophil-to-lymphocyte ratio.)
      ii. Get a general number: usually you see one to four leukocytes per field at this magnification, so a lot fewer or a lot more than this might be abnormal.
      iii. Cell morphology specifics (see results below for examples)
         1. Mature vs immature neutrophils
         2. Toxic changes in neutrophils
         3. Reactive lymphocytes
         4. Abnormal lymphocytes
         5. Optional: are there any visible organisms (e.g., Babesia spp., Anaplasma phagocytophilum)? Note: visualization of some organisms requires 100× oil, so they could be missed if you are only using a 40× objective.
   c. Perform a 100-cell differential cell count by scanning back and forth across the short axis of the slide (Fig. 3). This will require either an actual differential counter, or a differential worksheet where you make a hash mark next to each cell type as you scan, then tally up the number in each category when you reach 100 (see Appendix 1).
5. Optional: change to 100× (oil) objective to evaluate:
   a. Erythrocyte morphology: size and shape, and presence of any parasites or Heinz bodies
b. Estimated platelet concentration: take an average over 10 fields (7–22/100× field is adequate)

c. Note: if you elect to use an oil immersion objective for higher magnification, take care not to go back to the 40× objective without thoroughly cleaning the oil off the slide by changing to a new coverslip, or by wiping the coverslip with lens cleaner and lens paper. If oil gets on the 40× objective, it will damage it and render it unusably blurry without removal and proper cleaning.

3. Results

The above methodology is recommended for use in the field because it is quick, inexpensive, and user friendly. Below are three case examples that exemplify applications of this technique and commonly encountered leukocyte abnormalities encountered in equine practice.

- Case 1: Peripheral blood smear from an 18-year-old Quarter Horse gelding with colitis (Fig. 4).
- Case 2: Peripheral blood smear from a 5-year-old Warmblood mare with fever and distal limb edema (Fig. 5).
- Case 3: Peripheral blood smear from a 9-month-old Saddlebred foal with fever and cough (Fig. 6).

Estimation of peripheral leukocyte counts from analysis of blood smears can be inaccurate. When seven experienced laboratory personnel in our laboratory analyzed the same 10 blood smears from different species in a prospective study, estimated WBC count varied substantially among personnel (coefficient of variation, 13–43%), and less than half the samples were acceptably consistent with WBCs determined with the gold standard automated cell counter. Sources for error include different counting areas, counting broken cells, and variation in microscope aperture size. As a result of our study, WBC estimates are now interpreted qualitatively in our laboratory (e.g., low/normal/high) and quantitative estimates are no longer given. Thus, implementation of this technique in your practice should complement rather than replace automated complete blood counts.

4. Discussion

Preliminary evaluation of a peripheral blood smear is a simple procedure than can be performed quickly in a field setting and does not require expensive equipment. Information regarding total leukocyte mass, leukocyte differential, platelet mass, and even detection of some infectious organisms can be gleaned with this procedure, facilitating clinical decision making about the need for additional diagnostics, and permitting initiation of appropriate therapy without having to wait for the results of send-out tests. The necessary microscope can also be used to perform
in-house fecal flotation, skin cytology, and mass aspirates, and if centrifugation is available, the above slide preparation and staining procedures can also be adapted for other cytologic specimens such as transtracheal wash fluid (Fig. 7), synovial fluid, and peritoneal fluid. Thus, although practitioners must understand the limitations of using this technique to estimate peripheral leukocyte counts and morphology in the field, investment in this equipment and the time to perform the technique can improve efficiency and accuracy of patient care and increase practice revenue in a number of ways.

Fig. 5. Peripheral blood smear from a horse with fever and distal limb edema, illustrating a cytoplasmic inclusion in the neutrophil (arrow) consistent with *Anaplasma phagocytophilum* infection (formerly *Ehrlichia equi*, equine Erlichiosis). Neutropenia and thrombocytopenia are also frequently seen in this disease. Although the diagnosis is confirmed with positive serology, recognition of the organisms on a blood smear can permit earlier initiation of appropriate antimicrobial therapy with oxytetracycline, which is generally quite effective when started early. 100× magnification, modified Wright’s stain.

Fig. 6. Peripheral blood smear from a weanling with fever and cough, illustrating a lymphocytosis. Note that all four leukocytes visible in this field are lymphocytes, in contrast with the expected finding of a 2–3:1 neutrophil-to-lymphocyte ratio in equine peripheral blood. The foal was ultimately diagnosed with bacterial pneumonia, and this blood smear was made 11 days into the course of disease. Lymphocytosis is not an uncommon finding in chronic inflammatory conditions in horses, particularly in younger animals. Other differential diagnoses for lymphocytosis would include recent viral infection or hematologic neoplasia (lymphoma or lymphocytic leukemia). 100× magnification, modified Wright’s stain.

Fig. 7. A, Transtracheal wash cytology from the weanling with fever and cough in Case 3, illustrating septic, purulent inflammation, and consistent with a diagnosis of bacterial pneumonia. Note the large number of degenerate neutrophils with swollen, pale, puffy nuclei (black arrows) and intracellular bacterial cocci (white arrow). 100× magnification, modified Wright’s stain. B, Transtracheal wash cytology from a 12-year-old mare with cough and exercise intolerance, illustrating aseptic purulent inflammation and increased mucus. In these two images, note the nondegenerate neutrophils (white arrows), absence of bacteria, and preponderance of mucus (streaming blue/purple background) with the presence of Curschmann’s spirals (black arrow). This is consistent with a diagnosis of equine allergic/inflammatory lower airway disease such as recurrent airway obstruction (heaves) or inflammatory airway disease. Because this horse was symptomatic at rest, she was ultimately diagnosed with recurrent airway obstruction. 100× magnification, modified Wright’s stain.
### Appendix

Name(s) 
Species 

**WBC estimate:**
- NORMAL
- INCREASED
- DECREASED

<table>
<thead>
<tr>
<th>#CELLS/100 WBC COUNTED</th>
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<tbody>
<tr>
<td>Segs</td>
</tr>
<tr>
<td>Bands</td>
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<tr>
<td>Lymphs</td>
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<tr>
<td>Monos</td>
</tr>
<tr>
<td>Eos</td>
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<tr>
<td>Basos</td>
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### Acknowledgments

#### Declaration of Ethics

The Authors declare that they have adhered to the Principles of Veterinary Medical Ethics of the AVMA.

#### Conflict of Interest

The Authors declare no conflicts of interest.

### References and Footnote


*Camus M, Bush S. A method comparison study of two routinely used methodologies for performing nonmammalian complete blood counts. In: Proceedings of the Annual Meeting of the American College of Veterinary Pathology/American College of Veterinary Clinical Pathology, Montreal, Canada, 2013.*
How to Interpret Cytology From Body Cavity Effusions

Fairfield T. Bain, DVM, MBA, DACVIM, DACVP, DACVECC

Body cavity effusions are commonly sampled in clinical practice as a part of diagnostic evaluation. This presentation will review the gross appearance and common cytologic findings to aid the practitioner in rapid decision-making. Author's address: Washington State University, College of Veterinary Medicine, PO Box 646610, Pullman, WA 99164-6610; e-mail: fbain@vetmed.wsu.edu. © 2015 AAEP.

1. Introduction

Abdominocentesis and thoracocentesis are commonly used diagnostic tools in equine practice. Abdominocentesis is one of the standard diagnostic tests used in evaluating colic patients and is often performed to (1) differentiate strangulating from nonstrangulating lesions; (2) confirm gastrointestinal rupture; (3) document, evaluate, and monitor peritonitis as well as the identify-specific microorganisms involved; and (4) evaluate and determine the cell type of suspected neoplastic effusions. Pleural effusion samples may be collected when an effusion is suspected, e.g., when the patient has difficulty breathing because of a large volume of fluid or when a significant volume of effusion has been detected during diagnostic imaging. Pleuropneumonia is the most common pathologic process associated with pleural effusions in horses. Analyzing the pleural fluid is important for assessing the severity and pattern of inflammatory response as well as for detecting the presence and type of microorganisms. Cytologic evaluations are also important for detecting the presence and type of neoplastic cells in patients in which neoplasia is the underlying mechanism for the pleural effusion.

Evaluating body cavity fluids consists of an initial visual assessment, with more specific information obtained by a cytologic evaluation. The intent of this article is to provide the practitioner with information on how to perform a cytologic evaluation of a body fluid sample, specifically peritoneal or pleural fluid samples.

2. Materials and Methods

Sample Collection

Describing the techniques for collecting peritoneal fluid and pleural fluid samples is beyond the scope of this article. Fluid samples for cytologic evaluations should be collected into ethylenediaminetetraacetic acid (EDTA) anticoagulant tubes to preserve cellular morphology. The potential exists for false elevation of the total protein concentration of a fluid as measured by refractometry when the EDTA concentration is increased. This can occur when only a small volume of fluid is added to an EDTA-anticoagulated tube. If more than 2 hours pass before cytologic analysis, it may
be helpful to make smears on glass slides immediately for later evaluation. Heparin anticoagulant tubes will not preserve cell morphology, and serum tubes may allow for clotting of proteins and clumping of cells, making cytologic identification impossible.

Visual Assessment
The gross appearance of the peritoneal fluid is the first stage in evaluating a fluid sample. Normal peritoneal fluid appears clear and light yellow in appearance (Fig. 1). Fluid from patients with gastrointestinal rupture will often appear green-brown in color, with varying degrees of cloudiness and possibly particulates (Fig. 2). Patients with a strangulating intestinal lesion often have serosanguinous fluid as a result of varying amounts of erythrocytes (Fig. 3). Iatrogenic bleeding into the peritoneal fluid can result from a blood vessel that penetrates the skin or abdominal wall during the procedure or from an accidental splenic puncture during the tap, which results in a blood-tinged or bloody fluid sample. Patients with a peritoneal inflammatory response (peritonitis) will have a cloudy, opaque fluid that may vary from yellow to orange or red depending on the number of erythrocytes present (Fig. 4). Enterocentesis can occur by accidental perforation of a viscus during abdominocentesis. The gross appearance of enterocentesis can vary depending on the character of the luminal content and extent of admixing with peritoneal fluid.

Pleural fluid is often evaluated when an effusion associated with infectious pneumonia (pleuropneumonia) is suspected or when there is clinical and/or diagnostic imaging that indicates a pleural effusion of unknown cause. The gross appearance of pleural fluid with pleuropneumonia can vary depending on the extent of lung injury. Many will have a suppurative (cloudy yellow) appearance, whereas others with more severe lung necrosis may have a cloudy reddish appearance. Pleural fluid samples from patients with neoplastic disease of the pleural cavity can vary in appearance depending on the process. Lymphoma that involves the cranial mediastinal lymph nodes and other thoracic lymph nodes is often associated with a serosanguinous appearance. In some lymphoma patients, pleural fluid may appear yellow with variable cloudiness depending on the degree of cellularity. In some patients with primary neoplasia, suppurative inflammation can occur from necrosis of the neoplasm or secondary infection and obscure the diagnosis; thus, cytologic evaluation is critical in making the correct diagnosis.

Routine body fluid analysis before cytologic evaluation includes a total nucleated cell count and total protein concentration (or total solids via refractometry). Most normal body cavity fluids (peritoneal

![Fig. 1. Normal peritoneal fluid appearance is clear with variation in degree of yellow color.](image1.png)

![Fig. 2. Peritoneal fluid from a horse with gastrointestinal rupture. Note the green-brown color with turbidity.](image2.png)
and pleural) have a total protein concentration of less than 2.5 g/dL. Total nucleated cell counts are considered normal if there are less than 5,000 cells/μL. Peritoneal fluid from normal foals will have lower total nucleated cell counts (<1,500 cells/μL). The goal of fluid analysis has traditionally been to place the fluid into a pathophysiologic grouping: transudate, exudate, or hemorrhagic effusion (Table 1). Transudates have low total protein concentration (<2.5 g/dL) and low-cellularity (<5,000 cells/μL) fluids. Exudates have an increased total protein concentration (>2.5 g/dL) and high cellularity (>10,000 cells/μL). Hemorrhagic (serosanguinous) effusions by definition contain increased numbers of erythrocytes.

Beyond the visual assessment of a fluid sample, microscopic evaluation of the cytology can provide critical information of the pathophysiologic process and aid clinical decisions. Consideration should be given to whether the cytologic evaluation will be performed in house or transported to a reference laboratory for evaluation. If transporting the samples, it is helpful to prepare direct smears to be sent along with the fluid sample. If shipping biopsy specimens, formalin-fixed tissues should be transported in a separate container because formalin fumes will alter the cytologic features of cells on glass slides, making interpretation difficult.

Techniques for Slide Preparation and Staining
For most normal body cavity fluids, the cellularity will be low (<5,000 cells/μL), and cytologic evaluation of a direct smear will be difficult (Fig. 5). Con-
concentration of a specimen can be performed by using a regular centrifuge with low gravity (centrifuge 5–10 mL of fluid for 5 minutes at 1,000–1,500 rpm) or a cytocentrifuge (Fig. 6). For such normal fluid samples, it is rare that there will be clinically significant cytologic findings. More commonly, the identification of a transudate of low cellularity and low protein concentration implicates a particular pathophysiologic mechanism (venous or lymphatic obstruction, increased venous hydrostatic pressure, or systemic hypoproteinemia). For more cellular specimens, preparing a good-quality (cellular monolayer) smear on a glass slide is an important first step. The most common difficulty in evaluating highly cellular fluids is making a smear too thick such that staining is inadequate and observing cellular features is impossible. Slide preparation using the push technique, which is similar to what is used for peripheral blood smears, is nicely described in the literature. A drop of fluid is placed on a glass microscope slide, and the edge of a second slide is slid backward until the fluid spreads along the junction of the two slides. The spreader slide is then pushed forward to create a smear. It is useful to create a margin (“feathered edge”) that is at least 1/4 the way from the end of the slide to allow the microscope objective to reach it when the slide is placed on the microscope stage. In practice, it is helpful to prepare 3 to 5 slides so that a representative evaluation of the fluid can be made. Slides should then be air-dried and stained with a Romanowsky-type stain to allow for evaluation and observation of cellular features (Fig. 7).

### Table 1. Categories of Effusion and Pathophysiologic Mechanism

<table>
<thead>
<tr>
<th>Category</th>
<th>Total Protein</th>
<th>Nucleated Cell Count</th>
<th>Mechanism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transudate</td>
<td>&lt;2.5 g/dL</td>
<td>&lt;5 x 10^3/µL</td>
<td>Increased hydrostatic pressure</td>
</tr>
<tr>
<td>Exudate</td>
<td>&gt;2.5 g/dL</td>
<td>&gt;5 x 10^3/µL</td>
<td>Increased vascular permeability and inflammation</td>
</tr>
<tr>
<td>Hemorrhagic</td>
<td>&gt;2.5 g/dL</td>
<td>&lt; or &gt; 5 x 10^3/µL; RBC &gt; 1 x 10^9/µL; PCV &gt; 3%</td>
<td>Vascular injury</td>
</tr>
</tbody>
</table>

PCV, packed cell volume; RBC, red blood cells.

Cytologic Examination Process

The cytologic examination should take into account the total nucleated cell count and total protein concentration as well as the clinical parameters of the patient. To perform a cytologic examination, one should begin with a cursory screening of the slide on the low-magnification (4×) objective of the microscope. This will allow the examiner to gain an initial impression of the overall cellularity of the smear. Once regions of cellular interest or other features are identified, the examiner can apply immersion oil and then switch to the oil objective (100×) for closer viewing of the area or object of interest. Following this pattern in evaluating a slide will ensure complete evaluation of the entire smear.

Peritoneal Fluid Evaluation

The initial step in cytologic evaluation is to determine whether the sample is representative of the actual constituents of the body cavity effusion. Blood contamination during the procedure is often detected by observing the fluid as it is being collected. This usually results in increased erythrocyte numbers as well as platelets observed on the slide. Enterocentesis can also alter the cytologic findings and complicate the clinical evaluation when...
the patient is systemically compromised (signs of systemic inflammatory response syndrome or endotoxemia). Enterocentesis may result in the presence of protozoal organisms, plant material, and mixed bacterial organisms on the smear (Fig. 8). The task of the clinician is to differentiate enterocentesis from gastrointestinal rupture. The clinical condition of the patient combined with the discovery of some neutrophils with cellular features consistent with sepsis may be helpful in determining the presence of rupture if sufficient time has passed since the rupture for an inflammatory response to occur. In many cases of rupture, actual intact neutrophils are rare.

Cloudy peritoneal fluid samples typically have an increased nucleated cell count. The next step is determining the types of nucleated cells in the smear. Normal peritoneal (Fig. 9) and pleural fluids contain a slight predominance of nondegenerate neutrophils with lesser to almost equal numbers of mononuclear cells (which can be made up of macrophages and mesothelial cells) followed by lymphocytes. With inflammation, the percentage of neutrophils increases according to the intensity of the inflammatory stimulus (Fig. 10). Cytologic differentiation between a septic and nonseptic exudate is a traditional process based on identifying microorganisms in the cytology. An extensive microscopic search should be undertaken for evidence of sepsis in fluid samples with elevated total nucleated cell counts. Degenerative changes in the neutrophils that suggest the presence of sepsis include changes such as karyolysis, which is characterized...
by swelling and disruption of the nuclear membrane (Fig. 11, A). When observed, it is important to perform a detailed search for microorganisms. Although this classification of septic versus nonseptic fluids is based on identifying microorganisms (Fig. 11, B) in the cytology, fluids with markedly elevated numbers of neutrophils are generally considered as potentially septic for clinical management. The presence of phagocytized microorganisms strongly supports body cavity sepsis. Neutrophils can engulf extracellular bacteria that are present from situations such as enterocentesis while in EDTA-anticoagulated tubes over time, thus creating artificial intracellular bacteria. This can happen if the fluid sample is stored for a prolonged period of time (truck or transport) prior to making a smear. Thus, it is valuable to make a smear as soon as possible after sample collection to avoid such artificial findings. In such circumstances, it is also important to search for other evidence of degenerative changes within neutrophils to differentiate ex vivo engulfment of bacteria from true bacterial phagocytosis associated with a septic process. The type of microorganisms present may serve as a guide to the origin of the peritonitis. Mixed types of organisms suggest gastrointestinal origin or contamination from enterocentesis. Single organisms such as paired cocci may suggest an internal abscess. In postpartum mares, uterine tears are a common problem, and cytologic evaluation should include a search for meconium fragments and squamous epithelial cells that could support the diagnosis (Fig. 12).

Mesothelial cells are occasionally seen in body cavity fluids as individual cells or in cohesive sheets. Reactive mesothelial cells are frequently seen in inflammatory effusions and often require differentiation from neoplastic cells and have thus been called the “great impersonator” (Fig. 13, A–C). Reactive mesothelial cells usually have intense cytoplasmic basophilia, large nuclear-to-cytoplasmic ratios, and multinucleate and binucleate forms—all features similar to those of neoplastic cells. Ruffled cytoplasmic borders are also often seen in reactive mesothelial cells.

Neoplastic effusions may appear as exudates or hemorrhagic effusions. Cytologic evaluation is important in attempting to make a diagnosis before exploratory surgery. Lymphoma is common in the horse; however, neoplastic lymphoid cells may not appear in the peritoneal fluid as commonly as they do in the pleural fluid (Fig. 14). In some cases, there is a secondary inflammatory response with a predominance of neutrophils. Squamous cell carcinoma of the nonglandular region of the stomach (Fig. 15) is another neoplasm that may involve the abdominal cavity and can be diagnosed while cytologically evaluating the peritoneal fluid. Because
of the tendency of these neoplasms to penetrate the gastric wall and metastasize, the peritoneal fluid may be seen as an exudate, sometimes septic. Malignant squamous cells can be identified by cytoplasmic keratin formation and often appear as large, bizarre cells that are asymmetrical in size with multiple nuclei. Nuclear features of malignancy are abnormal nuclear chromatin patterns and multiple nucleoli. Mitotic figures, some of which appear atypical, also indicate neoplasia.

Serosanguinous fluids can occur with strangulating intestinal lesions as well as with exudates. Cytologic evaluation can aid in differentiating an active hemorrhage from an iatrogenic one, in which
case platelets may be seen on the smear. A longer-standing hemorrhage may result in erythrophagocytosis (Fig. 16).

Biochemical analysis of peritoneal fluid creatinine and electrolyte concentrations is the common method for confirming uropertoneum in neonatal foals and, less commonly, in adults. Cytology is occasionally useful in supporting the diagnosis. The key cytologic feature of urine contamination of the peritoneal space is the finding of calcium carbonate crystals (Fig. 17).

### Pleural Fluid Evaluation

Evaluating pleural fluid most often involves animals with effusions that have been detected with diagnostic imaging techniques (ultrasonography or radiography). Horses with pleuropneumonia should have thoracocentesis to (1) remove the fluid if excessive volume is present such that lung expansion is compromised and (2) differentiate septic effusion from nonseptic parapneumonic effusion. Nonseptic, noninflammatory effusions can arise from neoplasia and cardiovascular disease. Cytologic evaluation can be useful in further defining the pathologic process.

Pleural fluid from horses with pleuropneumonia can change in gross appearance over time with maturation of the pleural inflammatory process. In the early stages, a parapneumonic effusion of a slightly cloudy, yellow appearance can be seen. Cytologic evaluation of this stage may characterize the effusion as a nonseptic exudate. Changes may differ between pleural spaces that depend on the inflammatory process within that lung (Fig. 18). With more chronicity, pleural effusions can become a thicker, purulent exudate (Fig. 19). In cases of a thick purulent exudate from the pleural space, a direct smear would be appropriate for cytologic evaluation. With chronic fibrinopurulent pleuropneumonia, it is also important to evaluate the pleural space with diagnostic imaging for the presence of loculation, because this could result in variable cytologic changes between fluid pockets. Detecting pleural abscess during diagnostic imaging might guide the fluid collection process for cytologic evaluation.

Pleural effusions associated with lymphoma within the thoracic cavity are often serosanguinous (Fig. 20). Neoplastic lymphoid cells can vary in size, but a predominance of large lymphoid cells or premature stages suggests lymphoma (Fig. 14).
3. Discussion

Cytology of body fluids is a valuable technique for clinical practice. Although often associated with personnel trained in pathology, it is also something clinical practitioners can perform themselves. Practice in slide preparation and learning the technique for slide scanning will allow for obtaining quality information. Performing cytology in house allows for a real-time assessment of a fluid instead of the time required to transport it to a reference laboratory. The time required for an assessment will become shorter as the practitioner gains experience evaluating cytologic smears. When beginning to evaluate cytology of fluid samples in practice, it may be useful to refer fluid samples to a clinical pathologist when there is some question as to the cellular inflammatory patterns or when there are cells that appear atypical or possibly having features consistent with neoplasia. Comparing the in-house observations to the pathologist’s descriptions can be a useful learning process. Currently available reference texts provide excellent photos of common cytologic findings that can help practitioners assess the smear.3,5 The goal of the practitioner is to classify the type of effusion based on total nucleated cell count and total protein concentration and then to pursue further information regarding the cytologic evaluation. As with other clinical examinations (lameness, reproductive, etc.), developing a routine procedure for evaluating a smear will give the clinician a sense of completeness. In certain situations, the smear will confirm clinical suspicions (e.g., suppurative peritonitis associated with intra-abdominal abscessation with Streptococcus equi subspecies equi or Rhodococcus equi, where the microorganism might be observed during the cytology). In some patients, the cytologic evaluation will provide the critical piece of information in making a correct diagnosis, such as a horse with cranial mediastinal lymphoma with voluminous serosanguinous pleural effusion, ventral edema, and weight loss. Finer details of smear evaluation of septic processes include

Fig. 19. Thick, opaque, purulent exudate from a horse with pleuropneumonia with pleural abscess formation.

Fig. 20. Serosanguinous pleural fluid from a horse with cranial mediastinal lymphoma.
differentiating granular chromatin from ruptured neutrophils, stain precipitates, and other debris from bacterial structures. The process of evaluating a body cavity fluid from the initial clinical examination to collecting the fluid sample and visually assessing and then cytologically evaluating it can provide a more complete understanding of the pathophysiologic mechanisms underway and guide the medical or surgical treatment process more directly.

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Declaration of Ethics
The Author declares that he has adhered to the Principles of Veterinary Medical Ethics of the AVMA.

Conflict of Interest
The Author declares no conflicts of interest.

References and Footnote


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How to Interpret Common Hematologic and Serum Biochemistry Differences Between Neonatal Foals and Mature Horses

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There are important and significant differences in normal hematologic and serum biochemical parameters between neonatal foals and mature horses. Failure to recognize these differences can lead to erroneous interpretation of neonatal clinical pathologic values. Author’s address: Department of Large Animal Medicine, H-302 College of Veterinary Medicine, University of Georgia, Athens, GA 30602; e-mail: bartonmh@uga.edu. © 2015 AAEP.

1. Introduction
The dynamic physiologic changes and unique diet during the neonatal period contribute to distinctive features in clinical pathologic parameters of healthy foals relative to healthy adult horses. When reporting results, most diagnostic laboratories only provide reference ranges for mature horses. Thus failure to recognize the unique differences that occur in foals relative to adult horses can lead to erroneous interpretation of neonatal clinical pathologic values. Methodology can also profoundly affect the values reported in a reference range, which can lead to erroneous interpretation when extrapolating results between laboratories. Ideally, normal reference ranges for foals should be established within each diagnostic laboratory. However, funding limitations typically preclude provision of this data. Thus, the main objective of this session is to review distinct features of common hematologic and serum biochemistry parameters in foals relative to mature horses.

2. Materials and Methods
Medline, Pubmed, Agricola, and CAB databases were reviewed. Inclusion in this review was based on clinical applicability. To avoid unnecessary duplication, common themes in difference or trends of differences were extracted for this review. Given that methodology can influence the reported value or range of values, reference to absolute values will be limited. When applicable, breed differences will be noted.

3. Results
Hematology
Red Blood Cell Parameters
Red blood cell parameters are highly dynamic during the neonatal period. In general, immediately after birth, the red blood cell (RBC) count, hemoglobin (Hgb) concentration, packed cell volume (PCV), and hemocrit (Hct) are similar to slightly greater than adult horses.1–4 However, in most breeds, within 48 hours, these values decrease and continue to decrease to the low end or below the reference range for mature horses.5 For example, it is not atypical for the PCV to decrease in the first 48 hours of life from values of approximately 45 to 50% at
birth to 35 to 40%, whereas absolute RBC counts do not decrease as dramatically. The higher PCV at birth likely represents terminal placental transfer of blood and the physiologic stress of parturition inducing splenic contraction. The initial rapid reduction in PCV is due to a combination of adaptation to extrauterine life and hemodilution from blood volume expansion after ingestion of colostrum. The PCV, Hct, and Hgb continue to gradually decrease through the first month of life, most often with values falling in the low-normal adult range or sometimes, slightly below the adult range. The absolute RBC count often stays within the normal adult range; thus, the decrease in PCV is mostly due to the fact that neonatal erythrocytes become smaller (i.e., microcytes) and remain relatively uniform in size compared with adult RBCs. This is reflected in the mean corpuscular volume (MCV) values, which are similar to adult values at birth, and then gradually decrease, reaching a nadir between 3 and 5 months that is below the adult range. The MCV can remain lower than adults for up to a year. Mean corpuscular hemoglobin concentration (MCHC) is derived by diving the Hgb concentration by the Hct and is more accurate than the mean corpuscular hemoglobin (MCH). In foals, the mean corpuscular hemoglobin concentration tends to be slightly lower to within normal limits relative to adults. Thus, collectively and compared with adults, foals often seem to be mildly anemic with smaller RBCs with less hemoglobin. This physiologic anemia is common and seems to be due to reduced stimulus for erythropoiesis and decreased iron availability. Hypoxemia is a strong stimulus for erythropoiesis. After birth, foal RBCs have greater amounts of 2,3-diphosphoglycerate, which typically occurs with immature RBCs. 2,3-diphosphoglycerate facilitates the release of oxygen delivery to tissue; thus, greater concentrations in the neonate may curtail erythropoiesis. Serum iron and ferritin concentrations decrease rapidly in the first few days and total iron-binding capacity is greater in the neonatal foal than the adult and may be due in part to the low iron content of milk versus colostrum and depletion of fetal iron stores. The concurrent presence of microcytes is supportive evidence for functional iron deficiency. Relative lack of iron availability and the physiologic anemia associated with it rarely result in clinical abnormalities in the neonatal period. However, it would be atypical for the PCV to decrease below 20% in a foal or to decrease rapidly, in which case additional diagnostics would be warranted. In light of the fact that foals have functional iron deficiency, they may be more prone than adult horses to the development of iron deficiency anemia, especially if concurrent disease is present.

It should be noted that breed differences have been noted particularly in RBC indices in horses. Although there have not been direct breed comparisons within the same laboratory setting, comparisons between studies suggest that physiologic anemia and the changes in RBC indices may be less dramatic in draft breeds compared with light-breed horses. In one study, the degree of anemia in Arabian foals during the first year of life was more pronounced than in Thoroughbred or Quarter Horse foals. Donkey foals follow the same trend as light breed foals.

**Leukogram**

Total leukocyte and absolute neutrophil counts tend to be the same or slightly exceed adult values whereas lymphocyte counts tend to be the same or below mature horse reference ranges during the first day of life. During the first few days, absolute lymphocyte counts may fall below 1000/μL. The higher neutrophil-to-lymphocyte ratio may be due in part to the endogenous release of cortisol at parturition. In fact, lack of the parturition cortisol surge in otherwise-healthy premature foals is typically accompanied by characteristic neutropenia at birth, wherein the severity of the neutropenia correlates with the likelihood of survival. Lymphocyte counts can remain at or below the lower end of the adult reference range for the first few months of life. However, foals are also more likely to experience stress when handled for venipuncture and physiologic lymphocytosis subsequent to catecholamine release can result in a rapid increase in the total lymphocyte count. Band neutrophils are expected to remain less than 250/μL during the neonatal period. Eosinophils are typically absent.

**Coagulation**

Coagulopathy is common in critically ill neonates with one recent study reporting at least one abnormal coagulation parameter in 64% of foals with septic shock. Standard testing of the coagulation system would include determining the platelet count; prothrombin and activated partial thromboplastin times; fibrinogen concentration; fibrin degradation products or d-dimer concentration; and perhaps, antithrombin activity. As for many clinical pathologic parameters, methodology can directly affect the absolute values determined, and this is particularly true for coagulation testing. Platelet counts are most accurate if the blood is collected into sodium citrate as the anticoagulant. Platelet counts are the same or slightly greater in foals during the first few days of life and then comparable with adult values. Likewise, the prothrombin and activated partial thromboplastin times are the same or longer and fibrinogen concentrations are lower than the adult horse in the first few days of life. Fibrin degradation products concentrations are significantly greater than adult horses for at least 2 weeks. Antithrombin activity is significantly lower during the first month of life, with mean values approximately one half adult values at birth.
Serum Biochemistry

Proteins
The total serum protein concentration varies considerably in the first 24 to 36 hours, depending on timing of absorption of colostral immunoglobulin. Presuckle total serum protein concentration is usually less than 5 g/dL and thus would fall below the normal adult reference range. Post-suckle total serum protein concentrations usually are greater than 6 g/dL, but may remain in the low to slightly below the normal adult reference range for several weeks. Albumin concentrations tend to stay within the same reference range as adult horses, thus the albumin-to-globulin ratio is usually normal or slightly lower than adult horses. Although absolute total serum protein concentration is not a reliable indicator of transfer of passive immunity, a low albumin-to-globulin ratio may be supportive evidence for partial or complete failure of passive transfer and should be verified by a more specific test for immunoglobulin concentration.

Electrolytes
The only subtle difference in sodium concentration in foals relative to adult horses is that the serum sodium concentration might be at the lowest end of the normal adult reference range in the first 24 to 48 hours. This most likely is due to mild hemodilution following osmotic fluid expansion after absorption of colostral immunoglobulin. Otherwise, serum sodium, potassium, chloride, bicarbonate, magnesium, and calcium values typically remain stable during the neonatal period with no significant differences relative to the mature adult horse. Serum inorganic phosphorus concentration is similar to adult values at birth, and then gradually increases over the first 2 months and may be slightly greater than adult values during the first year of life. For example, adult serum phosphorus values usually are less than 4 mg/dL, whereas foal serum phosphorus concentrations can range between 6 and 8 mg/dL in the first year of life.

Renal
Serum creatinine values are often greater than adult values in the first 24 to 36 hours of life with values of 4 to 5 mg/dL, and as high as 27 mg/dL, without concurrent evidence of renal dysfunction. Endogenous serum creatinine is removed from fetal circulation via the placental circulation. Thus, higher creatinine values in the first day of life more likely reflect placental dysfunction rather than reflect primary renal disease. Furthermore, foals usually do not urinate until they are between 6 and 12 hours old, which also delays the clearance of endogenous creatinine. Spurious hypercreatininemia is commonly reported in foals with neonatal encephalopathy. However, foals with spurious hypercreatininemia without renal disease typically have normal concurrent blood urea nitrogen concentrations, and the serum creatinine concentration usually steadily decreases to normal values within the first 72 hours of life. Creatinine concentrations frequently fall below 1.0 mg/dL in the well-hydrated and vigorously nursing foal. Blood urea nitrogen values are equivalent to adult values at birth and then tend to drop below the lowest end of normal adult range (12 mg/dL) from the first few days of life to 5 months of age.

Hepatic
The liver serves many diverse roles in normal homeostasis including protein, lipid, and carbohydrate metabolism, vitamin storage, hematopoiesis, detoxification, and excretion. Total bilirubin concentration, primarily consisting of unconjugated or indirect acting bilirubin is significantly greater in neonatal foals than mature horses, peaking in the first week (up to 5 mg/dL) and remaining increased during the first 2 weeks of life. Unconjugated bilirubin values are often two to four times the adult mean value during this time. Physiologic hyperbilirubinemia of neonates is primarily caused by reduced availability of bilirubin-binding protein that is responsible for hepatocellular uptake of bilirubin and can be further exacerbated by anorexia. Physiologic hyperbilirubinemia, coupled with physiologic anemia during this period can be misconstrued as evidence of hemolytic anemia. Bilirubin concentrations in donkey foals tend not to be as high as horse foals and often are within adult donkey reference range.

Foals have less stored hepatic glycogen than adult horses and are not yet hindgut fermenters. Thus glucose concentrations tend to be highly variable, depending on demand, stress, and nursing frequency. In general, glucose concentrations are usually greater than the normal adult values during the first month of life, often exceeding twice the adult upper range of values. Likewise, serum triglycerides are highly variable and reflective of nursing. Values may be as high as 340 mg/dL in healthy foals in the first few months of life, when adult values rarely exceed 50 mg/dL.

In general, liver-associated enzymes are greater in neonatal foals and have larger standard deviations from the mean compared with adults. The liver-specific enzymes sorbitol dehydrogenase and gamma glutamyltransferase (GGT) are either unaffected or slightly increased in the first 2 weeks and increased between 1 and 4 weeks of life, respectively. Unlike ruminant neonates, there is little GGT in colostrum and thus, GGT levels do not correlate with transfer of passive immunity in foals. Alkaline phosphatase (ALP) activity is very high the first week of life (up to 3000 U/L) and remains two to four times the adult mean range (64 to 214 U/L) for the first year of life. The relatively higher ALP activity of neonatal foals is primarily due to the bone isoenzyme and increased release associated
with osteoblastic activity during rapid bone growth and bone stress.

Bile acids concentration is frequently used as a functional assay of the liver. Bile acids concentrations are significantly greater than mean adult values during the first 6 weeks of life, with radioimmunoassay values exceeding enzymatically determined values. Increased serum bile acids concentration in the neonatal period may be due to upregulation of hepatic production, reduced excretion into the bile, unique intestinal floral effects on the bile-acid composition of the neonate, or enhanced intestinal absorption or uptake from the portal circulation.

Collectively, greater bilirubin and serum bile acids concentrations and greater serum GGT, sorbitol dehydrogenase, and ALP activities in the neonatal period, could erroneously lead to a diagnosis of liver disease.

Muscle Enzymes
Aspartate aminotransferase (AST) activity is primarily associated with muscle, although some AST activity is also found in the liver. In foals, AST activity tends to be the same or slightly lower than adult values during the first week of life, but values tend to remain within adult reference ranges as they continue to exercise and grow. Increased creatine phosphokinase activity is reported in 62% of foals with neonatal encephalopathy.

Endocrine
At birth and during the first few days of life, thyroid hormones (T3 and T4) are at least ten times (991 ng/dL and 29 μg/dL) greater than adult horse values. Both T3 and T4 values gradually decrease, approximating adult values by 2 weeks (T4) to 1 month of age (T3), although values may remain two times greater than adult values for the first year of life. A cortisol surge in response to adrenocorticotropic hormone is an important physiologic event at parturition in both the mare and foal. Adrenocorticotropic hormone concentrations are greatest at birth (up to 968 pg/mL), rapidly decreasing within hours to approximate adult values within 48 hours. Likewise, cortisol concentrations are also highest in the first 30 minutes after birth (6 to 13 μg/dL), but drop within 48 hours, often falling below mean adult horse values during the first few weeks of life. Premature foals have significantly higher adrenocorticotropic hormone and lower cortisol concentrations at birth (often < 1 μg/dL) than full-term foals, indicating dysfunction of the hypothalamic-pituitary-adrenal axis.

4. Discussion
Relative to adult horses, there are several unique features of neonatal clinical pathologic parameters that must be considered for accurate interpretation.
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How to Interpret Serum Amyloid A Concentrations

Rose Nolen-Walston, DVM, DACVIM

1. Introduction
The role of the clinical veterinarian encompasses many features of medical practice, but in many cases starts with a simple dichotomization: is this animal normal or abnormal? This can include the question, “is it lame or sound,” but often also includes, “is it sick or well?” A thorough history and physical examination will often reveal how to stratify the patient, but in cases of subtle disease especially for horses in high level competition, mild and early signs of infection and inflammation may be occult yet significant. A reliable test for infection or inflammation, therefore, can have an extremely valuable place in the clinician’s armamentarium. Good tests allow for some degree of quantification both to allow the practitioner to assess the severity of the process and also to follow and document its response to therapy and track its resolution over time. The earliest of these tools was rectal temperature, in which fever signified a secondary indicator of increased cytokines such as tumor necrosis factor (TNF)-α and interleukin (Il)-1. However, over the past century, blood analysis has allowed us to quantify multiple inflammatory markers including the acute phase proteins such as fibrinogen, haptoglobin, α1-acid glycoprotein, C-reactive protein (mainly in humans), serum amyloid A (SAA), and many others, as well as secondary indicators such as white blood cell count and serum iron levels. Of these, fibrinogen has probably been the most heavily relied on for horses. It can be easily and inexpensively measured, but may be confounded by in vitro pre-analytical microclot formation. However, its concentration only slowly increases in the 24 hours after induction of inflammation and often does not peak until 48 hours. In addition, there is often only a small increase (often only a 1–2-fold difference) from baseline, and thus mild inflammation cannot reliably be distinguished from normal values. Nonetheless, any method that detects inflammation in the horse probably must outperform fibrinogen in one or more of these factors: accuracy, ease of interpretation, cost, and ease of use.

SAA is the major acute-phase protein of the horse (and most other mammals), and is produced predominantly by the liver as a systemic manifestation of the body’s response to inflammation. It exists in equine plasma as one of three isoforms of apolipoprotein and is complexed to high-density lipoprotein in circulating blood. First investigated in horses in the 1980s, its clinical use as a marker of inflammation is probably eclipsed by fibrinogen as a function of assay availability rather than diagnostic inferiority. The advantages of SAA over fibrinogen include that it has both low/undetectable constitutive expression in normal animals but reaches levels of 100-1000-fold baseline values in clinical disease
In addition, its rapid increase in concentration over 6–12 hours combined with a 30–120-minute half-life means that serum values track disease severity closely, and subsequent relapse or secondary infections result in similar response to primary infections. SAA is stable both at room temperature and refrigerated, can be measured using a relatively inexpensive stallside test or a variety of laboratory-based assays, can be performed using plasma as well as serum, and may be assessed using noninvasive samples such as saliva. Although there is some difference in precision and accuracy between assays, most available tests seem to be accurate enough within clinically relevant ranges to be acceptable to the practitioner.

2. Materials and Methods

A review of the literature reveals many publications that evaluate the use of SAA as a tool for distinguishing healthy horses from those with local or systemic inflammation, and as a diagnostic and monitoring tool for specific conditions. To maximize utility of this compilation of clinical equine veterinary publications, they are presented by body system or disease process, and the review focuses on the most clinically relevant studies. The astute reader will notice that these references refer to SAA in mg/L, μg/mL, and ng/mL; the first two of these units are equivalent and the third represents one thousandth the concentration of the first. The authors’ original units are maintained throughout.

3. Results

SAA to Determine Infectious/Inflammation Versus Normal

Horses that are “not quite right” or performing poorly are often diagnostic challenges, and identifying mild inflammation and distinguishing it from noninflammatory differential diagnoses before its clinical signs declare themselves can stymie even the most meticulous clinician. A recent large study evaluated the SAA concentrations of hospitalized horses that had either local inflammation (gastric ulceration, abscesses, Streptococcus equi subspecies equi infection), systemic inflammation (disease accompanied by fever, tachycardia, leukopenia/leukocytosis) or were otherwise healthy or had noninflammatory conditions. Patients with systemic inflammation had significantly higher SAA (mean, 1583 mg/L; range, 688–4000 mg/L) than horses with local or no inflammation, which had mean SAA concentrations of 168 mg/L (range, 37–1609 mg/L) and 5.6 mg/L (range, 1.8–14.5 mg/L) respectively. This discrimination was more distinct than that of fibrinogen, in which the mean values of the three groups were 124, 181, and 128 mg/dL, respectively. Using receiver operator curve analysis, SAA had the highest accuracy for diagnosing inflammation (Fig. 1), but predictive modeling failed to generate useful algorithms. A similar study dichotomized horses into “clinically normal” and “clinically abnormal,” the latter of which included conditions as diverse as pneumonia, cholangiohepatitis, Streptococcus equi subspecies equi infection, meningitis, enterocolitis, various forms of colic and neoplasia, and orthopedic infections. The clinically normal horses had a mean SAA of 6.8 mg/L (range, 0.1–26.6 mg/L), whereas the clinically abnormal horses had a mean SAA of 71.7 mg/L with a range of 0.1–3,800. In the same cases, mean fibrinogen values (ranges) were 349 mg/dL (100–800 mg/dL) and 514 mg/dL (100–1200 mg/dL), respectively. For discrimination of clinically normal horses from clinically abnormal horses, SAA had sensitivity of 53% and specificity of 94% (diagnostic accuracy, 75%), whereas using white blood cell count, and plasma fibrinogen concentration and mean albumin:globulin ratio, accuracy ranged from 59 to 62%. The authors also showed data from six cases comparing the resolution of inflammatory markers over time (Fig. 2) and concluded that “SAA concentration can provide valuable information regarding the clinical state of horses and may be more useful for patient monitoring and as a prognostic indicator than are traditional markers of inflammation.”

Fig. 1. Receiver-operating characteristic curves for myeloperoxidase index, fibrinogen, SAA, and iron for discerning horses with from those without inflammatory disease. Reprinted with permission by Hooijberg EH et al. 14

Foals have been shown to have similar baseline values of SAA compared with adults, the kinetics of its rise and resolution seems grossly similar, and SAA is higher in animals with bacterial infections than in those with nonbacterial or uncertain diagnoses. In the largest study looking at SAA in foals, 226 healthy Thoroughbred neonates had mean plasma concentrations of 345 mg/L (range, 100–1400 mg/L), and the highest SAA concentration was 3,800 mg/L, which was also the highest fibrinogen concentration (3,800 mg/dL).
dian SAA concentrations of 0.9, 4.5, and 2.5 mg/L on
days 1, 2 and 3 of life, with the values on day 2 being
significantly higher than baseline. In 136 foals
with clinical disease, median SAA concentrations of
cases with focal infections such as omphalitis were
195 mg/L and those with septicemia higher still at
280 mg/L. Foals with noninflammatory abnormalities
such as failure of passive transfer and noninfectious
disease had low SAA concentrations at 5.1 and
3.1 mg/L, respectively. These data suggest
that SAA can be used in foals even at young ages as
an indicator of infectious or inflammatory processes,
especially given that its quicker rise allows abnor-
malities to be identified in the first few days of life.
Increased fibrinogen levels in neonatal foals often
indicate intrauterine inflammation; whether SAA
rises during prepartum exposure to infectious or
inflammatory agents is not known.

SAA and Gastrointestinal Disease
The main applications for the use of SAA in colic
would be to assist in the ability to distinguish sur-
gical vs nonsurgical disease, to identify infectious
complications, and to gauge prognosis and response
to therapy. A study assessing SAA in colic cases
admitted to two university teaching hospitals found
that concentrations of SAA were significantly higher
at admission in horses with colic attributable inflam-
matory causes (e.g., enteritis, colitis, peritonitis,
or abdominal abscesses). This was most useful
in separating enteritis cases (median, 65.5 μg/mL;
interquartile range, 3–500 μg/mL) from strangulat-
ing obstructions (median, 4.8 μg/mL; interquartile
range, 0.3–58.6 μg/mL). A significant difference
was also seen in the SAA value between horses that
survived the colic episode (median, 1.4 μg/mL) and
nonsurvivors (median, 10.8 μg/mL). Looking at
SAA levels in the peritoneal fluid of horses with
abdominal pain, cases with various etiologies of colic
had a mean SAA concentration of 249 mg/L in serum
and 97 mg/L in peritoneal fluid, compared with less
than 1 mg/L in both samples obtained from a
healthy control population. Furthermore, SAA
was elevated in horses with equine grass sickness
(median, 50 mg/mL) compared with surgical lesions
and noninflammatory colics (median, ~0 mg/
ml). A small experimental study found that inoc-
ulation with equine coronavirus resulted in SAA
levels that mirrored clinical disease; although all
three challenged horses shed large quantities of vi-
rus, only those that showed clinical signs of diar-
rhea, fever, and anorexia had elevated SAA, which
peaked at 200–400 μg/mL. Interpreting these
data is tricky given that there is not clear consensus
on the value of SAA in colics. However, based on
these studies and clinical experience, SAA does not
offer clear guidance in making the decision if a colic
is surgical, nor should it be relied upon for prognos-
ticating survivability when euthanasia is being con-
dered. However, colic cases that are admitted
with SAA well above the reference range (> 20
mg/L) should have inflammatory gastrointestinal le-
sions such as enteritis and colitis higher on the
differential diagnosis, and SAA values in the hun-
dreds should prompt a search for an infectious
etiology.

SAA and Respiratory Disease
SAA has been investigated as a diagnostic tool for
both noninfectious inflammatory airway disease and
viral and bacterial infectious diseases. Horses with
severe bacterial pneumonia often present with SAA
values well into the thousands, and even road
transportation, the primary risk factor for pneumo-
nia in horses, increases SAA anywhere from ~30 to 500 μg/mL for 24 to 48 hours after shipping healthy Thoroughbreds 1200 km by road over 26 hours. This increase was abrogated by administration of antimicrobials,24 and shorter-distance shipping (4 h) did not have an effect on SAA concentrations.25 In equine influenza, serum SAA increases during the first 48 h of clinical signs and returns to baseline values in 11 to 22 days barring secondary infections, with maximum values of ~450 mg/L during the acute phase.26 In ponies experimentally infected with EHV-1, serum levels peaked at 100 to almost 1000 mg/L in the week after inoculation.5 Various methods have been evaluated to screen foals for sub-clinical Rhodococcus equi pneumonia including sonographic examination, hematology, and physical examination, but each have been found to be imperfect with regard to either diagnostic accuracy or cost effectiveness. Acute phase proteins, especially fibrinogen, have been used as moderately effective screening tools for foals on endemic farms. It would seem that with its greater sensitivity and more labile kinetics, SAA would be a better biomarker for this disease that often exhibits an extended preclinical latency where detection is often difficult. Two recent studies have investigated SAA as a possible predictor of R. equi pneumonia in at-risk populations. The first of these studies used a large, well-selected population of affected foals and age-matched controls from endemic farms. No predictive value was found in SAA levels in 212 foals 7 to 14 days and 196 foals 21 to 28 days of age, nor at the onset of clinical signs of pneumonia.27 The authors conclude that “monitoring concentration of SAA is not useful as a screening test for early detection of R. equi.”27 A subsequent smaller study also assessing weekly screening with SAA to identify pre-clinical R. equi infections found similar results. SAA concentrations did not show an association with the development of sonographic evidence of lung abscessation, and of six foals on an endemic farm diagnosed with R. equi pneumonia, only two demonstrated elevated SAA concentrations and this was around the time that the disease became clinically evident.28 These results are surprising given that R. equi generally results in a robust increase in fibrinogen and leukocyte count, which for other inflammatory diseases tend to be less sensitive than SAA. In conclusion, one must wonder why SAA is not a better aid in early identification of R. equi pneumonia; no completely satisfactory explanation is apparent for these results.

Recurrent airways obstruction (“heaves”) is a disease of the small airways characterized by neutrophilic exudate within the alveoli and bronchi. Although it is not associated with explicit signs of inflammation such as fever or peripheral neutrophilia, SAA has the potential to be more sensitive for subtle alterations. A prospective, observational study used six healthy and six heaves-affected horses challenged with hay and straw to examine a variety of acute-phase proteins.29 Although haptoglobin concentrations were higher in the heaves horses both before and during an exacerbation, the SAA did not reliably increase, although there was a small but significant difference between heaves-affected horses and controls on day 7 of exacerbation (15.75 vs 3.22 μg/mL, respectively).29 Nonetheless, probably the main use of SAA in horses with recurrent airways obstruction is distinguishing them from pneumonia cases, in which the SAA is likely to be much higher.

SAA and Surgery

However much attention is paid to careful tissue handling and correct technique, surgery is an inflammatory stimulus and this fact is revealed by elevations in SAA after even minor, uncomplicated procedures.4,5,30,31 Therefore, its more useful application is probably identifying postoperative infections both earlier and with more accuracy than other methods. A study looking at standing castrations, for example, found that all horses had elevations of SAA to the 400–600-mg/L ranges at day 3 postoperatively, but those that went on to develop infections (as evidenced by fever, swelling, serohemorrhagic or purulent discharge) still had SAA values in this range at the eighth day whereas horses recovering without complication were in the ~200–400-mg/L range by this point. The increased SAA values associated with infection were not reliably reflected by increases in rectal temperature, leukocyte count, or fibrinogen, suggesting that SAA was a superior marker for infection.31 A subsequent study found that perioperative treatment with penicillin reduced the SAA in horses undergoing castration from a mean of 708 to 543 mg/L at day 3 postcastration, and from 515 to 125 mg/L on day 8,12 supporting the idea that even mild infections result in appreciable differences in SAA concentration. Looking beyond orchidectomy, the effect of minor surgical procedures on SAA shows that levels of 100 to 400 mg/L that peak at approximately day 3 after surgery can be expected in cases uncomplicated by infection. These include tibiotarsal arthroscopy and osteochondrosis fragment removal, laryngoplasty, and ventriculectomy (peaked at 50–150 mg/L at d 2; normal by d 7)30 carotid exteriorization and flexor tendon division (peaked at 100–400 mg/L at d 2; normal by 7–14 d)5 and a variety of elective procedures including minor airway and orthopedic surgeries (peaked at 16.4 μg/mL at 24 h, 15.5 on d 2).16 SAA was also significantly lower in elective (defined as noninflamed) vs nonelective (pre-existing inflammatory foci) cases16 as well as being able to delineate differing levels of surgical trauma based on the invasiveness of the procedure.30 In several of these studies14,30 SAA response was found to be a more sensitive indicator of inflammation than a variety of other acute-phase protein or leukocyte responses, and decreased more quickly in response to resolution than fibrinogen (Fig. 3). This is particularly useful to the practitioner who must decide whether hematologic ev-
Although septic arthritis is rarely a diagnostic challenge, SAA may provide the clinician with supporting information regarding degree of inflammation and allow monitoring of resolution of time in conjunction with traditionally measured variables such as synovial fluid analysis.

SAA and Laminitis/Endocrinopathy

The question of how SAA changes in laminitis is complicated by the myriad inflammatory as well as noninflammatory etiologies of laminitis, as well as its varied chronicity and severity. A review of the literature in this area does not show a consistent story. For example, although SAA mRNA is increased in laminitic hoof tissue samples, SAA levels in previously laminitic ponies currently in remission showed no elevation from baseline. However, all of the horses had SAA levels that would fall within the reference range of the clinical assay, with highest values obtained topping out at 3845 ng/mL, or 3.8 mg/L. However, even at these low values, the authors concluded that “SAA concentration is a better marker of obesity-associated inflammation and laminitis [and] ... is possible that SAA is a component of laminitis pathophysiology.”

SAA and Exercise

In Standardbred trotters and Arabian racehorses, SAA levels were unchanged in response to racing. However, significant findings have been identified looking at the acute phase response of horses undergoing long-distance endurance riding competitions and training. Although horses evaluated before an endurance ride all had SAA values within normal limits, SAA was lower in horses that successfully finished a 120–160-km competition compared with those that were eliminated based on intra- and postrace lameness and metabolic examinations (411.7 ng/mL in finishers vs 5809.5 ng/mL in eliminated horses; N.B. these units are equivalent to 0.4 and 5.8 mg/L). This study found that “serum SAA level was the only laboratory parameter that indicated most (66.6%) of the eliminated horses before entering the competition.” After long-distance (> 100 km) but not shorter (30–60 km) rides, a 10-fold or greater increase in SAA to ~13,000 ng/mL (13 mg/L) was found, although other acute phase proteins such as C-reactive protein and haptoglobin remained unchanged. Looking beyond the massive efforts of competitions at this level, there were no changes in SAA concentrations after race and endurance training sessions in experienced horses. Interestingly, SAA values also increased during training levels for inexperienced, early-career endurance horses (from ~1 to ~3.5 mg/L), but not in experienced competitors. This suggests that using SAA after training sessions may be a method of determining preparedness for advanced competition, but this has not yet been explicitly evaluated.
SAA and Reproductive Disease
There is conflicting evidence on SAA in the periparturient period of healthy mares. One study found that SAA levels were low in the 8 weeks prepartum although increased slightly in some mares in the week preceding foaling, whereas other studies have found no change in SAA associated prior to normal parturition. At 12 and 36 hours postpartum, mean SAA increases to 62 mg/L (range, 0.7–305 mg/L) and 189 mg/L (range, 0–1615 mg/L) and returns to basal concentrations by 60 hours. In mares with experimentally induced placenta, SAA values peaked between 274 and 4385 mg/L within 2 to 6 days after intracervical inoculation with Streptococcus equi subspecies zooepidemicus; mares generally aborted within 2 to 6 days of the increase in SAA. Abortion was more likely in mares with high SAA compared with mares where the SAA remained within the reference range, and values increased steadily until abortion, when they then decreased rapidly. In comparison, fibrinogen and white blood cell count were not found to be useful markers of placentitis.

SAA and Parasites
In a study using horses experimentally infected with both small and large strongyles, acute-phase proteins were monitored over 161 to 164 days. Although haptoglobin, iron, and albumin/globulin ratios were associated with strongyle burden, SAA was not and remained low throughout the study. In addition, no significant change in SAA was seen after anthelmintic treatment in a group of heavily parasitized horses. This provides the practitioner with useful information as larval cyathostomiasis is a diagnosis difficult to make antemortem, and low SAA may steer the differential diagnosis away from inflammatory causes of colopathy.

SAA and Vaccination
After vaccination using two different influenza and tetanus toxoid products, horses showed variable acute-phase responses with six of 10 developing SAA concentrations greater than −5 mg/L which peaked at 48 hours after vaccination. Maximum values ranged from −30 to 175mg/L, and increased white blood cell counts and fibrinogen concentrations, and decreased serum iron were also noted. By 96 hours, SAA levels were returning to normal but had not quite reached baseline values.

4. Discussion
SAA is a sensitive predictor of early inflammation, and due to its rapid onset and short half-life, tracks the course of disease closely. In most studies it outperforms the common variables of both fibrinogen and white blood cell count, and also often the other measured acute-phase reactors of haptoglobin, C-reactive protein, and serum iron. In a general sense, SAA can be used in most situations to obtain early identification of an inflammatory process, to assess the effectiveness of a chosen antimicrobial or other treatment, to monitor the rate of improvement, and to mark resolution of disease. SAA should be considered as an adjunct to diagnosis of a wide variety of inflammatory conditions in the horse, and may well replace fibrinogen in coming years as the main acute phase protein monitored in clinical medicine. However, it is not useful to diagnose specific diseases, and should not replace careful physical examination and identification of the etiologic causes of the inflammatory response. Table 1 shows a compilation of approximations of SAA values for common scenarios encountered in both primary care and referral equine practice.

The main disadvantages of SAA at this time are a lack of complete standardization of assay techniques, which imposes on the practitioner the need to commit to the same assay throughout evaluation of a particular case; values obtained from different assays may not be completely interchangeable. Although there are differences in results and precision between laboratory-based and in-clinic or stall-side tests, studies comparing the different forms of assay have found that the results given by each are clinically comparable. A newer stall-side assay can be read visually for semiquantitative measurement or using a handheld reader for a quantitative results. No peer-reviewed data have been published demonstrating the accuracy of this kit.

Although SAA has many advantageous characteristics, it is still not a diagnostic panacea. It seems to have no validity in assessing foals for R. equi pneumonia, although it often increases to extremely high concentrations in pleuropneumonia in adults and can be very valuable in assessing response to treatment in these cases. SAA may reliably distinguish surgical from nonsurgical colic cases, although it is probably superior to fibrinogen in identifying postoperative infections because it follows a standard increase-and-decrease pattern of peaking at day 2 postoperatively and then returning to normal after approximately 1 week. Any deviations from a steady decrease after the first 2–3 days after surgery might prompt a search for infectious complications.

Although published reference ranges of SAA vary somewhat, the vast majority of normal, healthy horses tested in all these studies had SAA concentrations of 0 mg/L, or at the most, low single digits; control horses that had SAA levels greater than this may have been suffering from subclinical conditions that were not evident on physical examination. Most instances of infectious or inflammatory disease show SAA levels greater than 50 mg/L, which leaves a gray area between where interpretation may be difficult. SAA is still a relatively new technique compared with other measured indices of inflammation, so less is known about how to interpret these values than older, more familiar parameters. Studies correlating SAA concentrations to a large number of diseases have been published every year over the last decade, and currently the body of liter-
nature is expanding rapidly in ways that assist veterinarians in the application of this parameter. Overall, practitioners should feel comfortable using SAA in lieu of fibrinogen for most cases in which infectious or inflammatory disease is suspected, although ordering both for a period of time is probably wise until the clinician develops the experience necessary to interpret the wide range of values seen with this marker.

Acknowledgments

Declaration of Ethics

The Author declares that she has adhered to the Principles of Veterinary Medical Ethics of the AVMA.

Conflict of Interest

The Author declares no conflicts of interest.

References and Footnote


Table 1. Approximate Peak SAA Values Seen in Common Equine Diseases With Their Time to Peak and Resolution

<table>
<thead>
<tr>
<th>Disease State</th>
<th>Approximate Peak Value, mg/L</th>
<th>Time to Peak; Time to Resolution</th>
</tr>
</thead>
<tbody>
<tr>
<td>SAA in normal horses and foals</td>
<td>0–7 mg/L&quot;</td>
<td>NA</td>
</tr>
<tr>
<td>SAA, inflammatory colic (enteritis, colitis, coronavirus)</td>
<td>50–500</td>
<td>Depends; decreases in tandem with disease resolution</td>
</tr>
<tr>
<td>SAA, surgical colic</td>
<td>5–50</td>
<td>NA</td>
</tr>
<tr>
<td>SAA, grass sickness</td>
<td>−50</td>
<td>NA</td>
</tr>
<tr>
<td>SAA, major bacterial infections (peritonitis, pleuropneumonia)</td>
<td>Up to 3000–5000 depending on severity</td>
<td>Depends; decreases in tandem with disease resolution</td>
</tr>
<tr>
<td>SAA, viral lung disease</td>
<td>500–1000</td>
<td>Peaks in first wk; 11–22 d</td>
</tr>
<tr>
<td>SAA, R. equi pneumonia</td>
<td>Variable; not reliable indicator</td>
<td>NA</td>
</tr>
<tr>
<td>SAA, recurrent airway obstruction (&quot;heaves&quot;)</td>
<td>≤15</td>
<td>Increased &gt; baseline only during acute exacerbation</td>
</tr>
<tr>
<td>SAA, castration</td>
<td>400–700</td>
<td>2–3 d; to &lt; 200 by 8 d if no complications</td>
</tr>
<tr>
<td>SAA, elective surgery</td>
<td>50–400</td>
<td>2 d; 7–14 d</td>
</tr>
<tr>
<td>SAA, septic arthritis</td>
<td>Up to 1500 in serum, 1100 in synovial tissue</td>
<td>Not reported</td>
</tr>
<tr>
<td>SAA, chemical arthritis</td>
<td>50–300</td>
<td>48 h</td>
</tr>
<tr>
<td>SAA, laminitis</td>
<td>No change in chronic, metabolic etiologies</td>
<td>NA</td>
</tr>
<tr>
<td>SAA, post-race (STBs, Arabians) (&gt; 120 km)</td>
<td>−13</td>
<td>No data; no data</td>
</tr>
<tr>
<td>SAA, shorter-distance endurance ride (&gt; 40 km)</td>
<td>No change</td>
<td>NA</td>
</tr>
<tr>
<td>SAA, before normal parturition</td>
<td>No change</td>
<td>NA</td>
</tr>
<tr>
<td>SAA, after normal parturition</td>
<td>50–600 at 12–36 h postpartum</td>
<td>36 h; 60 h; Rises until abortion; rapidly drops after abortion</td>
</tr>
<tr>
<td>SAA, placentitis</td>
<td>250–4500</td>
<td>NA</td>
</tr>
<tr>
<td>SAA, parasitism</td>
<td>No change</td>
<td>NA</td>
</tr>
<tr>
<td>SAA, after anthelmintic treatment</td>
<td>No change</td>
<td>NA</td>
</tr>
<tr>
<td>SAA after vaccination</td>
<td>30–175</td>
<td>2 d; probably between 4–7 d</td>
</tr>
</tbody>
</table>

Abbreviations: STB, Standardbred.

*Suggested reference ranges vary from <1.3 and <20 mg/L.

HOW-TO SESSION: CLINICAL PATHOLOGY


How to Differentiate and Diagnose Pituitary Pars Intermedia Dysfunction and Equine Metabolic Syndrome

Kelsey A. Hart, DVM, PhD, DACVIM (LAIM)

1. Introduction
Pituitary pars intermedia dysfunction (PPID; equine Cushing’s disease) and equine metabolic syndrome (EMS) are by far the most common equine endocrine disorders, affecting up to 30% of some breeds of horses and ponies during the course of their lifetimes. PPID and EMS are distinct diseases with different pathophysiology that typically require different therapeutic approaches. PPID results in dysregulation of the hypothalamic-pituitary-adrenal (HPA axis) due to increased activity of the pars intermedia of the anterior pituitary gland resulting in overproduction of adrenocorticotropic hormone (ACTH) and other related hormones. Progressive loss of dopamine-producing neurons in the hypothalamus with aging in some animals results in loss of dopamine-mediated inhibition of the pars intermedia, leading to hyperactivity, hyperplasia, or a functional adenoma of this portion of the pituitary gland. Specific mechanisms that cause this dopaminergic neuron loss in the hypothalamus are not well characterized, but long-term, low-grade oxidative injury may play a role and helps explain why PPID is a disease of older horses and ponies. Persistently increased ACTH concentrations theoretically should increase adrenal activity in horses and ponies with PPID, and some clinical features of equine PPID are often attributed to increases in cortisol levels or activity. However, increased circulating total cortisol is not a typical finding in horses and ponies with PPID, suggesting that other noncortisol-mediated hormonal pathways may play a role in the clinical features of PPID.

In contrast, whereas the pathophysiology of EMS is poorly understood, it is clear that it does not result from primary HPA axis or thyroid gland dysfunction. EMS seems to be at least in part an adipose-derived endocrinopathy, in which abnormal or excessive adipose tissue produces a hormonal and/or inflammatory environment that alters insulin responses to feeding and the activity of insulin in peripheral tissues. Hyperinsulinemia and exaggerated insulin responses to feeding is a key aspect of EMS pathophysiology.

However, despite their different underlying pathophysiology, these two diseases share several common clinical features. Most importantly, acute or chronic laminitis—often seasonal or pasture associated—is a common and typical presentation for both PPID and EMS, and the development of lam-
nitis seems to be associated with underlying insulin dysregulation in both diseases.\(^1\)\(^2\) Because of the potential career- and even life-ending effects of laminitis, and the distinct therapeutic strategies necessary to control PPID and EMS, accurate diagnosis of one or both of these disorders in affected animals is paramount to successful management of these disorders.

A number of different diagnostic strategies for PPID and EMS have been assessed in recent years. Although a single perfect test to diagnose and differentiate these disorders is not yet available, using a combination of clinical features, measurement of basal concentrations of several hormones, and dynamic tests to measure endocrine responses to various stimuli, can permit accurate diagnosis of PPID and EMS in most animals. This session will summarize currently recommended diagnostic strategies for differentiating PPID and EMS in horses and ponies.

2. Materials and Methods
The recent literature on diagnostic evaluation for PPID and EMS was reviewed to determine current and practical recommendations for field and in-hospital testing for these disorders. The first, and arguably the most important, step in the diagnosis of PPID and EMS is the clinical evaluation of the patient to detect typical clinical signs. In some cases, clinical findings alone are enough to make a diagnosis of one or both diseases. However, in most cases, to distinguish between and definitively diagnose these two disorders, and to assess response to therapy, further hormonal testing— including screening tests to measure resting hormone concentrations and/or dynamic testing to measure hormonal responses to specific stimuli—is necessary. These tests are discussed in detail below and summarized in Table 1.

Clinical Findings
Typical clinical features of classic presentations for PPID and EMS and predisposed breeds are outlined in Table 2. The classic presentation of PPID is an older animal (usually late teens or older) that presents with pasture-associated or seasonal laminitis, generalized muscle wasting/weight loss, and hair coat abnormalities (delayed, incomplete, or absent shedding). In fact, the clinical sign of failure to shed (typically called hirsutism, but more appropriately termed hypertrichosis) is a more sensitive and specific test for late-stage PPID than any hormonal assay studied to date.\(^2\) In contrast, the classic presentation for EMS is also an animal that presents with pasture-associated laminitis, but in contrast this is a younger-to-middle-aged animal with a normal hair coat that has always been an easy keeper and is clearly obese with obvious fat deposits in the crest of the neck and tail-head.\(^1\)

However, these classic presentations are most helpful in horses with rather advanced stages of either disease; in early-stage disease, the only clinical sign of PPID or EMS might be an episode of acute pasture-associated laminitis in a middle-aged animal. Further, both diseases can have atypical presentations, so testing for PPID and EMS should be considered in animals with unexplained laminitis even if no other typical clinical signs are present. In addition, it is important to note that PPID and EMS can occur concurrently, so individual animals might present with signs consistent with both diseases. Thus, unless the laminitic animal is very young and very obese (consistent with EMS) or very old, muscle-wasted, and completely fails to shed out (most consistent with PPID), hormonal testing should be performed to differentiate between these two diseases.

Screening Tests—Measurement of Resting Hormone Concentrations
The simplest approach to hormonal testing to screen for PPID and EMS in suspect cases is measurement of resting hormone concentrations. This can easily be performed in the field, and thus is not typically subject to confounding by stress or transport effects.

PPID Screening
At present, the best commercially available screening test for PPID is measurement of plasma adrenocorticotropic hormone (ACTH) concentration. Measurement of other hormones, such as plasma cortisol or insulin concentration, are not as sensitive or specific for diagnosis of PPID as measurement of ACTH. Measurement of another anterior pituitary hormone, \(\alpha\)-melanocyte stimulating hormone (\(\alpha\)-MSH) is also a good test for PPID diagnosis, but at present this test is only available in a research setting.

To screen for PPID by measurement of resting plasma ACTH concentration, blood may be collected at any time of day and the animal does not need to be fasted. However, extreme excitement, transport, illness, and routine change can transiently increase ACTH concentrations, so testing under these circumstances should be avoided. The sample should be collected into a plastic tube with EDTA as an anti-coagulant given that glass seems to falsely lower ACTH concentrations.\(^5\) ACTH is very labile at room temperature, so the sample should be refrigerated or placed on ice immediately after collection. For accurate results, the sample must be centrifuged to separate the plasma as soon as possible after collection (within 4 h), and only the plasma should be submitted for analysis (ideally in a plastic vial). It is imperative that the plasma remain cold on arrival at the testing laboratory; this can be ensured by either 1) freezing the sample and shipping it overnight on dry ice, or 2) freezing the sample and shipping it overnight with several frozen ice packs in a styrofoam shipping cooler.

ACTH is measured by immunoassay, several of which are well validated for equine samples. A number of commercial laboratories run equine
<table>
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<tr>
<th>Test Sample Collection, Test Protocols, and Interpretation and Helpful Hints for Hormonal Testing Options for PPID and EMS</th>
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<tr>
<td>Test</td>
</tr>
<tr>
<td>PPID testing</td>
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<tr>
<td>ACTH (baseline)</td>
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<tr>
<td>ODST (to measure cortisol)</td>
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<tr>
<td>TRH stim test (to measure ACTH)</td>
</tr>
<tr>
<td>EMS testing (assessment of insulin response)</td>
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Information on general costs and options for some US diagnostic labs that offer these tests for equine samples is also provided, although this is not intended to be a complete or exhaustive list of all laboratories offering these testing methods. Test fees are subject to change at anytime.

ODST, overnight dexamethasone suppression test
TRH, thyrotropin-releasing hormone
EDTA, ethylenediaminetetraacetic acid
ORT, oral sugar test
ACTH samples, but they use different assays that often have very different reference ranges. Thus, cut-off values for diagnosis of PPID can vary substantially among laboratories. It is important to know which assay your laboratory uses to accurately interpret your results.

Furthermore, recent studies have demonstrated a substantial effect of season on resting ACTH concentration in healthy and PPID horses. In summary, resting ACTH concentrations are increased in autumn (August through October in the northern hemisphere) in healthy horses and horses with PPID. This increase is greatly exaggerated, however, in horses with PPID. Thus, whereas just a few years ago testing for PPID by measuring ACTH concentrations was not recommended in the autumn months, with the recent development of season-specific reference ranges, autumn now seems to be the best time to screen for PPID with this test because the difference in resting ACTH concentration between normal and PPID horses is greatest during this season. More research is underway to determine the effect of specific geographic location, age, and breed on resting ACTH to develop location and population-specific diagnostic cut-off values.

In the author’s experience with clinical and research samples in the United States, it has been found that the veterinary endocrinology laboratories at Michigan State University and Cornell University offer comprehensive testing for equine endocrine disorders and offer helpful information on their Web sites to provide assistance with interpretation of results. There are certainly a number of other good veterinary endocrinology laboratories, but the author has found these to be especially helpful with test interpretation. In general, regardless of assay and season, ACTH concentrations greater than 50 to 100 pg/mL are typically consistent with PPID, and normal horses, even those sampled every 20 minutes over a 24-hour period, typically have ACTH concentrations less than 20 to 30 pg/mL. However, horses with ACTH concentrations that fall close to or in between these values can be difficult to interpret and might require additional dynamic testing, or repeat ACTH measurement in 6–12 months.

EMS Screening
Testing for EMS is more complicated than testing for PPID, because EMS is best described as a collection of hormonal abnormalities and clinical signs associated with the development of laminitis in horse and ponies, rather than as a distinct and well-defined disease with a single direct etiology. Ideally, diagnosis of EMS requires consistent clini-
eral features (Table 1), some evidence of acute or previous laminitis, and documentation of insulin dysregulation. However, some horses lack the typical clinical features of EMS (e.g., regional adiposity or obesity), and present with laminitis and insulin dysregulation when in normal or thin body condition (the “lean EMS phenotype”). Further, it seems that insulin dysregulation precedes the development of laminitis, so requiring laminitis as a diagnostic criteria limits EMS diagnosis to horses with more advanced and potentially career-ending or life-threatening disease. Thus, documentation of insulin dysregulation can be considered the minimum diagnostic criteria for making a tentative diagnosis of EMS.

However, it cannot be overemphasized that a number of scenarios can result in insulin dysregulation in horses, of which EMS is only one. Stress, endotoxemia, pregnancy, and dietary factors can all affect insulin regulation in horses, but most importantly, insulin dysregulation is a common component of PPID. Thus, to diagnose a horse or pony with insulin dysregulation due to EMS, these other potential causes must be excluded. Often, this can be done based on the animal’s signalment, history, and initial clinical exam, but in many cases (i.e., the 14-year-old horse in normal body condition with slight regional adiposity who founders when turned out on grass for the first time in September in the Southeast) PPID must be excluded as a cause of insulin dysregulation with the testing strategies described herein.

The most straightforward screening test for insulin dysregulation is measurement of a fasted insulin concentration. It is helpful to measure glucose on the same fasted sample given that accurate interpretation of the insulin concentration requires knowledge of the concurrent blood glucose level. A simple method for collection of this fasted sample is as follows:

1. Have the owner feed the horse normally in the late afternoon/evening, and then place it in a stall or dry lot.
2. At 2200 hours, give the horse a flake of grass hay, and then no further feed overnight (water is fine). Horses that will eat their bedding (or stall walls) should be placed in a grazing muzzle.
3. Arrive at the farm between 0800 and 0900 hours, and pull your blood sample, then have the owner feed the horse normally and return it to its normal routine while awaiting results.

Insulin and glucose can be run on serum or ethylenediaminetetraacetic acid (EDTA)-plasma; thus, if you are also testing for PPID you can just submit one frozen EDTA plasma sample as described above. Either way, it is still important to keep the sample cool after collection, and separate the cells from the serum or plasma as soon as possible (and within 4 h) to ensure accurate measurement of the glucose concentration. As long as the horse is fasted, season does not typically affect results of this test unless the horse has underlying PPID.

Most veterinary diagnostic laboratories use similar methodology for measurement of equine insulin, so across laboratories, a fasted insulin concentration greater than 20 \( \mu \text{IU/mL} \) is generally considered consistent with insulin dysregulation/inappropriate hyperinsulinemia. However, insulin concentrations should be interpreted in light of the glucose concentration; an animal with a high insulin concentration and a normal-to-high glucose concentration is exhibiting evidence of peripheral insulin resistance, whereas an animal with a low insulin concentration but hyperglycemia is actually more consistent with true diabetes mellitus (rare but reported in horses). Further, some animals with EMS will have a normal fasted insulin concentration but an inappropriate insulin response to feeding, which is not detected with this test but can be detected with dynamic testing described below.

Finally, as mentioned above, horses with stress or ongoing systemic illness can have transient insulin dysregulation and hyperinsulinemia, and could be falsely diagnosed with EMS based on a single increased fasted insulin concentration. When horses that are stressed (i.e., actively laminitic) or systemically ill must be tested for EMS, concurrently measuring plasma leptin concentration can be helpful. Leptin is a hormone produced by adipocytes, and it is increased in horses with EMS. Its secretion also seems less likely to be affected by stress and systemic illness, so it can be measured concurrently with insulin to provide additional information to support a diagnosis of EMS. Leptin can be measured on serum or EDTA-plasma processed and shipped as for measurement of insulin and ACTH described above.

Dynamic Testing

In general, dynamic testing measures the hormonal response to a specific stimulus to determine whether the endocrine response is appropriate or dysregulated. Currently recommended dynamic testing options for PPID and EMS are outlined below.

PPID Testing

A number of dynamic tests for PPID have been evaluated in horses, including the overnight dexamethasone suppression test (ODST), the thyrotropin releasing-hormone stimulation (TRH stim) test, the domperidone test, and the ACTH stimulation test. Current data do not support the use of the latter two tests as first-line testing for PPID, due to either lack of sensitivity and specificity for diagnosis of PPID or lack of large-scale studies to determine optimal testing strategies and diagnostic cutoffs.

The ODST has long been considered the gold-standard antemortem test for PPID in horses. The basic premise of the test exploits the negative feed-
back mechanism of steroid hormones on the pituitary gland. In a normal horse, administration of an exogenous adrenal steroid hormone (e.g., dexamethasone) exerts negative feedback on the pars distalis of the pituitary gland, resulting in decreased ACTH secretion. This decrease in ACTH secretion then results in a down-regulation of cortisol secretion from the adrenal gland, and causes the circulating cortisol concentration to decrease. In a horse with PPID, however, the hyperplastic or adenomatous pars intermedia in late-stage PPID is producing increased amounts of ACTH and does not respond via this negative feedback mechanism. Thus, administration of dexamethasone fails to cause a decrease in ACTH and cortisol concentrations in horses with PPID.

To perform the ODST, the horse is administered 40 μg/kg dexamethasone (0.04 mg/kg) IM between 1600 and 1800 hours, then housed and fed normally overnight. Nineteen to 20 hours later (~1100–1200 h the following day), a blood sample is collected into a red top tube for serum collection and measurement of cortisol concentration. A cortisol concentration greater than 1 μg/dL is consistent with a diagnosis of PPID.

This test is easy to do, as the owner can administer the dexamethasone, and thus only one trip to the farm is required. Cortisol is quite stable and no special sample handling or processing is required. However, this test should not be performed in the autumn (August to October in the northern hemisphere) because normal horses can have a false-positive response. This test also performs best in horse and ponies with overt and often late-stage clinical signs of PPID such as hypertrichosis/abnormal shedding patterns. Because these clinical signs are already very sensitive and specific for making a PPID diagnosis, confirmatory testing with the ODST in late-stage PPID may not be necessary. The performance of the ODST in early- and middle-stage PPID does not seem to be as good, but additional study is needed. Finally, given anecdotal reports of steroid-associated laminitis, some owners are concerned about administration of dexamethasone to horses at risk for or with active laminitis, which is often when testing for PPID is considered. Thus, the ODST, although still potentially useful for PPID diagnosis, has fallen out of favor as a test that is useful for early-stage disease.

Recent data suggest that the TRH stim test may be a more useful method for earlier diagnosis of PPID. In this test, the innate cross talk across endocrine axes is exploited. TRH is generally thought of as activating the hypothalamic-pituitary-thyroid axis, and that is its primary function. However, TRH also up-regulates the HPA axis by acting on anterior pituitary cells to induce ACTH secretion. In normal horses this response is modest, but in horses with PPID with hyperfunctional and/or hyperplastic pituitary tissue, the TRH-mediated ACTH response is greatly exaggerated.

To perform the TRH stim test, a baseline ACTH sample can be collected at the start of the test, although this is not necessary if the horse has already been screened for PPID with this test and found to be within normal limits. Administer 1 mg TRH IV per horse (or pony) and collect blood into a plastic EDTA tube 10 minutes and 30 minutes later. Adverse effects of TRH administration in horses seem to be minimal and most often include sweating, agitation, and phlegmen response in the author's experience. Handle the blood samples as for baseline ACTH measurement by keeping them cold and removing the plasma as soon as possible for frozen or chilled overnight shipment to the laboratory. ACTH concentrations at 10 minutes and 30 minutes greater than 100 and 35 pg/mL, respectively are consistent with a diagnosis of PPID. If desired, the 30 minute sample may be omitted to save time and decrease the test cost, as results at this time point may be most useful.

The TRH stimulation test using this protocol seems to have adequate sensitivity and specificity even in earlier stages of PPID, although false positives are still possible, and the effects of feeding and season are still not well characterized. Some studies document an effect of season on ACTH response to TRH, with higher values in the autumn even in normal horses, but large-scale studies to determine season-specific reference ranges are not yet available. Thus, at present, testing between December and June is recommended using the above cutoff values. Horses tested in the autumn with borderline or slightly abnormal results should be retested in the winter or spring until seasonal reference ranges are established.

One issue with performing this test in practice has been the availability of TRH. Pharmaceutical-grade TRH has been either not available or prohibitively expensive for equine use. The bulk of the published literature on this method has been performed using reagent-grade TRH that has been reconstituted with sterile fluid and then filtered in a research laboratory or large-scale pharmacy, then divided into 1-mg aliquots and frozen for individual patient use. We prepare it this way in our hospital and have had good results with the test on research and client animals. Until recently, because the necessary equipment for this process is generally limited to universities or large referral practices, this test has been impractical for general practitioners. Recently, however, compounded TRH has become available, and preliminary studies suggest that it has comparable efficacy with the previously used reagent-grade product, greatly expanding the clinical utility of this test in general practice.

EMS Testing
The gold-standard dynamic test for evaluation of insulin regulation in horses and ponies is the euglycemic-hyperinsulinemic clamp method, which is not practical for use in clinical practice due to its inva-
siveness, intense sampling protocol, and complex analysis. A number of other dynamic tests to assess insulin responses and document insulin resistance or dysregulation in horses and ponies with suspected EMS have been evaluated, and of these, the recently described oral sugar test is, in the author’s opinion, by far the simplest and most useful test for a field setting. Indeed, this is the test we currently use in our hospital for assessing insulin dynamics in horses and ponies because it directly assesses the animal’s insulin response to dietary carbohydrates, which is a key component of the pathophysiology of insulin dysregulation in animals with EMS.4

To perform this test, the animal should be stalled and fasted overnight, with one flake of hay offered at 2200 hours and nothing afterward as for measurement of resting insulin concentrations. At 0800 hours, pull a blood sample for measurement of baseline glucose and insulin as for resting insulin concentration above, then administer Light (not “Lite”) corn (Karo) syrup orally at 15 mL/100 kg. The simplest post-sugar sampling protocol involves pulling blood again 60 minutes later for measurement of the glucose and insulin response to the sugar challenge. Blood may be sampled more frequently (every 30 min for 2–3 h) to plot a response curve, but for most settings the single 60-minute sample will suffice. If the baseline sample insulin concentration is greater than 20 μIU/mL or the 60-minute sample insulin concentration is greater than 60 μIU/mL, the animal is considered to have an inappropriate insulin response to sugar consistent with EMS. Animals with 60-minute insulin concentrations between 45 and 60 μIU/mL are considered equivocal and should be retested.

A common question from owners is “will the sugar in the Karo syrup make my horse founder” by stimulating an increase in plasma insulin in an at-risk animal? To date, hundreds or more of these tests have been performed in normal and EMS horses at a number of institutions to validate the test in research animals and in client animals, and to our knowledge the risk of adverse effects seems to be very low.15

3. Results
In general, there are two scenarios in which testing for PPID and/or EMS should be considered: 1) in a laminitic animal, particularly when the development of acute or recurrent laminitis seems to be seasonal or pasture-associated; or 2) in a nonlaminitic animal that presents with some other clinical feature of these disorders. Because insulin dysregulation can be a component of both disorders and is associated with the development of laminitis, the goal of this diagnostic approach is to assess insulin responses and test for PPID. Even if the animal is ultimately diagnosed with PPID, the presence of concurrent insulin resistance in horses with PPID seems to be associated with an increased risk of laminitis and decreased survival time.2 Thus, insulin dysregulation is an important prognostic factor in a horse with PPID. Further, if the clinical signs of PPID are eventually well controlled with medication but the animal continues to have insulin dysregulation, that animal has both PPID and EMS, which requires additional therapy.

To illustrate the use of the above testing strategies in a clinical setting, Figure 1 outlines the comprehensive diagnostic approach to a horse or pony with acute pasture-associated laminitis, to rule in...
and out PPID and EMS. Portions of this approach can be applied to individual cases that require testing for one but not both of these diseases, but in general, testing for both disorders is important to avoid missing concurrent disease or atypical presentations.

Determining ideal timing for endocrine testing can be problematic in the actively laminitic animal given that the pain and stress associated with a laminitis episode can result in HPA axis up-regulation that raises circulating ACTH concentrations or can induce transient insulin resistance. Thus, test results in an animal with active laminitis can be difficult to interpret because high ACTH and/or insulin concentrations could be indicative of endocrine disease or a transient stress response. Unfortunately, the ideal timing for endocrine testing during or after a laminitis episode that minimizes the likelihood of false-positive results is not known, and likely varies in individual cases.

It is the author’s opinion that initial endocrine testing should still be performed in actively laminitic animals fairly early on, usually within 1–2 weeks of initiation of appropriate analgesic therapy and mechanical support of the affected feet, with follow-up testing at a later duration when the animal’s laminitis is under control. False positives are still possible during this time frame, but making an early diagnosis to help direct therapy can often ultimately limit the severity and duration of the laminitis episode. In addition, in chronic stress the HPA axis tends to be down-regulated over time, so the plasma ACTH concentration is unlikely to be falsely increased much beyond 1 to 2 weeks after the acute laminitis episode. Stress-induced insulin resistance may still be difficult to distinguish from true EMS at this time, but it is the author’s opinion that given the previously described links between hyperinsulinemia and laminitis, and the potentially fatal consequences of uncontrolled laminitis, even transient stress-induced insulin resistance likely warrants specific therapy with diet ± medication during an acute laminitis episode.

Thus, in actively laminitic animals and borderline-positive results (such as moderately increased ACTH and fasting insulin concentrations), the author generally institutes appropriate medical and dietary therapy for endocrine disease as well as treatment for laminitis, and recommends repeat endocrine testing in 6 to 12 weeks once the laminitis is (hopefully) under control. However, this brings to light the other problematic aspect of endocrine testing: if and how to test the animal that is currently on medications for PPID or EMS, such as pergolide, metformin, and/or levothyroxine. Such medical therapy is sometimes initiated at the request of the owner without confirmatory diagnostic testing, or as above during an acute laminitis episode when test results might be falsely positive.

There is no perfect one-size-fits-all approach for this situation, and specific strategies depend greatly on individual animal characteristics and owner perspective. Testing can be performed while animals are on medical therapy for PPID and EMS, but normal results do not distinguish between animals who do not have the disease and those who are well controlled on the current medical regimen. If the animal was started on medical therapy with no previous testing, and the signalment or clinical signs are not consistent with the “diagnosis”—such as an obese 6-year-old horse with laminitis diagnosed with PPID and started on pergolide, or a shaggy 28-year-old pony diagnosed with EMS and started on metformin or levothyroxine—the animal can most likely be safely tapered off the medication over a period of 3 to 6 weeks and then tested appropriately after 2 to 4 weeks off of medication. Use caution, however, in discontinuing medical therapy in an animal who was started on it during an episode of laminitis because a flare-up of laminitis is possible when the therapy is discontinued. In those cases, a discussion with the owner regarding the risks of another laminitis episode vs the difficulty of interpreting test results in an animal on medical therapy is warranted and individual owner and animal factors should be considered.

Once an animal has been diagnosed with PPID and/or EMS, it is important to consider strategies for monitoring and follow-up testing. In the author’s experience, monitoring clinical signs and serum insulin responses is most helpful in both these diseases. In horses and ponies with PPID, resting plasma ACTH concentrations and TRH stimulation test responses tend to improve with pergolide treatment, but do not always return to normal and the degree of improvement in endocrine testing does not always correlate well with clinical improvement. Thus, for animals with PPID, the author re-evaluates the horse in 6 to 8 weeks after initiating treatment to insure clinical signs of PPID, and insulin dysregulation, if present, have improved. Thereafter, annual or semi-annual monitoring for recurrence of weight loss, hair coat/shedding abnormalities, or insulin dysregulation/hyperinsulinemia is most useful for determining when the pergolide dose should be increased in the author’s experience. For a younger animal with EMS, if its weight can be managed well and serum insulin concentration and oral sugar test responses return to normal when it is in appropriate body condition, simply monitoring with biannual physical exams to ensure continued maintenance of an appropriate body weight is generally adequate, with hormonal testing only necessary if the animal gains weight or has another episode of laminitis. For animals that remain obese or hyperinsulinemic despite dietary and medical therapy for EMS, assessment of fasting insulin concentrations and/or oral sugar test responses every 3 to 6 months is helpful for adjusting therapy.
4. Discussion
Diagnostic testing for PPID and EMS is an area of active research with constantly refined understanding of the etiology and pathophysiology of these diseases. Thus, recommendations for diagnostic tests have changed over time. Both of these disorders have complex pathology that vary among cases and likely occur on a spectrum of severity that changes over time as the disease progresses. Thus, it is unlikely that a single diagnostic testing approach will ever be applicable to or useful in all presentations of these diseases. It is important to remember that all of the tests described above can have false positives and false negatives, and that test results may be negative in the early stages of both PPID and EMS. Repeat testing in clinically suspect cases with negative initial results is strongly suggested.

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Declaration of Ethics
The Author declares that she has adhered to the Principles of Veterinary Medical Ethics of the AVMA.

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How to Sample Dermatologic Lesions for Submission to a Diagnostic Laboratory to Maximize Results

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Properly obtained diagnostic samples from primary lesions submitted to a veterinary diagnostic laboratory where the veterinary clinician has established a strong working relationship with laboratory professionals will result in a diagnosis in the majority of skin disease cases. Author’s address: University of Georgia, Department of Large Animal Medicine, Athens, GA 30628; e-mail: slwhite@uga.edu. © 2015 AAEP.

1. Introduction
Dermatologic disease is a common client complaint yet veterinarians often have difficulty in establishing a definitive diagnosis. Techniques for sampling dermatologic lesions to aid in diagnosis are simple and require limited equipment. Sample selection site, sample collection procedures, and sample submission all influence the quality of results obtained from a veterinary diagnostic laboratory. The purpose of this paper is to review sample collection and submission to maximize the ability of the diagnostic laboratory pathologists to obtain and provide useful results to the submitting clinician.

2. Materials and Methods
Medline, Pubmed, Agricola, and CAB databases were reviewed. The instructions for sample submission for several veterinary diagnostic laboratories were obtained from their websites. A limited number of diagnostic laboratory microbiologists and pathologists were interviewed for best practices and common errors in sample submission. This information was coupled with the author’s experience in obtaining dermatologic samples and the value of information received from the diagnostic laboratory in managing dermatologic conditions.

Procedures
Submission Forms
Evaluation of samples submitted to a diagnostic laboratory is done with consideration of the history and clinical context. Most diagnostic laboratory professionals agree that sample interpretation is 50–80% context.1 Thus, useful results obtained from dermatologic samples are dependent on appropriate information accompanying submitted material. Submission forms should include all patient information: owner name and animal name, species, age, sex, and breed. Submission containers should be labeled with the owner’s name and sample identification. If multiple samples are submitted, each container should also be labeled with the sampling location. Abbreviations or anagrams should not be used. What may be familiar among working clinicians may not be to diagnostic laboratory personnel. The history should be legible...
and informative such that the reader has a complete understanding of the patient’s circumstances and disease progress. A good way to ensure complete information is provided on a diagnostic requisition form is to answer the following questions posed by Pinson.1

- What is the primary reason for evaluation? This helps to define the problem for the laboratory diagnostician. Is the current skin lesion unresponsive to previous therapy? Is the lesion or disease process very unusual?
- What is the duration and frequency of the problem? Words such as acute, chronic, rapid, or slow have no specific meaning. Be precise in describing duration, progression, or growth, and/or recurrence of the lesion(s).
- What are the objective clinical findings? These are things that can be observed, described, measured, or quantified. It is important to indicate whether the disease is affecting the total health of the horse or not. This information provides the pathologist with important background information.
- What are your differential diagnoses? It is helpful to list these in order of probability. If the lesions are unusual, or if you have specific concerns about a possible differential diagnosis, this information should also be supplied.
- What specifically was sampled? For example, the junctional edge of a bullous lesion, a complete excision of a closed pustule, or a shave biopsy of the coronary band provides needed contextual information. If multiple lesions in different stages of development are biopsied, this information should also be included.
- What is the appearance of the tissue or lesion? In addition to a precise and concise written description, photographs of the patient and a close-up of the lesions and or biopsy site are helpful. Photographs can be included on electronic submission forms or, should the laboratory use paper forms, the owner’s name and your name can be linked to photographs submitted separately via email.

If there is question about proper submission of samples for a particular differential diagnosis, or unusual lesions are present, calling the laboratory ahead of time for guidance could shorten turnaround time and decrease the need for additional submissions or tests.

Cytology
Specimens for cytological examination can be collected in a variety of ways. Glass slides with a frosted end are preferable for ease of labeling. A pencil, rather than a pen, should be used to label slides as ink will dissolve during the automatic staining procedures used in many laboratories. Similarly, tape or other labels should not be applied to the slides because they might interfere with staining procedures performed by machine. Tape that encircles the slide will not allow the slide to lie flat on the stage of a microscope and thus will inhibit complete examination. If multiple slides are obtained, particularly if they are from different sites, the slides can be labeled with numbers or letters of the alphabet and a key provided with the submission form.

Impression smears are obtained by pressing firmly one or more times on a lesion that is wet or moist. Alternatively, the slide can be pulled across a lesion once. Rubbing the slide back and forth may disrupt cells and interfere with evaluation. Impression smears may also be made from the underside of crusts (e.g., when looking for Dermatophylius), from the cut surfaces of excised masses (such as tumors) or from extirpated nodules. Alternatively, scrapings may be used to sample underneath crusts, vesicles, or peeling stratum corneum. Intact pustules can be opened with the tip of a small needle and contents applied to a slide and subsequently smeared. Cotton or nylon tipped swabs are usually used when imprints, scrapings, or aspirates cannot be obtained, such as when evaluating draining tracts. The swab should be pre-moistened with isotonic fluid to minimize cell damage during sample collection and slide preparation. The swab should be rolled onto the slide in a single line to distribute the acquired material.4

Fine-needle aspirates can be obtained using a 20–22-gauge needle and a 6–10-cc syringe.3 Enough suction should be applied to aspirate tissue into the needle, but not so much that red-cell contamination occurs. Once the aspiration is complete, the syringe should be removed and air drawn into the syringe to expel the contents of the needle onto a slide. Very thick samples can be gently “squashed” between slides. The top slide is then pulled off the end of the sample slide to smear the material.5 The objective, regardless of acquisition method, is to obtain a single layer of cells on the slide given that multiple layers of cells are difficult to interpret. Ideally several slides are made. Slides should be rapidly and completely air dried and submitted unstained. Slides should not be placed in a refrigerator or freezer. The slides should be dried completely before packaging to avoid artifacts that severely distort the cells.6 One may wish to stain a slide (with Diff-Quicka or new methylene blue) at the clinic to ensure an appropriate sample has been obtained. If the material collected is greasy or waxy, or if the slide is made from a moistened swab, the slide should be heat fixed prior to staining or shipping.3 Heat fixation can be accomplished with a blow dryer directly on the slide from a distance of approximately 10 inches, a cigarette lighter, or a match. The slide should be held over the flame for 5 seconds without burning the slide or damaging the sample by overheating. If soot accumulates on the slide it should be wiped off either before or after the slide is stained.
Slides should be packaged in a slide holder surrounded by bubble wrap or polystyrene packing material and submitted in a sturdy container to avoid breakage during transport. Simple cardboard mailers or thin padded envelopes, even if labeled “fragile,” “breakable,” or “glass” are not adequate to protect the slides. Alternatively, slides may be submitted individually in a pill container prior to protective padding. Slides should be protected against moisture to avoid artifacts and never submitted in the same container as formalin-fixed tissue. Formalin fumes will fix the cells and render them unstainable.

To collect hair for suspected dermatophytosis, hairs should be plucked from the leading edge of a lesion. Alternatively, hair and crusts may be collected by brushing with a clean, small, stiff brush (such a toothbrush) or scraped with a scalpel at a lesion edge. Collected material should be placed in a test tube with a cotton plug or in a paper envelope. Moisture in a sealed tube may result in a proliferation of saprophytic fungi. These samples may be used for both cytology and culture.

Microbiology

Bacteriologic culture and sensitivity testing is necessary when initial therapy does not work, in deep skin infections with draining tracts or granulomas, when other bacteria besides cocci are seen on skin cytology, or if methicillin-resistant Staphylococcus is suspected. Any moist lesion will contain bacteria after 24 hours; therefore, attempt to culture lesions as soon as they occur or by opening a closed pustule. Samples may be obtained with a moistened cotton or nylon-tipped swab, by aspiration or from a tissue biopsy. Sterile equipment and aseptic technique should be used. Swabs or tissue samples should be obtained from the tissue exudate interface where the proliferation of organisms occur. Aspirated samples of purulent exudate generally are sparsely populated or devoid of live organisms. Multiple swabs should be obtained from the lesion site given that the laboratory will often set up a number of different tests on day 1 based on the information provided by the clinician. Swabs should be immediately placed in a non-nutritive transport media (such as a commercial culturette, www.veterinarylabsupply.com/culturettes.htm), and kept cool, as microorganisms die rapidly in dry conditions. Whenever possible submit tissue rather than tissue swabs. Tissue samples should be aseptically collected, wrapped in sterile gauze moistened with saline or lactated Ringer’s solution (LRS) and placed in an airtight sterile container. A ring block or regional anesthesia is preferred when obtaining a biopsy for culture because lidocaine and epinephrine inhibit the growth of many Gram-positive and Gram-negative bacteria, mycobacteria, and fungi. An unstained air dried and or fixed slide for a Gram stain should accompany the samples for microbial propagation.

Samples should be transported to the laboratory as rapidly as possible; ice packs and overnight shipping are recommended. Knowledge of the laboratory schedule is helpful if one can plan sample collection. Samples should arrive, if possible, in the morning so that tests may be set up on the arrival date. Ideally the diagnostic laboratory would operate fully 7 days a week and on holidays; however, many laboratories have only one, or a few, personnel that set up tests received on Friday afternoon or on Saturday, and subsequent tests are often delayed until the following Monday.

Limitations of bacterial culture include an overgrowth of contaminating organisms, organisms that do not grow or are slow growing, deficient culture technique, delayed shipment of the sample, and concurrent antibiotic therapy at the time of sampling. Organisms that are hard to grow may be identified by molecular techniques from tissue samples. If any unusual results occur, or if no growth occurs, consultation with the diagnostic laboratory microbiologist is advisable.

Samples submitted for culture of dermatophytes should be collected as described above. If a deep fungal infection is suspected a tissue sample is best for culture and molecular diagnostic techniques. A sample of tissue for histopathological evaluation should also be submitted to confirm that fungal isolates are pathogenic.

Biopsy

“When the clinician and pathologist truly work together, the skin biopsy can reflect the dermatologic diagnosis in more than 90% of cases.” Yet many clinicians are frustrated with vague diagnoses or descriptions of biopsies that leave the clinician and owner with the impression of little useful information. The selection of biopsy site, procurement of the biopsy, and proper handling and submission of biopsies are crucial to an informative interpretation of submitted material. A skin biopsy should be taken of any unusual or significant lesions, lesions that do not respond to empirical therapy in 3 weeks, any persistent ulcerated lesion, all neoplastic or suspected neoplastic diseases, when the biopsy is likely to be the definitive diagnostic test (such as immune-mediated diseases), and any lesions(s) where the treatment is likely to be time consuming, expensive, or dangerous.

Biopsies are best obtained from early developing lesions and not chronic ulcerated lesions. This step may require a careful complete examination of skin, especially if there is a generalized distribution of lesions. Biopsies from excoriated or traumatized lesions, found in many pruritic conditions, are not likely to be informative. Anti-inflammatory medications, especially corticosteroids, will alter an inflammatory disease process, rendering a biopsy less informative. Most authors recommend discontinuation of oral corticosteroids 2–3 weeks prior to a biopsy and 4–6 weeks if long-acting corticosteroids...
were given parenterally, even if new lesions are occurring.\textsuperscript{2,12} Lesions that are secondarily infected with bacteria or yeast should be treated prior to biopsy so the primary disease process can be identified.\textsuperscript{2}

All biopsies should include the dermis. Do not prepare or clip skin if the lesion is in the epidermis because preparation may remove important components of the lesion. For deeper lesions hair may be clipped with scissors. Instruments should not remove any crusts or scales and should not touch the skin. After obtaining the biopsy, specimens should be fixed in 10\% buffered neutral formalin (BNF). Formalin containers should have a top as wide as the container (do not use glass), seal completely, and the formalin solution should be no older than 1 year. If any precipitates are present in the formalin it is unsuitable for use.\textsuperscript{13} Autolysis of biopsy samples begins in less than 5 minutes; thus, biopsies should be placed in fixative immediately after they are obtained. If multiple biopsies are obtained, each biopsy should be placed in formalin in sequence, rather than waiting until all samples are collected.\textsuperscript{14}

Punch biopsies are useful for lesions of the epidermis and dermis, but not if the disease extends into the subcutaneous tissue. A new punch biopsy should be used for each patient. A minimum of a 6-mm biopsy punch should be used, and an 8-mm or larger punch is preferred.\textsuperscript{15} When obtaining a punch biopsy only abnormal skin should be included and the punch should be centered over the lesion. Using a permanent marker (such as a Sharpie) to mark the desired biopsy site prior to acquisition ensures the biopsy contains the part of the lesion selected. The junctional edge of a lesion should not be sampled with a punch. During processing by the histopathology laboratory, the circular sample is cut down the middle and each half placed face down in the cassette. Sections for slides are made from these cut surfaces. Skin coloration or other features that may be obvious on clinical examination lose their color and distinction once fixed in formalin and cannot be used for orientation.\textsuperscript{9} If it is important to examine the junctional edge of a lesion then an elliptical biopsy should be obtained.

If a biopsy is obtained from an area of alopecia the direction of the hair in surrounding normal skin should be marked with a permanent marker before taking the biopsy. On the submission form, request that the tissue be cut along the marked line. This will ensure that entire hair follicles are present in the resultant slide and avoid cross sections of hair follicles.\textsuperscript{15}

To ensure the disease process is properly represented several punch samples should be obtained, preferably from lesions in different stages of development. A minimum of three samples is recommended and some authors recommend five to eight.\textsuperscript{15,16} Most diagnostic laboratories have a single charge for three biopsies; some laboratories do not limit the number of skin biopsies that can be submitted from a patient on one submission form.\textsuperscript{6,7}

Care should be taken to ensure that local anesthetic is placed in the subcutaneous tissue under the biopsy site as infiltration of the local anesthetic into the skin will result in artifacts that can obscure pathological changes.\textsuperscript{16} The biopsy punch should be held perpendicular to the skin and turned in one direction while gentle pressure is applied. The biopsy should be carefully lifted using small forceps or a small needle, handling the subcutaneous tissue only, and cut by a scalpel blade (preferably) or small scissors to avoid creating stretching or crushing artifacts. If the crust detaches it should be wrapped in microscope lens paper and included with the biopsy.\textsuperscript{12} The submission form should indicate that the crust is separate and should be cut in. To avoid curling artifact, punch biopsies 8 mm or larger should be placed subcutaneous side down onto cardboard or a piece of a tongue depressor for a few seconds prior to being placed upside down in formalin for fixation. Any wrapped crust should also be submerged into the formalin for fixation.\textsuperscript{12,15}

An elliptical biopsy is preferable when the junctional area between normal and abnormal skin and/or evaluation of structures deep to the dermis is needed. The biopsy should include approximately one third normal skin and two thirds abnormal skin and be perpendicular to the lesion edge. Elliptical biopsies should be placed on a tongue depressor or cardboard, cut side down, with gentle pressure applied to adhere the biopsy. Preferably, the sample should be pinned in place (25-gauge needles work well) to avoid curling artifact.\textsuperscript{15} The entire unit is then placed in BNF, biopsy side down. The ratio of BNF to tissue should be 10:1 or greater to ensure complete fixation.

Excisional biopsies may be preferred when a small, circumscribed lesion can be completely removed. A wedge biopsy may be used when a large lesion, such as a tumor or other mass, requires deep tissue for evaluation. To avoid distortion of the tissue with local anesthetic an “L” or ring block or regional anesthesia should be used. Electrocautery or laser should not be used when obtaining the biopsy. Submitted tissue should be no thicker than 0.5–1 cm, as that is the limit of formalin penetration. If a mass larger than 1 cm has been removed, slice through most of the mass at 0.5–1-cm intervals to allow proper tissue fixation. If the margins of a lesion must be examined to determine whether a tumor has been completely removed, the edges may be marked with India ink (http://www.americanmastertech.com/india_ink.htm) by first submerging the mass in ink prior to formalin fixation. The ink will dry in approximately 60 seconds and the sample is then placed in BNF. Alternatively, each border of the sample may be marked with different colors of ink (http://www.cancerdiagnostics.com/CDI_Products.aspx?pid=25) and a key provided on the submission form.
Shave biopsies are often used on the coronary band due to concerns of subsequent abnormal hoof growth. Biopsies should be taken toward the heel bulbs. These small and fragile samples are best submitted on a sponge designed for histopathological use and placed in a cassette (http://www.mercedesmedical.com) prior to immersing in formalin. Plastic cassettes and sponges may be able to be obtained from the diagnostic laboratory on request. Alternatively, the biopsy may be wrapped in lens paper. The sample should be pushed into formalin to ensure fixation.

All samples containing biological materials or materials considered injurious if leaked should comply with the latest U.S. Postal Service shipping requirements. At a minimum, biological samples should be double bagged and surrounded by absorbent material to contain any leakage that might occur in transit. Formalin containers should be taped shut, double bagged, and preferably shipped in leak-proof (plastic) containers. Packages should be labeled “biological material.” Shipping instructions may be found on many diagnostic laboratory websites, such as https://ahdc.vet.cornel.edu/docs/shipping.

4. Discussion
With careful attention to appropriate collection and submission practices, high-quality diagnostic samples can be obtained. Samples should be submitted to an accredited veterinary diagnostic laboratory that has a dermatopathologist or a pathologist with a special interest in equine skin on staff. However, the quality of diagnostic services provided by the laboratory is largely dependent on the clinical and epidemiological information provided by the veterinary clinician. The best results from a diagnostic laboratory are obtained when the veterinary practitioner and diagnostic laboratory professionals work together as a team. Time spent in developing good communication, both written and oral, with veterinary diagnostic laboratory professionals greatly enhances the effectiveness of diagnostic laboratory services.

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Declaration of Ethics
The Author declares that she has adhered to the Principles of Veterinary Medical Ethics of the AVMA.

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How to Interpret Antimicrobial Susceptibility and Minimum Inhibitory Concentration Reports

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1. Introduction
Empirical antimicrobial therapy can often be used in practice based upon the body system(s) affected, most likely pathogen(s) involved, and their usual susceptibility profiles. However, in many situations, culture and sensitivity are warranted prior to or in conjunction with starting treatment, or in cases that fail to respond as expected. Susceptibility profiles are particularly important for recognizing and monitoring antimicrobial resistance patterns that may develop at a farm or in an equine hospital setting. Interpretation of culture and susceptibility reports for clinical use is not always clear cut because efficacy of an antibiotic depends upon not only susceptibility of the microbe in vitro, but also upon whether it is likely to reach high enough concentrations at the site of infection when administered at a given dose and route. The objectives of this paper are to briefly clarify the designation criteria used on antimicrobial susceptibility reports and their relevance to clinical use.

2. Materials and Methods
Bacterial susceptibilities to antibiotics are determined by assessing inhibition of bacterial growth in vitro via one of three methods: 1) Disk diffusion (Kirby-Bauer method), 2) gradient method (e.g., Etest strips) or 3) broth (or agar) dilution. The method used is not standardized across the board and depends upon laboratory preference. Both gradient and dilution methods produce a quantitative minimum inhibitory concentration (MIC), whereas disk diffusion only provides categorical data. The MIC is the lowest concentration (µg/mL) of the antibiotic that inhibits growth of a specific bacteria. The sensitivity and accuracy of these methods are influenced not only by sample handling in the field, but also by the standards of quality control in the laboratory.

3. Results
A culture and sensitivity report typically lists the bacterial species against a variety of antibiotics along with the interpretive criteria of susceptible (S), intermediate (I), or resistant (R), and usually also an MIC. Susceptible (S) indicates that at the recommended dose and route, the drug should be effective in treating an infection due to the bacterial isolate. Intermediate (I) indicates that the drug should be effective clinically at high doses or if the infection is at body sites where the drug is physiologically concentrated. Resistant (R) indicates that concentrations of the drug required to treat an infection caused by the bacterial isolate are too high to be achievable in vivo and clinical efficacy has not been reliable. Whether a bacterial isolate is designated as S, I, or R depends upon whether the MIC obtained is above or below the Clinical Laboratory Standards Institute (CLSI) clinical resistance breakpoint. Clinical resistance breakpoints are...
evidence-based cutoffs that are specific to bacteria-drug combinations and take into account inherent resistance, species, pharmacokinetics and site of infection. This explains why a bacteria may be designated susceptible to a drug with an MIC that is numerically higher than that for a drug it is resistant to. Unfortunately, there are relatively few veterinary-specific CLSI resistance breakpoints and thus sometimes criteria are extrapolated from human derived breakpoints. As such, some laboratories also use the designation of no interpretation (NI) alongside an MIC result if there is insufficient evidence or lack of established guidelines to assign an interpretive criteria (S, I, or R) to the specific drug-bacteria combination in that species. Equine specific CLSI clinical resistance breakpoints are only available for a few drug-bacteria-infection site combinations: gentamicin and Enterobacteriaceae, Pseudomonas aeruginosa, and Actinobacillus spp., penicillin and Staphylococcus spp. and Streptococcus spp. in respiratory and soft tissue infections, ampicillin and Streptococcus equi equi and S. equi zooepidemicus in respiratory infections, cefazolin and Escherichia coli and beta-hemolytic Streptococci in respiratory and genital tract infections and ceftiofur and S. equi zooepidemicus in respiratory infections.4

4. Discussion
Wherever possible, drugs with the designation of S should be chosen in practice. However, if choices are limited due to resistance, availability or significant economic reasons a drug with the designation of I or NI may be useable. In these instances, taking account of the site of infection (i.e., if the antibiotic is known to be concentrated at the known site of infection such as beta-lactams in the urine1), administering by local delivery (e.g., intra-articular, intracocular), or increasing the systemic dose, if appropriate, may well facilitate clinical efficacy. For example, penicillin against a Klebsiella obtained from a urine culture in a case of cystitis may be designated > 8 (NI) because the MIC is high but it may well be effective due to the fact that penicillin is so concentrated in urine.4 The important factor to determine in a case like this would be what the actual MIC was (i.e., was it 16 or did the laboratory stop diluting after 8?). In all cases, the concept of the mutant prevention concentration (MPC) and mutant selection window should always be kept in mind. The MPC is a higher concentration than the MIC and the range of concentrations between these two values is termed the mutant selection window.5 For example, if the MIC is 2 µg/mL and the MPC is 4 µg/mL, the mutant selection window equals drug concentrations that fall between 2 and 4 µg/mL. If drug concentrations in vivo fall into this range then the risk of resistance developing to the drug is much greater than either side of it. In general, this is more likely to occur with drugs designated I than S because drug concentrations at the site of infection are likely to be closer to the MIC. Using the above example of a mutant prevention window for 2–4 µg/mL, antibiotic X is designated I on a susceptibility panel and in a random sample of three horses, reaches concentrations of 2–5 µg/mL at the site of infection depending upon the horse. In horse 1 it reaches concentrations of 5 µg/mL and in this horse, antibiotic X will be effective. In horse 2 it reaches concentrations of 3.5 µg/mL and in this horse, antibiotic X will also be effective; however, resistance is much more likely to develop during the course of treatment in horse 2 than in horse 1. In horse 3 antibiotic X reaches concentrations of 2 µg/mL. In horse 3, resistance is no more likely to develop than in horse 2; however, there is a greater chance of concentrations falling below the MIC due to administration errors, particularly if given orally. In contrast, for the same bacterial pathogen, antibiotic Y, designated S on the susceptibility panel, reaches concentrations of 7–10 µg/mL at the site of infection in the same three horses. Thus, if available antibiotic Y is the better choice because it will be effective in all three horses and has the least chance of resistance developing during treatment. It is useful to note that combining antibiotics can sometimes significantly decrease the MPC, e.g., a macrolide in combination with rifampin6 and thus improve the chances of treatment efficacy, especially in long-term treatment because the chances of resistance emerging is greatly reduced. Unfortunately, the MPC is not routinely tested, but understanding of the concept helps with rational and responsible selection of antibiotics.

Acknowledgments

Declaration of Ethics
The Author declares that she has adhered to the Principles of Veterinary Medical Ethics of the AVMA.

Conflict of Interest
The Author declares no conflicts of interest.

References

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Autologous vaccination is an effective and safe adjunctive treatment for sarcoids. Authors’ address: University of Pennsylvania, New Bolton Center, Kennett Square, PA 19348; e-mail: dglevine@vet.upenn.edu. *Corresponding and presenting author. © 2015 AAEP.

1. Introduction
Sarcoids represent the most common skin tumor in horses. Multiple modalities are described in the literature with no one treatment accepted as the gold standard.

2. Materials and Methods
The medical records of all cases undergoing the autologous vaccination procedure from January 2009 to May 2014 were reviewed. Using sharp dissection, the lesion was debulked and the removed sarcoid was sectioned into multiple small-tissue cubes. The pieces were placed within liquid nitrogen and then implanted in an area located just ventral to the nuchal ligament.

The presence of multiple initial sarcoids, the initial tumor area in cm², the type of historical treatment, and the use of topical historical treatments were analyzed by logistic regression to identify association with tumor regrowth or a decrease in tumor size.

3. Results
The autologous vaccination procedure was performed on 15 geldings and three mares. The median time to follow-up from the autologous implantation was 10.5 months. Twelve of 16 (75%) cases demonstrated a decrease in number of sarcoids with 15 of 16 cases (93.8%) demonstrating a decrease in size of sarcoids. Complete regression was noted in 11 of 16 (68.8%) cases.

Mild complications were noted in seven of 16 cases (43.8%). The most common complication reported was swelling.

4. Discussion
Although the mechanism of the autologous preparation is unknown, we suspect the tissue acts as an immunomodulatory agent to stimulate a host response not only against the debulked lesion, but on other lesions on the body.

Acknowledgments

Declaration of Ethics
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The Authors declare no conflicts of interest.

Research Abstract—for more information, contact the corresponding author
Idiopathic Hemorrhage Associated With Anticoagulant Rodenticide Exposure in Exercising Horses

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Anticoagulant rodenticide (AR) exposure has been associated with idiopathic hemorrhage in horses experiencing sudden death while exercising at four California racetracks. Strenuous exercise may alter the toxicity threshold for ARs in horses. AR use should be carefully restricted around exercising horses until further information is available. Authors' addresses: School of Veterinary Medicine, University of California, Davis, CA 95616 (Arthur); California Animal Health and Food Safety Laboratory System, University of California, Davis, CA – San Bernardino branch, Davis, CA 92408 (Carvallo, Kinde, Nyaoke, Uzal); Davis branch (Poppenga, Diab, Hill), Davis, CA 95616; and California Horse Racing Board, Sacramento, CA 95825 (Salmon); e-mail: rmarthur@ucdavis.edu. © 2015 AAEP.

1. Introduction
Six horses originating from four different racetracks in California were submitted for necropsy and diagnostic evaluation. The six horses had a history of collapse and sudden death during or immediately after exercise. All horses showed massive hemo-peritoneum and/or hemorrhages in other cavities or organs. Traces of the ARs brodifacoum, diphacinone, or bromadiolone were detected in liver tissue from all six horses. The detected concentrations of ARs in the six horses were considerably less than what is typically thought to be associated with toxicity, although diagnostic liver concentrations have not been determined in most species. Other frequent causes of massive hemorrhages in horses were excluded in five of the six cases. One horse had a pelvic fracture that did not seem to be related to the abdominal hemorrhage. It is speculated that exercise-related physiological alterations or a combination of exercise and other unidentified factors present in a racetrack environment may reduce the toxicity threshold for ARs in strenuously exercising horses. The objective of this paper is to present the basis for the conclusion that ARs pose a potential and previously unrecognized risk to racehorses. Analysis of liver tissue for ARs should be performed whenever idiopathic hemorrhage is found in equine sudden deaths associated with exercise.

2. Materials and Methods
A full necropsy was performed on all six horses as previously described. All horses dying within Cal-
California Horse Racing Board (CHRB)–regulated racing or training racetrack facilities are required to undergo a necropsy. Necropsies are conducted under standard protocols designed for racehorses from CHRB-regulated facilities by pathologists within the California Animal Health and Food Safety Laboratory System (CAHFS), which is managed through the University of California at Davis School of Veterinary Medicine. The necropsies in this study were conducted at the CAHFS laboratories at San Bernardino for southern California tracks and Davis for northern California tracks.

Between December 2012 and September 2014, six horses submitted for necropsy were diagnosed as having idiopathic hemorrhage, primarily in the abdomen. No obvious cause for the hemorrhage was identified and AR testing was performed on liver samples. Traces of ARs were identified through analysis of liver tissue. A total of 374 horses, including the six reported here, were submitted from CHRB racetracks for necropsy during the 22-month timeframe described. Three of the cases were Thoroughbreds dying during or immediately after racing, one was a Thoroughbred training at a gallop and the other two were pony horses working on the track. A pony horse in the racetrack setting refers to a full-sized working horse that accompanies competitive horses during the post-parade and pre-race warmup or during training workouts. One pony horse was a Quarter Horse taking a horse to the starting gate to race; the other pony horse was a Thoroughbred working in the morning. The Thoroughbred pony collapsed on the track, was removed to the stable area equine hospital, and died shortly thereafter. All other horses died on the track. The six fatalities occurred at four different racetracks. One horse had shipped from a fifth racetrack the morning of its fatal race.

Anticoagulant screens were performed on prepared liver samples by liquid chromatography–tandem mass spectrometry using a previously published method modified for tissue analysis. Any sample testing positive for an anticoagulant was then quantitated by high-performance liquid chromatography using either ultraviolet diode array detection or fluorescence detection. The reporting limits in liver tissue are 0.01 ppm for brodifacoum; 0.05 ppm for bromadiolone, warfarin, and coumachlor; and 0.25 ppm for chlorophacinone, diphacinone, and difethialone.

Liver samples from 27 racehorses euthanized due to catastrophic musculoskeletal injuries were analyzed for ARs in the same manner as controls. The proportion of horses with idiopathic hemorrhage that had detectable AR in the liver was compared with the proportion of control horses that did not have detectable AR in the liver using Fisher’s exact test.

CHRB personnel conducted field investigations to determine AR use and identify possible exposure routes at California racetracks. The specific objective was to develop an understanding of all potential AR sources, types, distribution methods, and the associated risk of accessibility to the horses. On-site inspections of the stable areas were conducted at each track except one, Track A, which had closed. One horse had shipped into Track D in the morning from a fifth track, Track E. That track was similarly inspected. Interviews were conducted with stable managers, trainers, barn personnel, and either commercial pest control vendors and/or track personnel responsible for rodent control programs. Findings were recorded and evaluated relative to necropsy results.

3. Results

Case number, breed, age, sex, activity at time of death, pathological findings, AR identified at necropsy, track, and AR in use by track are listed in Table 1. The ARs identified at necropsy were brodifacoum, diphacinone, or bromadiolone. The most significant pathological finding in five of the six horses in the current series was severe hemoperitoneum, with a variable combination of hemothorax, hemopericardium, and mesenteric, muscular diaphragmatic, and/or pulmonary hemorrhages. One horse (Case 6) had multi-organ hemorrhagic diathesis but minimal hemoperitoneum. The hemorrhage into the abdomen, thorax, and pericardium was characterized by the presence of large quantities of mostly free unclotted blood within the corresponding cavity. The hemorrhage was characterized by approximately 8–20 liters of blood in the abdomen (Fig. 1) except for Case 6, in which there was a minimal hemoperitoneum; approximately 4–10 liters in the thorax except for Case 6, in which there was a minimal hemothorax; and approximately 0.5 liters in the pericardial sac except for Case 6, in which there was an estimated 2 liters of free blood. Case 6 was unusual in that it was the only case that did not die immediately on the track and showed a general hemorrhagic diathesis in multiple organs and tissues. After careful gross examination and detailed dissection of the main thoracic, abdominal, and pelvic blood vessels, no tears or other vascular lesions could be identified. One horse (Case 4) had an acute pelvic fracture with evidence of a pre-existing stress fracture with extensive hemorrhaging into tissues surrounding the fracture, but after careful examination, the extra-peritoneal hemorrhage did not seem to communicate with the abdomen.

Trace levels of brodifacoum were found in the livers of three horses; trace levels of bromadiolone were detected in two horses; and a trace level of diphacinone was found in one horse. No traces of ARs were identified in the liver samples from 27 horses submitted for necropsy.
racehorses euthanized due to catastrophic musculo-skeletal injuries used as controls. A Fisher’s exact test showed the probability of detectable AR in horses with idiopathic hemorrhage (6/6) was significantly higher ($P < .000001$) than in control horses (0/27).

The field investigation identified the rodent control program at each track, the AR used in those programs and the specific ARs that were being used during the period during which each horse died. Track inspections were conducted at Tracks B, C, D, and E. Track A had closed but a previous investigation had gathered pest control program information. Tracks B, D, and E had pest control programs managed by commercial vendors. Track A had a pest control program that used ARs in the stable area managed by track personnel. Track C provided mechanical traps to trainers and barn personnel but did not use a commercial vendor in the stable area. Commercial vendors are licensed and regulated in California by the Department of Pesticide Regulation. The ARs were distributed in sealed and secured hard-plastic bait stations outside the barns. The bait stations were not located near stalls when horses were on the grounds. All commercial vendors reported that they had instances in which the bait stations had been broken open and the bait looted or otherwise removed.

**Track A**

Track A used track maintenance personnel to manage their pest control program with bromadiolone, a second-generation AR, within enclosed, hard-plastic bait stations. Diphacinone, a first-generation AR was identified in Case 1.

**Track B**

Track B contracts with a commercial vendor for their pest control program. The vendor uses brodifacoum in sealed and secured bait traps when horses are present. When horses were not on the grounds, liquid diphacinone stations, including in stalls, were added to the brodifacoum program. Case 2 died at Track B with a brodifacoum finding and Case 6 died at Track B with a bromadiolone finding.

**Track C**

Track C does not use a commercial vendor in the stable area. Upon request, Track C management provides traps and labor support on an as-required
basis. Both Case 3 and Case 4 died with brodifacoum findings.

**Track D**

Track D contracted with a commercial vendor to manage their pest control program. The vendor was using the second-generation AR difethialone at the time Case 5 died at the track. Case 5 had shipped over from Track E the morning of the race. Bromadiolone was found at necropsy in Case 5.

**Track E**

Track E contracts with a commercial pest control vendor that uses bromadiolone. Case 5 had shipped from Track E the morning of his death to race at Track D. Bromadiolone was found at necropsy in Case 5.

**Personnel Interviews**

Barn personnel interviews were conducted at Tracks B, C, and E. Barn personnel provided statements that unauthorized and uncontrolled distribution of ARs was common. From statements made to investigators, unauthorized AR distribution occurs because barn personnel considered rodent control efforts inadequate and believed it was necessary to take matters into their own hands. Based on interviews, barn personnel were unaware that ARs presented a risk to the horses.

4. Discussion

Sudden deaths at California racetracks have been a particular interest in recent years after an unusual cluster of cases led to national press attention. Sudden deaths in racing and race training are more frequent than generally recognized. A review of sudden death in racing horses identified a number of causes of death including internal hemorrhaging.4 Excluding accidents and musculoskeletal-related causes for Thoroughbreds, 23/475 racing fatalities and 55/507 training fatalities could be attributed to sudden death at CHRB-regulated tracks between July 1, 2007 and June 30, 2013. For Thoroughbreds racing in California between July 1, 2007 and June 30, 2013, that translates to one sudden death for every 8789 racing starts and an estimated one sudden death for every 158,000 Thoroughbred training days.5 Case 1 was one of the horses in the aforementioned cluster of sudden deaths associated with one trainer. The finding of diphacinone in the liver of Case 1 was thought to be coincidental at the time but led to an expansion of toxicology testing to include ARs whenever the pathologist observed unusual hemorrhage. Five additional cases of AR findings in liver tissue of horses with sudden death while racing or training and idiopathic hemorrhage were subsequently identified during the next 22 months.

At gross necropsy, the most significant finding in five of the six horses was severe hemoperitoneum. The most frequent causes of hemoperitoneum in horses are neoplasia, uterine artery rupture, mesenteric injury, and trauma. Idiopathic hemoperitoneum is either the first or second most frequent classification of hemoperitoneum described in horses.6,7 After gross and/or microscopic examination of the tissue samples, common causes for hemoperitoneum were eliminated with the exception of AR intoxication and thrombocytopenia. Thrombocytopenia is an unlikely cause of hemorrhage due to the lack of other pathological signs. In addition to AR intoxication and drug reactions,8 thrombocytopenia is associated with disseminated intravascular coagulation and some neoplasias,9 which were not present in these horses. Although the pathologist was unable to identify a lesion into the peritoneum with the pelvic fracture in Case 4, the possibility that a sharp bone fragment produced a vascular tear that was not detected cannot be completely excluded.

ARs function as vitamin K antagonists by inhibiting vitamin K epoxide reductase, the enzyme that activates vitamin K. Their main effect is due to inhibition of the prothrombin complex (coagulation factors II, VII, IX, and X) as a result of interference with the action of vitamin K. The normal synthesis of coagulation proteins (factors I, II, VII, IX, and X) are in the liver. There is a latent period after AR exposure during which clotting factors already present are depleted but are no longer being produced due to the AR inhibition of coagulation factor synthesis. ARs are classified as first-generation ARs and second-generation ARs. The ARs identified in this study are the first-generation AR diphacinone and the second-generation ARs brodifacoum and bromadiolone. First-generation ARs are generally less toxic and have shorter half-lives.10 As rodenticides, first-generation ARs require multiple feedings to be effective. Several first-generation ARs have been used medically, including in horses. In horses, these compounds have shown limited effectiveness in the treatment of thrombotic diseases or diseases suspected or perceived of being of thrombotic origin, such as navicular disease, thrombophlebitis, chronic laminitis, disseminated intravascular coagulation, and verminous aneurysm.11 Diphacinone is one of the more toxic first-generation ARs and does not seem to have been reported as being used medically. Second-generation ARs were developed to counter resistance of rodent target species to first-generation ARs. Second-generation ARs are highly toxic and have longer half-lives than first-generation ARs. As rodenticides, a single feeding of second-generation ARs can be effective. ARs can be detected for much longer periods of time in the liver than in serum. The half-life of second-generation ARs in the livers of horses is unknown, but has been reported to be greater than 300 days for brodifacoum in mice.10
cannot be definitively determined whether the ARs caused the idiopathic hemorrhage in these cases. The finding of unclotted free blood in multiple body cavities is consistent with an underlying coagulopathy. Blood clotting parameters such as activated partial thromboplastin time and prothrombin time can be performed antemortem, but these tests cannot be evaluated using postmortem samples. The concentrations of brodifacoum, diphacinone, or bromadiolone were all reported as trace levels in liver tissue. Toxic doses for brodifacoum, diphacinone, or bromadiolone in horses must be extrapolated from other species or from occasional clinical case reports and there is considerable species variability. Available toxicokinetics for ARs is primarily based upon acute intoxications and serum concentrations. There is little information regarding chronic AR exposure and toxicity and how that relates to liver tissue concentrations as reported here. Although the AR concentrations were considered low, liver AR concentrations indicative of intoxication are largely unknown, and they are certainly unknown for the horse. A common factor in these cases is that all six horses died while undergoing exercise or immediately after exercise. Whether an unrecognized exercise-associated factor or factors lowers toxicity thresholds is speculative, but horses undergo dramatic physiological changes in response to exercise. There are well-documented marked increases in blood pressure, heart rate, and body temperature in strenuously exercising horses, and other physiological changes including pH alterations, release of vasoactive amines, or other metabolic mediators and byproducts. A number of drugs increase AR toxicity including the commonly used nonsteroidal anti-inflammatory drug phenylbutazone. Nonsteroidal anti-inflammatory drugs increase AR toxicity by displacing ARs from protein-binding sites. All three racing horses had received phenylbutazone within 48 hours of their deaths; the other three horses had either a record of phenylbutazone administration or barn personnel recollections of having received phenylbutazone within 30 days of death. Other drugs used on the racetrack that alter AR toxicity include phenytoin, salicylates, potentiated sulfas, and steroid hormones, but there was no specific history of any of these drugs being used in the six cases reported.

ARs are highly effective, cost efficient, and widely used for rodent control. Commercial pest control vendors interviewed were dubious that incidental low-level exposure could be associated with toxicity. AR baits are made highly palatable by the addition of saccharose, grains, and other material meant to attract rodents. Domestic animals are typically intoxicated accidentally by direct access and ingestion of relatively large amounts of the AR bait. Extrapolating reported acute exposure toxic dosages at just 10% of the lower LD₅₀ reported in dogs to horses with their respective AR bait concentration, a horse would need to consume nearly half a pound of brodifacoum AR bait or greater than 5 pounds of diphenyldione AR bait. Based on the investigation and interviews, horses did not seem to have had direct access to large amounts of AR bait. An alternative explanation would be chronic, low-level AR exposure. Given the long half-lives for ARs in liver tissue, especially the second-generation ARs brodifacoum and bromadiolone, bioaccumulation of these ARs in liver from chronic, low-level exposure should be possible. Whether this would result in an AR toxicosis is unknown and heretofore, unreported.

The relationship between AR use at the tracks and the ARs found in necropsies from horses dying at those tracks is inconsistent. In acute toxicosis, there should be a close relationship between the track’s rodent control program AR and the AR found at necropsy. That was not the case except for Case 2 and Case 5, and only the latter if AR exposure is assigned to the track. Case 5 shipped from the morning of its race. Racehorses in California change stabling locations frequently, including periods at off-track locations during rest periods, about which no information concerning AR use was available. Given the long biological half-lives of ARs and the possibility of bioaccumulation, confidently identifying the location of AR exposure is problematic. None of the barn personnel interviewed could specifically name the AR product they had used and an unidentified environmental source of AR exposure separate from the AR use in stables as described cannot be completely eliminated. ARs have relatively long environmental half-lives and have been identified in many wildlife species, but usually in secondary and tertiary predators.

Rodent infestations in stables are common and difficult to control. Even with grains kept in rodent proof containers as required at California racetracks, there is enough unavoidable spillage and other food sources to support large rodent populations at racetrack stables. There are numerous rodent nesting locations in any barn, but especially older wooden barns such as at Tracks B and E. Interviews with barn personnel made clear their use of ARs near horses was out of frustration with their barn rodent problem and from a lack of appreciation of potential horse health risks. Unauthorized and uncontrolled use of ARs by barn personnel is described as “freelancing” in communicating the AR risks to regulators and horseman. The CHRB has initiated a backside education program in collaboration with track management and trainers at Tracks B, C, and E. All personnel have been provided information with regard to the potential risks associated with AR use and have been instructed to contact track management with all rodent control concerns rather than freelance AR use.

There are the options to eliminate AR use at racetracks. Track D, which has a relatively small rodent infestation, has already discontinued use of second-generation ARs. At other tracks with significant rodent populations, trapping operations would be labor-intensive.
intensive, expensive, and of questionable efficacy. Barn personnel interviews suggest that without a rodent control program, rodent control measures will be initiated independently. Alternatives to AR use besides trapping are the non-ARs bromethalin and cholecalciferol. The disadvantage and serious concern is that there is no antidote for either of these rodenticides and there is no information as to their toxicity levels in horses. Whether these non-ARs would pose a greater or lesser risk to horses at racetracks is unknown. Non-ARs should not be considered a safe alternative without careful consideration of their potential risks.

This study demonstrates a very strong association between sudden death with idiopathic hemorrhage in exercising horses at racetracks in California and AR findings in liver tissue at necropsy. Toxicokinetic information for ARs in horses is lacking, especially as related to liver concentrations and chronic, low-level exposure. AR toxicity thresholds may be lowered by exercise or by exercise in combination with other management practices at racetracks. AR exposure should be restricted at racetracks and other locations stabling strenuously exercising horses until further information is available. AR exposure should be considered and evaluated through analysis of liver tissue whenever idiopathic hemorrhage is found in equine sudden deaths at racetracks or other equine sporting events.

Acknowledgments

Declaration of Ethics

The Authors declare that they have adhered to the Principles of Veterinary Medical Ethics of the AVMA.

Conflict of Interest

The Authors declare no conflicts of interest.

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aThermo LXQ, ThermoScientific, San Jose, CA 95134.
bAgilent 1100 series, Agilent Technologies Inc., Santa Clara, CA 95051.
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eTalon Ultrablok, Syngenta, Greensboro, NC 27409.
fTomcat, Motomco, Madison, WI 53704.
Biomechanics of Equine Mastication During Acute Unilateral Inflammation of the Temporomandibular Joint

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Synovitis caused by injection of lipopolysaccharide into the temporomandibular joint (TMJ), significantly alters the equine masticatory cycle. Horses continue eating on the opposite side of the mandible but some horses begin to quid. Authors’ addresses: Department of Large Animal Clinical Sciences, Western College of Veterinary Medicine, (Smyth, Carmalt), and College of Kinesthesiology (Treen, Lanovaz), University of Saskatchewan, SK S7N 5B4, Canada; e-mail: tts350@mail.usask.ca. *Corresponding and presenting author. © 2015 AAEP.

1. Introduction
The biomechanics of the normal equine masticatory cycle are understood, but the effects of TMJ disease on mastication are still unknown. Using an existing in vivo model of TMJ synovitis, the objective of this study was to determine whether unilateral inflammation of the equine TMJ would lead to alterations in the chewing cycle.

2. Materials and Methods
Six horses free of dental abnormalities were equipped with an optical motion tracking system. Horses were observed chewing grass hay over 3-minute intervals. Regardless of the side of mastication, all horses were injected in the left TMJ with lipopolysaccharide (0.0025 µg). Six hours post injection the horses were reassessed. All data were compared using paired t tests.

3. Results
Four of six horses developed effusion of the injected TMJs; two also began quidding. All horses injected on the original side of the power stroke switched sides post injection, the two injected on the contralateral side did not. All horses showed reduced vertical pitch of the mandible (mouth opening) but not lateral movement post injection. Overall rostrocaudal movement of the mandible did not change, but timing relative to the opening and closing phases of the cycle was different.

4. Discussion
Changes induced by TMJ inflammation were subtle in most horses. Even in those with demonstrable aversion behavior, horses continued to eat.

Acknowledgments
Declaration of Ethics
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Conflict of Interest
The Authors declare no conflicts of interest.
Comparison of Medical and Surgical Treatment in Horses Presenting With Temporohyoid Osteoarthropathy

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Temporohyoid osteoarthropathy (THO) is a common cause of neurologic disease characterized by facial and vestibulocochlear nerve deficits in horses. This disorder can impair athletic performance. The prognosis is favorable for return to previous athletic performance in horses undergoing surgical intervention. Authors’ address: The William R. Pritchard Veterinary Medical Teaching Hospital, School of Veterinary Medicine, University of California, Davis, CA 95616; e-mail: pablosvet@gmail.com. *Corresponding and presenting author. © 2015 AAEP.

1. Introduction
THO is a disorder of the stylohyoid and petrous temporal bones. Common clinical signs include facial and vestibulocochlear nerve dysfunction, secondary corneal ulceration, and head tossing; and less common signs include dysphagia and seizures. Both medical and surgical treatment options are available.

2. Materials and Methods
Medical records of horses diagnosed with THO were retrieved and signalment, history, clinical signs, diagnostic methods, treatment, and outcome were recorded for assessment. Statistical analysis was performed to compare outcome between horses treated medically and surgically.

3. Results
Seventy four horses (median age, 14 y; range, 3 to 29 y) with THO were identified between 1990 and 2014. Surgery was performed in 34/74 cases as follows: 27/34 had ceratohyoid ostectomy and 7/34 partial stylohyoid ostectomy. Horses treated surgically were more often affected bilaterally and presented more advanced signs of THO. Follow-up was available in 28/34 horses treated surgically; of these, 23 had marked improvement or complete resolution of signs following surgery. Follow-up information was available in 20/24 horses treated medically. Clinical signs were either unchanged or worsened in 11 of these 20 horses. Follow-up data was a mean of 1405 days (surgical; SD 1329.7 d) and 975 days (medical; SD 987.3 d) after start of therapy. Horses undergoing surgery had significantly higher complete recovery rate than horses treated medically.

4. Discussion
Horses undergoing surgery had a significantly better outcome independent of severity of disease for both life and return to athletic performance than horses treated medically.
Acknowledgments

Declaration of Ethics

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The Authors declare no conflicts of interest.

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A similar good survival to discharge can be expected for both groups following surgical correction. Horses with gastrosplenic ligament entrapments (GLE) are similar to epiploic foramen entrapments (EFE) with regard to age and sex predisposition; however, at presentation horses with EFE tend to be more systemically compromised. Authors’ addresses: The William R. Pritchard Veterinary Medical Teaching Hospital, (Kilcoyne); and Department of Surgical and Radiological Sciences (Dechant, Nieto), School of Veterinary Medicine, University of California, Davis, CA 95616; e-mail: isabellekilcoyne@hotmail.com. *Corresponding and presenting author. © 2015 AAEP.

1. Introduction
Few studies regarding the prevalence, predisposing factors, and clinical findings of horses presenting with entrapment of small intestine within the gastrosplenic ligament have been reported. The objectives of this study were to review cases presenting with entrapment of intestine in the gastrosplenic ligament and compare the signalment, diagnostic findings, complications following surgery, and short-term outcome with EFE given that it is the authors’ impression that case presentations for these two types of colic at our hospital were similar.

2. Materials and Methods
Records were reviewed for horses presenting between 1994 and 2012 for colic. Horses were included if a definitive diagnosis of a GLE or EFE was diagnosed at surgery or at postmortem examination. Signalment was compared with the total hospital colic population, and clinical findings were compared between gastrosplenic and epiploic foramen entrapments using t tests or $\chi^2$ tests. Significance was $P < .05$.

3. Results
Forty-three horses with intestinal herniation through the gastrosplenic ligament and 73 horses with herniation through the epiploic foramen were included. Geldings were overrepresented in both groups. Mean presenting heart rate and systemic and peritoneal lactate were significantly higher for horses with EFE. Survival to dis-
charge was similar for horses following surgical correction for both groups.

4. Discussion

There are similar favorable short-term survival rates for GLE and EFE if appropriate surgical intervention is performed. A history of prior colic surgery may increase the risk of EFE.

Acknowledgments

Declaration of Ethics

The Authors declare that they have adhered to the Principles of Veterinary Medical Ethics of the AVMA.

Conflict of Interest

The Authors declare no conflicts of interest.
Variations in the Computed Tomographic Appearance of the Equine Temporomandibular Joint: A Multi-Institution Study of 1,018 Cases

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Morphological variations occurred in a large number of normal horse temporomandibular joints. Shape and density variations in young horses are presumed to be associated with maturation. Cyst-like regions of the mandibular condyle and mineralization of the intra-articular disc in older horses suggest age-related degeneration. Authors’ addresses: Department of Large Animal Clinical Sciences, Western College of Veterinary Medicine, University of Saskatchewan, Saskatoon, SK S7N 5B4, Canada (Carmalt); Diagnostic Imaging, Department of Small Animals and Horses, University of Veterinary Medicine, Vienna, 1210 Vienna, Austria (Kneissl); Department of Clinical Sciences, College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Fort Collins, CO 80523-1601 and Department of Clinical Sciences, College of Veterinary Medicine, Cornell University, Ithaca, NY 14853 (Rawlinson); Tierärztliche Klinik, Grasweg 2, 86459 Gessertshausen, Germany (Zwick); Department of Veterinary Clinical Sciences, Ohio State University, Columbus, OH 43210 (Zekas); Clinic for Diagnostic Imaging, Vetsuisse Faculty, University of Zurich, Winterthurerstrasse 285c, 8057 Zurich, Switzerland (Ohlerth); and Clinic for Horses, University of Veterinary Medicine Hannover, Foundation, Buenteweg 9, 30559 Hannover, Germany (Bienert-Zeit); e-mail: james.carmalt@usask.ca. *Corresponding and presenting author. © 2015 AAEP.

1. Introduction

Computed tomography (CT) of the equine temporomandibular joint (TMJ) is becoming more common. This procedure is typically performed to investigate a clinical problem; however, large studies investigating variations in the normal joint do not exist. The objective in this study was to characterize variations in the equine TMJ and identify age-related features.

2. Materials and Methods

We reviewed a total of 1,365 horses without reported clinical TMJ problems that had a CT examination of the head or neck; of these, 1,018 were retained for interpretation. Age, breed, gender, the reason for the examination, and slice width were recorded in

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addition to the presence of alterations in contour and density of the mandibular and temporal condyles and the intra-articular disc. Logistic regression was used to determine whether variations in morphology were significantly associated with the variables examined.

3. Results
Morphological variations were found in 40% of the horses. Consistent, predictable alterations in the shape and density of the mandibular condyle occurred in horses aged <1 year. Hypodense regions in the mandibular condyles and hyperdense regions of the intra-articular disc were frequent in horses aged >1 year and significantly associated with age.

4. Discussion
An unexpectedly large number of normal horses had morphological changes. Further work is necessary to correlate clinical findings with these changes.

Acknowledgments

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Conflict of Interest
The Authors declare no conflicts of interest.
Factors Associated With Treatment, Colic Recurrence, and Survival in Horses With Nephrosplenic Entrapment

Brad B. Nelson, DVM, MS, DACVS-LA*; Audrey A. Ruple-Czerniak, DVM, MS, PhD, DACVPM; Dean A. Hendrickson, DVM, MS, DACVS; and Eileen S. Hackett, DVM, PhD, DACVS, DACVECC

Horses with nephrosplenic entrapment (NSE) have excellent long-term survival rates regardless of treatment. Horses undergoing laparoscopic ablation had decreased colic frequency after the procedure compared to an untreated cohort. Authors’ address: Department of Clinical Sciences, Colorado State University, 300 West Drake Road, Fort Collins, CO 80523; e-mail: brad.nelson@colostate.edu. *Corresponding and presenting author. © 2015 AAEP.

1. Introduction
NSE of the large colon is a common cause of colic in horses. The objective of this study was to determine long-term survival and factors associated with treatment success following NSE of the large colon and colic recurrence frequency following laparoscopic ablation relative to an untreated cohort.

2. Materials and Methods
Medical records of horses treated for NSE between January 1, 1999 and June 1, 2014 were reviewed. Data collected included signalment, physical examination parameters, laboratory results, diagnostic findings, and treatments. Factors associated with surgical treatment and non-survival were examined with separate multivariable logistic regression models to determine the influence on outcomes. Change in colic scores between groups was examined with a Mann-Whitney U test.

3. Results
During the study period, 247 NSE events were recorded in 225 horses. Of horses admitted for NSE, 90.2% survived to discharge, 86.2% survived 1 year, and 81.7% survived 2 years. Increased packed cell volume after correction ($P = .027$) and increased age ($P = .039$) were associated with short-term and 1-year non-survival, respectively. Colic frequency in horses undergoing laparoscopic ablation decreased following the procedure when compared with horses without an ablation ($P = .003$). After discharge for laparoscopic ablation, all 34 horses with 1-year follow-up available were alive.

4. Discussion
Survival is high following NSE correction. Decreased colic frequency following laparoscopic ablation suggests successful colic prophylaxis.

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Conflict of Interest
The Authors declare no conflicts of interest.
How to Perform Percutaneous Cecal and Colonic Trocarization in Horses With Gastrointestinal Colic

Elizabeth M. Tadros, DVM, PhD, DACVIM

1. Introduction

Acute abdominal pain, commonly referred to as colic, is frequently encountered in equine medicine and is most often caused by gastrointestinal disease. The reported incidence of colic in the general population ranges from 3.5 to 10.6 cases per 100 horses per year.1–3 Most episodes of colic are managed without surgical intervention and involve diseases of the large colon.1–6 In a study of 604 horses admitted to an equine hospital in Canada, large colon impactions and displacements were the two most common causes of colic, with colon impactions accounting for 38.4% of medically managed cases.6 Of 935 horses admitted to a referral center in South Africa, medical colic was caused by large colon impaction, tympany, or displacement in 52% of cases.4 Although the specific gastrointestinal lesions are not identified in most cases in the primary care setting, 20% of colic episodes were attributed to intestinal impactions or primary tympany in one study.5

Cecal and colonic gas distention occurs secondary to simple and strangulating large intestinal obstruction or as primary tympany caused by abnormal fermentation of ingesta. Although gas can sometimes escape around simple large intestinal obstructions, complete obstruction results in rapid cecal and colonic distention as gas accumulates oral (proximal) to the lesion. If bowel distention is severe, increased intraluminal pressure is transmitted through the intestinal wall, causing venous occlusion, mural edema, and eventual reduction in arteriolar blood flow.7,8 Although ischemia of the bowel wall is more commonly associated with small intestinal distention, it has been observed after prolonged cecal distention, and experimental distention of the small colon to an intraluminal pressure of 40 mm Hg has been shown to reduce mural microvascular perfusion.9 In one study of horses with strangulating large colon volvulus, colonic luminal pressures greater than 38 cm H2O were associated with nonsurvival.10

Decompression of the cecum and colon can relieve pain associated with bowel distention and may prevent ischemia caused by high intraluminal pressure. Percutaneous trocarization of the cecum and large colon through both the right and left paralumbar fossae has been described,11 and a recent report suggests that transrectal trocarization is safe and effective in relieving cecal and colonic tympany.12 Historically, trocarization has largely been reserved...
for cases of severe abdominal discomfort when surgical exploration of the abdomen was not an option. Many practitioners perceive the risk of complications such as peritonitis and abscessation at the trocarization site as a significant impediment to routine application of the procedure. Although these complications can occur, they are infrequently reported when percutaneous trocarization is performed properly. In the author’s clinical practice, trocarization is routinely performed, and experience suggests that complications after the procedure are rare. This article will describe a method of percutaneous trocarization of the cecum and large colon that can routinely be used in the management of equine colic.

2. Materials and Methods

When performed on the right side of the abdomen, a 10 × 10-cm area overlying the right paralumbar fossa is clipped of hair and prepared with povidone-iodine surgical scrub. The trocar insertion site is identified at a point midway between the tuber coxae and the caudal rib, at the level of the cecal base (approximately 5 cm ventral to the tuber coxae in a 500-kg horse). Transabdominal ultrasonography is not routinely used to determine the location of trocar placement. The skin and body wall at the insertion site are infiltrated with 3 mL of 2% mepivacaine hydrochloride. Trocarization is performed using a 14-gauge, 133-mm-long polypropylene intravenous catheter with an attached intravenous tubing extension set (Fig. 1). Using aseptic technique, the catheter is inserted with the tip oriented perpendicular to the skin or slightly ventrally. The free end of the attached extension set is submerged in a small container of water, and the catheter is slowly advanced until gas bubbles are seen escaping from the tubing. This position is maintained until gas ceases to escape, and the trocar is not removed from the catheter during the procedure. If necessary, the catheter is partially withdrawn and repositioned several times to achieve maximal bowel decompression. A suction device can be attached to the end of the extension set to hasten the procedure, with periodic disconnection from the suction apparatus and submersion of the extension set end in water to ensure continued appropriate catheter placement. At the end of the procedure, 300 to 500 mg (3–5 mL) of gentamicin sulfate is infused through the catheter as it is removed from the abdomen.

Percutaneous trocarization on the left side of the abdomen is also performed within the paralumbar fossa; however, transabdominal ultrasonography is used to identify the precise location of trocar insertion and minimize the risk of inadvertently penetrating the spleen. Trocarization is only performed if the gas-filled colon is ultrasonographically visualized adjacent to the body wall.

3. Results

In the author’s experience, percutaneous trocarization and bowel decompression are effective in many cases, as determined by a reduction in abdominal circumference and a decrease in palpable large intestinal gas distention upon rectal examination. In addition, subjective assessment suggests that the procedure frequently alleviates pain, although this may only be temporary if the underlying cause of colic is not corrected.

Although retrospective analysis of records is convenient, determining the efficacy of percutaneous trocarization in resolving intestinal disease is difficult using this approach; owner willingness to undertake exploratory surgery, the likelihood that severe lesions more frequently prompt decompression, clinician preferences, and other undocumented factors confound any conclusions. Likewise, identifying complications of the procedure is problematic because peritonitis, intra-abdominal hemorrhage, and leakage of ingesta from the gastrointestinal tract could result from the underlying disease or concurrently performed procedures such as abdominocentesis or exploratory surgery. It is also possible that minor complications such as local cellulitis at the trocarization site are not always noted in the...
record, and subclinical peritonitis may not be identified unless other factors prompt an analysis of peritoneal fluid. That said, no catastrophic complications were thought to result directly from percutaneous cecal or colonic trocarization among approximately 100 horses medically managed for signs of colic at the author’s practice over the past 5 years, some of which underwent the procedure multiple times; the infrequent complications attributed to the procedure were minor and included local cellulitis at the catheter insertion site and mild peritoneal inflammation.

4. Discussion

Percutaneous cecal and colonic trocarization offers theoretical benefits in the treatment of nonstrangulating gastrointestinal lesions with large bowel distension and seems to be effective at reducing pain. Stress hormones and increased sympathetic tone caused by pain decrease gastrointestinal motility, making pain reduction important in these cases. Decompression of the colon could potentially facilitate correction of displacements without surgical intervention by decreasing colon size and allowing for more mobility within the abdomen; however, efficacy is difficult to assess using retrospective analysis of medical records. One report found that heart rate and the use of strong analgesic medication significantly decreased, and the number of horses with normal rectal findings significantly increased, after cecal decompression. Performing multiple percutaneous trocarization procedures did not increase morbidity or mortality in that study. Although another study demonstrated an association between repeated decompression procedures and mortality, a confounding factor is that horses with more severe disease require more aggressive care and are also less likely to survive. No serious complications were identified in either study; minor complications ascribed to trocarization included fever, diarrhea, peritonitis, local inflammation, cellulitis, and hematomas at the catheter insertion site. Although rare, life-threatening complications such as severe peritonitis, intra-abdominal abscess formation, and severe intra-abdominal hemorrhage have occurred on occasion. The overall complication rate is estimated at 5% to 10%. The risks of the procedure should therefore be weighed against its potential benefits in each individual case. The occurrence of complications may potentially be reduced by only performing the procedure when the gas-distended large intestine is palpable in close proximity to the body wall. Ultrasound guidance can also be used to avoid perforating organs other than the intestine while inserting the catheter.

Although surgical intervention should not be delayed in favor of bowel decompression, percutaneous trocarization may benefit horses that eventually require abdominal exploration. The procedure can even be performed while preparing for surgery. Intestinal viability may be preserved by decreasing intraluminal pressure, improving microvascular perfusion, and preventing bowel wall ischemia and necrosis until surgical correction is performed. Prolonged large colon distention can also injure myenteric plexuses and contribute to motility disorders postoperatively. In addition, high intra-abdominal pressure hinders venous return and ventilation while under anesthesia and positioned in dorsal recumbency. Anesthesia risk may be further increased by acute lung injury secondary to bowel compromise and systemic inflammation. One study in which experimental distention of the small colon was maintained at 40 mm Hg for 4 hours identified histological evidence of neutrophil infiltration and increased myeloperoxidase activity in the lungs of horses euthanized 12 hours later. Remote organ damage caused by systemic inflammation in these cases could also conceivably increase the risk of developing laminitis.

Penetration of the cecum or colon likely causes mild localized peritoneal inflammation, and possibly generalized peritonitis, even in many routine cases where no clinically recognized complications have arisen from the trocarization procedure. Although changes in peritoneal fluid nucleated cell counts have previously been attributed to “uncomplicated” cecal and colonic trocarization, the author is not aware of any studies that have systematically evaluated peritoneal fluid changes over time after these procedures. Ventral colon enterocentesis has been shown experimentally to increase peritoneal fluid nucleated cell counts and specific gravity within 4 hours of intestinal puncture, with abnormalities persisting for at least 2 to 4 days in peritoneal fluid collected from locations adjacent to the enterocentesis site. It is therefore conceivable that cecal and colonic trocarization could induce similar abnormalities and confound interpretations of subsequent peritoneal fluid analyses. However, the author has not recognized trocarization as a hindrance to interpreting peritoneal fluid analysis when determining the need for surgical intervention. It is possible that peritoneal inflammation remains localized dorsally at the trocarization site and is not reflected at remote locations in the ventral abdomen where routine abdominocentesis is performed, at least for the first few hours after the procedure, which is often when decisions regarding surgical intervention are made.

Although no controlled studies have examined whether cecal or colonic trocarization increases the risk of intraoperative or postoperative complications in horses that eventually undergo abdominal surgery, the author has not recognized such an association in surgical cases. It is unlikely that trocarization adversely affects surgical outcomes because the puncture site is small and often difficult to locate; if identified, it is associated with only mild focal intestinal wall inflammation. Routine administration of perioperative antibiotics likely mitigates
any additional risk of peritonitis attributable to the trocarization procedure itself. On the contrary, it is possible that trocarization reduces surgical risks by improving the safety of anesthesia in horses with severe abdominal distention. It may also hasten recovery by lessening intestinal wall injury.

In conclusion, the benefits of percutaneous cecal and colonic decompression include pain relief, potential improvement in intestinal wall perfusion, and safer anesthesia if abdominal surgery becomes necessary. Although not entirely without risk, anecdotal evidence and personal experience suggest that serious complications are rare when the procedure is performed correctly and appropriate cases are selected for treatment. Careful monitoring of the patient’s systemic stability and viability of the bowel is essential after trocarization to ensure that surgical intervention is not delayed in cases of strangulating lesions due to temporary pain relief provided by large intestinal decompression. This percutaneous trocarization procedure should be considered in the arsenal of therapies used routinely in the management of colic.

Acknowledgments

Declaration of Ethics
The Author declares that she has adhered to the Principles of Veterinary Medical Ethics of the AVMA.

Conflict of Interest
The Author declares no conflicts of interest.

References
Automated Fecal Egg Counting Using Fluorescence Labeling, Smart Phone Image Capture, and Computational Image Analysis

Paul Slusarewicz, PhD*; Christopher Mills; Gabriel Popa, MD; Martin Chow, PhD; Michael Mendenhall, PhD; David Rodgers, PhD; and Martin K. Nielsen, DVM, PhD, DEVPC, DACVM

Both strongyle and ascarid eggs in equine feces can be rapidly labeled by incubation with chitin binding domain conjugated to fluorescein. Fluorescent eggs can be imaged with a camera with an 8-mega-pixel sensor and accurately counted with image analysis software. Authors’ addresses: MEP Equine Solutions, 1105 Benjamin Lane, Lexington, KY 40513 (Slusarewicz, Mills); Gluck Equine Research Center (Slusarewicz, Nielsen) and Department of Molecular & Cellular Biochemistry (Popa, Chow, Mendenhall, Rodgers), University of Kentucky, Lexington, KY 40506; e-mail: pslusarewicz@mepequinesolutions.com. *Corresponding and presenting author. © 2015 AAEP.

1. Introduction
The inconvenience and poor performance of fecal egg counting methods has contributed to widespread prophylactic treatment and concomitant development of parasite resistance to anthelmintic drugs. The availability of a simpler, stall-side test could help veterinarians reverse this worrying trend of treatment without diagnosis. Chitin represents a potential universal egg marker that could be used to develop a simpler and more rapid test for parasite egg output.

2. Materials and Methods
Parasite eggs were fluorescently labeled with chitin binding domain. Eggs were imaged either by fluorescence microscopy or using a smartphone in conjunction with a fluorescence gel imager. Eggs were quantified computationally using a particle analysis algorithm.

3. Results
Chitin binding domain rapidly labeled ascarid, strongyle, trichostrongyle, trichurid, and coccidian ova collected from horses, cattle, goats, and cats when combined with a filter system to remove excess feces and trap the eggs. Labeling of eggs required brief bleaching in order to expose the chitin. Automated counting compared favorably with manual McMaster counts with regard to precision while appearing to be more sensitive.

4. Discussion
Fluorescent chitin-based labeling, imaging, and electronic counting seems to be a viable approach

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toward developing a rapid and convenient on-site diagnostic alternative to current manual flotation egg counting methodologies.

Acknowledgments

Declaration of Ethics

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Conflict of Interest

Drs. Slusarewicz and Nielsen and Mr. Mills hold stock in MEP Equine Solutions LLC, which is currently in the process of developing and commercializing a chitin-based fecal egg counting technology. Dr. Slusarewicz is an employee of MEP Equine Solutions LLC. Part of this work was supported by a grant from MEP to Dr. Nielsen.
Reevaluation of Rates for Maintenance Fluid Therapy in Horses: Some Help for the Fluid Shortage

David E. Freeman, MVB, PhD, DACVS*; Alexandra Abramson-Mooney, DVM; Steeve Giguère, DVM, PhD, DACVIM; Patricia Diskant, BSc; James Burrow, BSc; and Chloe Evetts, BSc

Maintenance water requirements for horses denied food or not eating are considerably lower than for fed horses, so water supplementation of these horses could be reduced accordingly to prevent overhydration and waste. Authors’ addresses: Department of Large Animal Clinical Sciences, College of Veterinary Medicine, University of Florida, Gainesville, FL 32610-0136 (Freeman, Diskant, Burrow, Evetts); 25 S. Tamarac St, Denver, CO 80230 (Abramson-Mooney); and Department of Large Animal Medicine, College of Veterinary Medicine, University of Georgia, Athens, GA 30602 (Giguère); e-mail: freemand@ufl.edu. *Corresponding and presenting author. © 2015 AAEP.

1. Introduction
Life-threatening losses of water and electrolytes in horses are restored with crystalloid replacement fluids similar in composition to plasma. Maintenance fluid therapy delivers fluids to meet daily needs for water in horses unwilling or unable to eat and drink or that are denied food and water because of their primary disease. Because maintenance requirements were established in fed animals, this study examined the effects of food deprivation on voluntary water consumption and correlated this with indices of dehydration.

2. Materials and Methods
Water intake, body weight, physical findings, and vital signs were recorded daily in eight healthy horses assigned 4 days of feeding and 4 days of food deprivation in a randomized crossover design. Packed cell volume (PCV), total protein (TP), electrolytes, osmolality, and triglycerides were measured daily in the trial periods and plasma and extracellular fluid volumes were measured in the last 8 hours. Data were analyzed with two-way ANOVA with repeated measures, with \( P < .05 \).

3. Results
Food deprivation immediately and persistently reduced water consumption to \( \sim 14.5\% \) of fed values, with laboratory evidence of mild dehydration on day 4.

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4. Discussion

These findings demonstrate the potential for overhydrating unfed horses when maintenance fluid therapy is guided by current recommendations. Such overhydration can be wasteful, create water and electrolyte imbalance, and interfere with recovery.

Acknowledgments

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Conflict of Interest
The Authors declare no conflicts of interest.
Review on Postoperative Ileus—What Can Equine Surgeons Learn From Other Species Including Man?

Michael Simon Kasparek, MD*; Martin Waselau, DVM; and Anja Carolin Kasparek, DVM

Many data exist concerning the pathophysiology and treatment of postoperative ileus (POI). For most drugs used to treat POI in man as well as in horses, conflicting data exist concerning the effectiveness of these drugs and very frequently there is more evidence that a drug is not effective than the other way round, but still we use these drugs because we do not have a better, more effective alternative. Furthermore, it is sometimes challenging to identify the species in which this information was generated. Therefore, POI remains a significant clinical problem in man and obviously a life-threatening one in horses. Given that the pathophysiology of POI is a multifactorial one, multimodal prevention and treatment of POI is probably the most effective way to address this problem in man as well as in horses. Authors' addresses: Department of Surgery, Ludwig-Maximilians-University Munich, Marchioninistrasse 13, 81377 Munich, Germany (M Kasparek); and Equine Hospital Aschheim, Gartenstrasse 14, 85609 Arschheim, Germany (Waselau, AC Kasparek); e-mail: dr.michael.kasparek@icloud.com *Corresponding and presenting author. © 2015 AAEP.

1. Introduction
In man, postoperative ileus (POI) after abdominal surgery remains a significant problem leading to patients’ discomfort, morbidity, and subsequently to additional costs in patient care estimated to be as high as $1B per year in the United States. During the last decades tremendous efforts have been made to improve our understanding of POI in man using basic science in animal models (particularly rodent models) but also in clinical studies in man. This extended our understanding of the pathophysiology of POI and enabled the introduction of mechanistic strategies to prevent and treat patients with POI (see below). Nevertheless, POI remains a clinical problem in man, probably due to its multifactorial pathophysiology. The purpose of this review is to first, give an overview about the current understanding of POI from the perspective of a human surgeon and scientist studying POI for several years (M.S.K.). Second, we want to summarize the present understanding and therapy of POI in horses to identify further treatment options for POI for equine surgeons.

2. Current Understanding of the Pathophysiology of POI
It is of note that our understanding of the pathophysiology of POI is based mainly on observations from rodent models in which POI was induced by standardized mechanical manipulation of the small intestine. POI in man is characterized by a biphase...
ic response to the operative trauma with an early neurogenic inhibition of intestinal transit followed by a later paralysis of gastrointestinal motility caused by an intramural inflammatory response.\(^2\)\(^-\)\(^4\) In the early neurogenic phase intestinal motility is inhibited by sympathetic reflex pathways as a response to trauma to shut down intestinal motility.\(^5\) The extent of this reflex inhibition depends on the severity of the surgical trauma. When the trauma is great enough, even vagal inhibitory pathways are activated and impair intestinal motility by the release of mediators such as nitric oxide or vasoactive intestinal polypeptide within the bowel wall.\(^3\)^\(^,\)\(^6\) Furthermore, intestinal manipulation leads to activation of afferent vagal pathways and sensitization of these vagal afferents, which then in turn leads to activation of vagal efferent neurons causing the release of inhibitory neurotransmitters from the enteric nervous system.\(^7\)^\(^-\)\(^11\)

A couple hours after the incident, neutrophils invade the muscularis externa of the bowel wall followed by the invasion of mast cells and macrophages 12 to 24 hours postoperatively.\(^7\)^\(^-\)\(^14\) These inflammatory cells cause the local release of inhibitory substances such as nitric oxide and prostaglandins that impair intestinal motility.\(^15\)^\(^,\)\(^16\) Interestingly, this happens also in unmanipulated parts of the intestine where activated inflammatory cells invade the muscular bowel wall. Dendritic cells, T-helper cells, and cytokines are involved in this homing of inflammatory cells to the intestinal tissue and the exact mechanisms behind this so-called "field-effect" have just recently been described in detail.\(^17\)^\(^,\)\(^18\)

In the later inflammatory phase an interaction between neurogenic and inflammatory mechanisms occurs, leading to an inflammation-induced activation of vagal afferent nuclei within the brainstem.\(^21\) Furthermore, the so-called cholinergic anti-inflammatory pathway has been described, which seems to enable the central nervous system to modulate and ameliorate the inflammatory response within the bowel wall using vagal cholinergic efferent fibers.\(^3\)^\(^,\)\(^19\)

### 3. Treatment of POI in Man

Besides normal laxatives and enemas a variety of treatment options were introduced to prevent and treat POI in man and most of them are at least somehow based on our understanding of the pathophysiology of POI as described above.

Peridural anaesthesia is used to interrupt the communication between the enteric nervous system, the spinal cord, and the central nervous system to prevent reflex inhibition of gastrointestinal motility.\(^20\)^\(^,\)\(^21\) Laparoscopic surgery minimizes the surgical trauma and reduces POI.\(^20\)^\(^,\)\(^22\) Furthermore, it has been demonstrated that restricted perioperative fluid regimens reduced POI.\(^23\) Early postoperative feeding is known to be safe and to increase intestinal motility in the postoperative period probably by vagal activation.\(^20\)^\(^,\)\(^24\)^\(^,\)\(^25\) Conflicting data exists concerning gum-chewing ("sham feeding") as a vagal stimulus that reduced the duration of POI only in some studies.\(^26\)^\(^,\)\(^27\) These interventions together with other crucial components of so-called fast-track or enhanced-recovery protocols that aim to speed up patient recovery, reduce the length of hospital stay, and subsequently reduce treatment costs without increasing morbidity of the surgical procedure.\(^28\)^\(^-\)\(^31\)

Although several pharmacological therapies are used to treat POI, the evidence for the positive effect of all these drugs is rather low. Neostigmine is frequently used and has at least shown some effect in one of our studies in patients after colorectal surgery.\(^32\) Some positive effect has also been shown for perioperative lidocaine, a drug that is also commonly used to treat POI in horses.\(^33\) Peripheral μ-receptor antagonists can reduce POI not only by preventing the motility-impairing effects of opioids administered as pain medication, but also by preventing the same detrimental effects caused by endogenously produced and released opioids.\(^34\)

Other drugs with prokinetic properties such as metoclopramide and erythromycin act mainly on the upper gastrointestinal tract whereas POI in man is a particular problem of the lower intestine. This explains the missing effect of these drugs on POI in man.

Other drugs have been used to modulate the inhibitory response within the bowel wall. Mast cell stabilization in man has little effect on POI\(^35\) whereas inactivation of macrophages in animals showed a more pronounced effect;\(^36\) however, it must be kept in mind that this interference with a physiological inflammatory response has the potential to cause other problems (e.g., in wound healing). This is supported by studies exploring the effect of inhibition of prostaglandin production by blocking cyclooxygenase (COX)-2. This treatment reduced POI but increased leak rates after intestinal resections in man.\(^37\)^\(^,\)\(^38\)

Vagal stimulation (e.g., by high lipid enteral feeding during POI) can ameliorate not only the intramural inflammatory response but also POI by the vagal cholinergic anti-inflammatory pathway.\(^39\)^\(^,\)\(^40\) Currently, potential beneficial effects of electrical stimulation of the vagus nerve on POI (e.g., by stimulating its branches in the area of the ear) are being explored.

### 4. POI in Horses

Although only little data exist on pathophysiology of POI in horses, the above-mentioned mechanisms contributing to POI in other species can probably also be applied to equine POI. A recent study determining the effect of different mechanical stimuli to the equine intestine demonstrated that the intramural inflammatory response as described in rodents can also be found in the intestinal wall of horses.\(^41\) This confirmed the clinical observation of Blikslager et al.\(^42\) made 10 years ago already, whereas POI in man can usually be managed conservatively with almost no increase in postoperative...
mortality rates, this seems to be different in horses where POI occurs more frequently (approximately 18% of horses after celiotomy \cite{33,44} and causes a high rate of repeat celiotomies and an almost 30-fold increase in postoperative mortality usually due to subsequent euthanasia \cite{45-47}. One reason why POI is more common in horses might be the fact that celiotomy is usually performed for colic surgery where parts of the intestine are obstructed or strangulated causing a mechanical stimulus that can initiate the intramural inflammatory response and also the “field effect” representing a pan-enteric inflammatory response and inhibition of gastrointestinal motility to a localized intestinal trauma that are both important in the pathophysiology of POI as described above \cite{41,48}. However, COX-2 inhibition and nonsteroidal anti-inflammatory drugs were not demonstrated to be effective in treatment of POI in horses although there is some beneficial effect in rodents and man \cite{49}.

Although several drugs are used to treat POI in horses, the evidence for the effectiveness of these drugs is usually low and the data are conflicting \cite{50}, a phenomenon that is shared by the data for the treatment of POI in man. The positive effects of the commonly used drugs can be summarized as follows: In a recent study intravenous lidocaine has been shown to improve POI as well as short-term survival after small intestinal colic surgery, which confirms the beneficial effect of systemic local anesthetics as described by others \cite{52}. Although metoclopramide, a peripheral dopamine antagonist and serotonin agonist that increases the acetylcholine concentration in the neuromuscular junction, has no effect on POI in man, it has been shown that metoclopramide can improve POI in horses and can even improve survival of POI in this species. The same is true for the macrolide antibiotic and motilin receptor agonist erythromycin that has no effect on POI in man, but seems to have at least some beneficial effect in horses. Cisapride has been shown to be very effective in the treatment of POI in several human studies; however, this drug is not available anymore, at least in Germany, due to its detrimental adverse effects in terms of cardiac dysrhythmia. Nevertheless, a few studies exist that describe a positive effect of cisapride on POI also in horses. Drugs acting on serotonin (5-HT) receptors such as mosapride and tegaserod have some potential to stimulate intestinal motility, but these drugs have only limited or no proven effect on POI. Although frequently used to treat POI in man as well as in horses because of its subjectively perceived effect on intestinal motility, there are very little data to support a real beneficial effect of neostigmine on POI in both species from basic-science and clinical studies.

Interestingly, a survey of Diplomates of the American College of Veterinary Surgeons on the use of prokinetic drugs for the treatment of POI showed that lidocaine is most commonly used followed by erythromycin, metoclopramide, and cisapride although the dosage as well as the route of administration varied significantly \cite{57}.

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#### Declaration of Ethics

The Authors declare that they have adhered to the Principles of Veterinary Medical Ethics of the AVMA.

#### Conflict of Interest

The Authors declare no conflicts of interest.

### References


Neurologic Examinations of Horses

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Neurologic examinations of horses can be done by the practicing clinician and must include the evaluation of behavior, state of consciousness, cranial nerves, posture and postural reactions, segmental reflexes, palpation, and gait evaluation. Examiners need to rely on sight (observation essential), touch (palpation), and hearing. Key points include safety first, observation, knowing what is normal, tailoring the exam to the individual (safety, domestication, cooperation), and performing more than one examination. Author’s address: School of Veterinary Medicine, Department of Medicine and Epidemiology, University of California, Davis, CA 95616; e-mail: mraleman@ucdavis.edu. © 2015 AAEP.

1. Introduction

This section is intended to provide some guidelines or recommendations on how to perform a neurologic examination and how to localize the observed deficits. The author strongly believes in learning functional neuroanatomy because it helps in understanding and interpreting the findings from the neurologic examination. This is essential for localizing the affected area within the nervous system. All clinicians follow their own method and order when performing a neurologic examination. The examination will be successful if it is thorough, follows a consistent order, and avoids overlooking abnormalities. Several authors have described how to perform neurologic examinations in horses; each description varies slightly, and a few references are provided in subsequent sections.1–7 A complete neurologic examination is warranted in horses with a suspected neurologic condition, unusual gait, nonlocalizable lameness, unexplainable weakness and muscle atrophy, and altered behavior or sleep, among others. The author also considers it important to include a neurologic examination in pre-purchase examinations to determine whether a horse is neurologically normal.

It is essential before performing a neurologic examination to obtain relevant information about the horse such as signalment (breed, gender, age); physical activity or intended use (athlete, companion, breeding, shows); a complete medical history that includes previous illnesses and/or any illnesses the horse may have been exposed to while on the farm (and the age and activity of the animals affected); and preventive medicine (vaccinations, deworming programs, oral/dental and hoof care). It is important to know how many animals are affected, how old they are, and what their diet, water source, and housing (e.g., stall, pasture, dry lot) consist of. Nutrition/toxic, infectious, and genetic disorders (in breeding farms) must be considered as possible causes of disease if several animals are affected in close proximity. Duration of disease (acute vs. chronic), progression of disease (nonprogressive vs. progressive), fever (especially important because of the potential risk of an infectious contagious disease), and the presence or absence of apparent pain
are important features that will aid in the investigation of possible causes of neurologic disease. A full physical examination followed by a thorough neurologic examination is essential because systemic illnesses can influence the neurologic status of the horse, particularly the status of neonatal foals. Furthermore, metabolic, nutritional, and/or toxic disorders might present with neurologic manifestations. Weakness is a sign that could present with systemic illness and neurologic disease. Therefore, it is important to determine the overall health status of the horse before beginning the examination.

2. Key Points of the Neurologic Examination

Key points of the neurologic examination are as follows:

1. Safety first.
2. Observation is essential.
3. Know what is normal.
4. Tailor the exam to the individual horse (safety, domestication, lack of cooperation).
5. Perform more than one exam.

It is necessary to emphasize the importance of safety. Because of a horse’s size, level of domestication, and neurologic status, not all components of the neurologic examination will be feasible (e.g., full gait evaluation in a severely affected horse or performing a close examination in wild horses). However, one essential component in performing a neurologic evaluation is observation. Examiners can learn so much about the neurologic status of any animal just by carefully observing it. Knowing what is normal is paramount, particularly when it comes to different gaits of various breeds. Tailor the exam to the individual horse. For example, if a horse is at risk of falling as a result of severe deficits, it is not necessary to evaluate its gait on a hill or curb; for safety reasons the examination for that particular horse is as complete as it can be. Finally, performing more than one neurologic examination is important and helpful. The examiner might observe deficits that have gone unnoticed (mild deficits), or, in the case of progressive disease, the neurologic condition might change. This last point is important because neuroanatomical localization depends on a thorough neurologic evaluation.

A Cautionary Note About Rabies

Even if the local prevalence of rabies is low or you have not seen it in your area, if the horse develops acute progressive neurologic signs you should consider it as a possibility. The presence of wildlife, lack of vaccination in endemic areas, and increased traffic of animals across geographical areas (from endemic areas) can pose a risk. Protect yourself and others, minimize the number of personnel handling the horse, and wear protective clothing (do not touch without gloves). In endemic areas with a horse for which routine vaccination practices are minimal or nonexistent, wear gloves, protective clothing, and face shields when dealing with horses with acute neurologic disease. Rabies often presents initially with signs that are not perceived as being neurologic, such as lameness, fever, and colic; however, if undiagnosed, more obvious neurologic signs will quickly begin to show.

Goals

The first goal of the neurologic examination is to determine whether a neurologic abnormality is present. The second goal is to determine the neuroanatomical localization, which refers to the affected area within the components of the nervous system. Remember that the nervous system consists of central and peripheral parts. The central components include the brain and spinal cord. The peripheral parts include the nerve roots and ganglia, nerves, and neuromuscular junction. The neuromuscular system consists of central and peripheral components. All nerves are peripheral (cranial nerves, spinal nerves). The cauda equina is formed by sacral and caudal nerve roots and nerves. Do not forget the autonomic nervous system, which includes the sympathetic, parasympathetic, and intrinsic/enteric plexuses. Functional neuroanatomies for neuroanatomical localizations are discussed later.

3. How to Perform a Neurologic Examination

This section is merely a guideline and does not represent the only way to perform a neurologic examination. The first thing to note is that minimal equipment is needed. Remember that the most important equipment is your senses (i.e., sight [observation], touch [palpation], and hearing). Useful tools include a strong light source (transilluminator, ophthalmoscope, or pen light) and hemostats or a pen to assess pain sensation and to induce segmental (spinal) reflexes. Additional tools could include a sound source such as car keys or a ringing object (bell) to evaluate for hearing deficits. Examination under saddle is not recommended because of safety concerns. However, complicated cases or gait deficits only observed under saddle might require a rider, but the horse must be evaluated in hand before being ridden to determine whether it is safe. An example of a neurologic examination form the author has used is provided in the Appendix.

The examination consists of evaluating the neurologic status while the horse is at rest (static) and during movement (dynamic). Some authors divide the examination in 4 broad categories: (1) evaluation of mental status and behavior; (2) cranial nerve examination; (3) standing exam of posture, segmental reflexes, postural reactions, and muscle; (4) examination of gait and posture during movement. The author divides the examination in the following manner:

1. Behavior and mentation
2. Cranial nerves
3. Posture (head, neck, trunk, limbs, tail)
4. Postural reactions (i.e., proprioceptive positioning)
5. Segmental reflexes
6. Palpation
7. Gait evaluation
8. Nociception (pain perception)

Behavior and Mentation
Try to observe the horse in its own environment and note its behavior and how it interacts with others (humans and animals). In referral institutions, allow the horse to get some habituation (sometimes not possible) to the surroundings and observe it in confined and open areas. Keep an open mind but listen to what the owner has to say about the behavior of the horse. Usually, subtle changes in behavior are first noted by the owner. Note that behavior alterations might not always be caused by a neurologic disease (i.e., pain, learned behavior). Behavior is important even if the horse is apparently bright and alert. For example, sudden aggression, fear, or docile behavior in horses that usually have the opposite behavior should raise a concern. Horses with encephalopathies (brain disease) can manifest compulsive behavior such as compulsive walking, yawning, biting, circling, head pressing, and appearing sleepy or blind.

The 4 states of consciousness (mental status) are as follows:

1. Normal: Bright, alert, responsive.
2. Obtunded: Quiet because of a neurologic disease—not a systemic one (this would be lethargic). Do not use the word “depressed” because this is a neurologic disorder in humans. In this state, the horse remains responsive to stimuli (visual, tactile, aural) and reacts to the environment. There could be degrees of obtundation such as mild, moderate, or severe on which the stimuli might have to be stronger for a response. These degrees of obtundation are subjective. In mild cases, the horse might not respond to someone walking in the stall but might react to a loud sound.
3. Stuporous: Severely altered mental status; unresponsive to minimal-to-moderate stimuli. Profound painful stimuli (pinching the skin or foot with hemostats) generates a response of the animal (“waking up”), but the response dissipates (goes back to stuporous) as soon as the stimuli stop. Horse appears as if dead.
4. Comatose: The horse is unresponsive to any kind of stimuli, including profound painful stimuli.

Do not confuse response and reaction with reflexive movement when applying a painful stimulus. Alterations in behavior and/or state of consciousness indicate intracranial disease (forebrain, brainstem).

Cranial Nerves
Responses, reactions, and reflexes can be evaluated in the standing horse at rest or in the recumbent horse. Cranial nerves can be evaluated in order from CN I to XII to avoid forgetting one or more or could be evaluated to include all functional regions. The author prefers functional regions, starting with sense of smell (subjective); all eye functions (menace, palpebral fissure, palpebral reflex, corneal reflex, dazzle reflex, pupillary light reflex, adaptation to light and darkness, eye globe position and retraction, physiologic nystagmus, tear production); jaw/facial motor, sensation, and symmetry; and eating/drinking (prehension, suction, tongue tone and movement, gag reflex). Do not change your method of evaluation, and always be consistent to avoid overlooking abnormalities. The examination specifically covers the following:

- Olfaction (smell): CN I, subjective evaluation and interpretation. Horses with interest in food might have normal olfaction.
- Menace response: CN II and VII, cerebral cortex, and cerebellum.
- Palpebral fissure: CN III and VII, sympathetic innervation.
- Palpebral reflex: CN V and VII (check medial and lateral palpebral).
- Trigeminal facial reaction/reflex: CN V and VII (touch face, nasal mucosa, and inner pinnae).
- Facial and nasal sensation: CN V (VII for inner pinnae).
- Pupillary light reflexes (direct and indirect): CN II and III.
- Corneal reflex: CN V, VI, and VII.
- Eye globe position: CN III, IV, and VI (VIII also contributes; rule out extraocular muscle disease or retrobulbar or periorcular mass). Upon head elevation, horses have mild ventral strabismus that is considered normal, but if all you see is the sclera that is considered abnormal.
- Eye globe retraction: Gently press the eye globe for retraction; do not perform this task if ocular disease (i.e., corneal ulceration) or eye contamination is a concern.
- Tear production: CN VII (Schirmer’s test on both eyes—compare).
- Physiologic nystagmus: Turn head side to side and observe ocular movements; movements should always be horizontal if head is moved from side to side and synchronous with the fast phase toward the direction of the head turn. Pathologic nystagmus, occurring at rest or when the head is held in a certain position, is an indication of vestibular disease (central or peripheral).
- Gag reflex: Offer food and observe swallowing or palpate the larynx externally, not directly (orally) like in small animals, to induce a gag—this is very subjective and might not reflect an obvious abnormality where there is one and definitively does not determine degree or stage of dysfunction.
Offering food also helps to test olfaction (subjectively—could cover eyes to see if the horse would smell), vision (visual perception of food), prehension (CN VII—the horse has particularly mobile lips, especially the upper lip), mastication (CN V), pushing bolus back to the throat (CN XII with contributions of V), and swallowing (CN IX–XII). Food packing can also be seen with CN VII dysfunction. The author provides small amounts of food of various kinds (pellets, cookies, grain, mash, hay) and water to look for abnormalities or difficulty from suction, prehension, masticating, and swallowing that might be apparent with one type of food but not with others. The author has found this very useful for identifying subtle deficits.

Do not forget to observe cervical musculature (CN XI). Do a fundic exam since the retina and optic nerve are a few of the structures from the nervous system that can be visualized. Nerves located within the guttural pouch can be visualized through endoscopy. The slap test in the horse to assess possible laryngeal function is subjective and can be evaluated in conjunction with endoscopy. Sympathetic innervation to the eye is also important (third eyelid, pupil size, palpebral fissure, eye lashes). Sympathetic denervation of the head in horses presents as miosis, ptosis, protrusion of the third eyelid with eyelashes pointing down, and sweating of the head ipsilaterally.

Posture and Postural Reactions
The posture of the head, neck, trunk, tail, and limbs is important. Head tilt, neck turn, whole body leaning, trunk turn or scoliosis, lordosis, or kyphosis, wide- or narrow-based stance, or tail down or up or pulled to the side could be observed with neurologic disease. Postural reactions, including proprioceptive placing and hopping tests, can be performed in the horse, but examiners must practice caution because of safety concerns. Proprioception is the sense of knowing the relative position of a particular point or region in space (e.g., limbs; Fig. 1). Foot placement is evaluated for the thoracic and pelvic limbs. Examiners can place one limb at a time in an unusual or uncomfortable position to assess recovery and how quickly the horse returns the limb to a normal position. This can be achieved by either placing one limb far apart from the other or by crossing one limb in front of the other and observing when and how the horse replaces the limb. The author does this when proprioceptive deficits are not obvious and prefers simply to observe the horse throughout the exam for foot placement. A repeated series of maneuvers followed by rest for several seconds to minutes to note foot placement provides reliable information of proprioception without exposing the handler and/or examiner to the possibility of being crushed if a horse were to collapse. This also applies for horses for whose lack of cooperation makes assessment difficult. Observing how these horses stand at multiple time points during the examination gives an idea whether proprioception might be abnormal. Note that horses with painful limbs might stand in an unusual or abnormal position to protect the affected limb. Furthermore, a compliant or trained horse might hold an abnormal stance and thereby complicate how the limb placement is interpreted. Horses with a mechanical lameness can also present with abnormal limb placement that does not result from neurologic dysfunction. In some cases, although not always, these problems may be obvious. Proprioceptive deficits alone are not a localizable neurologic deficit to a specific area because these areas can be seen in brain, spinal cord, and peripheral diseases.

Fig. 1. Horses displaying proprioceptive deficits. Note the abnormal limb placement.
Segmental Reflexes
The evaluation of segmental reflexes is limited in horses but should be tested whenever possible. Segmental reflexes that can be readily evaluated include cervicofacial/aortic, cutaneous trunci (panniculus), perianal, and perineal reflexes. Tendon and flexor (withdrawal) reflexes are not usually performed in ambulatory horses. If the horse is young or recumbent, reflex testing, which includes triceps, biceps, patellar, and gastrocnemius reflexes, can be performed. Tendon reflexes might be difficult to interpret in recumbent adult horses, but withdrawal reflexes should be assessed. In general, the larger the horse, the harder tendon reflexes are to test and interpret. Remember that the horse must be relaxed and recumbent for adequate assessment.

Palpation
Palpation of the horse’s body, including the head, can reveal abnormalities that are not obvious upon visual inspection. Check for symmetry, shape, pain, swelling, temperature, sweating, and masses. Palpate muscles, bones, and joints; flex and extend the joints to assess for pain and mobility. Examine the horse carefully for any muscle atrophy. Check tail and anal tones. Look for any loss of skin sensation (hypalgesia or analgesia), increased sensitivity (hyperesthesia), or abnormal sweating (sympathetic denervation).

Gait Evaluation
The causes of lameness or irregular gaits could stem from an orthopedic, musculoskeletal, or neurologic disease. More than one system could be involved, which complicates gait evaluation and interpretation. If safe, a full lameness exam should be performed, including observations at the walk, trot, and canter, to investigate how other systems involved contribute to the abnormal gait. Horses with pain might alter their gait to protect a painful limb. Caution must be taken when assessing gait because certain breeds of horses have been bred for a particular desired gait (e.g., “floaty” gait in Warmbloods, hyperextension gaits in Paso Fino and Peruvian Paso, pacing in Standardbreds). There could be a fine line between what is considered a desired (i.e., normal) and undesired gait when breeding horses for specific gaits. Examiners must be familiar with the gaits of different breeds and be aware of what is considered an acceptable gait for a specific breed. For example, a floaty gait in an American Quarter Horse will be considered abnormal. An acceptable (or normal) gait is a topic of discussion and debate among breeders, owners, trainers, and veterinarians. Gait evaluation could be challenging for the reasons previously outlined but also because horses could present with a concurrent orthopedic or muscle disease that can make gait evaluation even more challenging. There will be situations in which full gait evaluation might not be possible because of safety concerns, horses at risk of falling as a result of the severity of deficits, or lack of cooperation.

Movement abnormalities include dysmetrias (abnormal range of movement: hypermetria, hypometria), ataxia (incoordination), paraparesis (decreased voluntary movement), weakness (lack of strength), hyperextension/hyperflexion, and specific gait deficits associated with nerves deficits (e.g., radial, femoral, sciatic, peroneal, tibial). Horses can be evaluated at the walk, trot, and canter when safe. Helpful maneuvers include the following: walk and trot in straight line, walk in serpentine (zigzag), walk with head elevated, walk while pulling tail in each direction, spin in tight circles, walk on uneven ground (back and forth over curb or cavaletti, up and down hill), and walk backward. Using different surfaces (soft, hard, uneven, colored) can be very helpful to challenge locomotion skills, especially in horses with subtle deficits.

If ataxia is noted, try to determine whether it is caused by cerebellar, vestibular, or general proprioceptive (GP; commonly known as spinal) disease. To determine the type of ataxia, look at the rest of the neurologic status of the horse. Does the horse have cerebellar (hypermetria, intention tremors, lack of menace response) or vestibular (pathologic nystagmus, head tilt, body lean, circles in the direction of the head tilt) signs? If the answer is yes to one of these questions, then you can answer the first question. Horses with general proprioceptive ataxia can present with toe scuffing, dragging of the feet, delayed protraction, knuckling over, crossing over, stepping on itself, pivoting (leaving the foot stationary), circumduction (“swinging” of the limb), or uneven/irregular stride length. Examples of GP ataxia include horses with spinal cord disease caused by equine protozoal myelopathy (EPM), cervical vertebral compressive myelopathy, neuroaxonal dystrophy (NAD), and intervertebral disc disease with compression of the spinal cord, among others.

In addition to ataxia with spinal cord disease, paresis and upper motor neuron (UMN) or lower motor neuron (LMN) deficits depending on the location within the specific spinal cord segments can be seen. With UMN involvement, the gait/stride could be exaggerated (“upper” = hyper, exaggerated, elongated), whereas with LMN, the gait/stride is short and choppy with weakness that can result in a base-narrow stance and muscle fasciculations if severe. In addition, pronounced muscle atrophy can be observed. Horses with UMN deficits can also have muscle atrophy, but this will be more gradual over time compared with atrophy from LMN injury. In chronic cases, the presence of muscle atrophy alone cannot help in distinguishing UMN from LMN injury. See section on the spinal cord for more information.
Nociception (Pain Perception)
Nociception or pain perception (conscious perception of pain = cerebral awareness of pain) must be tested in horses with no voluntary movement or when voluntary movement is questionable or not observed. When testing for nociception, two things are normally expected: (1) a conscious response to painful stimuli (e.g., head turn or any other response that demonstrates that the horse feels what we are doing), and (2) a reflex movement away from the painful stimulus. To clarify, voluntary movement does not equal reflexive movement. Both are different, and each tests different things and final pathways. This means that if you see movement of a limb being tested in a recumbent horse, this might not necessarily be voluntary—it may be a reflex.

Neonatal Foals
It is paramount to become familiar with what constitutes normal neurologic status according to age, especially during the evolving neonatal period. A comprehensive clinical history for both mare and foal and periparturient events must be obtained in the case of neonatal foals. A full physical examination is essential for determining the overall health status of the foal. It is important to determine whether systemic disease is present and whether there is any cause or effect, association, or contribution to the neurologic status. Common neonatal disorders manifest with similar clinical signs such as weakness, reduced muscle tone, recumbency, reduced or absent suckle reflex, and dysphagia. Therefore, a meticulous clinical evaluation of the horse and diagnostic workup to rule out common neonatal disorders is crucial for directing proper therapy in addition to supportive care. There are important functional differences in the neurologic examination of foals, especially in neonatal foals as they mature, and adult horses. Therefore, recognizing what is normal according to age is essential. Alertness, responsiveness to the environment, and movement are different in utero, during birth, and in extra-uterine life. Foals respond and move in utero but not to the extent of extra-uterine life. In the birth canal, foals appear to be in a drowsy state and become minimally responsive; movement is also depressed. During the first few hours of extra-uterine life, neonates must hit key milestones that will result in successful functioning and survival. Furthermore, evolutionary differences between prey and predators have resulted in differences in neurologic function. For example, the menace response (a learned response) develops earlier in prey (e.g., 7–10 days in horses and cattle) compared with predators (several weeks). Prey such as horses and cattle are born with more developed brains than predators’ brains. Prey have functional vision and hearing and can stand and nurse in a relatively short time after birth compared with predators. Although brain development continues after birth, the cerebellar layers in prey are already distinct histologically at birth, whereas cerebellar layers are not completely differentiated at birth in predators. Cerebellar development and myelination in various parts of the nervous system might explain the “bouncy” gait in neonatal foals.

Appropriate neurologic function is not the only important component for successful effector functions such as suckling, swallowing, locomotion, and autonomic functions, among many others. All the anatomical and functional components must be intact (e.g., musculoskeletal, visceral). Failure of any of these components will result in dysfunction. The next section briefly reviews key points of the neurologic examination in neonatal foals. As mentioned for adult horses, the autonomic nervous system (parasympathetic, sympathetic, and intrinsic/enteric) is also part of the nervous system.

Neurologic Examination in Neonatal Foals
Here again, observation is paramount. The neurologic status of the normal neonatal foal goes through a transition from in utero to ex utero life. The APGAR (appearance, pulse, grimace, activity, respiration) score developed for the assessment of neonates in the postfoaling period (one minute postfoaling) consists of the following variables:

1. Heart rate (normal: regular, 60 beats per minute; abnormal: undetectable, irregular, or <60 beats per minute)
2. Respiration (normal: regular, 60 breaths per minute; abnormal: undetectable, irregular, or <60 breaths per minute)
3. Mucous membranes (normal: pink)
4. Muscle tone (normal: strong enough to be able to be in sternal recumbency)
5. Responsiveness: nasal stimulation (expected response: strong grimace, sneeze); ear tickle (expected response: head shake); back scratch (expected response: attempts to stand)

This evaluation can be repeated at 5 and 15 minutes postfoaling to determine whether veterinary intervention is needed. Important milestones include time to sternal recumbency within 1 to 2 minutes, alert and responsive to external (tactile, visual, auditory) stimuli within 5 minutes, suckle reflex present within the first 20 minutes, vocalizing in response to the dam’s nickering within 30 minutes, time to stand within 60 minutes (longer than 2 hours is considered abnormal), and time to nurse within 2 hours (more than 3 hours is abnormal) after birth. The author performs a neurologic evaluation concurrently with the physical examination in neonatal foals. Similarly, multiple assessments of the neurologic status are done per physical examinations. The neurologic examination must cover all areas cited for adult horses.

The determination of mentation, behavior, and posture can be done as the history is being taken or as the foal is being examined. States of conscious-
ness include bright alert and responsive, obtunded, stuporous, and comatose. Behavior includes bright alert and responsive to the environment, mare attachment, udder seeking, nursing, curious of the environment, and sleep. Head posture in neonatal foals has a “flexed” appearance at the atlanto-occipital joint compared with adults, and their stance is wide-based and becomes narrower within days after birth. Cranial nerve deficits might be apparent during the initial observation before approaching the horse. Palpation is essential for detecting areas of apparent pain, local temperature, muscle tone and symmetry, joint extension and flexion, and tail tone, among others. Tactile stimuli result in brisk exaggerated responses and reactions in normal foals compared with older animals. Segmental reflexes that can be evaluated in foals include cervicofacial, cutaneous trunci, bicep, triceps, patellar, gastrocnemius, flexor (withdrawal), anal, and perianal reflexes. The cross-extensor reflex may or may not be present in the neonatal period. If present, it is not considered abnormal and will not be apparent within a few days after birth. An extensor thrust reflex can also be seen in normal neonatal foals. Neonatal foals have a hypermetric gait that becomes more coordinated within 3 days after birth. Effects of systemic disease, orthopedic disease, congenital anomalies, motor deficits (from initiation of movement by the forebrain all the way to the nerves, neuromuscular junction, and muscle as the executors), and weakness can result in recumbency. Cutaneous sensation can be evaluated to investigate the presence or absence of sensory function. Nociception (conscious perception of pain) is only evaluated if voluntary motor function is absent or difficult to interpret. For the purposes of this article, diseases of neonatal foals will not be discussed, but consider congenital/hereditary disorders and other common neonatal diseases that affect the overall neurologic condition in foals.

**Neuroanatomical Localization**

There are several ways of classifying the nervous system. This section will divide the nervous system into its functional areas to ease the interpretation of neurologic findings and to localize the lesion. The functional areas and their subdivisions are shown in Fig. 2.

When localization to a specific or single area is not possible, consider diffuse or multifocal localization. Neuroanatomical localization along with the signalment and medical history (including information of duration, progression, fever, and apparent pain) of the horse will aid in the formulation of a list of possible causes of the disease. Based on this list of possible causes, a targeted diagnostic plan can be made. Without an appropriate neuroanatomical diagnosis, it is pointless to start thinking about diagnostic tests or treatments. As previously mentioned, do not forget the autonomic nervous system (parasympathetic, sympathetic, intrinsic/enteric), which is also part of the nervous system. Next, the most common signs of each of the functional areas will be mentioned. As a reminder, proprioceptive deficits alone are not a localizing sign because these deficits could be seen in brain (all major functional divisions), spinal cord, and peripheral diseases.

**Brain**

The brain has three primary divisions: prosencephalon (forebrain), mesencephalon (midbrain), and rhombencephalon (hindbrain). It consists of three main functional areas: cerebrothalamic, brainstem, and cerebellum (Fig. 3).

The cerebrothalamic (prosencephalon or forebrain) area includes the cerebrum, basal nuclei, limbic system, and thalamus. One or more signs might be observed, such as behavior alterations (compulsive, bizarre, manic); lack of initiation of movement; ignoring one side of the head and body (contralateral); central blindness; wide circles ipsilateral to the lesion; seizures; contralaterally decreased nociception; and/or contralateral proprioceptive deficits. Note that the thalamus belongs to the brainstem but behaves functionally more like the forebrain. The thalamus is an important relay center. Examples of diseases include hypoxic/ischemic encephalopathies, metabolic encephalopathies (ammonia, sodium disorders, bilirubin [neonatal isoerythrolysis in neonatal foals], viral encephalitis [Eastern, Western, Venezuelan equine encephalitis), trauma, neoplasia (rare), and hereditary epilepsies (Egyptian Arabian foals), among others.

The brainstem has components in the forebrain (thalamus), midbrain, and hindbrain (pons and medulla). Signs that might be observed include an altered state of consciousness or mental status (obtunded, stuporous, comatose), altered sleep, and multiple cranial nerve deficits. Ataxia can be observed with the involvement of tracts within the brainstem. Proprioceptive deficits are ipsilateral in brainstem disease (except for the thalamus—functionally include thalamus as part of the cerebrothalamic area). Vestibular (central) dysfunction can also be observed with brainstem disease because the vestibular nuclei and part of the vestibular tracts are within the brainstem. Examples of diseases in-
clude EPM, West Nile virus and other types of viral encephalitis, trauma, and neoplasia (e.g., melanoma in grey horses), among others.

The hallmark signs of disease in the cerebellum include intention tremors (tremors upon intended movement), hypermetria of all limbs (but particularly in thoracic limbs), ataxia, and plus/minus menace deficits. Furthermore, horses might rear up when being walked backward as a result of exaggerated and uncoordinated movement of the thoracic limbs. Examples of diseases include cerebellar abiotrophy in Arabian horses and cerebellar hypoplasia.

**Spinal Cord**

Gait deficits such as general proprioceptive ataxia, paresis, and UMN or LMN deficits depending on the location within specific spinal cord segments will be observed. With the involvement of LMN, weakness will also be observed. Lesions localized in the following spinal cord segments (not vertebral bodies) will present thusly:

- **C1-C5/6**: UMN deficits (normal to exaggerated) of thoracic and pelvic limbs (sometimes also tail)
- **C6-T2**: LMN signs of thoracic limbs; UMN of pelvic limbs
- **T3-L3**: Normal thoracic limbs; UMN of pelvic limbs
- **L4-S2**: Normal thoracic limbs; LMN of pelvic limbs (plus or minus urinary/rectal depending on location—sacral segments)
- **S-caudal**: Normal thoracic limbs; normal or LMN of pelvic limbs depending on location because the sciatic nerve originates from cranial sacral segments; cauda equina signs (e.g., urinary/rectal incontinence)

UMN deficits of the tail can be observed with lesions cranial to the sacral segments. A grading system for neurologic gait deficits and ataxia was developed by Dr. Mayhew and is currently the method used by most clinicians. The author follows his grading system but have modified it to help teach students and residents and to minimize disagreement among examiners. Even then, the grading is still subjective and prone to individual interpretation. The deficits in gait/locomotion in the author’s modified system includes postural reactions, paresis, and ataxia. Simple grading system is as follows:

- **Grade 0**: Normal.
- **Grade 1**: Subtle deficits visible only under special circumstances and not always consistent.
- **Grade 2**: Mild deficits but visible at all gaits and tests, including walking in a straight line.
- **Grade 3**: Moderate deficits visible to any untrained eye and from a distance. Anyone can tell that something is wrong with the way the horse walks.
- **Grade 4**: Severe deficits with risk of falling easily even if just standing. Do not get close.
- **Grade 5**: Recumbent and unable to stand.

Examples of diseases that affect the spinal cord include cervical vertebral compressive myelopathy, EPM, NAD/equine degenerative myelopathy (EDM), herpesvirus myelopathy, other infectious causes, trauma, disc disease, neoplasia, and vascular anomaly, among others. Polyneuritis equi is an example of a disease that affects the cauda equina.

**Neuromuscular System**

As mentioned earlier, the neuromuscular system has central (lower motor neurons) and peripheral (nerve roots, ganglia, nerves, neuromuscular junction) components. Neuromuscular disorders can be diffuse or can involve only a single nerve. Diffuse
neuromuscular disease induces generalized weakness, difficulty supporting weight, base-narrow stance, paresis or paralysis, muscle fasciculations, and tendency to become recumbent. Segmental reflexes can be decreased or absent in neuromuscular disease. The two most common diffuse neuromuscular diseases of horses are equine motor neuron disease and botulism. Focal LMN disease or neuropathy lead to specific signs that pertain to the affected region, such as specific gait deficits and focal muscle atrophy.

Acknowledgments

Declaration of Ethics
The Author declares that she has adhered to the Principles of Veterinary Medical Ethics of the AVMA.

Conflict of Interest
The Author declares no conflicts of interest.

Appendix
### References

Clinical Recognition of Some Confusing Neurologic Syndromes

Robert J. MacKay, BVSc (Dist), PhD, DACVIM-LAIM

Modern equine veterinarians, armed only with patience, knowledge, a penlight, and a hemostat, should be able to locate a lesion and ultimately make a specific diagnosis. Author’s address: Department of Large Animal Clinical Sciences, College of Veterinary Medicine, University of Florida, Gainesville, FL 32610; e-mail: mackayr@ufl.edu. © 2015 AAEP.

1. Introduction

Ambulatory equine veterinarians cannot carry sophisticated electrodiagnostic or cross-sectional imaging equipment with them, and their clients often could not afford these diagnostics even if they could.1,2 Fortunately, armed with a penlight, a hemostat, some patience, and a little knowledge, modern equine clinicians should be able to work logically toward determining where the lesion(s) causing the clinical syndrome is located. The author tried to select a few syndromes that seem to commonly create confusion among veterinarians as they go about this process. Hopefully, working once again through selected clinical syndromes will help reinforce long-ago-learned principles of neurologic examination and neuroanatomic localization.

2. Syndrome 1: Mentation—Level of Consciousness and Behavior

Level of consciousness and behavior are two distinct components that should not be confused; veterinarians, however, frequently conflate the former, i.e., how alert an animal is, with the latter, i.e., how appropriately the animal interacts with the animate and inanimate environment. Although there are several overlaps between these two modalities of mentation, they are generated at separate sites: consciousness in the brainstem and behavior in the cerebrum (with assistance from the thalamus).

Alertness: Consciousness “originates” in the reticular formation, a loose network of neurons that extend longitudinally deep within the brainstem and continuously from the diencephalon to the medulla oblongata (Fig. 1). The reticular formation controls or modulates many basic life-sustaining processes such as heart rate and vascular tone, breathing and swallowing, maintenance of body tone, balance and posture, habituation to repetitive stimuli, and modulation of pain. Among the more than 100 networks that make up the reticular formation is the ascending reticular activating system (ARAS), which gathers and integrates stimulatory and inhibitory information from the internal and external environments and converts all of this information into waves of signals that are projected rostrally via thalamic connections to the cerebral cortex to keep the horse awake, i.e., conscious. The sources of stimulatory input are illustrated in Fig. 1. Most “alertness” signals projected to the forebrain from the ARAS are carried by monoamine neurotransmit-
ters such as norepinephrine, serotonin, and dopamine.

**Lesion localization:** As described in Dr. Monica Aleman’s lecture, during the first hour of this session, level of consciousness or alertness is evaluated on a continuous scale from normal (i.e., bright and alert) to comatose. As shown in Fig. 1, the ARAS is most active in the midbrain and progressively less so in more rostral and caudal sections. This knowledge can be used to predict the effect of a model injury at different levels of the brainstem. For example, a moderate bleed in the midbrain produces the most profound obtundation (coma for a serious injury); the same injury to the rostral medulla may only cause lethargy, and there may be no effect on consciousness if the injury were caudally in the medulla near the foramen magnum. The effect cerebral lesions has on consciousness is often very little unless there is a severe multifocal or diffuse involvement of the forebrain. In other words, it is possible for a horse to have a large mass lesion on one side of its cerebrum (e.g., cholesterol granuloma, Streptococcus equi abscess) and be fully alert. More diffuse processes, especially metabolic encephalopathies such as those of hepatoencephalopathy or hypo-osmolality, are accompanied by more profound obtundation, although there is likely also an effect on the brainstem.

**Behavior:** Abnormalities of behavior may be termed dementia. Typical abnormal behaviors that result from central nervous system (CNS) disease include self-mutilation, seizures, head-pressing, compulsive walking (often in a circle), yawning, aggression (including unprovoked biting or kicking), timidity, loss of affinity of a foal for its dam, and loss of learned behaviors and skills. The inability or refusal of a trained horse to follow when being led is a form of dementia that may be obvious during a neurologic examination.

**Lesion localization:** It must be kept in mind that bizarre behavior may be seen in horses without organic CNS disease. For example, such behavior is an appropriate response to an extreme stimulus (e.g., stinging nettle reaction), and stereotypical or otherwise destructive patterns of behavior such as self-mutilation syndrome can be extremely abnormal and difficult to differentiate from true CNS disease syndromes. However, for those disturbances of behavior that are caused by brain disease, the forebrain is almost exclusively the location. Focal, asymmetric lesions usually manifest with asymmetric abnormal signs. For example, the horse may turn its head (usually, but not always, toward the side of the lesion) and walk compulsively in circles, often around the inside of an enclosure. Such lesions also may cause partial (focal) seizures that at least initially affect one side of the body. Although anatomic mapping of dementia syndromes has not been carried out in detail in horses, involvement of the various components of the nuclei of the extrapyramidal system in the forebrain (and possibly midbrain) likely explains compulsive, repetitive, or bizarre motor activities such as fluphenazine reaction, compulsive circling, and the orofacial dystonia of nigropallidal encephalomalacia. The frontal and temporal cortices are involved with learned behavior, and the limbic system is involved with inherent behavior.

3. Syndrome 2: Menace Deficit—Where Is the Neurologic Lesion?

Almost all neurologic lesions of the visual pathways can be localized by carefully evaluating menace responses and direct and indirect pupillary light reflexes (PLRs). Additional information on vision is provided by obstacle tests. Accurate neuroanatomic localization then is used to generate likely differential diagnoses and diagnostic plans. The basics of this approach are summarized in Table 1 and Fig. 2, which together illustrate the effects of 6 model lesions on left pupil size in ambient light; menace responses, vision, and direct PLRs, all performed on the left eye; and indirect PLRs performed on both eyes.
The menace response can be interrupted anywhere in its afferent (visual) pathway from the eye via the optic nerve to the contralateral optic tract, lateral geniculate nucleus, and optic radiation to the occipital cortex. From the visual centers in the occipital lobe of the cortex, the menace response pathway continues via the contralateral motor cortex and then projects to the facial nucleus in the ipsilateral (i.e., same side) medulla oblongata (Fig. 2B).

Lesion 1—contralateral forebrain: At least 80% of optic nerve fibers cross at the optic chiasm in horses; thus, for routine clinical purposes in horses, vision in one eye is assumed to be perceived completely by the opposite visual cortex. If the menace response is defective in the left eye but there is no sign of cerebellar involvement and palpebral reflex (a test of ability to blink) and PLRs (II) are intact, then the causative lesion is contralateral and most likely located in the forebrain. Look for other signs of asymmetric forebrain disease such as compulsive walking in circles, other abnormal behavior, head and neck turn, obtundation, seizures, or reduced perception of touch to the side of the face opposite the lesion (i.e., the same side as the defective menace). Subtle proprioceptive deficits may also be appreciated on the side opposite the lesion.

Clinical examples: (1) large cholesterol granuloma in the right lateral ventricle in an aged mixed-breed mare and (2) a suckling foal kicked in the frontal/parietal area by a mare that is not its dam.

Lesion 2—ipsilateral cerebellum: In this situation, the horse has a defective menace response but nor-
nal vision. Before terminating on the facial nucleus in the ipsilateral medulla oblongata, the menace pathway passes through the ipsilateral cerebellum (Fig. 2A). For example, in normal neonates up to 2 weeks of age and in Arabian foals with cerebellar abiotrophy, there is no menace response, but the horse can see. In these settings, vigorous threatening gestures toward the eye may cause retraction of the eyeball and evasive movements of the head but not blinking. Head-bobbing, limb hypermetria, and ataxia are expected in horses with abnormal menace responses that result from cerebellar dysfunction.

**Clinical examples:** (1) melanoma in caudal fossa of skull that puts pressure on the cerebellum and (2) *Halicephalobus gingivalis* aberrant parasite migration.

**Lesion 3**—contralateral optic tract: Because “vision” fibers functionally cross en masse at the chiasma, transection of the right optic tract causes blindness and menace deficit in the left eye. However, enough “light” fibers project ipsilaterally to the midbrain to sustain a normal PLR, and because many of these fibers cross to the other side in the midbrain, both direct and indirect reflexes remain intact.

**Clinical examples:** Pressure from (1) expanding local tumor (pituitary adenoma, meningioma) and (2) *S. equi* (strangles) cerebral abscess.

**Lesion 4**—ipsilateral optic nerve: With injury to the left optic nerve, sensory input to all reflexes from that eye are lost, so the horse has defects in direct PLRs, menace and vision in the left eye, and indirect PLRs to the right eye. In contrast, motor innervation of the pupil is intact, so pupil size (responding to ambient light in the right eye) and indirect PLRs to the left eye are present.

**Clinical examples:** (1) a yearling colt that has flipped over backward while being handled, striking the poll, causing the cerebrum to “bounce” and tearing its connection to the left optic nerve; (2) a small ethmoid hematoma that grows backward in a Mammoth Mule molly and compresses the optic nerve against the sphenoidal bone.

**Lesion 5**—ipsilateral oculomotor nerve: Motor function to the sphincter of the right pupil is lost, so left PLRs (direct and indirect from the right eye) are absent, and the pupil is dilated and fixed; all other functions are normal.

**Clinical examples:** (1) peripheral: horse is kicked in the skull and fractures skull through the sphenoidal bone; the pupil is dilated and fixed, the eye is rotated laterally and down, and the upper eyelid is drooped; (2) central: after trauma to the left side of the skull, a portion of the left cerebrum herniates caudally (transstentorially) and compresses the oculomotor nerve.

**Lesion 6**—ipsilateral facial nerve: Inability to blink affects only the menace on the same side and is detected independently by performing the left palpebral reflex.

**Clinical examples:** (1) distal: horse cast in a stall overnight while wearing a halter and after thrashing in lateral recumbency injures the facial nerve behind the vertical ramus of the mandible; (2) proximal: great petrosal branch of the facial nerve damaged within the petrous temporal bone when that bone is fractured when temporohyoid osteoarthropathy is precipitated by placing a stomach tube in a horse with a fused temporohyoid joint.

### 4. Syndrome 3: Exposure Keratitis Versus Dry Eye—Different Levels of Facial Nerve Paralysis With Very Different Consequences for the Cornea

Maintenance of a tear film over the corneal surface is essential for normal corneal function and viability and requires both production of tear components (i.e., water, lipids, mucus) and their distribution across the corneal surface. Functions controlled by motor components of the facial nerve (VII) are involved in both phases: (1) a parasympathetic branch (great petrosal nerve) of VII exits the facial canal within the petrous temporal bone and innervates the water-producing lacrimal and nictitans glands, and (2) after the facial nerve emerges from the stylomastoid foramen of the petrous temporal bone, it gives rise to the auriculopalpebral nerve caudal to the vertical ramus of the mandible; palpebral branches of this nerve innervate the striated muscle that provides the blinking action—the orbicularis oculi. Blinking in turn serves to distribute the tear film and to squeeze out lipids from meibomian glands along the rim of the eyelids. Thus, injury of the facial nerve distal to the great petrosal nerve branch and proximal to the auriculopalpebral nerve causes eyelid paralysis and exposure keratitis while more proximal injury additionally causes dry eye (also known as keratoconjunctivitis sicca) because of the loss of lacrimal gland secretions.

**Exposure keratitis (somatic motor VII denervation):** In bright and otherwise healthy horses, eyelid paralysis frequently has no clinical effect. Presumably, in response to the irritation of incipient desiccation of the cornea in affected horses, there is frequent reflex retraction of the eyeball (Figs. 3 and 4). This is part of the corneal reflex that may be elicited during the neurologic examination and requires the trigeminal nerve (V) for its afferent component and abducent nerve (VI) for the efferent or
motor portion. Retraction of the globe pushes the third eyelid laterally across the entire cornea, an action that likely provides adequate tear film distribution. In obtunded animals, these protective reflexes are much less active, and a topical ophthalmic lubricant should be applied several times daily to provide additional lubrication and protection.

Dry eye (parasympathetic motor VII denervation): This is a medical emergency. Without preventive treatment, a deep horizontal corneal ulcer will form within 24 hours after cranial nerve VII denervation, and the eye will be irretrievably damaged within several days. Before the cornea is injured, tear film evaporation should be minimized by securing a partial third eyelid flap or split-thickness tarsorrhaphy that allows frequent topical medication. Reinnervation of the lacrimal gland by axonal sprouting from the uninjured segment of parasympathetic nerve is possible within a 6-month window after the time of injury. If natural tearing does not return, parotid salivary duct transposition could be considered, or the globe may need to be surgically removed.

5. Syndrome 4: Head Tilt and Circling—Differentiating Vestibular, Forebrain, and Musculoskeletal Causes

Combinations of a head tilt (i.e., rotation of the long axis of the head and not to be confused with a head turn caused by lateral bending of the neck) and repetitive walking in circles, usually in one direction, are often seen in horses suspected of having neck injuries and/or brain dysfunction. The explanation for these signs usually can be determined by successfully answering the three questions that follow.

Is the head tilt of vestibular or musculoskeletal origin? If it is possible to straighten out the head of a horse with rotational torticollis (i.e., rotation of the head originating in the neck), the eyeballs are found to sit correctly within the palpebral fissures, as shown in Fig. 5A. In contrast, the head of a horse with vestibular dysfunction, when forced into an upright position with the chin elevated, reveals vestibular strabismus; i.e., the eye on the side toward which the head was tilted is now rotated down (i.e., infraducted), and the other eye is in a normal position or rotated upward (i.e., supraducted; Fig. 5C). If the head of this horse is allowed to rotate back to its tilted position, the eyes will be in normal positions relative to the long axis of the skull (Fig. 5B).

Is the circling of vestibular or forebrain origin? A true head tilt is a consistent sign of asymmetric vestibular disease but is not a feature of forebrain dysfunction; thus, a horse that circles and tilts its head in the same direction shows asymmetric vestibular signs, whereas a horse that circles with its head upright likely has asymmetric forebrain disease. Additional distinguishing features include the following:

- Circle size: Small for vestibular and larger for forebrain.
- Circle completion: Incomplete for vestibular and complete and continuous for forebrain.
- Balance: Vestibular horses stagger and fall toward the inside of the circle, whereas forebrain dysfunction is associated with coordinated but compulsive locomotion.
Additional neurologic signs: Look for other cranial nerve dysfunctions (especially facial paresis) and/or signs of brainstem involvement (e.g., obtundation, limb weakness) in horses with suspected vestibular circling. A horse that is compulsively circling because of organic disease on one side of its forebrain (usually the side to which it is circling) may have additional signs of asymmetric forebrain dysfunction such as central blindness in the eye and/or hypoesthesia of the face on the side on the outside of the circle, abnormal behavior, and focal seizures.

Are the vestibular signs caused by a CNS or a peripheral lesion? Spontaneous and positional nystagmus are always pathologic and indicative of a lesion in the vestibular system. Peripheral vestibular lesions typically cause horizontal or rotatory nystagmus, although vertical nystagmus also is possible. With central lesions, nystagmus is less likely but may be in any direction when it occurs. In the case of a peripheral or pontomedullary vestibular lesion, the fast phase should be away from the side of the lesion. (This is shown in Fig. 5, which demonstrates vestibular strabismus with infraduction of the left eye and a head tilt to the left, consistent with a left-sided peripheral vestibular lesion. Such a horse may have horizontal nystagmus with the fast phase away from the side of the lesion, which is indicated by the green arrows.) By contrast, in horses in which the inhibitory vestibular components of the cerebellum are involved, the fast phase is toward the lesion. This is known as paradoxical central vestibular syndrome. With central but not peripheral lesions, the direction of nystagmus may vary with changing head position. Signs caused by central lesions are not likely to show significant clinical improvement with visual compensation, as may occur 2 or 3 weeks after peripheral vestibular lesions. If vestibular signs are accompanied by CNS-specific signs such as obtundation, limb weakness, nonvestibular ataxia, or dysfunction of cranial nerves other than the facial nerve, a central lesion should be suspected.

Acknowledgments

Declaration of Ethics
The Author declares that he has adhered to the Principles of Veterinary Medical Ethics of the AVMA.

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The Author declares no conflicts of interest.

References
Laboratory Diagnostics for Neurologic Disease—Equine Protozoal Myeloencephalitis, Lyme, and More!

Amy L. Johnson, DVM, DACVIM-LAIM

Knowledge of the best samples to collect and tests to request will improve antemortem diagnosis of neurologic disease in horses. Appropriate interpretation of results is critical to avoid misdiagnosis, particularly for pathogens that most frequently cause subclinical infection (e.g., Sarcocystis neurona and Borrelia burgdorferi). Author’s address: Department of Clinical Studies, New Bolton Center, University of Pennsylvania School of Veterinary Medicine, 382 West Street Road, Kennett Square, PA 19348; e-mail: amyjohn@vet.upenn.edu. © 2015 AAEP.

1. Introduction

Antemortem diagnosis of neurologic disease remains a frustrating challenge for practitioners, particularly in field settings. Even in hospital or referral settings, definitive diagnosis frequently requires postmortem examination. However, clinicians can improve accuracy of diagnosis by applying a methodical approach to horses with potential neurologic disease. This approach begins with a thorough clinical neurologic examination to assess whether the horse is truly showing signs of nervous system disease and the neuroanatomic localization (if appropriate). Based on this neurolocalization, the clinician can formulate an appropriate list of potential causative diseases. Season, geographical location, vaccination history, management/farm history, and individual horse history can all influence this list of differential diagnoses.

After establishing a list of likely diseases, ancillary testing is usually indicated. Neurodiagnostics for living horses can be grouped into imaging studies (radiography, computed tomography, magnetic resonance imaging, etc.), laboratory tests (cerebrospinal fluid [CSF] analysis, serology, polymerase chain reaction [PCR] assays, etc.), and electrodiagnostics (electromyography, electroencephalography, nerve conduction testing, etc.). These proceedings describe available laboratory tests, specifically those for infectious neurologic diseases given that they are often most accessible to the equine practitioner.

Although laboratory tests for infectious diseases are relatively easy to perform, interpretation of results can be difficult. For example, the protozoal organisms that cause equine protozoal myeloencephalitis (EPM) and the bacteria that cause Lyme neuroborreliosis (LNB) are endemic in many areas of the country. In these areas, equine exposure rates can be very high, with up to 80 to 90% of horses becoming serologically positive during their lifetimes. The vast majority of exposed horses never develop clinical signs of disease. However, some horses will develop central nervous system (CNS) infection with accompanying neurologic signs. Differentiating these horses from those that have been exposed but whose clinical signs can be attributed to a different disease is a perpetual challenge. These proceedings focus on EPM and Lyme testing, reviewing recommended diagnostic criteria and labo-
ratory testing, while also providing a brief review of tests for other infectious neurologic diseases.

General Approach

The majority of neurologic horses can be divided into those with brain disease, spinal cord disease, or multifocal CNS disease (neuropathies and neuromuscular disease will not be discussed here).

- **Brain disease**: Adult horses develop brain disease most commonly due to metabolic, infectious, and traumatic causes. Recommended laboratory testing often includes serum biochemistry, blood ammonia level, CSF analysis (if available), and specific infectious disease testing (Table 1).

- **Spinal cord disease**: Adult horses develop spinal cord disease most commonly due to vertebral problems (developmental, degenerative, or traumatic) and infectious causes, although degenerative myelopathies also occur. Recommended laboratory testing often includes CSF analysis (if available), vitamin E level, and specific infectious disease testing (Table 1).

- **Multifocal CNS disease**: Adult horses most commonly develop multifocal disease due to infectious causes. Recommended laboratory testing often includes CSF analysis (if available) and specific infectious disease testing (Table 1).

Specific Diseases

**EPM**

EPM is most commonly caused by the protozoan parasite *Sarcocystis neurona*, although infection with other protozoa such as *Neospora hughesi* has also been reported. Horses are infected by *S. neurona* when they consume food or water contaminated with opossum feces containing sporocysts. The life cycle of *N. hughesi* is not well elucidated but vertical transmission likely plays a role. When these protozoa invade the central nervous system, they can affect any part, causing highly variable clinical signs that might manifest insidiously or suddenly and subsequently progress slowly or rapidly. General proprioceptive ataxia is one of the most common clinical signs of disease, and is often asymmetric, with a mixture of upper and lower motor neuron paresis. Muscle atrophy (again, often asymmetric) is also common.

Antemortem diagnosis of EPM remains challenging and is always presumptive; definitive diagnosis requires postmortem confirmation of protozoal infection by microscopic identification, immunohistochemistry, culture, or PCR. Depending on geographic location, a high percentage of horses can be exposed to and infected by *S. neurona*, although only a small percentage of horses (estimated at < 1% of exposed horses) develop clinical signs of neurologic disease. Factors that govern whether any particular horse is able to eliminate the infection without developing clinical signs are unknown. Therefore, performing serology, regardless of test chosen, will only reveal whether the horse has been exposed to *S. neurona* or *N. hughesi* and does not indicate the current disease status of the CNS. To achieve the best possible presumptive diagnosis in the live horse, three criteria must be met: presence of neurologic disease, exclusion of other likely causes, and confirmation of *S. neurona*– (or *N. hughesi*)– specific antibodies in the CSF, serum, or both.

All of the commonly used diagnostic tests for EPM assess whether the horse has antibodies against the protozoa in serum, CSF, or both. The differences among the tests include their methodologies (Western blot [WB], indirect fluorescent antibody test [IFAT], or enzyme-linked immunosorbent assay [ELISA]) as well as which antibodies are recognized. A summary of commercially available testing options for antibodies against *S. neurona* and reported test performance is shown in Table 2. There are two testing options for antibodies against *N. hughesi*: an IFAT at University of California at Davis and an ELISA at Equine Diagnostic Solutions. Neither has been fully evaluated using clinical EPM cases.

General principles for interpretation of EPM test results are as follows:

- A positive serum test indicates exposure to the organism but does not confirm CNS infection, regardless of the magnitude of the titer.
- A negative serum test usually indicates that the horse has not been exposed to the organism. Exception: rarely, a recently infected horse might show clinical signs prior to seroconversion, in which case repeated testing in 10 to 14 days should yield a positive result.
- A positive CSF test is more likely to correlate with an EPM diagnosis than a positive serum test. However, false positives occur due to blood contamination (particularly with WB, less so with IFAT and ELISA) or normal diffusion of antibodies from blood to CSF.
- A negative CSF test usually means EPM is not the cause of disease. Exception: as mentioned above, in rare circumstances a recently infected horse will show clinical signs prior to developing a measurable antibody level in CSF; retesting 10 to 14 days later should yield a positive result.
- The best way to diagnose active EPM is to submit serum and CSF for quantitative testing, which allows detection of intrathecal antibody production.

The author’s current approach is to submit paired CSF and blood samples for an *S. neurona* SAG2, 4/3 ELISA (or *Neospora* ELISA) serum:CSF titer ratio, which allows identification of intrathecal antibody
Table 1. Common Infectious Causes of Neurologic Disease in Horses and Testing Recommendations

<table>
<thead>
<tr>
<th>Disease/Pathogen</th>
<th>Neurolocalization + Other Signs</th>
<th>Samples to Collect for Recommended Test</th>
<th>Available Tests</th>
<th>Recommended Test (Author’s Choice)</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Equine protozoal myeloencephalitis (Sarcocystis neurona or Neospora hughesi)</td>
<td>Variable: multifocal, spinal cord, or brain</td>
<td>Blood (serum) CSF</td>
<td>WB (S. neurona only) IFAT (S. neurona and N. hughesi) SnSAG1 ELISA SnSAG2, 4/3 ELISA SnSAG1, 5, 6 ELISA NhSAG1 ELISA PCR</td>
<td>SnSAG2, 4/3 ELISA serum: CSF titer ratio (± NhSAG1 ELISA serum:CSF titer ratio)</td>
<td>See Table 2 for more information about available EPM tests PCR on CSF rarely positive for clinical cases</td>
</tr>
<tr>
<td>Lyme neuroborreliosis (Borrelia burgdorferi)</td>
<td>Variable: multifocal, brain, or spinal cord; fever, uveitis, synovitis</td>
<td>Blood (serum) CSF</td>
<td>ELISA WB IFAT C6 SNAP Multiplex PCR</td>
<td>Multiplex on serum + CSF (+/- PCR on CSF)</td>
<td>See Table 3 for more information about available Lyme tests PCR on CSF not very sensitive but highly specific</td>
</tr>
<tr>
<td>Equine herpes virus 1 (EHV-1) myeloencephalopathy</td>
<td>Spinal cord ≥ brain; fever, urinary/rectal incontinence, abortion/ neonatal death, respiratory</td>
<td>Blood (EDTA) Nasal swabs</td>
<td>VI SN PCR</td>
<td>PCR on EDTA blood and nasal swabs</td>
<td>PCR can distinguish “neuropathogenic” from “non-neuropathogenic” strains Both strains can cause neurologic disease and affected horses should be managed similarly</td>
</tr>
<tr>
<td>West Nile Virus (WNV) encephalomyelitis</td>
<td>Spinal cord ≥ brain; fever, muscle tremors or fasciculations</td>
<td>Blood (serum)</td>
<td>SN IgG capture ELISA IgM capture ELISA</td>
<td>IgM capture ELISA</td>
<td>Testing serum alone is as good as testing CSF SN, IgG capture ELISA detect vaccinal antibodies (so IgM capture ELISA preferred for natural disease)</td>
</tr>
<tr>
<td>EEE</td>
<td>Brain; fever</td>
<td>Blood (serum)</td>
<td>IgM capture ELISA IgM capture ELISA</td>
<td>IgM capture ELISA</td>
<td>All horses that die/are euthanized after short course of unexplained progressive neurologic disease should be tested</td>
</tr>
<tr>
<td>WEE</td>
<td>Brain; fever</td>
<td>Blood (serum)</td>
<td>IgM capture ELISA IgM capture ELISA</td>
<td>IgM capture ELISA</td>
<td></td>
</tr>
<tr>
<td>Rabies</td>
<td>Variable: brain, spinal cord, or multifocal; lameness, colic</td>
<td>Brain</td>
<td>Direct FAT</td>
<td>Direct FAT</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: EDTA, ethylenediaminetetraacetic acid; FAT, fluorescent antibody test; Nh, Neospora hughesi; SAG, surface antigen; Sn, Sarcocystis neurona; SN, serum neutralization; VI, virus isolation; WEE, Western equine encephalomyelitis; EEE, Eastern equine encephalomyelitis.
Table 2. Commercially Available Immunologic Tests for Antibodies Against *Sarcocystis neurona*

<table>
<thead>
<tr>
<th>Test (Literature References)</th>
<th>Labs Performing Test</th>
<th>Interpretation Provided by Laboratory</th>
<th>Reported Performance in Literature</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>WB (4–7)</td>
<td>EDS</td>
<td>Band pattern read and interpreted visually (subjective)</td>
<td>Serum 80–89 CSF 87–89</td>
<td>Not fully quantitative</td>
</tr>
<tr>
<td></td>
<td>UC Davis</td>
<td>Results usually reported as negative, weak positive, low positive, or positive</td>
<td>Serum 87–89 CSF 44–89</td>
<td>Negative result more useful than positive (high negative predictive value, low positive predictive value)</td>
</tr>
<tr>
<td></td>
<td>IDEXX</td>
<td>Band pattern read and interpreted visually (subjective)</td>
<td>Serum 87–89 CSF 44–89</td>
<td>Minimal blood contamination of CSF can cause false-positive result</td>
</tr>
<tr>
<td>mWB (7–8)</td>
<td>Michigan State</td>
<td>Similar to standard WB (above)</td>
<td>Serum 89–100 CSF 69–98</td>
<td>Reported performance likely artificially high due to study design; in practice similar to standard WB</td>
</tr>
<tr>
<td>IFAT (7, 9–12)</td>
<td>UC Davis</td>
<td>Serum positive at ≥1:80 has ≥55% probability* of EPM</td>
<td>Serum 59–94 CSF 71–100</td>
<td>Probability values do not necessarily correlate well with disease status in all areas of country</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Serum negative at ≤1:40 has ≤33% probability* of EPM</td>
<td>Serum 65–100 CSF 90–99</td>
<td>Cross reactive with <em>S. fayeri</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td>CSF positive at ≥1:5 has 92% probability* of EPM</td>
<td>Serum 65 CSF 98</td>
<td>CSF results less affected by blood contamination</td>
</tr>
<tr>
<td>SAG1 ELISA (11, 13–14)</td>
<td>Antech</td>
<td>Serum positive at ≥1:16 but recommended cutoff ≥1:32</td>
<td>Serum 13–68 CSF 71–97</td>
<td>Low sensitivity limits usefulness of test; not recommended in mid-Atlantic region; performance in other regions unknown</td>
</tr>
<tr>
<td>SAG2, 4/3 ELISA (12, 15–16)</td>
<td>EDS</td>
<td>Serum positive for exposure at ≥1:250</td>
<td>Serum 30–86 CSF 37–88</td>
<td>CSF results less affected by blood contamination</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CSF correlates well with EPM if ≥1:40</td>
<td>Serum 77–96 CSF 58–96</td>
<td>Serum:CSF titer ratio using this test outperformed other test options in 3/3 comparison studies</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Serum:CSF titer ratio very predictive of EPM if ≤100</td>
<td>Serum 86–93 CSF 83–100</td>
<td>Author's choice</td>
</tr>
<tr>
<td>SAG1, 5, 6 ELISA (17)</td>
<td>Pathogenes</td>
<td>Serum positive at ≥1:8, indicating infection</td>
<td>Serum N/A CSF N/A</td>
<td>Has not been critically evaluated in literature</td>
</tr>
</tbody>
</table>

**Abbreviations:** EDS, Equine Diagnostic Solutions (Lexington, KY); EPM, equine protozoal myeloencephalitis; mWB, modified Western blot; SAG, surface antigen.

*Based on pre-test probability of 10%.
production and correlates best with active central nervous system disease.\textsuperscript{12,16,20}

**LNB**

Horses are frequently infected with *Borrelia burgdorferi*, the Gram-negative spirochete that causes Lyme disease. The significance of *B. burgdorferi* infection, however, remains unknown. As with EPM, one of the major problems in diagnosing Lyme disease is that many horses seem to be infected with *B. burgdorferi* and undergo seroconversion without clinical signs of disease. Historically, clinical signs attributed to *B. burgdorferi* infection include chronic weight loss, sporadic lameness, stiffness, arthritis, joint effusion, muscle soreness, hepatitis, laminitis, fever, abortion, uveitis, and encephalitis.\textsuperscript{21–23} More recently, several reports describing LNB in horses have been published.\textsuperscript{24–26} Although certainly less common than many neurologic diseases, including EPM, LNB is regularly diagnosed at the author’s practice (New Bolton Center, University of Pennsylvania), frequently with postmortem confirmation. Similar to EPM, LNB can cause a variety of neurologic deficits, including changes in mental status (dullness to increased anxiety and hyperesthesia), gait abnormalities (generalized stiffness to severe general proprioceptive deficits and paresis), cranial nerve deficits (reduced lip tone, bilateral aetnyenoid paralysis, and dysphagia), neck stiffness, muscle atrophy, and muscle tremors or fasciculations.\textsuperscript{27}

As with EPM, ante-mortem diagnosis of LNB is best achieved by fulfilling several criteria: potential *B. burgdorferi* exposure (via residence in or travel to an endemic area), presence of neurologic signs, diagnostic testing to rule out other potential diseases, and immuneologic evidence of infection.\textsuperscript{28} Although identification of the organism (via culture or PCR) in tissue or CSF samples from the patient provides strong evidence for active infection, positive results are uncommon even in horses with confirmed LNB, and immuneologic testing remains the mainstay of laboratory diagnosis. Ideally, this immunologic testing documents intrathecal antibody production, which is achieved by submitting paired serum and CSF samples for quantitative antibody testing.\textsuperscript{27} CSF cytological analysis is strongly recommended for horses with suspected LNB, as results are often abnormal (neutrophilic or lymphocytic pleocytosis with increased total protein).\textsuperscript{27}

Commercially available tests for *B. burgdorferi* include ELISA, IFAT, WB, C\textsubscript{6} ELISA SNAP test, and fluorescent bead-based multiplex assay (Multiplex).\textsuperscript{22,29–37} Table 3 summarizes relevant information on currently available tests. The SNAP test can be performed in-clinic or stall-side, whereas the other tests are performed at reference laboratories. As with available EPM tests, all of these tests detect antibody production by the host—the differences are in test methodology and which specific antibodies are detected. Although the pattern of antibody production might help elucidate the chronicity of the horse’s infection, none of these tests will tell you whether the infection is significant (e.g., whether it is related to the horse’s clinical signs or is just a red herring).

General principles of interpretation for Lyme test results are similar to those outlined for EPM testing. A positive test usually indicates past or present infection with *B. burgdorferi*, although vaccination can also cause positive results for some tests. Regardless of test utilized, clinical Lyme disease cannot be confirmed—there is no gold standard test. Horses living in an endemic area are frequently seropositive, so positive results will have a low positive predictive value of disease. However, with the exceptions of acutely infected or immunocompromised horses, negative results will have a high negative predictive value. None of the tests demonstrate causation of clinical signs or predict whether disease is likely to develop in the future. There is no known correlation between magnitude of titer and likelihood of disease. It is unclear how well tests that predict stage of infection perform with horses that have had repeated exposures. Several additional caveats should be mentioned:

- Knowledge of vaccination history will often aid in interpretation of results. There are four canine vaccines that are occasionally used in horses. Three\textsuperscript{a,b,c} are two-strain bacterins that could induce antibody production against multiple antigens, including OspA and OspC (to varying degrees). One\textsuperscript{d} is a recombinant pure nonadjuvanted protein that only includes the OspA antigen.
- Although in theory documentation of intrathecal antibody production is recommended for the diagnosis of LNB, in practice it becomes difficult. Unlike with EPM, there is no established optimal cutoff for LNB using a serum: CSF antibody ratio. Horses with LNB frequently have abnormal CSF cytology and hence might have an abnormal blood-brain barrier, confounding results. Finally, sample dilution can complicate interpretation of results (e.g., the Multiplex assay tests serum at a dilution of 1:400 whereas CSF is generally run undiluted or at 1:2, and these dilution factors are not reported with the results).
- Finally, the author knows of several horses with concurrent diagnoses of neuroborreliosis and common variable immunodeficiency; these horses (due to low B-cell numbers and hypogammaglobulinemia) have unreliable serologic results and might test negative for anti-*Borrelia* antibodies despite CNS infection.

**Viral Diseases**

In general, laboratory testing for viral neurologic diseases is more straightforward than that for EPM and LNB (Table 1). Diseases of most concern in the United States include equine herpes virus 1 myeloencephalopathy, West Nile virus en-
cephalomyelitis, Eastern equine encephalomyelitis (EEE), Western equine encephalomyelitis, and rabies. No antemortem rabies tests for horses have been validated; direct fluorescent antibody testing of brain tissue is required. Equine herpes virus 1 testing has been revolutionized by the development of PCR tests; submission of nasal swabs and whole-blood samples allows rapid diagnosis of active infection. Finally, IgM-capture ELISAs are available for West Nile virus, EEE, and WEE; these tests are highly sensitive and specific for active disease due to the kinetics of antibody production after viral infection.

2. Discussion

Although laboratory testing is often an important part of the neurologic evaluation, it cannot be viewed in isolation. Results must be interpreted in light of the clinical neurologic examination as well as in concert with other test results (including imaging studies, clinical pathology, additional infectious disease testing, and even electrodiagnostic testing). The author highly discourages running EPM or Lyme tests as a first step in a neurologic workup because the results are meaningless without a clinical exam. Even in the context of a neurologic case, serologic test results can be difficult to interpret without an understanding of test methodology and reported performance. Knowing which tests to submit and how to interpret the results will lessen the stress involved in working on neurologic horses and improve antemortem diagnostic accuracy.

Acknowledgments

Declaration of Ethics

The Author declares that she has adhered to the Principles of Veterinary Medical Ethics of the AVMA.
Conflict of Interest
The Author declares no conflicts of interest.

References and Footnotes

IN-DEPTH INTERACTIVE: NEUROLOGY


*LyvexVax, Zoetis, Inc., Kalamazoo, MI 49007.
3Recombitek Lyme, Merial, Duluth, GA 30096.
How to Perform Field Anesthesia and Utilize Pain Management

Keith R. Branson, DVM, MS, DACVAA

1. Introduction
There are a variety of surgeries that can be performed in the field. Most are performed on relatively healthy patients. Some procedures are done on patients that are awake using sedation and often a local anesthetic technique. Other procedures require general anesthesia.

2. Materials and Methods
Short procedures can be done under $\alpha_2$ agonist-dissociative combinations, which are widely used. In general, the $\alpha_2$ agonist is administered first, and then, after the horse is sedated, the dissociative combination is administered. These combinations are summarized in Table 1.

If longer general anesthesia is going to be used, the most commonly used regimens include some combination of guaifenesin, ketamine, and an $\alpha_2$ agonist. There are many combinations, but the most common is 5% guaifenesin, 1 mg/mL ketamine, and 0.5 mg/mL xylazine, which is commonly called “triple drip.” To make this drip, 10 mL of ketamine and 5 mL of xylazine are added to 1 L of 5% guaifenesin. This can be used as a maintenance general anesthetic after induction with xylazine and ketamine. Immediately after induction, a small bolus of triple drip is often needed, and then it can be administered at the maintenance rate of approximately 2.2 mL/kg/h (1 mL/lb/h). If a long anesthesia is anticipated (more than 1 hour), the author will double the ketamine and slightly decrease the administration rate. The xylazine can be replaced with detomidine (0.01 mg/mL or 1 mL/L) or romifidine (0.05 mg/mL or 5 mL/L).

The use of propofol for equine anesthesia is increasing as its cost decreases. It can be used as an induction agent (2.0 mg/kg intravenously [IV]), a maintenance agent alone (0.2–0.22 mg/kg/min IV), or with other anesthetic drugs such as ketamine.\(^1,2\) The quality of maintenance and recovery are comparable to recoveries after triple drip.

3. Results and Discussion
Combinations of an $\alpha_2$ agonist and ketamine do not always work as well on donkeys and mules; often a surgical plane of anesthesia is not achieved or is of a shorter duration than that seen in horses. Donkeys have a more rapid clearance of ketamine than horses, and mules seem to be less sensitive to ketamine and often require a higher preanesthetic dose for adequate sedation. One induction technique that works well for mules is premedication with an $\alpha_2$ agonist, and then 5% guaifenesin is administered.
IV until there is an obvious effect (sagging). At that point a bolus of ketamine is administered IV. If possible, an assistant should monitor the depth of anesthesia and try to maintain the patient at a light plane of anesthesia. Minimal supportive care is usually provided for these patients because they are healthy and the anesthesia is of short duration. Care should still be taken to correctly position the patients to minimize the chance for neuropathies. Although intubation is not always indicated, it can sometimes be useful. In particular, nasotracheal intubation assures an airway during oral surgeries. Having the ability to intubate a patient if needed can be beneficial. Intubation and the use of a demand valve provides oxygen as well as maintaining an open airway. A standard E tank contains enough oxygen to provide assisted ventilation to an adult horse for 20 to 30 minutes or supplemental oxygen for approximately 1 hour when using a demand valve. There are high-flow demand valves available for equine use. Obviously the demand valve must be compatible with the endotracheal tube being used (Fig. 1). Human resuscitation kits can be purchased that contain a small oxygen tank, demand valve (lower flow for human use), flowmeter, and even a gas-operated suction unit.

Table 1. Three α2 Agonist-Dissociative Combinations

<table>
<thead>
<tr>
<th>Combination</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. 1.1 mg/kg xylazine, 2.2 to 2.75 mg/kg ketamine, 0.02 mg/kg butorphanol, or 0.06 mg/kg diazepam or midazolam can be added; this can be extended by administering a half dose of both the xylazine and ketamine if needed; detomidine (0.02 mg/kg) or romifidine (0.01 mg/kg) can be substituted for the initial dose of xylazine</td>
</tr>
<tr>
<td>2. 1.1 mg/kg xylazine and 1.65 mg/kg Telazol</td>
</tr>
<tr>
<td>3. 0.02 to 0.04 mg/kg detomidine and 2 mg/kg Telazol</td>
</tr>
</tbody>
</table>

* Doses are in milligrams and kilograms and are administered intravenously.

b For draft horses the doses should be decreased by 10%.

IV until there is an obvious effect (sagging). At that point a bolus of ketamine is administered IV.

If possible, an assistant should monitor the depth of anesthesia and try to maintain the patient at a light plane of anesthesia. Minimal supportive care is usually provided for these patients because they are healthy and the anesthesia is of short duration. Care should still be taken to correctly position the patients to minimize the chance for neuropathies. Although intubation is not always indicated, it can sometimes be useful. In particular, nasotracheal intubation assures an airway during oral surgeries. Having the ability to intubate a patient if needed can be beneficial. Intubation and the use of a demand valve provides oxygen as well as maintaining an open airway. A standard E tank contains enough oxygen to provide assisted ventilation to an adult horse for 20 to 30 minutes or supplemental oxygen for approximately 1 hour when using a demand valve. There are high-flow demand valves available for equine use. Obviously the demand valve must be compatible with the endotracheal tube being used (Fig. 1). Human resuscitation kits can be purchased that contain a small oxygen tank, demand valve (lower flow for human use), flowmeter, and even a gas-operated suction unit.

**Acknowledgments**

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**Conflict of Interest**

The Author declares no conflicts of interest.

**References**

How to Utilize Standing Restraint and Local Anesthesia for Field Surgery

Keith R. Branson, DVM, MS, DACVAA

1. Introduction
There are a variety of surgeries that can be performed in the field. Most of them are performed on relatively healthy patients. Some procedures are done on patients that are awake using sedation and often a local anesthetic technique.

2. Materials and Methods
Short standing procedures can often be accomplished with a single injection of an α2 agonist and butorphanol tartrate. Drug combinations commonly employed include xylazine (0.25–1.0 mg/kg) or detomidine (0.005–0.02 mg/kg) given intravenously (IV) or intramuscularly. An opioid such as butorphanol (0.01–0.05 mg/kg) or buprenorphine (0.006 mg/kg) can be added for additional sedation and analgesia. If an opioid is used, the lower dose of the α2 agonist should be administered initially to prevent excessive sedation. If longer-term sedation is needed, xylazine can be administered as a constant rate infusion (CRI) at the rate of 0.55 mg/kg/h or detomidine at the rate of 6 μg/kg/h. Dexmedetomidine has also been given as a CRI at the rate of 2.5 μg/kg/h after a loading dose of 2.5 μg/kg IV. If an α2 agonist CRI is used, an opioid such as butorphanol can be given intermittently or as a CRI infusion. Butorphanol is administered as a CRI at the rate of 13 μg/kg/h. Morphine has also been used at a rate of 30 μg/kg/h after a loading dose of 50 μg/kg.

Often local or regional anesthesia is used in conjunction with sedation. Although local blocks on the distal limbs are widely used, the author would encourage practitioners to also become familiar with regional anesthetic techniques on the head. Mental and infraorbital nerve blocks are useful for procedures that involve the incisors and rostral head (Fig. 1). Likewise, sensory blocks around the eye can facilitate standing ophthalmic procedures.

Caudal epidurals are often used in conjunction with sedation for perineal surgeries. This is done in the traditional manner at the sacrococcygeal or first intercoccgeal intervertebral space. A dose of 4 to 10 mL of local anesthetic is usually recommended. The author usually uses the lower end of this dose range and prefers 4 mL of 2% lidocaine or carbocaine with 1 mL of 100 mg/mL xylazine added. The needle can be inserted perpendicular to the skin surface over the space or at a 30- to 45-degree angle, as shown in Fig. 2. Correct needle placement can be confirmed by a loss of resistance as the needle enters the epidural space or the use of the “hanging drop” technique. An al-
ternative to a caudal epidural for perineal laceration and rectovaginal fistula repair consists of a local infiltration of the nerves lateral to the rectum. After appropriate skin preparation, a long needle is inserted lateral to the rectum along either side to a point cranial to the surgical area. A hand inserted in the rectum can assist needle placement. Local anesthetic is infiltrated as the needle is withdrawn. Local anesthetic (20–40 mL) is used on each side (Fig. 3).

Epidural administration of opioids works well to provide analgesia to the hind limbs and perineal region. Epidural morphine is used most commonly and typically provides 12 to 18 hours of analgesia; the most commonly used dose is 0.1 to 0.2 mg/kg. The addition of 15 to 30 μg/kg detomidine may increase the effectiveness and duration of analgesia provided by the morphine. If repeated doses are to be administered, an epidural catheter can be placed, as shown in Fig. 4. The introducer needle is placed in a manner similar to a conventional caudal epidural, and when the tip of the Touhy needle is in the epidural space, the catheter is advanced cranially approximately 10 cm. The catheter is then secured to the patient. The author prefers the noncoil-reinforced catheters because they can be shortened to ease maintenance.

In localized areas, placement of a perineural catheter for continuous or frequent intermittent administration of a local anesthetic can be an excellent method of providing analgesia. There are some difficulties maintaining these catheters long term.
An epidural catheter is used for the perineural infusions.

Alternatively, a diffusion catheter can be placed (Fig. 5). A 0.125% bupivicaine solution with 0.1 mL of 8.4% sodium bicarbonate added to each 20 mL of bupivicaine and 1:200 000 epinephrine can be administered at the rate of 2 mL/h.\textsuperscript{1} A novel liposomal-encapsulated bupivacaine formulation is being used for postoperative pain in humans with effective analgesia lasting 24 hours or more. It has been used as a local infiltration postoperatively with effects seen for as long as 2 days.

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Reference

Minimizing the incidence of postoperative complications associated with equine castration should be the primary goal of the practitioner. Proper case selection, knowledge of pertinent clinical anatomy, perioperative physical examination, and correct surgical technique will minimize the incidence of complications. When complications do occur, early recognition and treatment will maximize the chance of a successful outcome. Author’s address: University of Georgia, College of Veterinary Medicine, Athens, GA 30602-7385; e-mail: emueller@uga.edu. © 2015 AAEP.

1. Introduction

Castration is one of the most common elective surgical procedures performed in the field. The procedure is most often performed to abate unwanted aggressive, masculine behavior in horses not intended for breeding. Although the procedure is considered routine, various postoperative complications including excessive swelling and edema, hemorrhage, infection, omental herniation, entervation, hydrocele, or septic peritonitis may occur. In one recent retrospective study, 10% of equids undergoing routine, elective castration experienced complications related to the procedure. Seventy-six percent of these complications were classified as mild and did not require emergency treatment. Although the vast majority of horses that experience complications are successfully treated on the farm with no long-term adverse effects, the increased morbidity and cost associated with additional veterinary care for a procedure that is perceived as “routine” often results in additional concern of the practitioner and client dissatisfaction.

For any surgical procedure, it is more desirable to minimize the occurrence of postoperative complications rather than to have to deal with the associated morbidity, time, and effort in treating them. A thorough understanding of pertinent clinical anatomy, strict attention to asepsis and surgical technique, and proper postoperative exercise recommendations will minimize the incidence of complications associated with castration. However, when complications do arise, the practitioner must be able to quickly recognize and correctly and aggressively treat them to assure a rapid and successful outcome. Although a comprehensive discussion of pertinent equine male anatomy is beyond the scope of this presentation, the principals of proper surgical technique, perioperative care and recognizing and treating common complications associated with equine castration will be discussed.

2. Materials and Methods

Perioperative Evaluation

A complete history should be obtained including a query of any medical conditions that may predispose
Surgical Technique and Postoperative Care

Castration may be performed in the standing horse, with the addition of incisional and intra-testicular lidocaine administration, or under IV general anesthesia. The complication rate between these two approaches is not significantly different.4,6 The approach utilized is dependent on practitioner preference, and the size and disposition of the horse.

From the initial scrotal incision to the final stretching of the surgical incision to allow adequate drainage, meticulous attention to asepsis and surgical technique will help minimize many of the complications that can be associated with routine castration. When making the scrotal incision both testes should be pushed firmly into the scrotum, tensing the skin, tunica dartos, and scrotal fascia tightly over the testis (Fig. 1). This will minimize incorporating multiple tissue planes in the incision and the chance of excessive subcutaneous tissue or scrotal fascia becoming edematous and protruding beyond the skin edges during early healing. Each scrotal skin incision should be made approximately 1 cm from the median raphe, along the most dependent aspect of the scrotum, and extend from pole to pole of each testis. This will allow easy exteriorization of the testis and minimize the amount of manipulation necessary to separate the scrotal fascia from the underlying parietal tunica in preparation of application of the emasculators. In addition, long incisions allow for adequate drainage and prevent premature healing of the skin incisions that can result in accumulation of contaminated debris and localized infection.

A variety of emasculators may be used to perform equine castration. The author prefers the Serra emasculators because of their vertically oriented serrated crushing surface that provide excellent hemostasis and long handles to provide adequate leverage for emasculating larger-diameter spermatic cords of older stallions. Correct application of the emasculators is essential to provide adequate hemostasis. The emasculator is assembled to provide crushing of the cord vasculature proximally while simultaneously transecting the cord, distal to the crush site. Four simple principals of emasculator application: 1) nut-to-nut, 2) applying the emasculator perpendicular to the cord, 3) minimal tension, and 4) leaving the emasculators on for 2–3 minutes will minimize complications associated with postoperative hemorrhage. Regardless of the type of emasculator, when correctly assembled, the prominent external assembly nut on the crushing apparatus should be positioned adjacent to the testis to be removed (nut-to-nut) (Fig. 2). This assures that crushing of the cord occurs proximal to the transection site. If the emasculators are applied in the reverse orientation, the crush site would be distal to the transected cord, and profuse hemorrhage would ensue. The emasculators should be oriented perpendicular (90°) to the cord to prevent premature cutting of the testicular artery before it is fully engaged by the crushing apparatus. Minimal tension refers to the tension of the spermatic cord during emasculation. The emasculators should be placed, closed just enough to engage and secure the cord, then the emasculator and cord relaxed toward the inguinal area (proximally), ensuring no skin is incorporated in the emasculation, before fully closing the emasculator. This prevents the elastic testicular artery from recoiling proximally beyond the grasps of the emasculator during incomplete or slow application of the emasculator. Leaving the emas-
Calculators in place for 2–3 minutes is sufficient for most horses to provide adequate hemostasis. In older stallions or donkeys, the author will leave the emasculators in place for 4–5 minutes, or transfix the cord with 2–0 polyglecaprone 25 before emasculcation. In addition, donkeys are reported to have relatively large testicular vessels; therefore, the author routinely ligates the cord proximal to the emasculcation site with a single absorbable transfixation suture.

Following emasculation, the parietal tunic of the spermatic cord should be grasped with Ochsner forceps before releasing the emasculator. Care should be taken not to clamp across the entire width of the cord, because this would preclude being able to identify a bleeder should it be present. The emasculator is released, the cord gently replaced into the scrotal incision to relieve any remaining tension on the cord, and the end of the stump examined. If no bleeding is evident, it can be released. The end of the transected cord should not be blotted or manipulated because this could disturb the existing clot and predispose to hemorrhage.

After both testes are removed, the scrotal incisions should be examined for any excessive or pulsatile hemorrhage. If excessive hemorrhage is identified, it may be clamped with a Carmalt forceps or ligated (see hemorrhage). Excessive scrotal swelling is usually due to the scrotal incisions being too small or not being stretched adequately after surgery, or noncompliance of the owner related to active postoperative exercise recommendations. This can result in premature closure of the incisions and accumulation of serous fluid in the scrotum. Excessive swelling may also be a sign of a localized infection or abscess (see infection). Excessive noninfectious swelling is most easily treated on the farm by sedating the horse, aseptic preparation of the scrotal area, manually opening and stretching the scrotal incisions with sterile gloved hands to facilitate drainage, and a 3–5-day course of nonsteroidal anti-inflammatory drugs (NSAIDs). The owner should follow this up with cold-water hydrotherapy and active exercise or

### 3. Results

When complications do occur it is important that the client or veterinarian recognize them early, and that they are treated promptly and correctly to minimize prolonged morbidity and client dissatisfaction.

#### Edema/Swelling

Some degree of postoperative swelling and edema is expected after routine castration; being most evident 3 to 4 days after surgery with complete resolution in 12 to 14 days. More severe swelling that results in clinical signs such as a stiff gait, reluctance to ambulate, or abnormal urination is considered abnormal and should be evaluated. Excessive scrotal swelling is usually due to the scrotal incisions being too small or not being stretched adequately after surgery, or noncompliance of the owner related to active postoperative exercise recommendations. This can result in premature closure of the incisions and accumulation of serous fluid in the scrotum. Excessive swelling may also be a sign of a localized infection or abscess (see infection). Excessive noninfectious swelling is most easily treated on the farm by sedating the horse, aseptic preparation of the scrotal area, manually opening and stretching the scrotal incisions with sterile gloved hands to facilitate drainage, and a 3–5-day course of nonsteroidal anti-inflammatory drugs (NSAIDs). The owner should follow this up with cold-water hydrotherapy and active exercise or
lunging twice daily to promote drainage and prevent premature wound closure.8

Hemorrhage

It is not unusual to have several drops of blood or a slow drip of blood associated with a castration for 5 to 10 minutes after the procedure. This is especially true if the median raphe has been excised or the bottom third of the scrotum has been removed to facilitate drainage. The source of this blood is often small, subcutaneous vessels. However, hemorrhage in the form of a fast drip or pulsatile stream, or of any kind that persists for more than 15 minutes after the procedure is completed is considered abnormal and should be addressed.2,4 Although the testicular artery is the most common source of significant hemorrhage, superficial branches of the external pudendal vessels and the cremaster artery may also be a source. Significant hemorrhage most often occurs because of improper application of the emasculator (Fig. 4). Initial treatment is aimed at identifying and stopping the source of hemorrhage. If the horse is still anesthetized, anesthesia should be prolonged with additional anesthetic and the stump should be closely examined under minimal tension before correctly concluding it is not the source of hemorrhage. If the distal stump is identified to be the source of hemorrhage, it may either be re-emasculated, or more commonly ligated with a transfixation suture of 1-0 or 0-0 absorbable suture.3

Fig. 4. Photograph demonstrating arterial hemorrhage from the scrotum following improper application of the emasculator during castration.

ternally directed tension on the transected stump during manipulation may be sufficient to significantly slow or stop the hemorrhage associated with the testicular or cremaster artery. Therefore, the stump should be closely examined under minimal tension before correctly concluding it is not the source of hemorrhage. If the distal stump is identified to be the source of hemorrhage, it may either be re-emasculated, or more commonly ligated with a transfixation suture of 1-0 or 0-0 absorbable suture.3 If the stump has retracted too far proximally into the inguinal canal and cannot be exteriorized for ligation or emasculcation, or the horse has already recovered from anesthesia, a large, curved crushing forcep(s) (Carmalt) may be used to isolate and secure the distal end of the stump. The horse is then recovered with the clamp(s) in place and maintained in a stall for 24 to 48 hours for close observation, after which time the clamps can be removed with the horse standing. In the standing horse, the author prefers to identify and secure the source of hemorrhage with curved Carmalt forceps rather than dealing with the frustration of trying to isolate and ligate the specific source of hemorrhage.

If the source of hemorrhage cannot be identified, the scrotum should be packed with a 5–7-meter-long piece of continuous sterile gauze (crypt packing), sutured closed, and transported to the nearest referral facility. Referral is also strongly encouraged in cases where considerable external blood loss has occurred or internal hemorrhage is suspected. Clinical signs associated with significant blood loss and hypovolemic shock include tachycardia, tachypnea, pale mucous membranes with an increased capillary refill time, weak pulses, cold extremities, and sometimes colic. Intra-abdominal hemorrhage may be confirmed in the field by trans-abdominal ultrasonography (swirling free peritoneal fluid) or an abdominocentesis. Monitoring pack cell volume and total solids will not reflect the severity of acute blood loss because of fluid distribution across intravascular and extravascular fluid spaces and hypovolemia induced-splenic contraction. Changes in pack cell volume and total protein (TP) may not be evident for 6 hours and 12 to 24 hours, respectively.2 Before transport, the practitioner should consult the personnel at the referral facility for recommendations on initiating intravenous fluid and broad-spectrum antimicrobial therapy, as well as additional considerations necessary to stabilize the horse sufficiently for shipping.

Infection

In a recent report, infection or abscess formation at the surgical site occurred in 2% of equids undergoing routine castration, with clinical signs developing from 3–21 days after surgery.1 Local infection most often results from premature closure of the surgical incisions secondary to the incision being too small or not being in the most dependent part of the scrotum, or insufficient postoperative exercise.
The most common organism associated with these infections is *Streptococcus zooepidemicus*; however, other organisms may be involved. It is always recommended to obtain a culture and sensitivity to direct appropriate antimicrobial therapy. Clinical signs associated with localized incisional site infection include fever, swelling ± purulent discharge, lameness, or reluctance to ambulate. Treatment is focused on opening the scrotal incisions sufficiently to establishing ventral drainage and irrigation of the site with sterile, balanced, isotonic fluids. This can be accomplished on the farm with the horse standing and sedated with xylazine (0.2–0.4 mg/kg, IV) or detomidine (0.005–.01 mg/kg, IV) and butorphanol tarate (.01–0.02 mg/kg IV). Broad-spectrum antimicrobial therapy is initiated until results of the culture and sensitivity are available. Nonsteroidal anti-inflammatory drugs are administered for analgesia and to promote ambulation. The owner should actively lunge the horse twice daily until there is complete resolution of clinical signs. For the majority of horses, the above protocol should result in resolution of clinical signs in 7 to 10 days.

More chronic or systemic infections usually require referral. Chronic *Streptococcus* sp. infection of the cord (Champignon) becomes clinically evident weeks to months after surgery. These horses usually present with a history of fever, a granulating inguinal wound with purulent drainage, and a thickened spermatic cord remnant. Historically these infections have been associated with ligation of the spermatic cord with nonsterile suture materials. However, with the use of commonly available sterile absorbable suture materials and proper aseptic technique, ligation of the spermatic cord does not seem to be associated with an increased risk of infection. In these cases, infection is more established in the tissues and usually requires surgical exploration of the area under general anesthesia and resection of the residual infected cord deep in the external inguinal canal.

Long-standing chronic diffuse infection of the spermatic cord with *Staphylococcus* sp is known as a Scirrhous cord. In these horses, the scrotal incision heals normally and the infection extends proximally along the spermatic cord remnant. Clinical signs may not be apparent for months to years after castration, and are dependent on the extent of infection. Horses usually present with fever and diffuse, firm, inguinal swelling ± single or multiple draining tracts (Fig. 5). These infections may ascend beyond the level of the internal inguinal ring and involve the peritoneal cavity, being palpable per rectum as a firm, thickened swelling associated with the internal inguinal ring. Horses with peritoneal involvement may develop septic peritonitis secondary to the primary disease or subsequent to surgical treatment. Treatment involves en bloc resection of the affected spermatic cord and associated infected tissues to the most proximal extent of the infection, establishing ventral drainage, long-term intravenous broad-spectrum antimicrobials, and NSAIDs. Horses with infection that extends beyond the internal inguinal ring have a guarded prognosis because of poor surgical access and the high probability of the septic peritonitis resulting in a severe systemic inflammatory response.

**Omentum Eventration (Prolapse)**

Eventration of inguinal or abdominal omental or inguinal fat following castration is a rare and less serious complication that, if it occurs, is usually evident within the first 24 hours after castration. Acute prolapse (Fig. 6) can be quite extensive and alarming to the owner, whereas more chronic prolapse usually presents as cold, thickened, edematous tissue protruding beyond the skin margins (Fig. 7). Affected horses demonstrate no additional clinical signs except for the prolapsed tissue, in contrast with horses with intestinal eventration, which demonstrate signs consistent with moderate to severe acute abdominal pain. A thorough physical examination of the patient and tissue is necessary to assure that no important anatomical structures (intestine) are contained within the tissue. Manual removal by external traction should not be attempted because of the risk of enlarging the internal inguinal ring and predisposing to intestinal eventration. Rather, the horse is sedated, the tissue is aseptically prepared, ligated with absorbable suture, and transected or emasculated distal to the ligation suture and deep to the level of the skin margin. Antimicrobials and NSAIDs may be administered but are generally not necessary.

**Hydrocele**

Hydrocele is an accumulation of serous or peritoneal fluid contained within the remaining closed vaginal tunic. Horses present with a flocculent, nonpainful
swelling of the scrotal area. It is a rare complication that may become evident months to years after castration. It seems to occur more commonly after open (with respect to the vaginal tunic) castration. Treatment is not necessary unless the owner finds the swelling aesthetically displeasing. In these cases the redundant tissue should be removed en bloc under general anesthesia. Fluid aspiration with a sterile needle should not be attempted because of the risk of causing a secondary infection and inevitable likelihood of recurrence.

4. Complications in Which Referral Is Highly Encouraged

Intestinal Eventration

Eventration of intestine is a rare but serious complication of equine castration, occurring in 0.3 to 4.6% of castrations, with Standardbred, draft breeds, and horses having had a congenital inguinal hernia at an increased risk. Herniation usually occurs within the first 4 to 12 hours after castration but has been reported to occur as late as 12 days after castration.\(^{10,11}\) There does not seem to be a significant difference in risk of eventration between open and closed castration techniques.\(^{12}\) Partial or complete inguinal/scrotal herniation should be suspected in any horse that demonstrates signs of acute abdominal pain within 12 hours after castration. Ligation of the spermatic cord with a transfixation suture has been shown to reduce, but not completely prevent, the risk of eventration.\(^{9}\) Horses deemed to be at increased risk of intestinal eventration should be referred for castration and primary closure of external inguinal rings. If a small section of intestine herniates through the inguinal ring during surgery, the intestinal segment should be thoroughly lavaged with sterile, balanced, isotonic solution, and replaced into the abdomen. A second surgical approach directly over the effected inguinal ring will be necessary to replace the intestinal segment and close the external inguinal ring (slit) in the aponeurosis of the external abdominal oblique muscle. The external inguinal ring should be closed with No. 2 absorbable suture material.\(^{b}\) These horses should be administered intravenous broad-spectrum antimicrobials and NSAIDs, and confined to a stall for 7 to 10 days for close observation. They may be hand walked after 48 hours.

If intestinal eventration is evident after the horse recovers from anesthesia or a significant length of intestine has protruded through the inguinal ring (Fig. 8), the immediate objective should be to protect and preserve the exposed intestine and prevent further eventration of the intestine. The principals of “clean, replace, and retain” the intestinal segment should be employed by the practitioner in preparation for transport to a referral facility. The exposed intestine should be thoroughly lavaged with sterile, balanced, isotonic solution. If the segment is small enough to be contained within the scrotum, it may be replaced in the scrotum and the scrotal skin apposed with suture or several towel forceps. For larger lengths of exposed intestine, a moist bed sheet or large towel maybe used as a sling to support

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*Fig. 6. Photograph demonstrating an acute (< 12 h) omental prolapse. This was the only abnormal finding upon complete physical exam.*

*Fig. 7. Photograph demonstrating omental prolapse of 4 days’ duration. The tissue is cold, edematous, and thickened.*
the intestine during transport. These horses should be administered intravenous broad-spectrum antimicrobials and flunixin meglumine, and immediately transported to the nearest referral facility for general anesthesia and ventral midline exploratory celiotomy. The prognosis is dependent on the amount of intestinal compromise, degree of peritoneal contamination, and overall systemic condition of the horse at admission, with reported survival rates from 36 to 87%.2

Septic Peritonitis

Septic peritonitis is a rare but serious complication of castration. Clinical signs associated with septic peritonitis include fever, depression, anorexia, tachycardia, tachypnea, colic, and diarrhea. Because the common vaginal tunic surrounding the testes is an extension of the parietal and visceral peritoneum and directly communicates with the peritoneal cavity, a transient nonseptic peritonitis is commonly associated with uncomplicated castration. Blood contamination during the procedure is believed to elicit the inflammatory response. Intrapitoneal nucleated cell counts of greater than 100,000 cells/µL have been reported for 5 to 7 days after normal castration.13 Nucleated cell counts and peritoneal protein concentration are not helpful in differentiating between nonseptic and septic peritonitis in the early postoperative period (< 5–7 d). Therefore, cytological evaluation for the presence of degenerative neutrophils and intracellular bacteria as well as bacterial culture of the peritoneal fluid should be performed to confirm the diagnosis. Horses with septic peritonitis should be immediately referred for treatment with intravenous broad-spectrum antimicrobials and supportive therapy including intravenous fluids, anti-endotoxemia therapy, and in advanced cases, standing intraperitoneal lavage.

Iatrogenic Penile Damage

Iatrogenic penile damage has been reported and usually occurs when an inexperienced surgeon mistakes the shaft of the penis for the testis. This should be completely avoidable with a thorough understanding of the pertinent clinical anatomy and sufficient surgical experience of the practitioner. As with any surgical procedure, meticulous, atraumatic handling of the tissue with minimal unnecessary tissue manipulation will minimize postoperative morbidity. Excessive manipulation and dissection of the surrounding tissue may result in excessive inflammation, swelling, and paraphimosis. This is treated with aggressive hydrotherapy, NSAIDs, and mechanical support of the penis until the inflammation subsides. If inadvertent direct penile damage (laceration or transection) does occur, the horse should be transported to a referral facility for further evaluation and treatment.

5. Discussion

Castration is one of the most common elective surgical procedures performed in the field. Although the procedure is considered routine, various postoperative complications including excessive swelling and edema, hemorrhage, infection, omental herniation, eventration, hydrocele, or septic peritonitis may occur. It is more desirable to minimize the occurrence of postoperative complications rather than to have to deal with the associated morbidity, time, and effort in treating them. A thorough understanding of pertinent clinical anatomy, strict attention to asepsis and surgical technique, and proper postoperative exercise recommendations will minimize the incidence of complications associated with castration. However, when complications do arise, the practitioner should be able to quickly recognize and correctly and aggressively treat them to assure a rapid and successful outcome.

Acknowledgments

Declaration of Ethics

The Author declares that he has adhered to the Principles of Veterinary Medical Ethics of the AVMA.

Conflict of Interest

The Author declares no conflicts of interest.

References and Footnotes


*Monocryl, Ethicon, Somerville, NJ 08876.
Vicryl or PDS, Ethicon, Somerville, NJ 08876.
How I Perform an Island (Punch/Pinch) Skin Graft

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Free island skin grafts such as a punch or pinch graft can be easily performed in the field and are a helpful adjunct therapy to facilitate wound closure in the distal limb of horses. Case selection, preparation of the graft bed, meticulous technique, and perioperative care will greatly enhance successful graft acceptance. Author’s address: University of Georgia, College of Veterinary Medicine, Athens, GA 30602-7385; e-mail: emueller@uga.edu. © 2015 AAEP.

1. Introduction
A free skin graft is a section(s) of skin that has been completely detached from its vascular supply and relocated to a distant site. The graft must establish a new vascular connection to the recipient wound bed to survive. Skin grafts are also classified according to the source of origination. An autograph or isograph is a graft that is relocated from one area to a distant site on the same animal. Due to the minimal immune response induced by the host, this is the graft type most commonly performed in horses. An allograft is transferred between two animals of the same species, and a xenograft is transferred between members of different species.1

The use of island skin grafts in horses should be considered for full-thickness wounds that cannot be closed primarily or heal by epithelialization and contraction. In horses, these wounds typically occur at or distal to the tarsus and carpus. These areas have a paucity of soft tissues and the wounds are subject to extreme tension and movement. It is important to communicate to the horse owner that the primary purpose of skin grafting of the distal limb is to increase the available surface area of epidermis from which epithelialization can proceed, thereby decreasing the time to complete epithelialization and wound closure, not necessarily to significantly improve the end cosmetic appearance of the wound. Improper graft bed or donor skin preparation, hemorrhage, infection, and movement are the most common causes of graft failure, regardless of the type of graft performed. Two types of free skin grafts that are commonly performed in standing horses are the punch graft and the pinch graft. With both techniques, careful case selection, meticulous preparation of the graft bed and surgical technique, and proper perioperative care are imperative to optimize graft acceptance and outcome.

Graft Acceptance
To maximize the acceptance of a graft, the wound bed must be well vascularized and free of infection. Although fresh wounds can successfully receive graft, the severe degree of trauma and contamination that accompany the majority of distal limb wounds in horses precludes them from being good candidates for grafting. Therefore, it is much more commonplace to graft a wound later in the healing process, after an established bed of healthy granulation tissue has formed.

Initial adherence of the graft to the recipient wound bed occurs through formation of a thin fibrin clot. Vessels and fibroblasts from the wound bed
invade the fibrin clot within 48 to 72 hours, with the graft firmly adhered to the recipient wound bed by the tenth day after grafting. For the first 48 hours the graft is nourished by plasmatic imbibition, during which small vessels in the graft passively absorb plasma via capillary action. The graft has no inherent circulation during this period and becomes edematous. After 48 hours new capillaries in the center of the wound form and extend into the graft, anastomosing with existing capillaries in the graft. This process is called inosculation. During this time, additional new capillaries from the recipient site penetrate the graft and establish new microvascularity, a process called neovascularization. Effective revascularization and lymphatic flow occurs by the fifth day after grafting, leading to resolution of the edema. The epidermis of the graft becomes hyperplastic and often dies shortly after grafting, leaving exposed pale-pink dermis on the wound surface. These dermal tissue plugs resemble granulation tissue but may be differentiated by their pale-pink color as compared with the deeper, darker red color of granulation tissue. Epithelial cells migrate from the hair follicles and sweat glands within the dermis and soon cover the entire wound. Pigmentation of the graft site becomes evident approximately 4 weeks after grafting, with the appearance of hair 4 to 6 weeks after grafting.1

Causes of Graft Failure

The most common causes of graft failure are infection, fluid accumulation under the graft, and motion. Granulation tissue is inherently resistant to infection because of its abundant blood supply and high population of phagocytic cells. However, when the concentration of bacteria within the granulation tissue exceeds innate humoral and cellular defense mechanisms, the result is a localized infection. Although various types of bacteria may cause an infection, β-hemolytic Streptococcus sp. are a particular common and virulent isolate. β-hemolytic Streptococcus organisms produce proteolytic enzymes that degrade the early fibrinous attachment of the graft to the recipient bed, thereby preventing early adherence and subsequent inosculation. This results in early graft failure. Clinical signs associated with β-hemolytic Streptococcus sp. infection include a glossy, friable appearance of the granulation wound bed, a wound surface that bleeds easily with minimal manipulation, and in more advanced stages, suppurative exudate. Although the gold standard for diagnosing infection in chronic wounds is a quantitative tissue biopsy with greater than 10^5 cfu per gram of tissue of any organism, β-hemolytic Streptococci sp. are the exception, with any level of the organism indicating infection.2

Fluid accumulation in the form of hemorrhage, serum, persistent inflammatory exudate, or excessive and prolonged edema prevents graft adherence and vascularization by physically separating the graft from the recipient wound bed. Minimizing inflammation, controlling hemorrhage, and firmly compressing the graft to the recipient bed with a bandage will help minimized excessive fluid accumulation. As opposed to full-thickness sheet grafts, island grafts such as punch or pinch grafts allow excessive fluid to escape from the wound surface between the individual grafts, and therefore are less susceptible to failure secondary to fluid accumulation.

Excessive motion at the graft site is another cause of graft failure. Movement leads to shear forces at the graft-recipient wound interface, causing disruption of the fibrin seal and new vessel anastomoses and growth, interfering with the vital processes of plasmatic imbibition, inosculation, and neovascularization. This is of particular concern on wounds over high-motion areas such as the fetlock and tarsal joints. When grafting over high-motion areas it is optimal to immobilize the graft site with a half-limb or tube cast.

2. Materials and Methods

Case Selection/Preparation of the Graft Bed

In horses, skin grafts are most commonly utilized in chronic, slow-healing wounds of the dorsal distal limb. It is important that the granulation tissue recipient bed stays well vascularized and free of infection. If there is evidence of persistent inflammation, exudate, or a draining tract, it is imperative that the wound be thoroughly evaluated to rule out the presence of an underlying sequestrum or foreign body. Radiographic evaluation of the underlying osseous structures or ultrasound examination may be necessary to fully assess the suitability of the wound for grafting. Wounds that appear grossly abnormal or proliferative should be biopsied and examined histologically for the presence of cutaneous neoplasia such as equine sarcoid or squamous cell carcinoma. Healthy, vascular, granulation tissue should be uniform, smooth, deep red in color and free of exudate. More chronic, fibrous granulation tissue has a pale pink to gray appearance, representing the fibrous nature of the tissue and is not a suitable graft bed without additional preparation.

Once the recipient wound bed is deemed ready for grafting (healthy, adequate vascularity and free of infection), it should be lightly debrided with a scalpel blade or non-guarded disposable facial razor to a level just below the skin edge. This is performed approximately 36 to 48 hours before performing the grafting procedure. The sharp debridement serves two primary purposes: 1) removing surface contamination and resident bacteria, and 2) stimulates and provides time for new capillary growth to develop in the recipient bed. Fresh debridement of the wound bed can reduce the time from initial grafting to inosculation (plasmatic imbibition phase) from 48 to 24 hours.3 Following the debridement, a sterile, nonadherent, semi-occlusive pressure ban-
Dagea is applied to the wound to promote hemostasis and protect the wound bed. The bandage is changed daily until grafting. The author often applies Dakin’s solution (0.5% dilute sodium hypochlorite solution), a topical antiseptic to the recipient wound bed for 1 to 2 days prior to grafting. This solution is relatively safe for living tissues, reported to hasten the separation of dead cells from living tissue, and is effective in the presence of blood and serum. The solution can be applied directly to the wound bed, or applied to a sterile non-adherent dressing incorporated directly on the wound during bandaging.

Grafting Technique

Both the punch and pinch graft can be performed in the standing sedated horse. Horses should be up to date on tetanus prophylaxis. Preoperative procaine penicillin G (22,000 IU/kg, every 12 h, IM) and phenylbutazone (2.2–4.4 mg/kg, every 12 h) is administered for 24 to 48 hours. Horses are most often sedated with detomidine hydrochloride (0.01–0.02 mg/kg IV) and butorphanol tarate (0.01–0.02 mg/kg IV). The donor site is clipped and both the donor site and the graft wound bed are surgically prepared. The donor site is desensitized with 2% lidocaine hydrochloride, usually in an inverted “U” block so as not to directly infiltrate the skin that will be incorporated in the graft. The common donor sites for directly obtaining punch grafts from the horse are the ventrolateral abdomen, perineum, or portion of the neck that is concealed by the mane. Alternatively, an elliptical, 10-cm long × 4-cm wide full-thickness sheet of skin may be harvested from the cranial-pectoral area. The segment of skin is stretched and secured epidermal side down to a sterile piece of styrofoam or polypropylene block. The subcutaneous tissues are sharply excised away from the graft and the punch grafts obtained from the stretched sheet of skin.

Punch Grafts

Punch grafts are small, full-thickness plugs of skin that are harvested and implanted into the granulation wound bed using commercially available skin biopsy punches. The recipient sites in the granulation wound bed are created first to allow time for hemostasis prior to implantation. Because the skin graft plugs will undergo contraction after they are harvested, the biopsy punch used to make the recipient holes should be slightly smaller that the punch used to harvest the grafts. The author prefers to use a 5-mm punch to create the recipient holes and a 7-mm punch to harvest the donor grafts. It also is important to work from distal to proximal so that hemorrhage from creation of the recipient holes will not impair visualization of the wound bed. Starting at the most distal aspect of the granulation bed, recipient holes are made approximately 5 to 7 mm apart and 5 to 7 mm deep in a symmetrical pattern. A common mistake is to make the recipient holes too deep, in which case exuberant granulation tissue will cover the grafts and impede epithelialization. A single horizontal row of recipient holes are created, then each site is filled with a broken-off cotton-tipped swab to help control hemorrhage (Fig. 1). This process is continued until all the recipient holes have been created. The cotton-tipped swabs are left in place until they are replaced with a graft plug.

The donor punches are either removed directly from the horse (Fig. 2) or from a sheet of skin removed from the cranial pectoral area using a 7-mm biopsy punch (Fig. 3). Removing the graft leaves a small circular defect at the excision site, so they should be harvested in a symmetrical pattern, approximately 1 cm apart to optimize cosmetic appearance. The grafts are harvested, the fascia and subcutaneous fat sharply excised from the dermal side of the plugs to facilitate plasmatic imbibition and revascularization, and then placed on sterile, saline-soaked gauze until they are placed in the recipient holes. The cotton-tipped applicator is removed, and the graft placed into the recipient hole. It is impractical and of little importance to attempt to line up the direction of the hair follicles of each plug given that the wound will heal primarily by epithelialization with minimal hair growth. After all the graft plugs are inserted (Fig. 4), the wound is covered with a nonadherent...
dressing\textsuperscript{a} and pressure bandage. The author leaves the initial bandage in place for 4 to 5 days to allow for undisturbed adherence and revascularization during the plasmatic imbibition, inoscullation, and neovascularization phases. If the site is over a highly mobile area such as a joint, a cast or cast bandage should be utilized for 2 to 3 weeks to immobilize the area and optimize graft adherence. The cast can be bi-valved to allow access to the wound for bandage changes (Fig. 5).

The donor sites can either be closed with a single suture\textsuperscript{d}, staples, or left to heal by second intention. When a sheet of skin is removed from the cranial pectoral region en bloc, the incision should be sutured closed with an appositional or sometimes a tension-relieving pattern.
Pinch Graft

Pinch grafting is performed in a similar manner as punch grafting; however, instead of harvesting the plugs of skin, small 3–5-mm cones of skin are elevated with a curved cutting suture needle or a sterile 18 g hypodermic needle with the point bent 90°, and sharply excised with a scalpel. The grafts are placed on saline-soaked gauze sponges until implantation. The horse, donor site, and recipient bed are prepared as for punch grafting. A No. 15 scalpel blade is then used to create small pockets in the granulation recipient bed, in a symmetrical pattern to receive the grafts. It is important to work from distal to proximal so that hemorrhage from creation of the recipient pockets does not impair visualization of the wound bed. Starting at the most distal aspect of the granulation bed, recipient pockets are made approximately 5 to 7 mm apart and 5 to 7 mm deep in a symmetrical pattern. The scalpel blade is introduced at an approximately 45° angle, in a proximal-to-distal direction (Fig. 6). With the pinch technique, hemorrhage from the graft bed is difficult to control and the recipient sites difficult to identify soon after the recipient pockets are created. Therefore, the pinch grafts are immediately placed into their respective recipient pockets one by one as they are created. After all the pinch grafts have been inserted, the wound is covered with a nonadherent dressing and pressure bandage. If the site is over a highly mobile area such as a joint, a cast or cast bandage should be utilized for 2 to 3 weeks to immobilize the area and optimize graft adherence. Perioperative care is identical to that of punch grafting.

Both punch and pinch grafts are relatively easy to perform, economical, and can be performed on a standing, sedated horse. Each graft is independent of the others, and failure of one graft does not adversely affect the remaining grafts. Pinch grafting can be more tedious to perform and often results in an inferior cosmetic appearance compared with punch grafting; therefore, punch grafting is the method most often preferred by the author.
3. Results

With both techniques, 60 to 75% of the grafts can be expected to take (survive). The superficial, pigmented, epidermal portion of the grafts will usually slough within the first 1 to 2 weeks after grafting, exposing the pale underlying dermis and making it difficult to distinguish between the graft plugs and the surrounding granulation tissue. This should not be misinterpreted for graft failure. By 3 weeks the grafts become more readily apparent with pink rims of epithelium migrating centrifugally from the grafts (Fig. 7). Epithelial migration from the wound periphery and circumferentially from each graft coalesces to cover the entire recipient bed with epithelium. The wounds heal primarily with an epithelial scar with variable amounts of pigment and hair as the scar matures. The time required for the wound to become completely covered with epithelium is inversely related to the number of grafts placed within the recipient bed. As a rough guide, epithelialization of the distal limb usually progresses at a rate of 0.09 mm per day.4

It is important to note that because the initial epithelial layer covering of the wound is thin and extremely friable, the horse should be restricted to stall or small paddock confinement for an additional 4 to 6 weeks after the wound has healed to allow for maturation and thickening of the epithelial scar. Premature return to unrestricted turnout or athletic activity is a common cause for complete disruption of the epithelial scar, especially when located over high-motion areas, such as the fetlock, carpus, or hock joints.

4. Discussion

The use of island skin grafts in horses should be considered for full-thickness wounds that cannot be closed primarily or heal by epithelialization and contraction. There are several advantages of punch/pinch grafting. They are relatively easy to perform; can be performed in the standing sedated horse; and are economic alternatives to more expensive sheet grafting techniques. Additionally, each graft heals independently of one another and thus 100% graft acceptance rate is not necessary to effectively enhance the healing of the wound.4 It is important to communicate to the horse owner that the primary purpose of skin grafting is to decrease the time required for complete epithelialization and wound healing, not to significantly improve the end cosmetic appearance of the wound. The veterinarian must emphasize to the horse owner that the wound will heal with an epithelial scar and variable amounts of pigmented epithelium and hair.

Although the grafting techniques are relatively straightforward, improper graft bed or donor skin preparation, hemorrhage, infection, and movement can result in graft failure. With both punch and pinch grafting techniques, careful case selection, meticulous preparation of the graft bed and surgical technique, and proper perioperative care are imperative to optimize graft acceptance and outcome.

Acknowledgments

Declaration of Ethics

The Author declares that he has adhered to the Principles of Veterinary Medical Ethics of the AVMA.

Conflict of Interest

The Author declares no conflicts of interest.

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*Telfa Pad, Covidien, Minneapolis, MN 55432.*
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*d2-0 Prolene, Ethicon, Somerville, NJ 08876.*
1. Introduction
Intrasynovial sepsis in horses results in severe lameness and can potentially be career- and/or life threatening. In foals, infectious arthritis secondary to the hematogenous spread of bacteria is the most common form of intrasynovial infections encountered, whereas traumatically induced septic tenosynovitis and infectious arthritis seem to be more common in adults.1–3 Successfully treating an infected synovial structure requires rapidly eliminating the infecting organisms from the synovial structure before irreversible damage occurs.1–3 Recommended treatments include systemic antimicrobials and anti-inflammatories, synovial and endoscopic lavages, and arthrotones.1–3 Local antimicrobial administration has also been used to improve drug delivery to the site of infection in the form of intra-articular injections,1 intraosseous perfusion,4 regional limb perfusion,5,6 antimicrobial-impregnated polymethyl methacrylate beads,7 antimicrobial-impregnated collagen sponges,8 and continuous-rate infusion (CRI) via indwelling synovial catheters.9–11 Direct intra-articular antimicrobial injections can deliver concentrations greater than 100 times the minimum inhibitory concentration (MIC) of commonly isolated bacterial pathogens.12 To continuously deliver and maintain high intrasynovial antimicrobial MICs, a commercially available continuous-rate infusion systema is available to treat a multitude of joints, tendon sheaths, and bursas (Fig. 1).10

2. Materials and Methods
Pre-CRI Catheter Placement
Synovial fluid should be aspirated for cytologic examination, bacterial culture, and sensitivity. Infected synovial structures should be drained, lavaged, and/or debrided before placing the indwelling, intrasynovial CRI system. The intrasynovial CRI catheter placement system comes with a 14-gauge trochar that contains a peel-away introducer; this trochar can be used only once to lavage a synovial structure for a thorough lavage just before inserting the CRI catheter.

The pump of the CRI system, which is an elastomeric balloon, should be filled with a concentration-dependent antimicrobial solution 10 to 15 minutes before placing the catheter to allow time for the solution to fill the delivery tubing. The CRI administration pump holds a maximum of 100 mL and delivers antimicrobials at a rate of 0.5 mL/h. It is recommended that the administration pump be filled with 48 mL of either 100 mg/mL gentamicin sulfateb or 250 mg/mL amikacin sulfatec, thus providing a 4-day supply of antimicrobials. The pump
can be refilled if needed. It is likely that other antimicrobials (including time-dependent antimicrobials) could be used as the CRI perfusate based on bacterial culture and sensitivity results; however, no current referenced information is available. Avoid infusing air bubbles into the administration pump to prevent air from entering the flow control tubing within the CRI system. Air bubbles can obstruct the antimicrobial solution through the flow control tubing.9 Before placing the CRI catheter, ensure that the antimicrobial solution is beading at the end of the infusion tubing and flowing correctly before placing it within the synovial structure.

CRI Catheter Placement
The intrasynovial catheter can be placed after synovial lavage while under general anesthesia or using sedation and local anesthesia. A synovial pouch should be chosen that positions the catheter away from weight-bearing surfaces wherever possible (i.e., the palmar metacarpal joint pouch, plantar tibiotarsal joint pouch, proximal aspect of digital flexor tendon sheath). Aseptic intrasynovial CRI catheter insertion is imperative.

Insert the trochar that contains the peel-away introducer into the infected synovial structure all the way to the hub; it is easier to thread the indwelling catheter if the introducer is fully inserted into the synovial structure. Remove the trochar and pass the crush-resistant 16-gauge indwelling catheter through the introducer until at least 5 cm of the catheter is estimated to be within the synovial structure. Once the catheter is placed, pull the blue tabs on the introducer apart, up, and out of the synovial structure (Fig. 2). Next, attach the CRI administration pump that contains antimicrobials and its infusion tubing to the CRI indwelling catheter. The indwelling catheter and infusion tubing should be secured to the skin with a 2-0 monofilament suture using a simple interrupted pattern (Fig. 3). A sterile, protective bandage is applied over the indwelling catheter and infusion tubing. The excess tubing can be rolled up and placed along the outside of the bandage; position the administration pump on the limb so the tubing is pointed down toward the insertion site to reduce air bubbles from forming.
entering the tubing (Fig. 4). Additional bandaging can be applied to each side and over the top of the CRI administration pump. This will allow the pump to be secured to the outside of a sterile bandage using elastic tape. The tubing that exits the CRI pump should point distally to reduce air bubbles from entering the tubing.

Most intrasynovial CRI catheters are left in place for 5 to 10 days. The longest time period that Lescun et al. left an intrasynovial CRI catheter in place was 15 days. The catheter can be removed once treatment has been completed. A sterile bandage is then applied for at least 2 days depending upon the initial injury. Note that synovial fluid may be observed egressing from the catheter site after removal when the catheter has been in place for greater than 5 days.

3. Results and Discussion

Following appropriate medical or surgical therapies for infected synovial structures, the use of an indwelling, intrasynovial CRI catheter is an excellent adjunctive modality of therapy. It has been shown that the mean steady-state synovial fluid gentamicin concentration during continuous infusion is 1,069 μg/mL, which is greater than 100 times the MIC of commonly isolated equine bacterial pathogens. Because it is cost-effective and yields a high MIC, gentamicin is the author’s antimicrobial of choice when using the intrasynovial CRI system. This antimicrobial delivery method avoids repeated injections, articular damage that results from antimicrobial impregnated beads, damage associated with retrieval of nonabsorbable beads, and the anatomic limitations of regional limb perfusion. No significant effects on articular cartilage or synovial membrane histologic scores occurs with the continuous infusion of gentamicin. Two separate retrospective studies that investigated the efficacy of intrasynovial CRI antimicrobial infusion yielded resolution in 93% of horses with synovial infections.

The advantages of using an intrasynovial CRI catheter include its ease of placement, ability to maintain a high intrasynovial level of antimicrobials while periodically instilling other medications, and ease of removal when therapy is discontinued. If necessary, it is possible to lavage the infected synovial structure while the indwelling CRI catheter is in place; however, you cannot lavage directly through the catheter system because the intraluminal diameter of the indwelling CRI catheter is too small. Disadvantages of using this CRI catheter include the inability to aspirate synovial fluid through the lumen for synovial fluid analysis because of the small intraluminal-diameter of the CRI catheter, unknown effect on articular cartilage if the indwelling catheter is placed against a weightbearing surface, and potential of developing an ascending infection. Indwelling CRI catheters should be placed within joints distant enough to avoid contact with weight-bearing articular surfaces.

The intrasynovial CRI system should be considered when acute synovial infections are refractory to systemic antimicrobial therapy, synovial lavage, drainage, and regional limb perfusion. Intrasynovial CRI catheters have proven to be very effective clinically, and the author recommends implementing them in cases that present with chronic and complicated synovial structure infections.

Acknowledgments

Declaration of Ethics
The Author declares that he has adhered to the Principles of Veterinary Medical Ethics of the AVMA.

Conflict of Interest
The Author declares no conflicts of interest.

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How to Apply Foot Casts for Managing Distal Limb Injuries

John C. Janicek, DVM, MS, DACVS

1. Introduction
Distal limb injuries are frequently encountered emergencies in equine medicine. More specifically, heel bulb lacerations, pastern lacerations, hoof wall avulsions, collateral ligament injuries, and coffin bone fractures can be managed using a distal limb or foot cast. The most common distal limb injury is heel bulb or palmar/pastern lacerations. Primary or delayed primary closure should be performed, when appropriate, before applying a foot cast; however, distal limb wounds may be allowed to heal by second intention under a cast. Casts that incorporate the foot and extend upward to the distal fetlock can substantially decrease the length of convalescence in horses by more completely immobilizing the distal interphalangeal and proximal interphalangeal joints than bandages or just casting the hoof wall. Although the initial treatment of a distal limb injury may be performed under general anesthesia, it can be more advantageous to apply a foot cast while the horse is under general anesthesia may result in an improper hoof-pastern axis as a consequence of hyper- or hypoelevation of the distal portion of the limb.

2. Materials and Methods
Precasting Considerations
A considerable number of synovial structures (distal interphalangeal joint, proximal interphalangeal joint, navicular bursa, digital flexor tendon sheath) are present in the equine distal limb; therefore, all synovial structures close to a distal limb injury should be thoroughly examined to determine whether there is communication with the wound. Arthrocentesis and synovial structure distention with sterile saline may be necessary to determine synovial structure involvement (Fig. 1); radiographs, contrast radiographs, and ultrasonography are adjunctive diagnostic modalities that may help assess the severity of the injury and whether synovial structures are involved. If a synovial structure is involved, then appropriate therapy to treat the infected synovial structure should be performed. It is important that the clinician is confident that the infected synovial structure has been eliminated before a distal limb cast is applied.
Patient Preparation
The most common distal limb injury is heel bulb or palmar/pastern lacerations.\textsuperscript{1,2} Primary or delayed primary closure should be performed, when appropriate, before applying a foot cast; however, distal limb wounds may be allowed to heal by second intention under a cast. A light bandage that consists of a nonstick pad,\textsuperscript{a} elastic gauze,\textsuperscript{b} and a small length of elastic bandage material\textsuperscript{c} should be applied over the lacerations. Before a cast is applied, the shoe should be removed from the affected limb, and the foot should be trimmed to avoid focal pressure along the edges of the cast. All casting materials should be gathered in one location to expedite the cast application (Fig. 2).

General Casting Recommendations
General casting recommendations are as follows:

- Wear latex or nitrile gloves.

- Casting material should not be opened until ready to use.
- Submerge casting material in water for 10 seconds to activate cast resin.
- Shake off excess water from casting material.
- Warmer water typically results in faster casting material cure times.
- Avoid wrinkles when applying casting material.

Forelimb Cast
With the horse sedated, an assistant should hold the affected limb off the ground along the distal metacarpus so that the foot can position itself into a neutral position (Fig. 3). A double layer of stockinette\textsuperscript{d} (3–4 inches wide) is placed over the foot and extended to a level 2 inches above the metacarpophalangeal joint. A premeasured length of stockinette should be cut, and each end should be rolled toward the middle; one end is rolled inward, and the other end is rolled outward (Fig. 3). When applying the stockinette, it is important that it covers the sole (after one end of the stockinette is unrolled, twist the last roll of fiberglass casting material can be fanfolded upon itself 5 to 6 times in 6-inch increments; this fanfolded portion of fiberglass casting material can be placed on the solar surface of the foot and secured to the cast using the remaining portion of the casting material.

Fig. 1. Arthrocentesis and synovial structure distention with sterile saline may be necessary to determine synovial structure involvement. The distal interphalangeal joint is communicating with the palmar pastern wound in this horse.

Fig. 2. Materials needed to apply a distal limb cast include gloves, stockinette, felt, white tape, 3-inch casting material, acrylic, aluminum foil, and elastic tape.

Fig. 3. With the horse sedated, an assistant should hold the affected limb off the ground along the distal metacarpus so that the foot can position itself into a neutral position. A premeasured length of stockinette is applied, and orthopedic felt is taped around the pastern.

Fig. 4. The last roll of fiberglass casting material can be fanfolded upon itself 5 to 6 times in 6-inch increments; this fanfolded portion of fiberglass casting material can be placed on the solar surface of the foot and secured to the cast using the remaining portion of the casting material.
the stockinette at the toe 360 degrees and unroll the second end), extends just above fetlock, and is applied wrinkle-free to avoid unnecessary focal pressure under the cast. A 1-inch strip of felt is placed around the pastern just below the metacarpophalangeal joint (Fig. 3). Three rolls of 3-inch fiberglass casting material are applied to include the foot and extend proximally to overlap only half the width of the felt strip. The palmar aspect of the cast should be slightly lower than the cranial aspect of the cast to allow for metacarpophalangeal joint extension. It is important to fold the stockinette distally at some time during cast application so that it becomes incorporated into the casting material. The last roll of fiberglass casting material can be fan-folded upon itself 5 to 6 times in 6-inch increments; this fan-folded portion of fiberglass casting material can be placed on the solar surface of the foot and secured to the remainder of the cast using the remaining portion of the casting material (Fig. 4). The foot is placed on the ground during the final curing process to allow the cast to conform to the normal hoof-pastern axis (Fig. 5). After the cast is cured, acrylic is placed on the bottom of the cast and formed around the edges of the cast to reduce wear. Once the acrylic is mixed in a disposable cup, it can be poured onto a piece of aluminum foil and placed onto the bottom of the cast; this allows even spreading of the acrylic (Fig. 6). Once the acrylic begins to set, the foot can be placed on the ground, allowing the acrylic to fit the form of the bottom of the cast. Last, elastic bandage material should be applied along the top of the cast to prevent dirt, bedding, or debris from entering between the limb and the cast (Fig. 7).

Hindlimb Cast
Stockinette and felt strip application principles are the same as in the forelimb. Once the stockinette and felt strip have been applied, the horse’s hind foot should be positioned with the toe of the affected limb resting on a board that is at least 2 inches thick and 4 inches wide; the contralateral foot should be placed on a board with the same dimensions (Fig. 8). The affected hindlimb should be maintained in a neutral position, with as much of the foot as possible hanging off the back of the board. It is important that the solar portion of the foot remains parallel with the ground and that the toe does not become raised off the board during positioning. Properly placing the foot to be casted is crucial because it allows most of the cast to be applied while the limb is fully bearing weight. Two rolls of 3-inch fiber-
glass casting material are applied with the horse standing on the board and should encompass all of the foot except for the toe (Fig. 9). It should extend proximally to encompass the proximal pastern. The proximal aspect of the cast should overlap only half the width of the felt strip. It is important to fold the stockinette distally at some time during cast application so that it becomes incorporated into the casting material. The third roll of casting material should incorporate the toe and be applied with an assistant extending the limb cranially off of the board (Fig. 10). It is important to extend the limb cranially to keep the limb in extension as much as possible; pulling the hindlimb caudally to place the last roll of casting material will result in too much flexion of the distal limb. Flexing the limb while the casting material is not cured will result in wrinkling; as a result, this will cause focal pressure to the limb under the cast and not allow proper hoof-pastern alignment. A figure-8 pattern may be used to adequately cover the toe and ground surface of the

Fig. 7. Elastic bandage material should be applied along the top of the cast to prevent dirt, bedding, or debris from entering between the limb and the cast. Acrylic can be seen conforming around the cast edges.

Fig. 8. Once the stockinette and felt strip have been applied, the horse's hind foot should be positioned so that the toe of the affected limb rests on a board that is at least 2 inches thick and 4 inches wide; the contralateral foot should be placed on a board with the same dimensions.

Fig. 9. Two rolls of 3-inch fiberglass casting material are applied with the horse standing on the board and should encompass all of the foot except the toe.

Fig. 10. The third roll of casting material should incorporate the toe and be applied with an assistant extending the limb cranially off of the board. A figure-8 pattern may be used to adequately cover the toe and ground surface of the foot.
foot. After the toe has been sufficiently covered with casting material, the foot should be placed back onto the board, and the remainder of the third roll of casting material should be applied proximally. The cast should be allowed to cure while the horse is fully bearing weight and with the casted limb in a neutral position. After the cast is cured, acrylic is placed on the bottom of the cast as described for the forelimb cast.

3. Results and Discussion
Treating distal limb injuries should be specific for each horse; therefore, the original injury should determine the length of time the cast is worn. In general, only a single cast is required, and most distal limb casts are worn for 3 weeks. It is important that horses be confined to a clean, dry stall while wearing a cast to minimize cast complications. Elastic bandage material around the proximal cast should be changed every 3 to 4 days but should be changed immediately if it becomes wet. Casts need to be monitored daily for signs of fluid discharge from the proximal cast or through the cast, cast breakage, cast sore development around the proximal cast, excessive cast “wearing” around the toe or solar surface, or lameness development. If any of these abnormalities are noted, cast removal should be considered. Foul odor alone without the presence of these complications is not enough to warrant distal limb cast removal; the foul odor could be the result of sweating, production of granulation tissue exudate, or bacterial fermentation from the solar surface of the foot. Distal limb casts less commonly develop rub or pressure sores when compared to half- or full limb casts; cases requiring half- or full-limb casting yield 50% development of cast sores.

Cast immobilization can be used to manage a wide variety of distal limb injuries and offers many advantages over bandaging. Distal limb cast immobilization enhances healing by limiting the movement of the injured tissue, decreasing tension on sutures, limiting the development of excessive granulation tissue, providing a moist environment for re-epithelialization, and eliminating the time and cost associated with frequent bandage changes. Treating uncomplicated heel bulb lacerations that do not involve synovial structures is associated with a good prognosis for return to soundness (90%) and an acceptable cosmetic appearance (90%). When used appropriately, distal limb casts can be applied efficiently and effectively in the standing horse to manage distal limb injuries. Being able to apply a distal limb cast while the horse is standing is advantageous because this allows the proper hoof-pattern axis to be established within a well-conforming cast.

Acknowledgments
Declaration of Ethics
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How to Modify Hoof Boots to Treat Chronic Laminitis

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1. Introduction

Equine hoof boots have come a long way since the first widely used and commercially successful hoof boot was unveiled by nuclear physicist Dr. Neel Glass in 1970.1 Developed to help his daughter’s navicular horse, this original boot is still in production today. There are now numerous hoof boot manufacturers producing a countless array of boot styles and sizes. Boots have become commonly used for riding. This allows horses to remain barefoot when not on the trail or in the arena. Horses have won the grueling Tevis Cup 100-mile endurance race in a type of glue-on hoof boot the last 4 years in a row. Veterinarians often use hoof boots therapeutically. From the author’s personal observations, the most common therapeutic use of hoof boots is for the treatment of acute and chronic laminitis. Their ease of use, adaptability, and cost effectiveness make them a logical choice for this disease process. Paired with the right sole support option hoof boots can offer a laminitic horse much-needed comfort.

Improved comfort is important from a humane standpoint and owners tend to use comfort as a barometer for treatment success or failure. A more comfortable horse following boot application can, however, be deceiving. It is not uncommon for a horse with chronic laminitis to get more comfortable in boots, but deteriorate radiographically. As the structural integrity of the digit diminishes, pain often remanifests. This deterioration may be prevented or halted with a hoof boot that provides more optimal mechanics for the digit. Mechanics is a term commonly used by foot care professionals. Generally speaking, altering the mechanics of a foot involves trimming the hoof or applying a shoe or a device in an effort to alter foot function. A wedged shoe, rolled-toe shoe, and extended heel shoe for example, each apply a different type of mechanics to a foot. What might constitute optimal mechanics for one disease process or injury may be precisely the wrong mechanics for another. Hoof boots can be modified to optimize their support and mechanics. The objective of this approach is to achieve comfort while providing mechanics that improve the health and integrity of the compromised digit. The author realizes there are vastly differing views on what constitutes appropriate mechanics for the treatment of laminitis. The efficacy of the many shoeing approaches as they relate to chronic laminitis remains anecdotal.2,3 The beauty of this approach is that hoof boots can be modified to provide any support and mechanical combination you desire. With a well-fit boot you can glue on, screw on, or bolt on any shoe you can think of. Recently, interchangeable boot soles have been developed commercially (Figs. 1A and B) to sim-
plify and improve the modification of hoof boots. Boot bottoms are modified by attaching one of five interchangeable sole options with screws.

The objective of this paper is to familiarize the practitioner with the modification of hoof boots and the utilization of the commercially available interchangeable boot soles. Photos and descriptions that follow show mechanical choices that the author commonly uses. The author encourages practitioners to experiment with shoes/mechanics they have had success with. Addressing the underlying cause or causes of the laminitic episode is key to success and quite possibly more important than the application of any trim, shoe, or boot.

Fig. 1. A, Hoof boot with interchangeable soles. B, Hoof boot with a variety of interchangeable soles.
2. Materials and Methods

There are several key elements to the successful use of hoof boots for the treatment of chronic laminitis: 1) Choose boots with a perfect fit and solid construction, 2) choose case appropriate sole support, 3) modify the boots to improve their mechanics (anterior breakover, mediolateral breakover, and heel elevation when necessary), and 4) mitigate rubs and maintain skin and hoof health while in boots.

Choosing a Boot With a Perfect Fit and Solid Construction
A snug-fitting boot is critical to the success of this approach. Because of the cylindrical nature of the horse’s hoof, boots that are too large tend to twist and turn. This tendency is compounded when shoes are applied to the bottom of a boot. Imagine the consequence of a boot that spins 180° with a 10° wedged shoe on the bottom. Instead of heel elevation and reduced tension in the deep digital flexor tendon (DDFT), you now have toe elevation and increased tension in the DDFT. Boots should be selected after the foot has been trimmed appropriately. Most boot manufacturers have sizing charts and other recommendations on their web sites. As you become accustomed to certain boots, fitting becomes second nature. A well-constructed hoof boot is of paramount importance. From experience when you add a shoe to a boot, you are asking that boot to perform a task that it really was not designed for. For example, the author uses heel elevation on many horses with laminitis. The wedge places added strain on the toe of the boot and many boots fail at the toe region over time.

Choosing Case-Appropriate Sole Support
Hoof boots are commercially available with a variety of supportive insole choices. This gives practitioners the ability to customize sole support on a case-by-case basis. The author commonly uses ethylene vinyl acetate (EVA) foam pad material. This material can be purchased in bulk in a variety of densities and is surprisingly lightweight. The author has sourced a variety of EVA foam pad materials from gym mat manufactures, stall mat manufactures, and even archery targets. Orthopedic felt cut to fit the boot bottom is an excellent choice as well. The author typically uses felt when he feels a horse can handle the firm support that felt provides. The author has found that some horses prefer felt over impression materials, silicones and urethanes, because they do not apply much pressure to the medial and lateral sulci of the frog and sometimes bars (depending on hoof conformation). Hoof emollients, hardeners, and medications can also be applied to felt pads. Felt padding for carpet and area rugs can be obtained from your local carpet store. Carpet felt is a plentiful and inexpensive felt option. Just as with more traditional shoeing approaches, tube-based silicones and urethanes can be used to make custom sole and frog supports. Jar-based two-part dental impression materials can be used as well. The author prefers to use two-part dental impression materials in boots over tube-based support materials because they are neater, more user friendly, and do not tend to stick to the boots or hoof as readily. Regardless of your preference, it is important to understand that most support materials are available in a variety of durometers (hardnesses). In general, the harder the material, the higher the durometer, and the more support it offers. For example, a support material with a marshmallow-like consistency offers little support to the sole whereas a support material with a rubber-like consistency offers more support. The author uses dental impression materials in the 50-durometer range on most horses with laminitis. The author keeps material in the 25-durometer range on hand for horses that do not tolerate the firmer impression material (Fig. 2).

Modifying Hoof Boots to Treat Horses With Chronic Stable and Chronic Unstable Laminitis
Chronic laminitis is defined by the presence of mechanical collapse of the lamellae and displacement of the distal phalanx. Simplistically, horses with chronic laminitis are those that have displacement of the distal phalanx that is radiographically apparent (be it dorsal capsular, mediolateral displacement, distal displacement, or any combination thereof). Chronic laminitis is further subcategorized as chronic compensated (stable) and chronic uncompensated (unstable). A chronic stable foot is no longer actively displacing and is growing wall and sole whereas a chronic unstable foot is actively displacing. Both stable and unstable horses can be sore but the unstable horse often suffers the consequences of the digital instability with secondary abscessation, seromas/hematomas, osteomyelitis, coronary band shear lesions, solar penetration, prolapsed solar corium, etc. Shoeing mechanics are
similar for treating the chronic stable and chronic unstable horse (with dorsal capsular rotation) but special attention must be placed on managing secondary infections and complications in the chronic unstable horse. Horses with mediolateral and/or distal displacement (sinkers) often require a different mechanical approach and will be covered separately from those with dorsal capsular rotation.

Modifying Hoof Boots to Treat Horses With Chronic Stable and Chronic Unstable Laminitis That is Predominantly Dorsal Capsular in Nature

With experience, hoof morphology (growth rings, stretching of the white line, solar prolapse, etc.) can give the practitioner great insight into what is going on inside the hoof capsule. A horse with unstable laminitis can displace in a matter of days and even hours. It can take weeks to months before hoof morphology will reflect these changes. Considering this, the author prefers to take quality radiographs prior to trimming horses with laminitis.

The author approaches the treatment of dorsal capsular rotation with well-recognized biomechanical principles as described previously by Morrison and O’Grady. The hoof boot is simply going to provide a novel means of applying shoeing mechanics. It bears repeating that the efficacy of shoeing approaches as they relate to chronic laminitis remains anecdotal. Generalizing when talking about laminitis is dangerous given that each case is different and deserves an individualized approach. Having said that, the process can be broken down into the trim (wall sculpting and heel trim), support, and the application of mechanics (altering anterior breakover, mediolateral breakover, and heel elevation when necessary). When treating horses with dorsal capsular displacement, the author trims the heels in an effort to recruit the healthier caudal portion of the foot to share more load (Fig. 3). Excessively long toes and flares are sculpted with a rasp. Because this technique is not dependent on the hoof wall for shoe attachment, the ground surface of the wall can be rounded in an effort to move weight bearing axially (Fig. 4). The frog and bars are often recruited to bear weight by adding support material (covered in the section above, “Choosing Case-Appropriate Sole Support”). The foot is wedged to varying degrees in most cases. This is in an effort to get the solar margin angle of the distal phalanx near its pre-trim state (Figs. 5A and B). This negates the effects that the heel trim has on tension in the DDFT and consequently the dorsal lamellae. A rolled and/or rockerced toe is used to enhance digital breakover and reduce the moment arm around the distal interphalangeal joint. This is thought to decrease stresses within the dorsal lamellae. In most cases the author chooses to place the point of breakover as far back as possible without the horse tipping forward onto the rocker in the stance phase. The anterior coronary band can be used to approximate this point (Figs. 5A and B).

A shoe is chosen or modified that fits the boot and eases mediolateral breakover in an effort to reduce torque placed on the lamellae in turns. Clogs, rail shoes, and half-round shoes, for example, accomplish this well. The shoe is rockered, rasped, or ground to provide the desired breakover. Methylmethacrylate hoof glues adhere well to boot bottoms constructed of polyurethane. You must also be sure that the glue will adhere to your shoe choice. Methylmethacrylate bonds well to aluminum, polyurethane, and wooden shoes. Approximately 5
ounces of glue is necessary to secure most shoes to a boot. The author prefers a swift-setting methylmethacrylate with a 2–4-minute cure time. Shredding one square inch of fiberglass cloth material into every 2 ounces of glue increases the tensile strength and will make it a nicer consistency to work with. It is helpful to place the boot in a clamp or vice with the ground surface facing up. Masking the boot off with duct tape prior to mixing glue ensures a nice, clean, finished product. Glue should be mixed thoroughly and applied to both the boot bottom and shoe. The shoe is then pressed firmly into place and the excess glue is sculpted to a nice cosmetic look. Excess glue can be wiped away with cotton gauze (Figs. 6A–D). The glue will go through an exothermic phase. When the glue has cooled, imperfections can be rasped or ground if necessary. The boot can then be applied to the foot. Radiographs taken following boot application ensure the desired mechanics have been achieved (Fig. 5B).

To apply mechanics using commercial interchangeable boot soles, select a compatible boot and choose a sole that provides the desired mechanics (Fig. 1). The interchangeable soles are currently available in five options as follows: 1) 10° wedge with rolled toe, 2) 5° wedge with rolled toe, 3) clog, 4) flat with rolled toe and heel extension, and 5) 5° wedge with rolled toe and heel extension. Select the desired sole and secure with three-quarter-inch No. 14 Phillips head screws. Again, it is recommended to take radiographs following boot application to ensure that the desired mechanics have been achieved. Breakover can be adjusted with a bench grinder or a rasp.

Modifying Hoof Boots to Manage Horses Receiving a Deep Digital Flexor Tenotomy

Deep digital flexor tenotomy is indicated for horses that continue to rotate despite the application of shoeing mechanics to address the collapse of the digit. It is also indicated in horses that stabilize radiographically but remain significantly painful. Sole and wall growth is typically diminished in these horses. Finally, tenotomy is indicated for horses that suffer from severe acquired flexural deformity that can occur with chronicity. Transection of the DDFT is a serious decision that should not be taken lightly. Complications include distal interphalangeal joint subluxation, distal interphalangeal joint arthritis, acute overloading of the heels and/or quarters with further digital collapse, retraction of the muskulotendinous unit, and overcorrection resulting in a chronic negative solar margin angle and heel pain.

There are two different approaches commonly used to shoe for a deep digital flexor tenotomy. Both methods use a heel extension to prevent hyperextension of the distal interphalangeal joint. Both methods advocate trimming heel and propping the toe in an effort to achieve a near-0° solar margin angle relative to the surface of the shoe. Both techniques are commonly referred to as a form of “derotation shoeing.” The difference lies in the application of heel elevation to minimize distal interphalangeal joint subluxation. Some practitioners use a flat shoe (usually an egg bar) leaving the solar margin angle of P3 flat relative to both the shoe and the ground. Horses shod in this manner tend to exhibit at least some degree of distal interphalangeal joint subluxation. Other practitioners apply varying degrees of heel elevation until distal interphalangeal joint subluxation is eliminated post surgically (Fig. 7B). There is a commercially available shoe for this purpose. It is a rail shoe with 5° of wedge and a sizable heel extension. Elevating the heels to mitigate joint subluxation is thought to prevent secondary distal interphalangeal joint arthritis. The author has used both methods extensively. Anecdotally, the
author believes he achieves a lower incidence of post-tenotomy heel pain using heel elevation. Consequently, the majority of the author’s tenotomies are now performed with heel elevation. The author has not appreciated distal interphalangeal joint arthritis of major clinical significance with either method. Both forms of derotation shoeing are technically challenging requiring some experience with glue-on technologies. The use of a flat egg bar shoe following tenotomy was described by Morrison. The use of a 5°w edged rail shoe with a heel extension was described by Redden. Either type of shoe can be glued to a hoof boot with methylmethacrylate as discussed previously (Fig. 8). Commercial interchangeable soles can be used post tenotomy as well. Select and secure either the 5°wedged or flat heel extension (Fig. 1B).

Fig. 6. A, An aluminum rail shoe being glued onto a hoof boot that is being held by a vice. The boot is masked off with duct tape to ensure a cosmetic outcome. B, Aluminum rail shoe glued onto hoof boot. The finished product is shown following duct tape removal. C, Polyurethane rail shoe in the process of being glued on. D, Polyurethane rail shoe glued on. Photo shows the finished product.

Fig. 7. A, Distal interphalangeal joint subluxation following tenotomy with derotation shoeing. The shoe is a flat egg-bar shoe with a heel plate. B, The same foot as pictured in panel A was switched to a 5°rail shoe with a heel extension due to heel pain 8 weeks later. Notice the improvement in distal interphalangeal joint subluxation provided by heel elevation.
Trimming remains the same regardless of the method you prefer. With radiographic guidance, the heels are trimmed aggressively without invading the live sole. Most cases are rarely trimmed at all forward of the widest part of the foot. This ensures as much foot mass as possible remains beneath the tip of P3. Some horses may require additional toe elevation to achieve a 0° solar margin angle relative to the boot bottom. Cutting a wedge pad to match the interior of the boot and placing the thick portion at the toe accomplishes this well. The support option of choice can then be placed on top of the wedge pad. Radiographs should be taken to ensure that the desired phalangeal alignment has been achieved. An extremely well-fit boot is required for this technique. Without a snug fit, the toe can flip up within the boot resulting in serious heel bulb rubs or sores. In addition, the heel extension provides leverage that can predispose the boots to twisting on the foot. The author prefers to use glue-on shoes following a tenotomy because they require less maintenance and he is comfortable with glue-on techniques. Boots with heel extensions are used by the author most commonly to give horses a break from shoes. This is done for a variety of reasons including soreness, hoof wall deterioration under glue, etc. (Figs. 9A and B). Modified hoof boots may provide practitioners an alternative method of derotation shoeing. This could be especially beneficial in situations where the farrier and/or practitioner are not comfortable with the traditional glue-on derotation technique.

Using Hoof Boots to Treat Distal and Mediolateral Displacement (Sinkers)

Distal and mediolateral displacement is the most challenging manifestation of chronic laminitis to...
Managing Secondary Infections and Complications in the Chronic Unstable Horse

With more traditional shoeing approaches unstable horses often require the fabrication of specialized treatment or hospital plates to manage areas of secondary subsolar abscesses, seromas, hematomas, osteomyelitis, solar penetration, prolapsed solar corium, etc. Hoof boots can be completely removed giving total access to the solar surface. Similarly, boot removal allows easy and frequent access to the coronary band and hoof wall. This allows the practitioner access to submural abscesses that drain at the coronary band, partial hoof wall resection sites, or shear lesions. Boot removal allows totally unimpeded follow-up radiographs and venograms to be completed. Easy and frequent access to the entire digit is a significant benefit to this approach.

Larval therapy has been described as an adjunctive therapy to treat secondary infections arising from chronic laminitis. The author has utilized larval therapy with light foot bandages under hoof boots successfully. A light foot bandage is necessary, given that the larvae tend to escape the boot without it. Care must be taken to ensure that the larvae are not crushed. Felt pads with material removed at the site of larval placement is helpful if there is not a subsolar or submural cavity to place them in.

Mitigating Rubs and Maintaining Skin and Hoof Health While in a Boot

Horses maintained in hoof boots for an extended period of time are prone to dermatitis, skin irritation, rubs, and sores. It cannot be emphasized enough how important a perfect-fitting boot is. Hooves can become soft and water logged from sweat and environmental moisture. Commercially available hoof hardeners and sole paints are commonly used to combat this. Baby powder applied to a felt pad works surprisingly well and is the author’s preferred method. In very moist environments it is beneficial to have a spare set of boots allowing caretakers to rotate to a dry pair.

Interestingly, some horse owners use human socks on their horse’s hooves prior to applying boots. There are several benefits to using socks with hoof boots. Socks minimize rubs and sores and the moisture-wicking properties of some socks seem to promote better overall skin health. The author routinely dusts socks with baby powder prior to applying them for even better moisture control. In addition, the upper portion of the sock can be folded down over the boot’s straps. This protects the straps from chewing or loosening by the patient (Fig. 11).

3. Results

Over the period of 1 full year from March 2014 to March 2015 the author used modified hoof boots to treat 16 horses with chronic laminitis. Modified hoof boots were used primarily when other shoeing...
or boot options proved unsatisfactory. Both methods of boot modification were used (gluing on shoes and the commercial convertible soles). Both methods proved to be equally functional and reliable. Three horses were euthanized for humane reasons in that time period. Two of the euthanized horses were severe mediolateral sinkers and the other was suffering from dorsal capsular rotation with severe osteomyelitis. Boots with a 5° wedge and heel extension were used post tenotomy in three cases. The remainder of the horses received boots with a 5° wedge and rockered toe. Given the chronic nature of this disease many of the cases were still ongoing at the time of paper submission. Of the 13 surviving horses eight were transitioned to various glue-on shoes when hoof health and comfort allowed. The remaining five horses were still in modified hoof boots at the time of paper submission.

4. Discussion

Laminitis is a disease that requires the practitioner to react and adapt quickly to the changing clinical picture. Hoof boots allow sole support changes to be made by loosening a couple of straps or buckles. This is a distinct advantage of using hoof boots over traditional therapeutic shoeing methods. For instance, to change the density of dental impression material under a heart bar or heel plate, the shoe must be removed and reapplied in most instances. Similarly, to change the type of mechanics of many commonly used therapeutic shoes, the shoe must be removed, altered, and reapplied. With this approach practitioners can make changes to support and mechanics independent of each other and with ease.

Pros of this approach:

- Modified hoof boots can provide treatment that is both comfortable and mechanically favorable for the treatment for chronic laminitis.
- Modified hoof boots allow relatively easy application of good mechanical treatments without the need for advanced farrier skills.
- Modified hoof boots allow you to be nimble. Changes to both mechanics and support can be made quickly if current set up is not working.
- Modified hoof boots are applied with minimal trauma. Boots can be altered off of the horse eliminating the need for a horse to stand on one leg while being shod.
- Modified hoof boots are cost effective when compared with common glue-on methods. Quality hoof boots have approximately 3–4 months longevity with continuous use.
- Modified hoof boots allow the application of good mechanical treatments to feet that are sometimes too debilitated for nail-on or even glue-on shoes.
- Modified hoof boots allow easy and complete access to the entire digit.
- Modified hoof boots can expand your ability to help horses that reside well beyond your practice area. You can modify boots and ship to other veterinarians, farriers, trainers, and owners.

Cons of this approach:

- There is the potential for needing a large inventory of boots given that there are many types and sizes.
- Rubs, sores, dermatitis, etc. can develop with prolonged or improper use of hoof boots.
- Modified hoof boots are relatively high maintenance. Owner and staff compliance is critical to success. Boots should be reset frequently (at least every other day). This is challenging because owners often want a “set it and forget it” approach.

Modified hoof boots as they relate to this paper were predominantly used when other methods had already failed. It is interesting to note that many horses became more comfortable in boots with no changes to the previous mechanical approach. Meaning, the same shoe was often removed from the foot and glued to a boot bottom. The author spec-
ulates that using a boot to indirectly attach a shoe to the hoof wall allows the boot to act as a buffer against forces that are painful to some horses including shock, vibrations, and torque. The semi-soft construction of the boot bottom is likely dampening shock and absorbing vibrations pro-

Fig. 12. A, Chronic laminitic foot in an unmodified hoof boot. Support material was EVA foam pad. Mare was 2 months from foaling, Obel grade 3 in the boot above. B, Same mare in panel A now in a hoof boot with a 5° interchangeable sole. Support material was EVA foam pad. Mare was Obel grade 1 to 2 for the remainder of her pregnancy. She delivered a healthy, full-term foal. She has been transitioned to glue-on shoes. C, Chronic laminitic foot. Mare was Obel grade 3. Mare had a large subsolar seroma. A modified hoof boot was chosen to provide mechanics and keep the solar defect clean and protected. D, Same mare in panel C, shown in a hoof boot with a 5° interchangeable sole. Support material was felt pad soaked in betadine solution. Mare was Obel grade 2 in the modified hoof boot. She has been handed over to her farrier and is in and out of boots depending on her comfort level.

Fig. 13. A, Chronic laminitic mare 3 years post tenotomy of the DDFT. She has a chronic, negative solar margin angle of the distal phalanx due to overcorrection. She suffers from heel pain and occasional abscesses in the caudal portion of her foot. B, Same mare in panel A, shown in a hoof boot with a 5° polyurethane rail shoe glued on. She has been managed for > 1 year in a modified hoof boot. She is turned out 12 hours a day in a small paddock. She has dedicated owners, a good quality of life, and is able to successfully perform her duties as a broodmare.
duced by the shoe’s impact with the ground. Importantly, hoof boots allow impression material, EVA foam, felt, etc. to be placed between the hoof wall and the boot/shoe. The support material’s location there also allows it to dampen shock and absorb vibrations as they make their way to the hoof wall. The somewhat elastic nature of the boot’s attachment to the hoof wall is likely reducing torque that is transferred from the shoe to the wall when pivoting or turning.

The author’s overall impression of using modified hoof boots to treat chronic laminitis is very favorable. In general, horses became more comfortable and hoof morphology improved. Due to the demographics of the author’s practice area the majority of cases were Thoroughbred broodmares. Modified hoof boots allowed many of these mares to carry their pregnancies to term with greater comfort (Fig. 12, A–D). Modified hoof boots require a lot more effort on the part of caregivers than more traditional approaches. The author views them primarily as a temporary treatment for chronic laminitis. As hoof morphology improves, he tends to transition them to shoes or barefoot. However, with a dedicated caregiver, modified hoof boots may provide a long-term solution for severely compromised feet. The author has been managing a chronic laminitic mare with post tenotomy complications for over a year in modified hoof boots (Fig. 13, A and B). This technique is in its infancy but does seem to be a viable and useful treatment option for chronic laminitis.

Acknowledgements

Declaration of Ethics

The Author declares that he has adhered to the Principles of Veterinary Medical Ethics of the AVMA.

Conflicts of Interest

The author is co-inventor of the interchangeable boot sole concept. This technology was licensed to Easy Care for commercial production.

References and Footnotes

1. Conversation with Mr. Garrett Ford of Easy Care, Inc., Tucson, AZ 85755.
Plasma, Soft Tissue, and Bone Concentrations of Ceftiofur After Regional Limb Perfusion in Horses

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Regional limb perfusion (RLP) in healthy horses using ceftiofur produced adequate plasma concentrations for 12 hours and soft tissue concentrations for 24 hours. Bone concentrations were not adequate beyond the initial perfusion time. Authors’ addresses: Department of Clinical Sciences, (Dr. Cox’s address at the time of the study), Gail Holmes Orthopaedic Research Center, Department of Clinical Sciences, (Nelson), Flint Animal Cancer Center (Wittenburg), College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Fort Collins, CO 80523; Department of Population Health Sciences, Virginia–Maryland Regional College of Veterinary Medicine, Virginia Tech, Blacksburg, VA 24061 (Burgess); and Department of Clinical Sciences, Washington State University, College of Veterinary Medicine, Pullman, WA 99164 (Gold); e-mail: shamrider12@hotmail.com. *Corresponding and presenting author. © 2015 AAEP.

1. Introduction
RLP is a treatment option for injuries and infections of the distal limb in horses. RLP using ceftiofur sodium has been studied but it is unknown whether this antimicrobial drug significantly penetrates soft tissue and bone. The objective was to determine the concentration of ceftiofur in plasma, soft tissue, and bone in healthy horses after RLP.

2. Materials and Methods
Six healthy horses were used. Under standing sedation, a tourniquet was applied to the proximal metacarpus. RLP was performed in both forelimbs of each horse by injecting either 2 g ceftiofur sodium expanded to 60 mL with sterile saline (experimental) or 60 mL saline (control) intravenously in the lateral palmar digital vein. The tourniquet was left in place for 30 minutes post injection. Plasma, soft tissue, and bone samples were collected at times 0, 12, and 24 hours post-RLP. Concentrations of ceftiofur and its metabolites were analyzed using high-performance liquid chromatography.

3. Results
The median plasma concentrations were greater than 1 μg/dL at time 0 and 12 hours. The median soft tissue concentrations were greater than 1 μg/g at the three time points. The median bone concentrations were greater than 1 μg/g at time 0 and were below 1 μg/g at 12 and 24 hours.
4. Discussion
Ceftiofur sodium administration via RLP achieved adequate plasma concentrations for 12 hours. Adequate soft tissue concentrations were achieved for 24 hours but bone concentrations were not adequate beyond the initial perfusion time. Further research is needed to understand ceftiofur sodium administration via RLP in disease states.

Acknowledgments

Declaration of Ethics
The Authors declare that they have adhered to the Principles of Veterinary Medical Ethics of the AVMA.

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Olin K. Balch, DVM, MS, PhD*; Greg G. Habing, DVM, MS, PhD; and Harold C. Schott II, DVM, PhD, DACVIM

Fatality rates in endurance rides are low; however, risk increases with ride distance. Colic is the most common clinical presentation. Authors’ addresses: North Fork Veterinary Service, 514 P31 Sawyer Street, Cascade, ID 99163 (Balch); Department of Veterinary Preventive Medicine, The Ohio State University, Columbus, OH 43210 (Habing); and Department of Large Animal Clinical Sciences, Michigan State University, East Lansing, MI 48824 (Schott); e-mail: olinbalch@gmail.com. *Corresponding and presenting author. © 2015 AAEP.

1. Introduction
Fatalities are a recognized consequence of prolonged endurance exercise but data documenting incidence and causes are limited.

2. Materials and Methods
Numbers of starts and fatalities were obtained from the American Endurance Ride Conference Web site and details regarding fatalities were obtained from attending veterinarians. Annual incidence rates and odds ratios for region and ride length were calculated.

3. Results
Seventy-one fatalities attributable to the demands of endurance exercise occurred among 270,070 starts in American Endurance Ride Conference–sanctioned rides (2002–2014). Fifty horses were eliminated during competition while 21 completed the ride distance and were deemed fit to continue, thereby receiving completion awards. Fourteen horses died and euthanasia was performed in the remaining 57, of which 50% had necropsy examinations performed, and 75% developed colic and necropsy findings included gastric rupture in nine horses. Nineteen riders declined fluid therapy, referral, and/or surgery when recommended. There were no differences between years or regions. In contrast, the risk of fatality was ~2 (0.26 fatalities per 1,000 starts) and ~10 (1.46 fatalities per 1,000 starts) times more likely for participation in 50- and 100-mile rides, compared with competition in limited-distance rides (25-30 miles, 0.14 fatality per 1,000 starts).

4. Discussion
Overall fatality rates of endurance horses were low but increased with ride length. Fatalities associated with 100-mile rides were comparable with those for flat racing but considerably less than that for steeplechase racing.

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Declaration of Ethics
The Authors declare that they have adhered to the Principles of Veterinary Medical Ethics of the AVMA.

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The Authors declare no conflicts of interest; Dr. Balch is a member of AERC Veterinary Committee, Research Committee, and Board of Directors; Dr. Schott is a member of the AERC Research Committee.

Research Abstract—for more information, contact the corresponding author
Strategies for Promoting Success for Women in Equine Veterinary Medicine

Carol Sabo, DVM

Haymarket Veterinary Service opened its doors in 1997, when it was just me and one part-time associate. I started practice life in a conventional equine practice with a 5-day workweek. I was the first female vet that stayed in that practice after having a child. When the reality of having a kid and trying to balance it all hit me, I negotiated a half day off instead of a pay raise. When my second child was born 6 years later, I negotiated the second half day, allowing me to have a 4-day week. As fate would have it, I started my own practice when that child was 9 months old. Of course, I was back to square one, but I knew that my goal would be to have a practice with vets working 4-day weeks.

We currently have 8 veterinarians, 8 technicians/assistants, and 4 administrative personnel. We are different from most large equine veterinary businesses in that all of our employees are females. We have weathered internally many of the same challenges that face most equine veterinary practices: personal health issues, change of marital status, births, and deaths. Our external pressures are shared by others in the profession as well and include declining horse populations paired with higher customer expectations, financial uncertainties among our clients, and increased costs that affect our ambulatory practice. Despite these internal and external challenges, we feel that we are doing something right in creating an excellent working environment. For example, our employee turnover is low—we have had two vets leave us in nearly 20 years of practice history. Our support staff also mirrors that statistic.

This complex environment clearly requires simplifying strategies and tactics to meet our goals of providing timely, predictable, competent, and professional service while still nurturing the best working environment for our veterinary and support staff. Perhaps counterintuitively, we’ve found that by standardizing shared resources and processes we have created a work culture that maximizes flexibility. For example:

- Our ambulatory service trucks carry equipment and medications organized in a standardized layout. Trucks are cleaned and restocked each evening after service and remain at the practice overnight except when needed on call. Although each of our vets has a favorite truck, all trucks are available for use.
- Veterinary assistance is provided by any of a group of trained staff technicians so that the
absence or unavailability of one person does not impede workflow.

- Veterinary assistants are cross-trained to work in the office, which provides experience in the office environment, mitigates an “us versus them” mentality, and gives the staff a broader view of the practice.

- Clients accept more than one doctor treating their horse. This system allows for vets to share cases and have real days off when they are off duty. Regular updates and communication between vets promotes confidence that there is continuity of care. A good software system and laptops keep us all up to date on the latest bloodwork and client communications. Updates to patient records are completed before a vet leaves for the day, alleviating lost information and lost revenue as a result of forgotten charges.

- Practice-owned cell phones are kept in the trucks to minimize the personal cell phone umbilical cord often seen between vet and client. Most communications come through the office during regular business hours. Of course, office staff must be very well-trained and familiar with clients to personalize the experience.

- Use of an iPad in the office allows easy communication between the vets on the road and the team in the office. Group texting allows for all the trucks to know what emergencies have come in during the day and which vets may be getting bogged down with calls and may need help to finish their day. Texting the veterinarian while the client is on the phone reduces the need to call the client back and gets the client answers sooner.

All of these strategies have been fine-tuned over the years with an eye for exceptional customer service, patient care, and employee work–life balance. Specifically:

1. It allows veterinarians to work a 4-day week while taking great care of cases.
2. It allows for illness, injury, pregnancy, and child and elderly caretaking without a loss of continuity in patient and client care, which increases our clients’ confidence in us.
3. It allows for vacations, horseshows, family events, farms, second careers, and just about anything that may catch the fancy of a group of professionals who wants to be equine veterinarians but not to the detriment of pursuing outside interests.

Most veterinarians in the practice were hired out of internships. This makes them especially grateful for the workweek, and they see the value of time away from their job. When interviewing, the practice culture is emphasized, and we strive to hire veterinarians and staff that we feel would fit in with our group. I think the practice works well because the employees are committed to having personal time despite the fact that a different structure may be more economically lucrative.

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Declaration of Ethics
The Author declares that she has adhered to the Principles of Veterinary Medical Ethics of the AVMA.

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The Author declares no conflicts of interest.
Ambulatory Practice and the TEAM Approach

Mary Beth Hamorski, VMD

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1. Introduction

Creating a consistent and sound business philosophy begins with how you envision the growth of your practice while maintaining the client base you already have. Female practitioners will likely have even more challenges in achieving this goal when factoring in family matters and career choices. Many of us would not have chosen a career as an equine practitioner if we didn’t love working with horses as well as medicine and surgery. The purpose of this article is to demonstrate how to successfully chart your own path to achieve a solid work and family life.

To achieve this in my equine ambulatory practice, I instituted the “together everyone achieves more” (TEAM) approach about 2 years ago. I have owned this practice since 1994 with a partner who does companion animal practice. Our practice started out primarily with racetrack and Standardbred breeding horses and now focuses on sport and pleasure horses. We currently employ 3 full-time veterinarians, 1 part-time veterinarian, and a staff of 4 receptionists and technicians plus 1 office manager. The TEAM approach was utilized at the middle school where my children attended. It has been utilized in many settings and can be adapted to any business setting. The goal of the TEAM approach is to have the day fall into such a groove that it leads to more time during the off-hours. The TEAM approach focuses on all members to contribute to a positive work atmosphere and mutual respect. Although we all have different personalities, our goals are the same—providing the best care for our patients and their owners. We rely on our strengths and help each other when needed. When team members have the next day off, they relay information about ongoing cases to each other so that when the client calls everyone is on the same page.

The doctor knows what information has been passed along and what the expectations are. We also keep a phone log with the receptionists showing what time the call came in, what the message was, and to whom it was directed to (vet, office manager, receptionist). The reply is then logged in the same place with the date and time the call was made. On a rare occasion, some information misses the book—after all, we are human. But all in all this system works quite well. The TEAM approach also helps with the billing by providing checks and balances. By reviewing the Google calendar and spot-checking transactions, especially with lab work, missed charges can be identified. This contributes to the bottom line. The client who drops off a fecal but does not get charged may be spotted when the receptionist adds the results to the patient record or when the office manager is paying the lab bill and sees that the charges have not been entered. As
required by law in New Jersey, all prescriptions must have a label, and by printing a label at the clinic, charges there will not be missed (plus they are a vital part of the medical records). In our ambulatory trucks we carry premade labels with all the required information: name, practice name and address, and license number.

2. The TEAM Approach in Action

Receptionist Staff

A receptionist staff is a critical component of the TEAM approach in veterinary practices. As such, clear guidelines should be established with your receptionists so they understand what is expected of them. Although veterinarians generate income through the services they provide, it is the receptionist who often creates that first impression for clients when they contact your office. You therefore must have the right person in that position. This person must be someone who understands basic veterinary and client terms, such as colic or squinty eye, while also being sensitive regarding health matters. This person should also not be a pushover because they will be training your clients about your expectations of them.

The receptionist should gather as much information as possible during the initial phone call to improve organizational effectiveness and ultimately to help all involved parties, including the client, patient, and veterinarian. Our staff routinely adheres to the following procedure when accepting a new client:

1. Obtain or review all client information, including home address, e-mail address, all relevant phone numbers, and credit card information. Explain payment policy and which payment methods are acceptable.
2. Obtain the name, breed, age, and sex of the horse. Does the horse have any vices or allergies? Are there any past medical or surgical problems? Record the show and barn names of the horse. Make arrangements for the owner to send any pertinent records to the office before the first visit. Ask if the horse is insured and, if so, with which insurance company.
3. Obtain the name of the trainer, if applicable, the name(s) of any barn help, and the facility where the horse is boarded (which may or may not be at the owner’s home).
4. Send a welcome e-mail or packet to new clients.
5. Provide a timeframe for the appointment. Avoid giving exact times, however. Because veterinarians routinely examine more animals than scheduled on any given day, they rarely can stay on a precise schedule. This can be mitigated by asking the client if the veterinarian will need to see additional animals during their visit. (If clients start to fuss with the window provided, we joke that repairmen give us wide time windows, and they don’t see the life-threatening issues that we see!) When running late, either the staff or the veterinarian should call with an updated timeframe. It helps to mention that you value their time too. No one likes waiting at the doctor’s office or at their farm for the veterinarian, farrier, or feed man. Be respectful of your clients’ time.
6. Set expectations with the client that the animal is to be caught with a halter on and cleaned up (a quick brushing will suffice) before the doctor’s arrival. This is important for avoiding those instances when you arrive to photograph for a Coggins test, perform a yearly exam, and administer vaccinations only to find that the horse has just been pulled out of the field and covered with dirt or mud. If a client habitually is unprepared for your visit, you may wish to inform that client that a $250 per hour charge will apply in the future if assistance is needed to catch and clean the horse. Make a note in the record so that when future appointments are scheduled, your staff can remind the client that this fee will be incurred if the animals are not ready.

Scheduling

A schedule that allows all veterinarians and staff real-time access to changes helps everyone on the team know how the day is evolving. Some practices use a Google calendar. Whatever scheduling system is used, it is important to have the staff record as much information in the calendar as possible, including the reason for the call, the services to be performed, special equipment that might be needed, and medications or supplies that are likely to be dispensed. The address of the farm and necessary phone contacts should also be accessible in the schedule.

Personalized Service

It is beneficial to use a form for the client to complete with their credit card information. This can be filled out at the farm or sent back to the office in advance. These forms can be electronic and available on a website as well. Having the client’s signature on a form that gives you permission for treatment and agreement to pay for such treatment is recommended. Remember that credit card numbers must be kept secure and that there are rigorous guidelines for such security; using a bank or other vendor for storing these numbers may thus be the best solution.

Clients may wish to share other information if they cannot be reached in times of emergency. They may wish to record their preference of referral hospitals if their horse needs to go to a clinic. They
may have a financial limit on what they would be willing or able to spend. If the horse is insured, the insurance information should be present in the record. If a client does not want referral under any circumstances, it is helpful to know the extent of treatment they are willing to support on the farm if they cannot be reached. It is best, of course, to have this communication prior to an unforeseen event. Clients will frequently relay this information readily if they are asked. A good newsletter item to include is a reminder to clients who might be going on vacation to provide this information to the practice or to their horses’ caregivers.

Giving Back
In return for their dedication, consider allowing all staff members 1 day off during the week, and try not to bother them during this time. Who wants to have a doctor’s appointment after a long day of work? Wouldn’t it be nice to have your hair done and come out of the salon during daylight hours? Wouldn’t you like to ride your horse when other people are around instead of closing up the barn at night? Team members generally enjoy this policy and treasure days off. When team members are refreshed, the practice runs more smoothly. Many practices also stagger their veterinarians’ workdays so that each doctor also has a weekday off. Together, all team members take care of those items that need to get done during their day off on a weekly basis.

Self-Care
As the leader, you too must follow these principles and have a day off to take care of personal matters. Your health is important, and yearly exams should not be skipped. You also need to attend family activities and events. It is perfectly acceptable to say no to a client’s party or to request a service to be with your family. You will undoubtedly have some clients who won’t appreciate your outstanding service and commitment. At times, it is appropriate to simply inform them you will be unavailable on certain days. There is no need to get into an explanation. Simply have them call the office the next day to be scheduled. If you have a bad vibe about a client, you are usually right. Just as you instinctively move out of the way of a striking or kicking horse, you can sense when something is not right with a client. Many “problem clients” have used all the other veterinarians in the area already. Why do you want to be next? Go with your gut feeling. Call your colleagues to find out their experiences with a particular person that you are uncertain about.

Organization
Being organized makes the day flow more efficiently. Take adequate time to put things back neatly and exactly into the same spot. File paperwork promptly. Do your billing regularly because as time goes by, charges are missed. The same applies to medical records because information may become inaccurate or obsolete as time goes by. By accomplishing these tasks, you can enjoy your day off.

Organization also is needed for your home. Map out plans for the family for the week. Consider a whiteboard where everyone can write down their activities. Make sure that any major events go on a big wall calendar. In this way, there is no lack of communication among everyone in the family. Make sure that you schedule time for your family. Plan vacations in advance so coverage can be easily arranged.

Being on emergency call is a serious commitment. Communicating that commitment to your partner is a key element to a successful relationship. Spend time with them. Tell them what your schedule is like and how much you love your job. They must be willing to understand this aspect of your profession. Likewise, you must also be flexible with their wants and needs. Isn't that why you are together? Set realistic goals for your future but don’t be afraid to dream big.

3. Summary
As you strive for the TEAM approach that incorporates clients, staff, family, and you, hopefully the practice will thrive and be a place everyone is proud to say they are a member. Your practice philosophy and training are key to getting the job done. But remember—it all starts with you!

Acknowledgments

Declaration of Ethics
The Author declares that she has adhered to the Principles of Veterinary Medical Ethics of the AVMA.

Conflict of Interest
The Author declares no conflicts of interest.
Women Partners in Veterinary Practice

Rhonda A. Rathgeber, DVM, PhD

It is possible and pleasurable to have a happy family and be a successful partner in an equine practice. There are some time management strategies that may help make this easier. Author's address: Hagyard Equine Medical Institute, 4250 Iron Works Pike, Lexington, KY 40511; e-mail: rrathgeber@hagyard.com © 2015 AAEP.

1. Introduction

I was invited to be a partner at Hagyard Equine Medical Institute just before my second daughter was born. After the birth of my first child, I decided I needed to work smarter, not longer, to try to be able to be a mom and spend time with my family. I was able to maintain my busy equine practice and still have time for my children.

2. Solutions

One of the most helpful things I did was to manage my clients' expectations. They were used to being able to reach me 24/7. I told them that I was going to work fewer nights and not be available on the weekends while my kids were young. Some clients dropped me right away and found a new veterinarian. I tried to manage those clients and keep them with another veterinarian within the practice. Other clients were, and still are, very respectful of my personal life. They were happy to wait until the next morning for certain things or allow me to send another veterinarian from the practice to help them.

I also had to manage the clinic's expectations. At Hagyard Equine Medical Institute, we are lucky to have an excellent and huge support staff, and I utilized them as often as possible. I became more comfortable with allowing our support staff to help me and as a result began to delegate more work to them. There is definitely a learning curve to delegating tasks that you are used to doing yourself. The next step that I took was to hire a full-time technician and nanny. The nanny lived close by and was available for nighttime emergencies. As time went on, and the kids were at school, I used her to help me run errands. She went to the cleaners, the grocery, the post office, etc.

I made it a priority to do my best veterinary work and to be extremely organized and efficient. Hiring a full-time technician helped me achieve that. Veterinarians play a “who can work the longest” game that can be exhausting. I wanted to spend time with my children and not be exhausted for work or for fun. I feel strongly that I am a better veterinarian and a better mother for making this a priority. I tried to make the most of my driving time by helping clients with scheduling, keeping track of records for them, and calling them to check on horses or cases and to see if they needed anything. I was surprised how helpful it was to contact clients instead of waiting for them to contact me. I could schedule them easily and when I was in their location. I also tried to finish all of my reports, billing, and correspondence letters while my technician

NOTES
drove. This way when I got home I could focus on being a mom.

I also utilized technology more than I ever had before. It is very easy now to have Internet access anywhere you are. However, 17 years ago it was not as easy, and I tried every new bit of technology. I got a computer, and my technician used it all day to keep track of records, billing, and appointments. I would schedule phone calls and recheck examinations in the calendar to help me keep track of cases. This technology was not as popular then as it is now but can be a very powerful and timesaving tool. It allowed me to keep efficient and detailed medical records. This was helpful for the client, the practice, and for me when I needed to reexamine a horse or send another veterinarian to examine it.

The next thing that I tried to do was “job crafting.” I wanted to craft or create a practice or job where I loved everything I did, so I started customizing my services. I did not want to do any reproductive work or dentistry, so I would refer clients to someone within the practice. I focused on lameness, ultrasound, and radiography. I also tried to build an acupuncture practice. I was amazed at how appreciative all the associates were when I delegated the things that I did not enjoy to them, and I was more surprised at how well the clients received it. I had built a relationship with most of them such that they trusted me to allow another veterinarian to help care for their horse.

The hardest thing I had to do was to learn to say no and to say it nicely. I think women in particular are afraid to say no—somehow considering it impolite or unacceptable. Then they are left with too many tasks and not enough time to do the things they like to do. I learned to say to clients that what they were requesting was a great opportunity but that I was already working on another project. It is still difficult for me to say no, but practice makes perfect.

Last, the part that I still struggle with every day is the balance between work and life. I love my career, but there still must be something else. No one can balance this scale for you; you have to do it yourself, and you have to do it almost every day. It is different for all of us. You have to have something you enjoy doing. For me, I find that enjoyment in cooking, yoga, running, and riding. It has been proven repeatedly that this makes us more productive, creative, and energized in our careers.

The combination of these changes amazingly increased my productivity despite working fewer hours. This was the measurable parameter that made me feel like the investment in the veterinary technician and technology were worth it. I feel that I am a good partner in a large equine practice and am able to participate in a large practice in a manner that I am proud of. I would be remiss not to acknowledge that I have a wonderful husband and parents that have been supportive every step along the way.

3. Pricing for This Strategy

Veterinary Technician

Calculations for insurance, a 401k plan, and time off are based on $12.00/h and a work schedule of 40 hours per week. All employees who receive benefits are classified as regular (not seasonal) full-time employees and must work a minimum of 30 hours per week to qualify for insurance and time-off benefits.

Insurance: Benefits include medical, dental, a $30,000 life and accidental death policy, and long-term disability at an annual cost of $4,907.

401k retirement plan: Employees are eligible on the first day of the quarter following 1 year of employment and must be 21 years old. The annual cost for the plan is $751.

Time off: Paid time off for those who have less than 5 years of service include the following: 80 hours of vacation; 16 hours to be used as a floating holiday; 32 and 48 hours, respectively, for personal leave and sick leave; and 64 hours of designated holidays (New Year's Day, Memorial Day, July 4th, Labor Day, Thanksgiving Day, Christmas Eve, Christmas Day, New Year's Eve). This is a total of 240 paid hours off per year. Employees are allowed to build up 480 hours of sick time in their time-off bank, which equates to 12 weeks of paid time off and is intended to serve as a short-term disability benefit. It requires 10 years of not using any sick leave to build up these hours. After completing 5 years of service, vacation time increases to 120 hours annually; after completing 10 years of service, it increases to 160. The annual cost for these benefits is $2,880.

Payroll taxes are not a benefit but an expense and are calculated at ~0.0765% of the annual compensation. Continuing education can also be an expense. For example, we send technicians to the Hagyard Bluegrass Symposium or Kentucky Veterinary Medical Association meeting in Louisville for those who are seeking a veterinary tech license. Mileage at the current adopted rate can also be an expense. Providing a cell phone could be considered as an expense or benefit.

Nanny/Full-Time Childcare Provider

Initially, I paid a full-time nanny $400 per week plus free housing; eventually I increased her wages to $500 per week. I used an accountant to help with the taxes, and that was an additional fee of $1,200. We had a place on the farm that she lived and where she could be available for nighttime emergencies. I did not include health insurance for the first few years but finally got ahead enough to be able to purchase it. The nanny was responsible for laundry, feeding the kids, and sometimes driving them to activities. I usually tried to come back home and take them but often could not make it. As the kids
got older and were at school longer, the nanny was able to run errands and help around the house. It is really hard to find someone to leave your kids with, but it can be done, and the young lady that worked for me is still part of our family even though she does not work here anymore.

Computer and Technology
This will depend on your personal preferences for Internet services and for a computer or tablet. I have a billing program called HVMS. It works well, but there are several other programs that may work even better for your type of practice. The total cost was $1,500–$5,000; however, this could vary. Computer software also may already be part of your practice and therefore not an additional cost.

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Declaration of Ethics
The Author declares that she has adhered to the Principles of Veterinary Medical Ethics of the AVMA.

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The Author declares no conflicts of interest.
Strategic Negotiation: Babies, Bosses, and Pregnancy Plans

Charlotte Lacroix, DVM, JD

This article focuses on empowering female veterinarians and staff to more effectively conduct strategic negotiations with employers. Examples provided largely (but not entirely) focus on topics related to pregnancy and childbirth, but these techniques can be applied to a far wider range of issues. Strategies discussed include recommendations on how to prepare for a negotiation, defining appropriate accommodations to request, and presenting a strong case. Author’s address: Veterinary Business Advisors, Inc., 24 Coddington Road, Whitehouse Station, NJ 08889; e-mail: clacroix@veterinarybusinessadvisors.com. © 2015 AAEP.

1. Introduction

In 2009, the American Veterinary Medical Association (AVMA) reported that females outnumbered male counterparts for the first time. In 2010, the Association of American Veterinary Medical Colleges shared that 78% of veterinary students were females.1 With this evolution in demographics, increasing numbers of veterinarians and their staffs must negotiate how to deal with accommodations needed because of pregnancies, which include medical leave and more. And yet, research shows that females, when compared to males, lack the confidence needed to effectively negotiate.2

This article therefore shares empowering strategies that women can use to confidently approach their employers to discuss difficult issues and/or to ask for accommodations. Although the examples largely focus on pregnancy and childbirth, these techniques can be applied to a far wider range of issues, including salary negotiations, sexual harassment, and more.

2. Strategies

Preparation

Before a woman approaches her employer with a topic that could prove challenging to discuss, she should first consult her employee handbook to determine practice policies that relate to her situation, which could include accommodations and medical leaves of absence. In the case of pregnancy, this should be done early so that steps can be taken to safeguard fetus health.

It can also be helpful to understand basic rights provided under the Family Medical Leave Act (FMLA), which applies to people who work for private companies with 50 or more employees. The FMLA provides up to 12 weeks of unpaid leave for a serious health condition that prevents an employee from performing essential functions of her job. The FMLA prohibits employers from attempting to prevent a qualified person from taking this leave and from terminating an employee who legally takes this leave. An understanding of FMLA is crucial if a woman plans to discuss pregnancy or newborn child care issues, adoption or foster care, and serious health issues of her own or that of a spouse, child, or parent.

The American Disabilities Act applies to people who work for private companies with 15 or more people and prohibits discrimination against people with disabilities in employment, among other areas. Pregnancy, in and of itself, is not considered a disability, but complications that occur during preg-
nancy or childbirth could qualify. Many states also have laws that provide protections, including some for companies with fewer employees.

Once a woman has gathered relevant information, which could include consultations with an attorney, medical personnel, and more, then it is time for her to outline what she wants to achieve when she meets with her employer and what she needs to say to effectively state her case. Some people find it helpful to rehearse their presentations in front of a trusted friend or family member who is willing to give honest feedback. This will help to make sure that important details are included and extraneous details are pruned from the presentation.

Accommodations
An accommodation in this context is a request to modify the work environment or the circumstances under which a job is being performed. Generally speaking, these accommodations focus on physical demands of the job that the woman cannot currently perform, such as lifting, standing for extended periods of time, exposure to certain chemicals or temperatures, and so forth. Accommodations may also involve a change in schedule, the ability to eat or drink more frequently throughout the day, taking breaks to elevate the feet, the wearing of a fetal monitor, a temporary transfer to another department or duty, and so forth. If a woman is pregnant, Tara Mah shares the following potential risks to a fetus in a typical practice:

- Radiation exposure
- Exposure to hazardous chemicals/drugs (e.g., pesticides, hormones, chemotherapeutic agents, etc.)
- Exposure to anesthetic gases, especially during “hard-to-scavenge” procedures such as masking, and waste anesthetic gases
- Exposure to infectious or zoonotic diseases, especially when handling fractious animals (e.g., rabies, tetanus, Lyme disease, salmonellosis, leptospirosis, chlamydiosis, etc.)
- Overexertion associated with lifting/restraining patients

Presenting the Case
The employee should then schedule a meeting with her employer to be held in a private place, preferably when no time crunch exists for the employee or employer. It can be helpful to bring notes of key points to be made, and it is important to present any documentation provided by medical personnel in relation to the situation. It is also important for the employee to adopt the right mindset before the meeting, “detaching from the emotional impact of charged discussions with careful preparation ahead of time... enter into the discussion with an open mind, and the ability to listen to the other party.”

The woman should explain her point of view clearly, sharing specifically what she is asking from her employer while not providing more information than is necessary. In the case of a medical condition, this could serve as an opening statement: “Doctor Morton, I’m sharing this information because it’s important for you to know. I have been diagnosed with overactive bladder and I am receiving treatment for the condition that should help alleviate symptoms but won’t cure the condition. I will need more frequent restroom breaks and it’s possible that I may sometimes need to briefly leave a meeting without much notice.”

For clearer communication, an employee should also give her employer specific requests in writing. If a situation is covered by state or federal laws, then the employer is required to participate in an interactive process to make accommodations. Note that the employer can request reasonable documentation about a disability or limitation before making accommodations, so the employee should be prepared to comply.

An employee should listen carefully to the employer’s response and try to avoid being defensive or argumentative. She should reframe any objections to requests made and focus on the main goal: to negotiate a solution that is satisfactory to both employee and employer and that takes into account any medical restrictions or other challenges involved in the particular situation.

3. Discussion
This approach typically works well because of the elements of careful preparation and clear communication—2 key components of successful negotiations. By preparing herself by carefully reviewing the employee handbook and applicable state and federal laws while gathering documentation and getting advice from medical and legal experts, a woman will have a clearer understanding of her rights. This helps her to craft reasonable requests that can be formulated in conjunction with medical and legal professionals and empowers her to make a more confident presentation.

Rehearsing the presentation in front of an appropriate test audience helps to identify its weaknesses and hone its strengths before the employee requests specific accommodations and/or makes other requests. Choosing the right time and place is key, as is the right mindset and the use of nondefensive communication.

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References


Overview of Selected Pathologic Conditions in Breeding Stallions: Hemospermia, Poor Semen Quality, Enlarged Scrotum

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1. Introduction

Equine veterinarians can be faced with challenging cases that are outside the scope of their usual clinical paradigm. Issues with breeding stallions oftentimes fall into this category, as breeding stallions are not a common patient for many practicing veterinarians. This paper addresses three reproductive conditions of stallions that may require veterinary intervention. The accompanying interactive presentation will highlight aspects of these topics through discussion of bona fide cases.

2. Hemospermia

Hemospermia refers to the presence of blood in semen. The amount of blood in the semen can vary from minute to large, with the amount of blood giving some indication of the cause of the hemospermia. Blood-laden ejaculates are generally detected easily by their pink to reddish to frank red color. Less obvious contamination is detected by microscopic analysis. A disproportionately elevated ratio of neutrophils to erythrocytes in semen or a brown or reddish brown semen color (often indicating presence of non-fresh blood) is suggestive of an internal genital infection, such as that associated with seminal vesiculitis, epididymitis, or orchitis.

Stallions with overt blood in ejaculates have been reported to be highly subfertile to infertile\textsuperscript{1,2}; however, this is not always the case. For one Thoroughbred stallion breeding by natural cover, per-cycle pregnancy rate was 67\% (97/144) for covers with no blood in dismount semen and 64\% (29/45) for covers in which the dismount semen contained blood. The following year, per-cycle pregnancy rates were 70\% (78/1111), 78\% (21/27), 65\% (17/26), and 50\% (4/8) when dismount semen contained no blood, slight blood, moderate blood, or extensive blood, respectively (D. Varner, personal observations). This demonstrates that a certain amount of blood contamination of stallion ejaculates is compatible with good fertility. Laboratory studies on the effect of whole blood on in vitro fertilizing capacity of bull semen revealed that the presence of added blood had
a negative effect as amounts of added blood increased from 0 to 1.5%. The effect of hemospermia on equine sperm quality or fertility following cooled storage has not been critically studied. When blood-contaminated semen of one stallion was mixed with an extender prior to artificial insemination, a high pregnancy rate was achieved. Pregnancy rate was also not hindered for one stallion with frank blood in semen when extender was placed in receptacle of artificial vagina prior to semen collection (T. Blanchard, personal communication, 2015).

There are many causes of hemospermia, but the condition is generally related to lacerations/abrasions on the exterior penis or urethral process, urethral defects (rents), or infection/inflammation of the prepuce, penis, urethra, accessory genital glands, epididymides, or testes. Tumors of the penis (e.g., squamous cell carcinomas [SCC] or, uncommonly, papillomas) and active penile lesions associated with equine coital exanthema may also result in hemospermia.

Penile SCCs are identified more frequently in geldings than stallions. This is likely due, in large part, to the fact that most male horses are castrated; and that geldings may accumulate more smegma in the prepuce than actively breeding stallions. Smegma has been found to have carcinogenic properties. Although the glans penis, penile body, and prepuce are the most common sites for male genital SCC, primary SCC involvement of the urethral process resulting in hemospermia has been reported. There has been a flurry of research in recent years on the pathogenesis and relationship between equine penile papillomas, in situ carcinomas, and invasive SCC. The gross appearance of penile and preputial surfaces with these lesions vary from smooth raised hyperplastic plaques to wart-like growths to irregular masses with ulceration and secondary bacterial infection. Using molecular techniques, the equine papilloma virus, EcPV2, has been found in all three lesions. (Two other equine papilloma viruses are associated with classical equine papillomas, EcPV1 and equine aural papillomas, EcPV3).

Although bacterial urethritis in the region of the ejaculatory ducts has been described as a common etiology for hemospermia, this cause may have been misdiagnosed. Habronema-induced granulomas of the urethral process (cutaneous habronemiasis) were once a relatively common cause of hemospermia, but their prevalence has reduced considerably in the past three decades, probably owing to the widespread use of ivermectin and, more recently, moxidectin, as parasitcides.

The most common cause of profuse contamination of semen with blood is an idiopathic urethral defect (or rent) that communicates with the surrounding corpus spongiosum penis. The defect is generally associated with hematuria in geldings, but the presenting complaint is generally profound hemospermia in stallions. Bleeding is profuse because the lesion communicates with the underlying corpus spongiosum penis. As such, bleeding typically occurs at full erection, when the glans penis and communicating corpus spongiosum penis (which surrounds the urethra) are fully engorged. Mean peak pressure in the corpus spongiosum penis at the time of coital ejaculation in a pony stallion is reported to be 762 mm Hg, compared with 17 mm Hg during the quiescent state. The lesion(s) is (are) typically small (2–5 mm in length) and is (are) almost always located on the posterior surface of the urethra near the pelvic ischium.

Diagnosis of hemospermia begins with an assessment of the quantity of blood in the semen given that minute amounts of blood are oftentimes associated with abrasions or small lacerations on the surface of the penis. The penis should be visually inspected and palpated, paying particular attention to the distal end of the penis, including the urethral process and the fossa glandis. Lesions within the dorsal urethral sinus or the ventrolateral sinuses can be difficult to detect. Within the fossa glandis, the dorsal urethral sinus seems to be particularly susceptible to SCC or to epidermal hyperplasia with associated submucosal neovascularization, lymphoplasmacytic inflammation, mucosal ulceration, and associated hemorrhage at the time of ejaculation.

To aid visualization of the fossa glandis, the penis can be firmly grasped at the time of stallion dismount from a mare or breeding dummy immediately following ejaculation to maintain distension of the glans penis. This will allow easier visual and digital examinations of the fossa glandis. The area can also be balloted with a paper towel to remove any standing blood so that the origin of any bleeding can be more easily recognized. Maintaining engorgement of the penis with digital pressure following ejaculation will also allow for better inspection of the surface of the glans penis for bleeding lesions. If profound hemospermia is detected and of urethral origin, then a urethral defect is a likely cause and should be included at the top of the list of differential diagnoses. If the blood contains a disproportionately large number of neutrophils, then seminal vesiculitis is a likely cause. Diagnosis of either of these conditions is best achieved using urethral endoscopy. The method for performing this examination was reported previously.

Treatment of the hemospermia is dependent on the underlying diagnosis. Surface lesions on the penile shaft can often be treated with locally applied emollient and sexual rest. Lesions that penetrate into underlying cavernous spaces, such as those occurring with use of wire brushes to curb masturbation, may require surgical closure. Lesions within the fossa glandis can be difficult to treat. Surgical ablation of the dorsal urethral sinus has been attempted experimentally (J. Schumacher, personnel communication, 2014), but the effects of this surgery
on mating ability or ejaculatory bleeding have not been investigated. A stallion with refractory SCC involving the fossa glandis has been treated successfully with intralesional injection of bleomycin in conjunction with electrotherapy to improve drug permeability (G. Kelly, personal communication, 2014). Although outcomes have been variable for more generalized penile tumors, both nonsurgical and surgical interventions have been attempted. Topical application of 5% 5-flourouracil (at 14-day intervals), with or without surgical debridement, for treatment of SCC of the external genitalia of stallions has been reported to be successful, as has intralesional injection of cisplatin. Electropulsation methods might further enhance the efficacy of less cell-permeable chemotherapeutic drugs such as bleomycin or cisplatin, but additional studies in horses are required to determine effectiveness. Electrochemotherapy has proved to be advantageous in several species. The possible long-term negative effect of some cytotoxic chemotherapeutic agents on spermatogenesis must be considered before implementing this form of therapy.

Surgical approaches are the best way to treat defects (rents) of the pelvic urethra, as sexual abstinence alone, even when prolonged, is generally unsuccessful. Subischiopelvic urethropotomy can be used as a corrective measure, as can subischial corpus spongiotomy (subischial incision that extends into the corpus spongiosum penis but does not enter the urethral lumen). Either of these methods is thought to allow mucosal healing by preventing escape of cavernous blood through the urethral defect at the time of micturition. With these surgeries, a minimum of 60 days of postsurgical sexual rest is generally recommended prior to recommencement of breeding activities. Three refractory cases were treated in our hospital (Texas A&M University) by primary closure of the defect through a subanal surgical approach combined with urethral endoscopy to readily identify the defect and monitor its surgical closure. Surgical correction was successful in all three cases. Buccal mucosal urethroplasty was reported to be successful for treatment of hemospermia in one stallion with a urethral defect, but the surgical approach included a subischial urethropotomy. Others have reported excellent results with laser treatment in combination with subischial corpus spongiotomy; however, the value of laser as a primary treatment for urethral defects remains open to question. Others have reported that endoscope-directed laser tissue soldering is an effective technique for surgical repair of hypospadias in humans.

Seminal vesiculitis can be difficult to treat successfully, but it seems to be most effectively treated with voluminous lavage of the affected vesicular glands via an endoscopic approach, followed by instillation of antibiotics that are appropriate for the offending etiologic agent. Subsequent massage of the vesicular glands per rectum may aid in distrib-

uting the antibiotic within the glandular luminae. The procedure can be repeated daily for 5–7 days. Care should be taken to avoid use of antimicrobial drugs that are caustic to mucosal tissues. Systemic administration of antimicrobials is oftentimes unsuccessful because of inability of many of these to gain access into the accessory genital glands. Those antimicrobials with high lipid solubility, high pKa (i.e., reduced tendency for proton dissociation), and low protein-binding capacities are reported to diffuse most effectively into the accessory genital glands. Fluoroquinolones reportedly meet these criteria, with good resulting concentrations in accessory gland fluids of rats, dogs, men, and horses, however, treatment of one stallion with systemic enrofloxacin did not correct the chronic seminal vesiculitis long term. In men, incorporation of specific oral nutritional supplements (saw palmetto extract, bearberry extract, and Lactobacillus sporogenes) with systemic fluoroquinolone treatment improved elimination and recurrence rates of bacterial prostatitis, compared with fluoroquinolone treatment alone.

3. Poor Sperm Quality (Teratozoospermia, Necrozoospermia, Oligospermia)

The equine breeding industry abounds with stallions with reduced semen quality, primarily owing to the fact that stallions typically become sires based on their pedigree, performance record, and conformation rather than reproductive health. In addition, many sires remain sexually active as they become aged, and such stallions tend to develop age-related deterioration in testicular/epididymal function. As such, veterinary intervention and more intensive reproductive management are needed to maximize the fertility of these stallions.

Poor semen quality generally presents itself in the form of oligospermia (reduced sperm number in semen), necrozoospermia (elevated percentage of dead or motionless sperm in semen), and/or teratozoospermia (elevated percentage of malformed sperm in semen). Logically, stallions that are affected by one condition oftentimes are affected by all three conditions.

Diagnosis is based on collection and microscopic analysis of semen. An important component of a breeding soundness examination is to determine the number of sperm produced by a stallion at daily sperm output (DSO). This entails depleting extragonadal sperm reserves (by collection of semen for 5–7 consecutive days) before determining actual DSO. Furthermore, measurement of testicular volume allows one to predict DSO. Efficiency of testicular function can then be determined using the following formula: (actual DSO/predicted DSO) × 100. Lowered spermatogenic efficiency is indicative of a disruption in spermatogenesis and is associated with reduced sperm output. This condition can be temporary if the underlying cause can be identified and eliminated. It can also be
permanent, as is the case with age-related testicular degeneration, where reduced spermatogenic efficiency is an early change in the progression of the disorder. It can be coincident with a reduction in the percentages of morphologically normal and progressively motile sperm.

Total sperm number is determined as the product of gel-free semen volume and sperm concentration. To maximize sperm contained in the gel-free semen, semen should be collected in a receptacle that is fitted with a nylon-micromesh filter, as this type of filter will effectively separate gel from gel-free semen, and will reduce loss of sperm within the filter. The most accurate method of estimating semen volume is by weight (1 g = 1 mL). Sperm concentration is typically determined with a hemocytometer counting chamber, spectrophotometrically, or with a semiautomated fluorescence-based sperm counter.

Reduced sperm motility can imply a reduction in the percentage of motile sperm, the percentage of progressively motile sperm, or the average sperm velocity. For objectivity, this analysis is best performed using a computerized sperm motility analyzer. If unavailable, a phase-contrast microscope equipped with a warming stage can be used for subjective analysis, with the magnification set at 200–400×. For most accurate assessment of progressive motility, the sample should be diluted with extender sufficiently (i.e., to 30 × 106 sperm/mL) so that one can more critically assess motion characteristics of individual sperm. As an adjunct to assessment of sperm motility, examination of sperm membrane intactness (a measure of sperm viability) can yield valuable information regarding sperm function. One study revealed that sperm “viability” may be a more accurate predictor of the fertility of cool-stored semen than sperm motility.

Automated analysis of equine sperm morphology is unreliable, in the author’s experience, but vivid images of sperm can be viewed using differential interference contrast microscopy at magnification of 1000–1500×. Such specimens are examined as a wet mount after fixation of sperm in buffered formal saline. Phase contrast microscopy can be used if differential interference contrast optics are unavailable. The authors consider either method to be superior to microscopic examination of stained dried-mounted specimens. A minimum of 100 sperm are evaluated, and the incidence of each type of morphologic defect is recorded. The sperm defects that are the most significant predictors of fertility are normal sperm (positively correlated with fertility) and abnormally shaped heads, detached tails, general mid-piece abnormalities, coiled tails, and premature germ cells (negatively correlated with fertility).

The etiology of oligospermia, necrozoospermia, and/or teratozoospermia is variable. Testicular dysfunction is a leading cause of these clinical findings. Although testicular dysfunction can be genetic, environmental causes are possible and include those associated with various medications and conditions that cause an increase in scrotal temperature (including an increase in body temperature). Age-related testicular dysfunction also results in a progression of reproductive changes, beginning with a reduction in spermatogenic efficiency and the number of morphologically normal, progressively motile sperm in ejaculates. These clinical changes generally occur prior to a marked decline in testicular volume in association with age-related testicular dysfunction.

One relatively common cause of oligospermia, necrozoospermia, and teratozoospermia is a condition called spermiostasis, or plugged ampullae. These stallions are sometimes referred to as sperm accumulators. With this pathologic state, sperm tend to accumulate in the extragonadal ducts, primarily in the ductus deferens and glandular ampullae in the terminal segments of the ductus deferens. The condition occurs following extended periods of sexual abstinence, and can affect sexually mature stallions of any age. Stallions with the condition are more likely than the general stallion population to have issues with recurrence. Although spermiostasis can result in necrozoospermia and teratozoospermia, in the presence of extraordinarily high sperm output, ductal blockage oftentimes results in concurrent oligospermia, or occasionally, azoospermia (no sperm in ejaculates). A pathognomonic finding of severe cases is a high percentage of detached heads (tailless heads) in the ejaculate. High percentages of hairpin bent tails and/or distal protoplasmic droplets are oftentimes indicative of stagnant sperm in the extragonadal ducts and represent a propensity for more pronounced clinical signs such as oligospermia, a high incidence of detached heads, or even complete ductal blockage if the stallion is not subjected to increased breeding frequency. Diagnosis is based on assessment of the ejaculate, in combination with transrectal palpation/ultrasonographic evaluation of the ampullar regions. Degenerating sperm may be ejaculated in numerous clumps (or casts). These can be missed if an in-line gel filter is discarded before its contents are examined. Palpation per rectum may reveal enlarged, turgid ampullae. Ultrasonographic examination will oftentimes disclose dilated ampullar luminae. The diameter of the ampullae in reproducitively normal stallions is quite variable (16.3 ± 3.6 mm) and the lumen can contain a small amount of echoluent fluid. The luminal diameter can increase following sexual stimulation, but dilated ampullar luminae in a nonsexually stimulated stallion are highly suggestive of spermiostasis involving the ampullar regions. Sperm-occluded ampullae are occasionally associated with cystic remnants of the Müllerian ducts, which can be visualized ultrasonographically at the terminations of the ampullae within the colliculus seminalis.

Treatment strategies for reduced semen quality are dependent on the underlying causes and the rules and regulations of the breed registry involved.
Although stallions subjected to only natural cover conditions cannot benefit from many laboratory techniques that would likely improve their breeding performance, one can still implement methods to critically assess their fertility and devise strategies to enhance their reproductive potential.\(^46\) An example is the use of “reinforcement breeding” to improve the fertility of stallions afflicted with oligospermia, necrozoospermia, and/or teratozoospermia.\(^47,48\) The technique involves collection of semen from the penis immediately upon dismount of a stallion from a mare following an ejaculatory mating. The protocol recommended by the authors is to maintain the dismount semen sample at body temperature, strain it through a filter to remove extraneous debris, and then mix the filtered semen with a small volume (5–10 mL) of warmed good-quality semen extender. The extended semen is then loaded into an all-plastic syringe and the filled syringe is affixed to a standard insemination pipet. Covered mares are immediately placed in stocks and the perineal area is prepared for insemination of the extended dismount semen sample. As the pipet and hand course through vagina, any fluids grossly free of urine or blood are also aspirated into the pipet, and then the contents are discharged into the uterine body.

Approval of artificial insemination by most horse registries has resulted in commercial application of a multitude of assisted reproductive technologies to maximize the reproductive efficiency of stallions. Centrifugation of semen is a central component of improving the fertility of some stallions. One type of centrifugation process (cushioned centrifugation) can be used to simply remove or reduce seminal plasma contribution to semen or to increase sperm concentration. This technique is typically used when sperm concentration in semen is low (i.e., \(< 100–125 \times 10^6/\text{mL}\) ) or when seminal plasma is known to possess factors that are deleterious to sperm. Another type of centrifugation process involves centrifugation of semen through a density gradient (e.g., a silica particle solution) in an effort to enhance sperm quality prior to insemination.\(^48\)

Cushioned centrifugation of semen has generally replaced traditional centrifugation because this procedure maximizes sperm harvest without associated sperm injury.\(^49,50\) Investigations regarding cushioned centrifugation of stallion semen with the product, iodoxanol, have demonstrated excellent yields of sperm with no detectable damage from the centrifugation process. Centrifugation can be performed using either plastic conical-bottom tubes or glass nipple-bottom tubes. The nipple-bottom tubes are recommended over the conical-bottom tubes for cushioned centrifugation when the sperm number in ejaculates is relatively low (e.g., \(< 2 \times 10^9 \) sperm), or when it is necessary to separate more seminal plasma from sperm following centrifugation than is possible with cushioned centrifugation in conical-bottom tubes.

Centrifugation of equine semen through a silica-particle solution has shown promise for “selecting” sperm with good motility, morphology, and chromatin quality, and enhancing the fertility of selected subfertile stallions.\(^48,51\) Given that this technique results in sperm separation based on sperm buoyancy or isopynic point, its use is most justified when an ejaculate contains a high percentage of sperm with morphologic defects, specifically sperm with abnormal heads, abnormal midpieces, bent midpieces, bent tails, coiled tails, or premature (round) germ cells. However, the technique will also improve chromatin quality in the recovered sperm population, regardless of sperm morphologic profile.

When processing semen by centrifugation through a density gradient, the resulting sperm pellet may only contain a relatively small percentage of the sperm originally in the ejaculate. As such, insemination doses may contain low sperm numbers and a low insemination volume. The threshold number of sperm that can be used to inseminate mares and yield a commercially acceptable pregnancy rate is not known, but this number is certainly affected by the fertility of a given stallion, and by the semen processing method that is applied prior to insemination. Using customary methods, mares are typically inseminated with 200 to \(500 \times 10^6\) progressively motile sperm deposited directly into the lumen of the uterine body when fresh semen is used. One study revealed no difference between insemination doses of 50 or \(300 \times 10^6\) progressively motile fresh sperm from selected stallions.\(^52\) However, high pregnancy rates can be achieved with a much lower inseminate number when low-dose insemination strategies are used. In one study using low-dose insemination, pregnancy rate was high in a stallion when only \(1 \times 10^6\) total sperm were used for insemination.\(^53\)

Treatment of spermiostasis (plugged ampullae) can take several forms. Increased ejaculatory frequency may, by itself, correct the problem. Administration of oxytocin (20–40 U, IV) at 2 to 15 minutes prior to semen collection attempts, or cloprostenol (125–250 \(\mu\)g, IV) at 15–60 minutes prior to semen collection attempts, may aid further in resolving the problem. In addition, manual massage of the ampullae prior to semen collection attempts can aid in dislodging the sperm occlusion(s).\(^41\) The author (D.V.) had one refractory case that did not respond to the treatments listed above, but the occlusions were subsequently dislodged when breeding the stallion immediately following administration of phenylephrine (20 mg in 600 mL saline, IV, \(> 5\) min).

4. Scrotal Enlargement

The etiologic basis of scrotal enlargement in sexually mature stallions is variable, ranging from blunt scrotal trauma, to orchitis/epididymitis, to testicular or scrotal area tumors to hydrocele to inguinal/scrotal herniation. Diagnosis is based on visual inspection of the scrotum and digital and ultrasonographic
examination of the scrotal contents. Transrectal palpation and ultrasound examination of the internal inguinal rings also aids in identification of inguinal herniation.

Blunt scrotal trauma is often the result of a breeding incident, most commonly from a mare kick at the time of natural mating. Scrotal contusions and lacerations also occur when stallions unsuccessfully hurdle fences or similar barriers. Blunt trauma to the scrotum can produce an array of local disturbances, including scrotal edema, dartos or scrotal hemorrhage, hematocoele (blood accumulation in the vaginal cavity), orchitis, or rupture of the tunica albuginea, which encapsulates each testis. Any local trauma may incite bilateral scrotal and preputial edema. Secondary bacterial infection is possible, especially when the skin is broken by lacerations or puncture wounds. Initial examination of the scrotal area may require animal sedation and physical restraint, as the area usually is quite painful. Phenothiazine tranquilizers should be avoided because of their propensity to induce penile paralysis or priapism. The scrotal surface should be examined thoroughly for injuries, including abrasions, lacerations, puncture wounds, and foreign bodies, such as thorns. The scrotal contents should be evaluated next. If the testes and attached epididymides are readily palpable, the swelling is probably unassociated with scrotal edema, dartos hemorrhage, or hematocoele, but originates in the testis (es), as with orchitis. Conversely, inability to manually define the testes and epididymides suggests one of the above (i.e., excessive scrotal edema, dartos hemorrhage, or hematocoele). Rupture of the tunica albuginea also may preclude identification of the involved testis and adjacent epididymis because of extreme hemorrhage emanating from the rent. It is sometimes difficult to differentiate massive scrotal edema from hemorrhage of scrotal, spermatic cord, or testicular origin. Ultrasound examination of an enlarged scrotum may help establish the underlying cause. Fresh blood that accumulates in the vaginal cavity (i.e., hematocoele) or scrotal wall typically has a homogenous anechoic appearance, even if freshly clotted. Conversely, a thickened scrotal wall resulting from edema usually has a more heterogeneous pattern. Needle aspiration of the contents of the vaginal cavity helps with the diagnosis, if hematocoele is suspected. It is mandatory, however, that the external and internal inguinal rings first be palpated thoroughly to ensure that the scrotal swelling is not associated with a scrotal hernia. The needle-aspiration technique should be performed aseptically, and care should be taken to avoid laceration of vessels. Torsion of a spermatic cord also should be included in the differential diagnosis of acute scrotal swelling.

Intrascrotal hemorrhage, whether from the scrotal wall, spermatic cord, or testis, becomes organized to form fibrous tissue. Fibrin deposition in the scrotal fascia and vaginal cavity leads to adhesions and immobilization of the associated testis. The fibrous tissue can also interfere permanently with thermoregulation of the spermatogenesis, especially when it envelops the testis. Degeneration of the seminiferous epithelium and progressive testicular atrophy are a result. Persistent scrotal edema also can promote tissue fibrosis; however, if arrested in the acute phase, edema generally subsides without this complication. Another consequence of uncorrected edema is local hypoxia and cellular undernourishment, causing cell death and sloughing of scrotal tissues.

Bacterial- or viral-induced forms of orchitis are rare in stallions. Local bacterial invasion of the testes can be secondary to infectious peritonitis (via the inguinal canal) or, more commonly, penetrating wounds of the scrotum. Retrograde transmission of bacteria to the testes is rare, but can occur via the extragonadal ducts (e.g., an extension of seminal vesiculitis) and is associated with ipsilateral epididymitis. Affected testes are hot, tense, slightly swollen, and acutely painful. Testicular enlargement, due to inflammation and edema, is constrained by the overlying inelastic tunica albuginea. Edema may be apparent during ultrasonographic examination. The testes remain freely movable within the scrotum unless peri orchitis also is present. Fever and scrotal edema often accompany orchitis.

Testicular neoplasia of stallions is uncommon, but its actual incidence cannot be measured because most male horses are castrated when young. Testicular tumors tend to be diagnosed more frequently in younger men (average age, 30 y). Interestingly, the spectrum of testicular tumors varies with the age at which they develop in humans. Furthermore, teratomas are generally benign in boys and malignant on older men. Primary testicular neoplasms occur more frequently than secondary neoplasms. Primary testicular neoplasms are classified as germinal or somatic (nongerminal), depending on their cellular origin. Germinal neoplasms originate from germ cells of the seminiferous epithelium and include seminomas, embryonal carcinomas, teratomas, and yolk sac tumors. Nongerminial tumors include Leydig cell tumors and Sertoli cell tumors. Secondary tumors include leiomyomas, leiomyosarcomas, mast cell tumors, fibromas, and lipomas. A mixed testicular tumor (sex cord-stromal tumor with elements of Sertoli cells and Leydig cells) in a stallion has also been described.

In horses, seminomas are one of the most commonly reported tumors. They have been described in retained and scrotal testes and are more frequently diagnosed in older stallions. These tumors are locally invasive, and progressive testicular enlargement occurs because of the sheets of neoplastic cells that gradually replace normal testicular parenchyma. Occasionally, the tumor breaches the tunica albuginea and spreads to adjacent tissues. Widespread metastasis of seminomas to the thoracic and abdominal cavities has also been reported.
Teratomas can occur anywhere in the body, but are most frequently located in the gonads in horses, and have been identified in foals as young as 3 days of age.\(^{60}\) They are classified as germinal tumors, as they are derived from pluripotent germ cells. In horses, teratomas are benign, but malignant teratomas (also termed teratocarcinomas) have been reported.\(^{61}\)

Leydig cell tumors (also termed interstitial-cell tumors) are relatively common in dogs and bulls (especially the Guernsey breed), but are rare in stallions.\(^{52}\) This tumor type is generally benign, has been associated with undescended testes,\(^{63}\) but can be associated with scrotal swelling and pain in descended testes.\(^{64}\)

Sertoli cell tumors arise from the somatic elements of the seminiferous epithelium (Sertoli or sustentacular cells). This tumor type is rare in stallions and is generally benign; however, a malignant Sertoli cell tumor with metastasis to the lungs, has been described.\(^{65}\)

The risk factors associated with testicular tumors of stallions are poorly understood. Predilections have been identified in men and include certain occupational and environmental exposures.\(^{56}\) In addition, genome-wide association studies have provided some likely genetic mechanisms involved in testicular oncogenesis.\(^{67}\) Antigen expression has also been used for investigating early testicular tumorogenesis in humans.\(^{68}\) In stallions, retained testes probably have an increased risk for development of seminomas, teratomas, or Leydig cell tumors, but genetic testing and molecular probes for early diagnosis have not been reported.

Hydrocele refers to an abnormal collection of fluid between the visceral and parietal layers of the tunica vaginalis. The condition can accompany inflammatory or noninflammatory types of scrotal edema and can also arise without concurrent scrotal edema. The vaginal cavity communicates with the peritoneal cavity, so abdominal fluid can transfer into the vaginal cavity, as seen in some cases of ascites. Local lymphedema has also been postulated to contribute to hydrocele.\(^{62}\) The fluid character is usually serous, suggesting that it may occur in response to trauma to the visceral or parietal vaginal tunics.\(^{69}\) Uncomplicated hydroceles can also develop, acutely or insidiously, without an apparent cause. Disruptions of the secretory/absorptive functions of the tunica vaginalis are thought to cause noninfectious primary hydrocele. The condition can be unilateral or bilateral, and temporary or permanent. Hydroceles can occur in hot weather conditions, resolving when the ambient temperature decreases. Initial diagnosis is based on palpation of the scrotal contents. When the condition is pronounced, the enclosed testis and epididymis may not be easily palpable, but tend to remain freely movable within the scrotum. Ultrasonographic examination reveals anechoic fluid surrounding the involved testes and epididymides. Definitive diagnosis is based on aseptic needle aspiration of fluid from the vaginal cavity, and the volume may range from 10 mL to greater than 1 L. Care should be taken to maintain asepsis and to avoid puncture of the testis or epididymis during this procedure.

Inguinal/scrotal herniation involves a protrusion of abdominal viscera, usually small intestines, into the inguinal canal or scrotum. Most inguinal/scrotal hernias are indirect, i.e., they protrude through the vaginal ring into the vaginal cavity. Occasionally, indirect hernias may occur, where viscera rupture through the parietal tunic and lie partially outside the vaginal cavity. Inguinal or scrotal hernias occur almost exclusively in intact males. They can be present at birth or develop soon after birth as a result of congenitally large vaginal rings. Acquired hernias may result from increased intra-abdominal pressure or alteration of the architecture of the inguinal canal, and predisposing conditions may include exercise or breeding. In some horses, the size of the vaginal ring seems to have no influence on development of the hernia.\(^{70}\) Acquired inguinal/scrotal hernias are most commonly attended by signs of acute abdominal pain, and should be considered in stallions exhibiting such signs. If the hernia is inguinal, percutaneous palpation of the herniated viscera may not be possible, but ultrasound examination may reveal the presence of intestine. Palpation per rectum will generally allow confirmation for the diagnosis by identification of viscera entering the vaginal ring. Strangulation of hernia contents is likely, and immediate corrective action is required.

Torsion of the spermatic cord refers to rotation of the spermatic cord about its longitudinal axis. Often, the condition is inappropriately called testicular torsion. Although only observed occasionally in stallions, it occurs more commonly than in other large domestic animals, probably because the long axis of the testes is positioned horizontally in the equine scrotum. Torsions usually occur in descended testes, either unilaterally or bilaterally, and can be transient or permanent. Chronic recurring spermatic cord torsions are also possible. Most spermatic cord torsions range from 180 to 360 degrees, but more pronounced torsions can occur. Clinical signs depend on the severity of the torsion. In most instances, 180-degree torsions are subclinical and found incidentally during examination of the external genital organs. In such cases, the torsion is usually not associated with pain, and semen abnormalities are not present. In some stallions, the torsion may only be transient. Infrequently, stallions with a 180-degree torsion of the spermatic cord display mild intermittent scrotal pain that becomes most pronounced during athletic activity. Spermatic cord torsions up to 180 degrees probably do not produce local circulatory disturbances and so are generally considered of minor consequence. A 270-degree torsion of the spermatic cord was reported to result in preputial/scrotal edema in a stallion, even
though scrotal pain was not evident. A 360-degree spermatic cord torsion generally causes acute scrotal pain and scrotal enlargement from local edema and hemorrhage from severe vascular compromise. Unrelenting signs of colic and a stilted gait may be evident. Typically this type of torsion is unilateral and permanent; however, temporary torsions are possible and can result in recurrent attacks. Factors predisposing to spermatic cord torsion are poorly understood. Elongation of the caudal ligament of the epididymis or proper ligament of the testis and/or an excessively long mesorchium reportedly encourage spermatic cord torsion. The torsion can be intravaginal (excluding the parietal layer of the vaginal tunic) or extravaginal (including the parietal vaginal tunic).

Diagnosis of 180-degree torsion is relatively easy and based on identifying the location of the bulbous cauda epididymis and caudal ligament of the epididymis within the scrotum. The caudal ligament of the epididymis is a remnant of the gubernaculum testis. It typically remains as a small fibrous structure attached to the cauda epididymis, and is generally palpable through the scrotal wall. Under normal circumstances, these structures are located on the caudal pole of the testis. Their presence in the cranial scrotum, with no attendant clinical signs, indicates a 180-degree torsion of the spermatic cord. With a 360-degree torsion of the spermatic cord, the cauda epididymis and caudal ligament of the epididymis are palpated in their proper scrotal position, but acute scrotal pain, often with scrotal enlargement and edema are evident. Palpation of the scrotal contents becomes difficult as scrotal edema and swelling progress. A primary differential diagnosis for torsion of the spermatic cord is inguinal/scrotal herniation. Examination of the suspect vaginal ring by palpation per rectum helps differentiate these conditions.

Treatment strategies for an enlarged scrotum are dependent on the underlying cause. Treatment of acute scrotal trauma initially is aimed at controlling local inflammation, edema and hematoma formation. Gentle cold-water irrigation of the site is an important, yet inexpensive, corrective action. Cold application sessions should not exceed 20 minutes and should be applied at 3- to 4-hour intervals. Local blood flow is reduced by using this cold application strategy, thus minimizing tissue edema and hemorrhage. Short-term topical cold application offers beneficial cooling effects that can last up to several hours, and also provides a degree of analgesia. However, continuous application of cold temperature can actually increase local blood flow and lymphatic permeability. These changes can negate the beneficial effects. Cold water sprays are not recommended given that they can cause additional skin damage if tissue has been compromised. Systemic anti-inflammatory and diuretic medications are useful adjuncts to local therapy for controlling inflammation and edema. Prophylactic antibiotic therapy also should be instituted to prevent secondary infection. A tetanus toxoid booster is recommended if the horse has not been vaccinated against tetanus within the past year. Emollients should be applied to the skin intermittently to protect it against maceration. Significant intrascrotal hemorrhage leads to permanent testicular damage due to the insulating effect of secondary fibrous tissue formation. To save the compromised testis, surgical removal of the organized blood clot can be attempted 4–7 days after injury, but the prognosis for the affected test is quite guarded. If the condition is chronic, with pronounced atrophy of the ipsilateral testis, the affected testis could be removed surgically, along with any attendant fibrotic tissue. This may promote some compensatory hypertrophy (and increased sperm production) of the remaining testis. Scrotal lacerations generally incite marked tissue swelling. Such wounds should be cleaned thoroughly then treated locally to control bacterial infection and progressive cellulitis. Lacerations in this tissue generally do not heal by first intention because of pronounced tissue compromise and swelling. Nonetheless, wound closure is generally indicated to cover exposed testes and their tunics. When lacerated, the parietal vaginal tunic should be sutured after thoroughly cleansing the vaginal cavity with balanced salt solution containing antibiotics. Surgical debridement of subcutaneous tissues should be followed by skin closure with nonabsorbable nonreactive suture material. If a seroma forms in the subcutaneous tissue, drainage should be established.

Although targeted treatment of testicular tumors has been attempted to prolong the breeding life of selected stallions with bilateral involvement, prompt orchiectomy is the primary treatment for all testicular neoplasms, regardless of type. Owners must be advised if the neoplasm is potentially malignant. As a precautionary measure, radical removal of the attached spermatic cord is advised and the excised spermatic cord should be evaluated histologically for evidence of metastatic lesions. Radical scrotal ablation should accompany orchiectomy if the tumor has invaded peritesticular tissues. Excision of adjacent lymphatic chains is indicated if metastasis is suspected. Chemotherapy is used in conjunction with surgery to treat men with metastatic testicular tumors, but this therapeutic strategy has not been perfected in the horse.

Treatment of hydrocele is aimed at removing the underlying cause of the hydrocele, if it can be identified. Hydroceles associated with some underlying conditions, (e. g., infectious conditions, ascites, or malnutrition) may take some time to resolve. The fluid can be removed by aseptic needle aspiration. Exercise may help control fluid accumulation in some cases; yet improvement may only be transient. Fluid usually reaccumulates when drained from the vaginal cavity unless an inciting factor for the hydrocele is identified and corrected. Mild cases of
hydrocele may not adversely affect fertility, but accumulation of a significant volume of fluid can cause heat-induced testicular degeneration. If a persistent hydrocele is unilateral, surgical removal of the affected testis and surrounding tunics may prevent/correct thermal injury to the contralateral testis.

Treatment of inguinal/scrotal hernias in newborn foals can oftentimes be corrected simply by application of a “foal diaper” with the foal sedated and placed in dorsal recumbency, with the area of external inguinal rings packed with cotton pledgets. Care should be taken to prevent occlusion of the anus, preputial orifice, or penile urethra. The diaper can be replaced at weekly intervals until the hernia is corrected. The external inguinal rings should be palpated intermittently under the diaper each day to ensure that herniation has not recurred. In foals, surgical intervention is generally not required.

For acquired inguinal/scrotal hernias in adult stallions, surgical correction of the hernia is almost always indicated. Retraction of entrapped viscera per rectum has been successfully performed in isolated cases where it was thought that the involved intestine was uncompromised. This method has potential complications such as damage to the rectum or entrapped viscera, lack of information regarding degree of intestinal compromise, and hernia recurrence rate after nonsurgical reduction has not been reported. Surgical reduction of the hernia is indicated if manual reduction fails, if there is evidence of enlarged or damaged vaginal rings, or if infarction of the entrapped viscera is suspected. To surgically reduce herniated intestine, the stallion is placed in dorsal recumbency under general anesthesia, and prepared for both inguinal exploratory surgery and for celiotomy at the ventral midline. Through a cutaneous incision made over the ipsilateral superficial inguinal ring, the herniated intestine can be surgically exposed, evaluated for viability, and reduced through the vaginal ring into the abdomen. Reduction of incarcerated bowel may require manual traction through the ventral midline celiotomy, and may also require surgical enlargement of the vaginal ring. Resection and anastomosis of devitalized intestine can sometimes be accomplished at the inguinal incision, but this procedure is generally accomplished more easily with exteriorization of affected intestine through the midline celiotomy site. Partial closure of the external inguinal ring can be attempted if retention of the ipsilateral testis is preferred; however, the testis could be compromised by this procedure. As such, unilateral orchiectomy and complete closure of the external inguinal ring may be the best option. Laparoscopic inguinal herniorrhaphy has been performed successfully to salvage the ipsilateral testis.

Treatment of 180-degree spermatic cord torsions is generally not necessary given that the condition usually does not interfere with reproductive or athletic performance. If necessary, orchiopexy can be performed with the horse under general anesthesia. The spermatic cord torsion is reduced manually, and then a small skin incision is made in the scrotum over the intended site of the epididymal tail. If the torsion is judged to be intravaginal, an incision is made in the underlying parietal tunic. The proper ligament of the testis is exposed through the incision in the parietal tunic, then sutured to the adjacent tunica dartos with nonreactive, nonabsorbable suture material. The vaginal tunic and scrotal skin are closed routinely. If the torsion is extravaginal, the parietal tunic can be sutured to the adjacent tunica dartos.

A 360-degree (or greater) spermatic cord torsion is considered to be a surgical emergency. Unilateral castration is generally recommended, as the affected testis is rarely salvageable. The spermatic cord is ligated and transected proximal to the site of torsion. Early diagnosis and treatment are imperative if the affected testis is to be saved. Studies of affected men indicate that permanent testicular damage is likely unless spermatic cord torsion is corrected within a few hours of occurrence. An early experimental study suggested that unilateral spermatic cord torsion can damage sperm produced in the contralateral testis by an immunologic mechanism whereby anti-sperm antibodies were produced to sperm liberated from the ischemic testis. More recently, other humoral and cellular immune-mediated factors have also been shown to be involved in damage to the contralateral testis. Others have reported that ischemia-reperfusion injury leads to extension of the original tissue lesion to the contralateral testis primarily though generation of reactive oxygen and nitrogen species. To this end, various pharmaceuticals have been shown to mitigate the detrimental effects of perfusion-reperfusion testicular injury in experimental laboratory models. Clinical studies regarding such treatment strategies in horses have not been reported, but experimental results are encouraging.

Acknowledgments

Declaration of Ethics

The Authors declare that they have adhered to the Principles of Veterinary Medical Ethics of the AVMA.

Conflict of Interest

The Authors declare no conflicts of interest.

References


Selected Topics in Reproductive Pathology: Mare I

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I. The Enlarged Ovary

1. Introduction

A mare is considered “normal” if she has regular estrous cycles, conceives, carries the foal to term, undergoes parturition, raises the foal, and continues to have regular estrous cycles. When any of these processes are associated with pathologic conditions, infertility may ensue. This article addresses common pathologic conditions that may disrupt these processes and require veterinary intervention.

2. The Enlarged Ovary

Normal ovarian function is essential for fertility and reproductive efficiency. Any ovarian disorder or pathology that disrupts the estrous cycle causes loss of time and the potential for pregnancy. Ovarian enlargement has been identified in barren, pregnant, and maiden mares during routine reproductive exams as well as in mares that present for anestruis, behavioral abnormalities, and colic.

Ovarian enlargement is usually a unilateral condition that can have numerous etiologies that are both non-neoplastic (hematoma, hemorrhagic anovulatory follicle, and abscess1–3) or neoplastic (granulosa theca cell tumor, dysgerminoma, teratoma, teratocarcinoma, cystadenoma, and cystadenocarcinoma4–9). Clinical signs, ultrasonographic findings, hormonal assays, laparoscopy, biopsy, and exploratory surgery can aid in differentiating these disorders.3

3. Ovarian Hematoma

An ovarian hematoma results from excessive hemorrhaging into the follicular lumen after ovulation.10 As one of the common causes for an enlarged unilateral ovary, it is imperative to differentiate it from neoplastic disease (i.e., granulosa cell tumors). Ovarian hematomas can be found in maiden, barren, and foaling mares, although in the authors’ experience foal heat ovulations tend to be the most prone to formation. Clinical presentation can include the presence of an enlarged ovary during a routine reproductive examination, abnormally aggressive behavior, or colic as a result of pain from the weight of the ovary.1

A diagnosis can be made by utilizing different techniques. Ultrasonographic evaluation of the enlarged ovary reveals hyper- or hypoechochogenic homogeneous blood within the lumen of an extremely large follicle or a “spider web” appearance that results from a fibrin strand formation that spans the...
or collapse (Fig. 1). After acute hemorrhage, the ovulation failure occurs when the follicle does not rupture. Hemorrhage into the dominant follicle with ovulation failure can be treated by ligating the ovarian vasculature. A standing-flank laparotomy approach can lead to identifying the ovary, draining the hematoma, and removing and visually inspecting the ovary. Hemorrhagic anovulatory follicles (HAFs) have also been called autumn follicles, persistent anovulatory follicles, and hemorrhagic follicles. The presence of the hematoma can make the ovary sensitive upon palpation, functionally compromised, and enlarged. Although the overall HAF incidence is 5% to 8% of the mare population, it can be a frustrating disorder because normal cyclicity is affected. It has been suggested that the induction of estrus with prostaglandin substantially increases the likelihood of developing HAFs. With prostaglandin-induced luteolysis, progesterone drops rapidly, allowing for the removal of the negative feedback of progesterone on the luteinizing hormone (LH) that causes an early rise during the beginning of follicular deviation. This LH surge could interfere with the intrafollicular metabolism of prostanoids and the proteolytic enzymes that are necessary for ovulation and follicular collapse. An increase in HAF incidence has been reported after the use of human chorionic gonadotropin to induce ovulation. However, the mechanism responsible for this increase is not clear.

Diagnosis can be difficult because HAFs can appear to be similar to a normal evacuated follicle and corpus hemorrhagicum (CH) (or hematoma) during an ultrasonographic transrectal examination. A differentiating feature that may help is the thickness of the luteal border, with an HAF <3 mm in contrast to >5 mm in CHs. Vascular perfusion has also been identified as being different in HAFs versus CHs. Hormonal assays are usually normal, and enlarged. Although the overall HAF incidence is 5% to 8% of the mare population, it can be a frustrating disorder because normal cyclicity is affected. It has been suggested that the induction of estrus with prostaglandin substantially increases the likelihood of developing HAFs. With prostaglandin-induced luteolysis, progesterone drops rapidly, allowing for the removal of the negative feedback of progesterone on the luteinizing hormone (LH) that causes an early rise during the beginning of follicular deviation. This LH surge could interfere with the intrafollicular metabolism of prostanoids and the proteolytic enzymes that are necessary for ovulation and follicular collapse. An increase in HAF incidence has been reported after the use of human chorionic gonadotropin to induce ovulation. However, the mechanism responsible for this increase is not clear.

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tion or seeding of the organism within the abdominal cavity can be a life-threatening complication.

6. Granulosa Theca Cell Tumor

Granulosa theca cell tumors (GCTs) are the most common ovarian tumor reported in mares and represent approximately 2.5% of all neoplasms in the horse.23 They are made up of granulosa cells or granulosa and theca cells.24 Mares aged 2 to 20 years can be affected25 and have been reported in maiden, barren, pregnant, and foaling mares.25,26

Clinically, diagnosing GCTs in mares is frequently based on behavioral changes (aggressive stallion-like, anestrus, or persistent estrus) depending on the predominant hormone, unilateral ovarian enlargement with atrophy of the contralateral ovary, ultrasonographic appearance of the affected ovary, and endocrine profiles.25,27 Ultrasonographic examination of the affected ovary usually reveals an enlarged multicystic structure but can vary, presenting in either the form of a solid mass or a single large fluid-filled cyst (Fig. 2).28,29 They are usually unilateral with a small and inactive contralateral ovary.25,30 Palpation of the ovulation fossa is not possible in the affected ovary because of the GCT emanating from that region.

GCTs are hormonally active and produce variable amounts of testosterone, estradiol, and inhibin.27,30 Increases in serum concentrations of inhibin and/or testosterone combined with low circulating levels of progesterone in nonpregnant mares have been used for endocrine diagnosis of GCTs in mares.29–33 Testosterone and inhibin were increased in 50% and 87%, respectively, of mares with GCTs.27 Inhibin produced by the GCT is responsible for the inactivity of the contralateral ovary through the suppression of pituitary follicle-stimulating hormone (FSH) release.32 Although inhibin has been very useful for diagnosis of GCTs, interpretation of serum inhibin concentrations can be confusing in pregnant mares (where both inhibin and testosterone may be increased), and early GCTs may require multiple sampling over time to detect increases in inhibin.34 Anti-Müllerian hormone is also normally produced by granulosa cells of small-growing and antral follicles.34 In a recent study, using histologically confirmed GCTs, serum anti-Müllerian hormone concentrations were significantly more sensitive than inhibin, testosterone, or the combination of inhibin/testosterone for detecting known GCTs in mares.34

Treatment includes surgical removal of the affected ovary; approaches include colpotomy, standing laparoscopy, and flank and ventral midline laparotomy.35,36 Testosterone and inhibin concentrations decline rapidly after surgical removal of the affected ovary, and the contralateral ovary returns to normal function on average 8.5 months after surgery in most mares.25,30–32

7. Dysgerminoma

A dysgerminoma is a germ cell neoplasia that is rare in mares; clinical history and presenting signs have not been well described.3,38 In two reports, horses presented for signs of abdominal pain and weight loss and an abdominal fluid sample that was serosanguineous.39,40 In another report, a mare presented with none of the described signs—only a large mass in the caudal abdomen.5 In mares as in humans, the mass may be associated with hypertrophic osteopathy of the lower limb and loss of condition and stiffness when walking; it is also thought to be a more aggressive neoplasm with a potential for metastasis.38,41,42

An abnormally large ovary can be seen during an ultrasonography and is characterized by an echogenic cavity surrounded by a thick wall.5 The contralateral ovary is active and of normal size. Transabdominal and thoracic ultrasonography as well as abdominocentesis may be warranted if metastasis is suspected. A definitive diagnosis is made by histologically examining the ovary. If metastasis is identified early, treatment can include unilateral ovariectomy with approaches that are similar to those described previously for GCTs.

8. Teratomas and Teratocarcinomas

Teratomas and teratocarcinomas are other germ cell tumors composed of multiple tissues that are foreign to the gonad.7 Tumors may be derived from 2 or 3 germ layers as well as sebaceous and sweat glands, respiratory epithelium, salivary glands, ganglion cells, nerve fibers, hair, skin, cartilage, bone, teeth, or muscle and may be solid or cystic (Fig. 3).3,43 Teratomas have been reported to be nonfunctional with regard to steroid hormone secretion; therefore, the contralateral ovary remains functional, and regular estrous cycles are noted.44 Their growth, how-

Fig. 2. Hemisection of an equine ovarian granulosa cell tumor. Note the presence of very characteristic cystic spaces and more solid areas. Hemorrhage into the neoplastic cell lined spaces is common. Rarely, solid or large single cystic masses occur. Malignant forms are rare.
commonly spread via the peritoneal cavity after a cyst ruptures or content leaks. Ascites may arise as a result of the obstruction of peritoneal lymphatics, peritoneal metastasis, or both. Removing the affected ovary is necessary for treatment. Other mesenchymal tumors such as hemangiomas, leiomyoma, fibroma, and lymphoma have been described but seem to be extremely uncommon.

Acknowledgments

Declaration of Ethics
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Conflict of Interest
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II. Abortions Caused by Leptospirosis, Equine Herpes Virus, or Equine Viral Arteritis

1. Introduction

Abortion, defined as the loss of a pregnancy between 150 and 300 days, has a severe economic impact on the equine industry. Not only is a mare rendered unproductive for that year but also may be more reproductively inefficient the following year. The incidence of abortion in mares ranges from 5% to 15%, with the upper limit becoming alarming. Older mares that potentially have decreased uterine defense mechanisms and increased fibrosis seem to be at higher risk. Although abortion “storms” are less common than sporadic abortions and noninfectious causes are diagnosed twice as frequently as infectious, when one does occur it is imperative that isolation protocols be implemented immediately. Clinical signs of impending abortion vary considerably from none—in which the mare is found empty during a late-term pregnancy check or immediately after abortion has occurred—to premature mammary gland development that commonly occurs with placental inflammation and separation. These mares should not be moved or mixed with others on the farm until testing for leptospirosis, herpesvirus, and equine viral arteritis (EVA) is negative. Close monitoring of temperatures, serum titers, and pregnancy status should be instituted on exposed mares. The reported causes of abortion and stillbirths in mares change with time, but the differentiation between infectious and noninfectious agents is still critical in the implications.

Infectious agents that cause abortion can be more devastating and produce the most distress among horse owners, managers, and veterinarians. Fortunately, improved methods of preemptive monitoring, vaccinating, testing, isolating, and treating provide a new arsenal with which to combat the diseases. Of the infectious agents, Leptospira spp., equine herpesvirus, and EVA will be discussed in this article.
2. Leptospirosis

Leptospirosis is a zoonotic disease that affects many domestic and wild animals worldwide. Horses are incidental hosts of several leptospiral serovars; only serovar Bratislava is suspected to be maintained in horses.11 Serological surveys demonstrate that leptospiral infection is common in equine populations. However, most leptospiral infections in the horse are subclinical.12 Clinical disease produces symptoms that include recurrent uveitis, fever, hemoglobinuria, jaundice, stillbirth, and abortion mainly in the last trimester.11 There are more than 250 serovars of Leptospira spp., but equine leptospirosis has been attributed primarily to L. interrogans serovar Pomona type Kennewicki in North America and serovars Bratislava, Grippotyphosa, Copenhageni, Autumnalis, Hebdomadis, Arborea, and Icterohaemorrhagiae in other parts of the world.11 Which serovar presents usually depends on the host in a specific area. Leptospiral infection as a cause of equine abortion has been reported as a diagnosis in 3.1% of cases in Hungary and 2.2% to 3.3% in the United States, whereas in Northern Ireland it has been reported in 35% of cases.11,13 In Kentucky, the source of equine infection is thought to come from the wild animal population, including raccoons, skunks, deer, opossum, and cattle and swine. The Leptospira spp. organisms are shed in the infected animal’s urine and contaminate water and feed, which are the probable sources of infection for the equine population. Environmental conditions with low-lying swampy areas and stagnant water such as ponds produce higher incidences of the disease.

Abortion or stillbirth usually occurs from 6 months of gestation to term, with fetal autolysis present as a result of death in advance of delivery. The mare usually displays no premonitory clinical signs before delivery but often has a high antibody titer against one or more leptospiral serovars. The mare is exposed and then becomes infected and bacteremic. The organism enters the placenta, causing fetal infection with placentitis and funisitis. The gross allantochorion lesions associated with equine leptospirosis consist of nodular cystic allantoic masses, edema, necrotic areas of chorion, and necrotic mucoid exudates that coat the chorion.14,15 The microscopic lesions in the allantochorion are thrombosis, vasculitis, mixed inflammatory cell infiltration of the stroma and villi, cystic adenomatous hyperplasia of the allantoic epithelium, villi necrosis, and calcification.2,15 The gross and microscopic lesions of the umbilical cord include mild-to-severe edema, focal-to-multiple sacculations filled with fluid, and coating of the surface with a fibrinous exudate, without visible involvement of the three primary blood vessels.2 A recent report revealed the surface of the umbilical cord diffusely coated by a dense exudate of mostly nondegenerate neutrophils (funisitis) that were mixed with fibrin. These neutrophils only infiltrated the cord surface and minimally into the Wharton’s jelly.15 Gross pathologic lesions of the fetus or stillborn foal include icterus and generalized petechial and ecchymotic hemorrhages. Livers are enlarged, mottled, and discolored yellow. Edema is evident in the kidney with pale white radiating streaks in the cortex and medulla.2,15 Microscopic changes show lesions in the liver and kidney to be the most severe. Liver lesions consist of hepatocellular dissociation, giant cells throughout the parenchyma, and leukocytic infiltration of the portal triads. The kidneys contain microabscesses with giant cells, dilated tubules, fibrosis, and multifocal areas of nonsuppurative interstitial nephritis.2

Diagnosis can be made by identifying Leptospira spp. in the allantochorion, umbilical cord, or fetal kidneys by fluorescent antibody tests (FATs), silver staining, or immunohistochemistry.2,15 A recent study indicated that real-time polymerase chain reaction (PCR) is an effective method for diagnosing leptospiral abortion in horses on both placenta and fetal kidney (and liver when available).16 Exposure usually occurs 2 to 4 weeks before abortion; therefore, the affected mares have high serological titers. Serology in the mare and fetus is based on enzyme-linked immunosorbent assay and microscopic agglutination tests. Positive diagnosis in mares occurs with serum titers of ≥1:6400.2 Detecting leptospires using a FAT is the method of choice for diagnosing leptospirosis in the kidney of aborted fetuses, although immunohistochemistry has shown to have a 78% sensitivity and 100% specificity when compared to the gold standard method of culture.2 Culture, however, is not practical because it takes 6 months for Leptospira spp. to grow.

Once an abortion has occurred and leptospirosis is suspected, the mare should be isolated so infective urine and uterine fluids will not expose other mares to the pathogen. Mares that have been pastured with the aborted mare should have Leptospira spp. titers drawn to try to identify potentially exposed mares so treatment can be initiated and abortion hopefully prevented. Serial serum titers may better identify exposed mares. Sources of infection such as wildlife (skunks for Kennewicki and raccoons for Grippotyphosa), water, and contaminated feed should be identified so further exposure does not occur. Treatment is successful with 5 mg/kg oxytetracycline intravenously once a day, doxycycline 10 mg/kg orally twice a day, and 20 000 IU/kg penicillin G procaine intramuscularly twice daily for 7 to 10 days.

Leptospira spp. titers remain high for long periods of time. In addition, naturally infected mares shed high numbers of nonhost-adapted leptospires in urine for up to 14 weeks; therefore, mares should not be reintroduced to other mares until shedding has ceased and a FAT, silver stain, or PCR shows that the mare’s urine is negative.216 To collect the urine, furosemide must be administered, and then
disorders, equine abortion, and neonatal death. EHV-1 and EHV-4, both viruses and the mechanism by which they persist in infect a susceptible host is the hallmark of herpes-viridae characteristically infect a susceptible host, replicate, and establish a life-long latent infection. This cycle of primary infection with periodic episodes of reactivation and shedding of virus to infect a susceptible host is the hallmark of herpes-viruses and the mechanism by which they persist in the host population. EHV-1 and EHV-4, both alpha herpesviruses, cause respiratory tract infection. However, EHV-1 is involved in neurological disorders, equine abortion, and neonatal death.

After respiratory infection or latent virus reactivation, uterine infection occurs via viremia. Transplacental spread of the virus then occurs at sites of uterine infarction after endometrial vasculitis and thrombosis, allowing infection of placental trophoblasts, whereby cell-to-cell spread or infected leukocytes transmit the virus to the intravillous endothelium and to fetal organs. A feature of spontaneous EHV-1 abortions in mares is the often sudden and explosive nature of the event, in which the fetus is expelled without warning while still enveloped within the fetal membranes.

Some experimental and field data suggest that the uterus and placenta become more susceptible to EHV-1 infection as pregnancy proceeds as a result of the local production of progesterone at the uteroplacental junction, resulting in immunosuppression. Transplacental spread of the virus occurs at sites of uterine infarction associated with thrombus formation in endometrial arterioles. Therefore, when the virus is in the placenta, it should be concentrated in those areas apposing sites of infarction.

Diagnosis can be made with PCR of fresh fetal tissues and paraffin-embedded placenta. Because the virus is transmitted by close contact via aerosol exposure, respiratory secretions, fetal tissues, placenta, and uterine fluids from mares that have aborted need to be disposed of and the mare isolated. The virus can be transmitted via organic material on clothes, shoes, and material inside stalls, trailers, water buckets, or feed. If mares have aborted in the field that area needs to be isolated from the remaining horses in the field; however, those horses should not be moved or mixed with other horses. Horses that have been exposed to infected horses but have not developed any clinical signs within 21 days of the potential exposure are unlikely to do so.

Vaccination has decreased the incidence of abortion storms dramatically, with most affected mares exposed coming from naive herds. Initial recommendations to reduce the risk of abortion from EHV-1/EHV-4 included using a killed vaccine at 5, 7, and 9 months of gestation. Recently, increasing the frequency to every 2 months year-round has been suggested on farms with large movements of mares or that have had endemic problems. Modified live virus vaccines have been used during gestation; however, at this time it is an off-label use. Immunity to the virus only lasts 4 to 6 months, so repeated abortions can occur in successive seasons.

3. Equine Herpes Virus

Equine herpesvirus 1 (EHV-1) was first isolated in 1932 in Kentucky and has been recognized around the world as a major cause of abortion. However, since the use of preventive vaccination, the incidence of loss has decreased over the years. EHV-1/EHV-4, both alpha herpesviruses, cause respiratory tract infection. However, EHV-1 is involved in neurological disorders, equine abortion, and neonatal death.

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4. Equine Viral Arteritis

The causative agent of the respiratory and aborto-genic disease equine viral arteritis (EVA) is a small single-stranded RNA virus (togavirus). Infection is believed to occur by direct contact via nasal droplet spray during the acute phase of infection and by shedding the virus in the semen of an infected stallion. Susceptible mares that are then bred to a shedding stallion acquire the disease. Blood, lachrymal fluid, urine, feces, vaginal secretions, aborted fetus, placenta, and amniotic fluid may also contribute to horizontal virus spread. Two carrier states exist in the stallion: a short-term state during convalescence (weeks) and a long-term condition that may persist for years. The virus persists in the vas deferens, ampullae, seminal vesicles, prostate, and bulbourethral glands and seems to be testosterone-dependent. Mares neither seem to become carriers and shedders nor have been shown to be infected venereally.

Clinical signs range from subclinical disease recognized only by seroconversion to acute illness and abortion. Signs are variable and include pyrexia, depression, anorexia, edema of scrotum, ventral trunk and limbs, conjunctivitis, lacerimation, serous nasal discharge, and respiratory distress. Adult horses usually make an uneventful recovery after a viremic phase that can persist for up to 40 days after infection. The incidence of abortion increases to approximately 50% of exposed mares and generally occurs 10 to 33 days after equine arteritis virus (EAV) infection. Abortion may occur with or shortly after infection as a result of fetal infection and death or myometrial necrosis and edema, which lead to placental detachment and fetal death.

EAV can be isolated from both the fetus and placenta by isolating the virus, especially from the placenta, fetal spleen, lung and kidney, and fetal/placental fluids. Semen samples with sperm-rich
fractions should be collected for isolating the virus suspected of infecting stallions. Antibodies to EAV can be demonstrated by complement fixation (CF) and virus neutralization tests. The CF test is most useful for studying immunity to arteritis during the first 4 months after exposure because the titer peaks 2 to 4 weeks after infection and decreases below detectable limits after 8 months. Virus neutralization antibody titers develop simultaneously, and CF titers are maximal 2 to 4 months after infection and remain stable for several years. Previously infected horses are immune to re-infection with the virus for up to 7 years.

A modified live virus vaccine is registered for use in some US states. The use of the modified live virus vaccine does not produce any side effects in stallions apart from a short-term abnormality of sperm morphology and a mild fever with no overt clinical signs. The virus can be sporadically isolated from the nasopharynx and blood for up to 7 to 32 days after vaccination, so vaccinated horses should be isolated for 1 month. Horses in contact with and mares served by vaccinated stallions are not infected with EAV. Vaccinated mares bred by positive stallions are protected from clinical infection. Prevention and control of the disease involves isolating and vaccinating stallions, mares being serviced by infected stallions, and maintaining diligent monitoring of seropositive nonshedding stallions. Stallions shedding the virus should be housed and bred in separate facilities.

5. Conclusion
Infectious abortions in the mare should be identified quickly and efficiently. Isolation protocols should be initiated immediately, and other exposed horses should be continually monitored and treated as necessary. These measures will potentially diminish the effect and spread of disease.

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Declaration of Ethics
The Authors declare that they have adhered to the Principles of Veterinary Medical Ethics of the AVMA.

Conflict of Interest
The Authors declare no conflicts of interest.

References and Footnote

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Selected Topics in Reproductive Pathology: Mare II

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This manuscript will review commonly encountered reproductive pathologic conditions in the non-pregnant and pregnant mare. Conditions include endometritis, placentitis, and retained fetal membranes. Authors’ addresses: University of Florida, College of Veterinary Medicine, Gainesville, FL 32610-0125, macphersonm@ufl.edu (Macpherson); and T6-020 Veterinary Research Tower, College of Veterinary Medicine, Cornell University, Ithaca, NY 14853, dhs2@cornell.edu (Schlafer). *Corresponding and presenting author. © 2015 AAEP.

1. Endometritis

Endometritis, or inflammation of the endometrium, is a condition that is costly and frustrating to mare owners. The condition is most commonly defined as either infectious endometritis or postmating-induced endometritis and can be caused by the introduction of pathogenic organisms, semen, urine, or air into the uterus. A variety of factors contribute to the development of endometritis, including age, perineal conformation, anatomic abnormalities, poor uterine contractility, defective lymphatic drainage, and degenerative changes in tissues that make up the reproductive organs. Elucidating the root causes of endometritis can be challenging.

Pathophysiology

Because endometritis is a complex condition, the pathophysiology of the disease is as well. Although all mares develop endometritis as a result of breeding,1 the response to inflammation differs between mares (Fig. 1). Most mares are intrinsically prepared to eliminate contaminants after mating, thus resolving inflammation and preventing infection.2 These mares are termed “resistant” to mating-induced endometritis. Other mares are more prone...
Risk Factors

Risk factors for endometritis include mare age and parity, perineal and birth canal conformation, clearance of contaminants, and immune response to antigenic stimuli. Pluriparous mares frequently experience trauma during delivery that can affect the uterus or anatomical barriers to uterine infection, including the vulva, vestibule-vaginal sphincter, and cervix. Aberrations in reproductive anatomy provide an opportunity for air, urine, and bacteria to contaminate and colonize the reproductive tract. If the mare is free of other conditions, such as dependent uterine position or impaired lymphatic drainage, she may be able to clear herself of any contamination and inflammation. However, older mares often experience overall conformational abnormalities that affect uterine position, uterine contractile, and lymphatic drainage. These mares are prone to developing pendulous uteri that fail to evacuate contents. These mares are susceptible to endometritis, and they are termed “susceptible.” Mares that are deemed resistant to endometritis have intact mechanisms for uterine clearance of contaminants after breeding via mechanical evacuation of uterine contents as well as lymphatic drainage. Mares that are susceptible to endometritis suffer one or more defects in the system such as an anatomic abnormality, impaired mechanical or lymphatic evacuation of the uterus, or compromised immunologic response to inflammation. Under inflamed conditions, resident bacteria become opportunists and infection ensues. Therefore, endometritis is further subcategorized as infectious or postmating-induced endometritis. In some instances, both conditions coexist. To properly diagnose and treat endometritis, risk factors common to all types of endometritis must be examined.

More recently, the role of mucus production and the presence of biofilm have been examined in persistent uterine infections (Fig. 2). Mucus can both aid and impair pathogen clearance in systems with a mucociliary apparatus. It has been postulated that normal mucus production facilitates cilia-propelled removal of contaminants from the uterus after breeding. Under persistently inflamed conditions, excess or reduced mucus production alters mucociliary function and impairs pathogen clearance. Excess mucus production has been identified in mares with chronic endometritis and has been specifically associated with microorganisms (Escherichia coli and β-hemolytic Streptococcus). Biofilm has similarly been postulated to contribute to conditions that favor bacterial proliferation in the uterus of some mares. Under natural conditions, bacteria exist in either a free-floating (planktonic) or sessile (adhered) state. The free-floating form allows bacteria to proliferate and grow, whereas the sessile state promotes persistence and long-term survival. The formation of a biofilm is a complex process that allows a bacterial population to transition from planktonic to sessile states, thus promoting survival. Through the formation of biofilm, bacteria are enclosed in a biopolymer matrix that allows them to be adherent to inert surfaces (table tops, glassware, vascular catheters, orthopedic prosthetic devices, etc.) or to each other. Once attached, bacteria grow and produce extracellular polymers that facilitate long-term survival of the sessile bacterial cells. In addition, the host produces antibodies to the bacteria (both planktonic and sessile), but the immune response eliminates the planktonic bacteria only. Sessile bacterial antigens, although recognized, are impenetrable within the extracellular polymeric substance (EPS) layer of biofilm. As a result, general immune responses to pathogenic bacteria are diminished when biofilm is present. In addition, antimicrobial susceptibility is affected by biofilm production. The thick EPS layer as well as proteins and lipids provide an effective barrier for antimicrobial penetration of sessile bacteria. Disease symptoms are often ameliorated after antimicrobial-induced death of planktonic bacteria, but infection persists after treatment because the sessile bacterial population is protected by the biofilm. Recent data confirm biofilm production by bacteria (E. coli, Pseudomonas aeruginosa, and Klebsiella pneumoniae) isolated from the equine uterus. Additional data
uterine swabs also produced biofilms. Ongoing work is examining effective treatments against biofilm producing bacteria in the equine uterus.

Diagnostic Tools for Endometritis

The evaluation process always starts with an accurate history of both general and reproductive health, a full physical examination, and a thorough examination of both the external and internal genitalia. Examination tools that are commonly used include evaluation of the perineal area, transrectal palpation and ultrasonography of the reproductive tract, sampling for uterine culture, cytology, endometrial biopsy, and vaginal examination. Results from these tests help identify obvious, and often correctable, problems. Additional tests such as low-volume uterine lavage for cytology and culture, hysteroscopy, and karyotyping are sometimes necessary to gain needed information.

General Physical and Perineal Examination

Overall, good physical health and conformation are important for reproductive health in mares. Although perineal anatomy is often the focus of attention when evaluating a mare for fertility, it is important to consider the impact overall body condition has on perineal anatomy. Underweight mares frequently have a pelvic tilt that results in a dependent uterus and sinking of the anal sphincter, both of which indicate risk factors for uterine contamination. A first step toward good reproductive health is good body condition, which can help correct perineal defects.

Vulvar anatomy is a critical first-line defense against uterine contamination. Poor perineal conformation predisposes the mare to “wind sucking,” fecal contamination, and pooling of urine in the vagina. The long axis of the vulva should be vertical, with no more than a 10° angulation. Most of the vulva should be ventral to the pelvic brim. When 2 fingers are placed on the pelvic ischium, no more than 2.5 cm of the vulva should be dorsal to the pelvic floor. The vulvar lips should not have obvious irregularities, and apposition should be good. When the vulvar lips are gently parted, no obvious influx of air should be heard. The vestibule-vaginal seal can be evaluated through the parted vulvar lips and should be intact.

Transrectal Ultrasound Examination

Ultrasound examination is an important and useful component for evaluating reproductive health in mares. Intraluminal uterine fluid around the time of breeding and ovulation has an established relationship to reduced pregnancy rates in cycling and postpartum mares. Postmating-induced endometritis is the most common cause of uterine fluid accumulation during the periovulatory period of mares. Mares with delayed uterine clearance often have defects in intrinsic myometrial contractility and drainage through the lymphatics that is evidenced by lymphatic stasis. The conditions frequently manifest themselves as intraluminal fluid accumulation and abnormal uterine edema patterns during estrus. Specifically, these mares often have an unusually large amount of uterine edema starting early in the estrus period and persisting after ovulation. Interestingly, mares with bacterial endometritis do not consistently develop intraluminal fluid. Recent studies reported that mares that had uterine infections caused by E. coli were less likely to have intrauterine fluid accumulation identified by ultrasound and less evidence of cytologic inflammation. Mares with uterine infections caused by β-hemolytic Streptococcus, K. pneumoniae, Enterobacter cloacae, or yeast had a higher incidence of ultrasonographically detectable uterine fluid. These findings can be useful when determining the need for additional diagnostic tests.

Uterine Culture and Cytology

Culture and cytology should be performed to evaluate for potential endometritis. Uterine culture and cytology can be obtained from either a double-guarded swab, cytology brush, endometrial biopsy, or low-volume uterine lavage. Culture and cytology should be performed together because both tests have a high prevalence of false negatives when used alone. Because the vagina is not a sterile environment, false-positive culture results are common as a result of contamination from the perineum or the vagina. Conversely, aerobic culture may fail to diagnose fungal or yeast infections, which are more easily detected upon cytology. In general, any bacterial growth in conjunction with neutrophils upon cytology is considered diagnostic for uterine infection. In addition, pure growth of a single organism, particularly those that commonly contribute to endometritis, could arguably be considered diagnostic for infection even in the absence of positive cytology. Commonly described reproductive pathogens in the mare include S. equi subsp. zooepidemicus, E. coli, K. pneumonia, and P. aeruginosa.

Historically, the presence of more than two neutrophils per high-powered field is diagnostic for endometritis. A recent study examined the cytologic sampling methodology (cytology brush vs. endometrial biopsy), interpretation of results, and correlation to endometritis in mares. Conclusions from this study were 2-fold: first, endometritis diagnosis was more accurate when assessing the percentage of polymorphonuclear leukocytes (PMNs) in the total cells evaluated (>2% PMNs in 300 cells counted) versus the number of PMNs per high-powered field; second, endometrial biopsy offered no advantage over cytology brush for diagnosing endometritis. These interesting data may help lead to more accurate endometritis diagnoses using traditional
tools. However, it has been shown that uterine infections with \textit{E. coli} and \textit{P. aeruginosa} are not easily detected by positive cytology using traditional methods. Therefore, the growth of one of these pathogens warrants further investigation in the mare with a history of chronic subfertility. Procedures such as low-volume uterine lavage can be helpful in identifying infectious endometritis caused by gram-negative organisms.

\textbf{Low-Volume Uterine Lavage}

Low-volume uterine lavage can be performed to obtain a more representative sample of the uterine contents.\textsuperscript{10,19} It has been postulated that this method allows for sample collection from the entire uterine lumen rather than an isolated sample using an endometrial instrument (swab, brush, or biopsy). Low-volume lavage is best performed during diestrus to allow complete dilation and exposure to recesses between endometrial folds. When the technique is performed during estrus, fluid tends to collect in between edematous endometrial folds and can be difficult to retrieve.

After fecal evacuation and preparation of the perineal area, a sterile Foley-type catheter is aseptically inserted through the cervix and into a uterine horn. Using transrectal guidance, the catheter is fed into the tip of one uterine horn. The catheter balloon is not inflated so that the catheter tip can be manipulated easily to aid in fluid evacuation. Sterile saline (150–250 mL), lactated Ringer’s solution (LRS), or phosphate-buffered saline are infused into the uterus. The fluid is agitated throughout the uterus using rectal massage for at least one minute. Oxytocin (10–12 IU) is administered intravenously to promote uterine contractility for fluid collection. Using transrectal manipulation, fluid is moved to the tip of the uterine horn where the catheter tip is placed. With the operator’s hand cradling the tip of the uterine horn around the catheter, the fluid is allowed to drain out through the catheter and back into the bag so that the system remains “closed.” Fluid is then transferred into conical 50-mL tubes. A minimum of 50 mL of efflux is needed for accurately representing uterine contents. Furthermore, fluid should be processed for evaluation within 1 hour of collection to avoid iatrogenic cellular changes.\textsuperscript{10} The clarity of the fluid is evaluated immediately for 10 minutes at 400x magnification. Determining the presence of microorganisms (\textit{E. coli} and \textit{\beta}-hemolytic \textit{Streptococcus}). The contents are allowed to settle for at least 1 hour, or the tubes are centrifuged immediately for 10 minutes at 400g. Determining g force on a standard benchtop centrifuge can be done with a nomograph and has been well described.\textsuperscript{21} After processing, fluid is decanted (if centrifuged) or aspirated (if allowed to settle), leaving 5 mL of supernatant and the pelleted cells. Two sterile cotton swabs are used to obtain samples from the pellet. One sample is placed in transport media for bacterial culture; the second sample is smeared directly onto a slide for cytological evaluation.

Low-volume uterine lavage provides a concentrated sample of cells and debris that can help identify subclinical infections. Results from low-volume uterine lavage should be interpreted cautiously; however, because the concentration process also increases the likelihood of isolating bacterial contaminants. It is best to interpret the samples from low-volume uterine lavage in conjunction with additional evidence of endometritis such as abnormal intraluminal fluid accumulation (especially in diestrual or early estral mares), exuberant uterine edema, and repeated pregnancy losses after using good breeding management techniques. Positive indicators of subclinical uterine infection obtained from low-volume uterine lavage include cloudy or mucus fluid, isolation of known uterine pathogens, and more than one PMN identified per high-powered field of concentrated cells. It is interesting to note that the cytological interpretation method recently reported by Kozdrowski et al.\textsuperscript{18} may be perfectly suited for evaluating samples from low-volume uterine lavage.

\textbf{Endometrial Biopsy}

Uterine tissue obtained through endometrial biopsy is considered to be the gold standard for detecting reproductive pathologies in the mare’s uterus.\textsuperscript{22} Microscopic examination of the endometrium provides the most accurate means of identifying inflammation and other pathologies in the mare’s uterus. The endometrial biopsy should be taken at the base of one uterine horn, and a single sample has been shown to be representative of the entire uterus when considering inflammation or periglandular fibrosis. The sample should be submitted to a laboratory in an appropriate fixative (Bouin’s solution, Davidson’s solution, or 10% formalin) along with a detailed history of behavioral, ovarian, and uterine findings at the time of biopsy. Based on the degree of change in the characteristics that are described in the sections that follow, a grade is given with the modified Kenney scoring system.

\textbf{Inflammatory Cells}

Types, location, and distribution of inflammatory cells in the endometrial sample are noted. Neutrophils often indicate acute inflammation, whereas lymphocytes indicate chronic changes. The presence of a significant number of plasma cells is a poor prognostic sign because it indicates long-standing persistence of antigenic stimulus—usually bacteria. The presence of eosinophils indicates uterine irritation such as urine pooling and/or pneumovagina. Treatment can resolve many types of inflammation.
Fibrosis

Uterine fibrosis is essentially connective tissue formation and is a normal degenerative part of aging. Fibrosis can be widespread or focal. It is important to note that fibrosis is untreatable and permanent. A mare with significant endometrial fibrosis has a much lower chance of carrying a foal to term.

Dilated Lymphatics

Older, pluriparous mares tend to have large, pendulous uteri that do not provide adequate lymphatic drainage. Dilated lymphatics seen in the endometrium may be clinically significant if paired with findings such as uterine cysts or poor uterine tone. These findings often are concurrent with a propensity for pathologic uterine edema, uterine fluid accumulation, or postbreeding endometritis.

Cystic Glandular Distention

This lesion, when not associated with periglandular fibrosis, may indicate an inability to empty uterine contents. Whether this is associated with deficient uterine contractility or tone has not been studied. Chronic distention that results in glandular epithelial atrophy probably renders the glands useless, thus affecting their ability to provide nutritional support to the developing embryo.

Vascular Elastosis

Degenerative lesions develop in vessel (arterial) walls in multiparous mares. Arteries in both the myometrium and endometrium are involved. Vascular degeneration has been shown to be statistically correlated to the number of pregnancies. Elastic fibers within vessel walls become increased in number and undergo fragmentation. Doppler flow studies have shown that mares with vascular elastosis have reduced uterine perfusion. The authors of these studies hypothesize that compromised uterine blood flow could affect endometrial development, uterine clearance, endometritis, and general fertility.

Endometrial biopsy samples can also be used for culture and cytology. Recent studies suggest that the incidence of false-negative results is significantly reduced when obtaining culture and cytology samples directly from an endometrial biopsy specimen. With this method, the endometrial tissue is obtained using the aseptic technique and a sterile equine biopsy forceps. Once the tissue is obtained, it is used to inoculate on a blood agar plate for culture and then smeared onto a glass slide for cytologic preparation. Samples are then processed using traditional methods for microbial isolation and staining. The sensitivity for bacterial growth using this method was almost 2.5 times greater when using the endometrial biopsy sample compared with a traditional double-guarded swab system.

Cervical Evaluation

The cervix is an important barrier to uterine infection. For multiparous mares, a thorough examination of the cervix can reveal abnormalities that contribute to reduced fertility. Cervical lacerations or damage to the cervical muscles after prolonged foaling prevent proper closure of the cervix, leading to uterine contamination. Conversely, mares that have experienced severe dystocia, long-standing endometritis, or pyometra may have cervical adhesions that compromise cervical patency and evacuation of uterine contents. More recently (M. Macpherson, personal observation), a population of middle-aged performance mares that were being bred for the first time and mares that had served only as embryo donors (i.e., had not carried a foal) revealed a phenomenon of poor cervical relaxation during estrus. The reduced muscular relaxation in these mares led to compromised uterine evacuation at the time of breeding, fluid accumulation, and endometritis.

The cervix of a mare is best evaluated during diestrus. However, the typical breeding soundness evaluation is performed during estrus when the cervix is relaxed and permits easy passage of diagnostic instruments. In many cases, digital examination of the cervix at the time of breeding soundness examination will be adequate for determining cervical patency, presence of adhesions, and large cervical defects. Estrus is the optimal time for detecting poor cervical relaxation in mares. For assessing muscular function or identifying smaller cervical defects, the mare’s cervix should be examined during diestrus (when under the influence of progesterone) and tightly closed. The operator’s index finger can be carefully passed through the lumen of the cervix using gentle digital pressure. Once the lumen is penetrated, the operator can circumferentially examine the cervix for defects with the index finger in the lumen and the thumb at the vaginal wall and external cervical os. If cervical damage is suspected, it is important to perform a thorough cervical exam before repeated manipulations, which can cause the cervix to soften and change shape.

The prognosis for mares with cervical damage is often poor. Depending on size, a cervical laceration often will require surgical repair. Scar tissue formation at the site of the repair compromises the elasticity of the muscles required for dilation during estrus and at the time of parturition. As a consequence, the tears frequently recur during foaling. Mares with cervical adhesions are at risk for loss of cervical patency and pyometra formation. Early, aggressive treatment with cervical breakdown and application of steroid-based creams to prevent reformation are the first line of defense with cervical adhesions. In more advanced cases, cervical wedge resections have been used to successfully maintain cervical patency and allow uterine drainage. Adhesions are prone to reformation even after complete.
breakdown, so owners should be advised that the presence of cervical adhesions significantly reduces the prognosis for fertility. When possible, assisted reproductive procedures are the best option for breeding mares with cervical abnormalities.

Hysteroscopy

Hysteroscopy is the direct visualization of the interior of the uterus using an endoscope. It is used to evaluate pathogenic conditions, including endometrial cysts, retained endometrial cups, focal lesions, masses, and foreign bodies. Uterine adhesions, which may severely impact fertility, are best diagnosed with hysteroscopy.

Hysteroscopy is performed with a 1-m flexible endoscope. Proper preparation of the endoscope is critical for avoiding iatrogenic introduction of pathogens to the urogenital system. Because of the delicate nature of the fiber optics in the endoscope, the equipment is generally cold-disinfected using a 2% glutaraldehyde solution. Immersion of the fiber optic portion of the endoscope in glutaraldehyde for 12 to 15 minutes will destroy most pathogenic bacteria, fungi, and viruses. Glutaraldehyde solution should also be aspirated through the biopsy channel and placed in the fluid receptacle of the system. Prolonged exposure of the endoscope to glutaraldehyde will result in deleterious effects on the fiber optics as well as the rubber outer coating. All parts exposed to glutaraldehyde should be rinsed with copious amounts of sterile water before being introduced into the genital tract. If sterilizing the equipment is necessary, ethylene oxide is the method of choice.

The perineal region of the mare is aseptically prepared before introducing the endoscope into the uterus. The operator's arm is covered with a sterile sleeve, and the hand is covered with a sterile glove. A very small amount of lubricant is placed on the back of the hand to avoid smearing lubricant onto the camera face and altering image quality. The endoscope, trapped in the hand, is carried to the external cervical os. Introduce one or two fingers through the cervix while ensuring that over dilation is avoided. The endoscope is carefully placed into the cervical os and passed into the uterus. Once the endoscope is in the uterus, the cervical os is held tightly closed around the instrument, and the uterus is insufflated with air. In some cases, particularly for laser-assisted removal of endometrial cysts, the uterus is insufflated with fluid such as LRS or sterile saline. After the uterus is insufflated, the endoscope is guided up each uterine horn and back to the uterine body for full evaluation. Culture and biopsy samples can also be taken through the endoscope using instruments specifically fitted for this purpose. Endometrial biopsy samples using an endoscopic instrument are small and difficult to interpret. Recently, Card and Eaton29 described a method for obtaining an endometrial biopsy using hysteroscopic guidance and a traditional biopsy punch. With this technique, the endoscope is used to visualize areas of interest in the endometrium. The biopsy punch is then inserted alongside the endoscope, and a sample is taken under visual guidance. The advantage of this technique over a biopsy through the channel of the endoscope is that the sample size is much larger and useful for diagnostic purposes. After hysteroscopy, air or fluid is removed from the uterus. Prophylactic treatments using uterine lavage and intrauterine antibiotic infusion are advised.

Polymerase Chain Reaction

Polymerase chain reaction (PCR) assays have recently been investigated for their usefulness in diagnosing uterine pathogens in mares.13 Using this sensitive technology, more than 30 uterine bacterial pathogens have been identified from mares. PCR has also been used to identify fungal organisms from uterine samples. An advantage of PCR for detecting fungal pathogens is the rapid turnaround time for results. Traditional fungal cultures take 5 to 7 days to grow organisms, whereas PCR can provide results in 48 hours. Because of the sensitivity of the test, PCR is best reserved for difficult-to-detect uterine pathogens.

Treatment Strategies for Endometritis

Treatment approaches for endometritis are built on the premise of correcting anatomic defects, removing contaminants, and treating pathogens. The fundamental principles have not changed dramatically over time. Anatomic abnormalities are frequently the source of genital tract contamination. Mares with poor perineal conformation and/or compromise to the vulvar, vestibular, and cervical barriers often benefit from having their defects surgically corrected. A Caslick suture is a simple remedy for most vulvar anatomic defects. Less frequently, mares may require perineal reconstruction or cervical repair.

Uterine Evacuation

Promoting uterine evacuation through the administration of ecbolic agents (oxytocin, prostaglandin) and uterine lavage is important for treating problem mares. Affected mares frequently experience poor evacuation of uterine contents after contamination. In some cases, the use of ecbolic agents is sufficient to evacuate uterine contents. Oxytocin is a potent ecbolic agent that promotes strong but short-lived uterine contractions.30,31 The traditional dose for oxytocin is 10 to 20 U administered intramuscularly or intravenously. The half-life of oxytocin is short (~7 minutes31), so it is generally used for its immediate effect (i.e., after uterine lavage). When administered intramuscularly, the interval is often every 3 to 4 hours a few times a day. Mares with significant compromise to uterine contractility as a result of poor muscle tone or lymphatic drainage abnormalities require the prolonged contractile action that prostaglandin analogs provide. Clopros-
tanol, a prostaglandin analog, has been shown to induce low-amplitude uterine contractions that last for 4 to 5 hours.\textsuperscript{32} Cloprostenol administered during the immediate periovulatory period has been shown to affect luteal function and reduce progesterone concentrations, which in some cases negatively affected pregnancy rates.\textsuperscript{33–35} The recommended dose for cloprostenol is 250 \( \mu g \) administered intramuscularly once or twice a day. A suggested protocol\textsuperscript{8} for mares with poor uterine clearance or drainage is to perform uterine lavage 4 to 8 hours after mating and to administer oxytocin to help completely evacuate uterine fluid. The lavage is followed by administering 250 \( \mu g \) of cloprostenol intramuscularly 12 to 18 hours after mating.

As described previously, ecobic agents are routinely used in combination with uterine lavage. Isotonic fluids such as 0.9% saline or LRS are infused into the uterine lumen in 500-mL to 2-L increments (depending on uterine capacity), and the fluid is siphoned off. The procedure is repeated until the effluent collected appears to be relatively clear. Uterine lavage can be safely performed 4 to 8 hours after breeding.\textsuperscript{36} Mares that have a history of postmating-induced endometritis or chronic subfertility may benefit from aggressive treatment, including uterine lavage immediately before\textsuperscript{37} and/or after\textsuperscript{38} breeding. Vanderwall and Woods\textsuperscript{37} showed that pregnancy rates were not negatively affected in mares undergoing uterine lavage using 1 L of LRS within an hour before mating. This procedure can be useful in problem mares that retain uterine fluid before mating or that are inseminated with a second dose of frozen-thawed semen. Mares with clearance problems often benefit from 1 to 4 L of uterine lavage (0.9% saline or LRS) 4 to 8 hours after breeding, when feasible. However, uterine lavage can be performed up to 12 to 24 hours after mating. Uterine lavage therapy is often combined with repeated doses of oxytocin or prostaglandin during the periovulatory period. Uterine lavage is also frequently repeated (for up to 3 days) in mares that retain fluid after mating and ovulation.

**Antimicrobial Therapies**

Antimicrobials are often the first line of defense in mares with infectious endometritis. Selecting the appropriate antimicrobial treatment should be based on culture and sensitivity results. Antimicrobial agents commonly used to treat mares with infectious endometritis are shown in Table 1.

**Solvents and Mucolytic Agents**

Several novel therapies have recently been investigated for treating problem mares. These therapies have been directed toward removing mucus or biofilm, which are thought to protect bacteria within the reproductive tract.\textsuperscript{8,39} Proposed uterine therapeutics for mares with persistent microbial infections that may be associated with biofilm or excessive mucus production include the use of mucolytics and/or chelating agents.

Several products, including dimethyl sulfoxide (DMSO), kerosene, and N-acetylcysteine (NAC), have been instilled in the mare’s uterus to promote the breakdown of mucus exudate seen in chronic infections. Uterine lavage using DMSO has been investigated for its anti-inflammatory effects and ability to reduce collagen formation in the equine endometrium. Inflammation was reduced in some mares after uterine treatment with DMSO while endometrial fibrosis remained unchanged.\textsuperscript{40,41} Intrauterine administration of DMSO caused desquamation of the endometrial epithelium that regenerated within 21 days of treatment. Pregnancy rates tended to improve in one population of barren mares.\textsuperscript{32}

Chemical curettage with kerosene has been demonstrated to produce glandular activation in mares, with improved conception rates in treated vs control mares.\textsuperscript{43} Biopsy grades improved from Category III to Category I after treatment in some mares. Of 11 mated grade III mares, 9 conceived, and 5 carried pregnancies to term.\textsuperscript{43} In a small, controlled study, intrauterine infusion of kerosene to mares resulted in the loss of ciliated cells 24 hours after treatment.\textsuperscript{44} From these studies, it is postulated that kerosene strips the endometrium of ciliated cells, mucus, and possibly epithelial cells, resulting in regeneration of the endometrium. The effects of kerosene treatment on fertility in mares remains poorly understood.

More recently, the use of NAC has been investigated as a uterine treatment for reducing mucus exudate.\textsuperscript{45} NAC, known as the “mucus dissolver,” is widely used in human cough preparations because of its ability to break disulfide bonds in mucus. Additionally documented benefits of NAC when used to treat human medical conditions include anti-inflammatory and antimicrobial properties.\textsuperscript{46} The infusion of NAC before breeding has been advocated in mares with exuberant mucus production or chronically barren mares.\textsuperscript{39,45} Thirty milliliters of 20% NAC solution is added to 150 mL of sterile saline to produce a 3.3% solution. This solution is infused into the mare’s uterus. Uterine lavage is performed the following day. If mucus is evident in the uterine lavage effluent, NAC is infused into the mare’s uterus again. NAC infusions are routinely followed with traditional uterine lavage 24 hours after treatment. Recently, the effects of NAC were tested in a population of barren mares to evaluate both positive and negative properties of this product.\textsuperscript{45} Evaluation of endometrial biopsies did not reveal negative effects of NAC on the endometrium of mares after uterine infusion with the product. Endometrial mucus thickness was found to be reduced in some treated mares. Limited data regarding pregnancy rates after NAC treatment are available, and additional studies are required to fully understand the value of this

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Antimicrobial Agents</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Penicillin (K⁺ or Na⁺ salt)</td>
<td>5 million units IU</td>
<td>Especially effective for streptococci and gram + bacteria; economical</td>
</tr>
<tr>
<td>Ticarcillin</td>
<td>1–3 g IU</td>
<td>Use for <em>Pseudomonas</em> but not <em>Klebsiella</em></td>
</tr>
<tr>
<td>Timentin</td>
<td>3–6 g</td>
<td>Ticarcillin + clavulonic acid to prevent degradation of ticarcillin by β lactamase enzyme producing bacteria; broad spectrum activity against gram + and gram – bacteria</td>
</tr>
<tr>
<td>Gentamicin sulfate</td>
<td>500–1000 mg IU</td>
<td>Highly effective for gram – bacteria; must be mixed with an equal volume of NaHCO₃ and diluted in saline to avoid irritation of the endometrium; economical</td>
</tr>
<tr>
<td>Amikacin sulfate</td>
<td>1–2 g IU</td>
<td>Use for <em>Pseudomonas</em>, <em>Klebsiella</em> and persistent gram – bacteria, not as caustic as gentamicin but still must be mixed with an equal volume of NaHCO₃ and diluted in saline to avoid irritation of the endometrium; more costly than gentamicin</td>
</tr>
<tr>
<td>Ceftiofur sodium</td>
<td>1 g IU or IM</td>
<td>Third generation cephalosporin with broad spectrum activity against gram + and gram – bacteria; empirical use for endometritis</td>
</tr>
<tr>
<td>Ceftiofur crystalline free acid</td>
<td>6.6 mg/kg IM q 96 h</td>
<td>Third generation cephalosporin with broad spectrum activity against gram + and gram – bacteria; excellent for <em>S. zooepidemicus</em>;</td>
</tr>
<tr>
<td>Enrofloxacin</td>
<td>5 mg/kg, IV, once daily, 3–5 days</td>
<td>Broad spectrum activity but especially Gram – bacteria (<em>Pseudomonas</em>); commercial preparation should only be used systemically as causes severe irritation to uterus</td>
</tr>
<tr>
<td>Polymixin B</td>
<td>1 million units IV or IU</td>
<td>Gram – bacteria, especially <em>Pseudomonas</em>; costly</td>
</tr>
<tr>
<td><strong>Antifungal Agents</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nystatin</td>
<td>500,000 U, IU or PO</td>
<td>Excellent for yeast but moderate for molds; dilute in 100–250 mL sterile water and mix well</td>
</tr>
<tr>
<td>Amphotericin B</td>
<td>200 mg IU or IV</td>
<td>Excellent for yeast but moderate for molds; dilute in sterile water for IU infusion as precipitation occurs; nephrotoxic when administered systemically</td>
</tr>
<tr>
<td>Ketoconazole</td>
<td>30 mg/kg (in 0.2 N HCl by NG tube; q 12 h)</td>
<td>Highly effective for yeast but not mold; irritating so requires naso-gastric administration; absorption may be improved with fasting</td>
</tr>
<tr>
<td>Clotrimazole</td>
<td>700 mg, IU</td>
<td>Good for yeast but poor for molds; best to crush tablets and dissolve in 40–60 ml sterile water</td>
</tr>
<tr>
<td>Fluconazole</td>
<td>14 mg/kg loading dose PO, 5 mg/kg maint for 6 d</td>
<td>Good endometrial concentrations with this dosing regimen; MIC for Candida spp. Susceptibility very poor for molds (0%)</td>
</tr>
<tr>
<td>Miconazole</td>
<td>200 mg, IU</td>
<td>Sensitivity limited for both yeasts and molds (&lt; 50% susceptible)</td>
</tr>
<tr>
<td><strong>Uterine Irrigation</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vinegar</td>
<td>2% (v/v) 20 mL/L saline</td>
<td>Daily uterine lavage for fungal infections; monitor for tissue irritation</td>
</tr>
<tr>
<td>Povidone iodine solution (%)</td>
<td>5mL stock Betadine® solution/ L lavage solution</td>
<td>Can be irritating to endometrium if too concentrated; indicated for treatment of bacterial and fungal infections</td>
</tr>
<tr>
<td>Tris-EDTA</td>
<td>250–500 mL IU</td>
<td>Buffered chelator that potentiates antimicrobials (antimicrobials can be mixed into Tris-EDTA solution); uterine infusion followed by uterine lavage at 24 h; can be repeated</td>
</tr>
<tr>
<td>N-acetylcysteine (20% NAC; 200 mg/mL)</td>
<td>30 mLs (6g) diluted in 150 mL sterile saline</td>
<td>Intruterine infusion with mucolytic and anti-inflammatory properties; follow with 1 L uterine lavage (saline or LRS) 24 h later; can be repeated</td>
</tr>
<tr>
<td>Dimethyl sulfoxide (99% DMSO)</td>
<td>50 mL in 1 L saline</td>
<td>Unspecific treatment for inflammation, effectiveness in the uterus not clear; follow with uterine lavage using 1 L saline or LRS</td>
</tr>
<tr>
<td>Hydrogen Peroxide (3% H₂O₂)</td>
<td>60 mL IU</td>
<td>Anecdotal reports for use with fungal infections: uterine infusion followed by uterine lavage at 24 h; repeat 3 d</td>
</tr>
</tbody>
</table>
treatment. For the most part, NAC infusions can be considered to be a harmless treatment that might impart positive effects for chronically barren mares with excessive mucus production.

Buffered Chelators

Buffered chelating agents have been advocated for targeting resistant bacteria, particularly those that produce biofilm. *P. aeruginosa*, *E. coli*, *S. epidermis*, and both fungal and yeast species have been identified as potent biofilm producers. In buffered chelators have been examined for treating persistent uterine infections in both cattle and mares. Early studies showed that first-generation Tris-EDTA–buffered chelators used in the uterus of mares had no negative effects on the endometrium. In vitro, Tris-EDTA reduced the minimum inhibitory concentration of *P. aeruginosa* isolates recovered from mares with endometritis. These studies showed that the intrauterine application of Tris-EDTA was safe and effective against a problematic reproductive pathogen in mares. Additional studies in cattle showed that Tris-EDTA combined with an antibiotic was more effective at treating bacterial endometritis than antibiotics alone. More recently, a third-generation–buffered chelator has shown to potentiate the effect of antifungal agents when applied to equine keratitis isolates in vitro. Based on these promising results, the use of Tris-EDTA has been advocated for treating chronic uterine infections. To date, information about the use of Tris-EDTA for equine uterine infections is largely anecdotal. The recommended intrauterine protocol is to lavage the uterus with an isotonic solution, infuse 250 to 1000 mL of Tris-EDTA (depending on uterine capacity) into the uterus, and leave overnight. The uterus is lavaged 24 hours after the chelator infusion. Antimicrobials may be mixed directly into the chelator solution at the time of infusion or infused after the removal of the chelator solution. This treatment is repeated as necessary. In most cases of chronic infection, intrauterine treatments are repeated for 3 to 5 days.

Immunomodulators

Both infectious and noninfectious forms of endometritis are thought to result from delayed uterine clearance and inflammation. The influx of inflammatory cells into the uterus is necessary for removing harmful byproducts of breeding. In some mares, inflammation persists and becomes pathologic. Although clearing uterine contents is a central component of treating this “susceptible” population of mares, controlling aberrant inflammation is also important. In recent years, immunomodulatory agents such as steroids (dexamethasone, prednisolone), *Mycobacterium phlei* cell wall extracts (MCWEs), and *Propionibacterium acnes* have been tested for immunomodulatory effects in mares with persistent inflammation. Studies have been highly variable in design, so the effect of these treatments is difficult to discern. A recent study that examined the effect of both glucocorticoids and MCWEs on the endometrial gene expression of proinflammatory and anti-inflammatory cytokines in susceptible mares showed a positive effect of glucocorticoids for modulating the inflammatory response after induction. Glucocorticoids both decreased proinflammatory cytokines and increased anti-inflammatory cytokines after uterine infusion with *E. coli*. Both glucocorticoids and MCWEs had a significant (positive) effect on fluid evacuation and clearance of pathogens after uterine infusion of *E. coli*. Evidence-based results from this study implied that immunomodulators had a positive effect in improving fertility in previously barren mares when administered at the time of breeding.

Autologous plasma has been used as an intrauterine treatment to supplement complement and immunoglobulins. These factors are essential for bacterial opsonization which aids in the post-mating immune response. An adequate response is essential for clearing spermatozoa and bacteria from the uterus. In a large field study, plasma was used in combination with antibiotics postbreeding. Pregnancy rates per cycle were improved in lactating mares but only tended to improve in barren mares and had no effect on maiden mares. More recently, platelet-rich plasma (PrP) was used as an intrauterine treatment in mares. Twenty milliliters of PrP was infused into the uterus of mares 4 hours after artificial insemination. Uterine fluid volume, neutrophil, and nitric oxide concentrations were all lower in mares treated with PrP compared with untreated mares. The authors concluded that treatment with PrP reduced the inflammatory response after breeding, particularly in mares susceptible to endometritis. In a similar study, mares defined as being susceptible to postmating-induced endometritis had better pregnancy rates after treatment with PrP.

2. Placentitis

Placentitis has been well established as a significant cause of pregnancy loss in mares. Data collected in North America shows that a third of foal deaths in the perinatal period were attributed to placentitis. A more recent study that examined records of 1800 mares during a 24-year period showed a 64% incidence of pregnancy loss as a result of placentitis. Of those cases, 80% were caused by bacterial infection. *S. equi* subsp. *zooepidemicus*, *E. coli*, *Klebsiella* spp., *Pseudomonas* spp., and *Staphylococcus aureus* are the organisms most often implicated in placentitis. Less commonly, fungal organisms (*Candida* and *Aspergillus* spp.) cause placentitis. In North America, particularly in central Kentucky, actinomycete bacteria such as *Crossiella equi* and *Amycolatopsis* spp. have resulted in preterm delivery from placentitis (termed nocardioform placentitis). More recently, a syndrome was described in Australia that has components of nocardioform placentitis and mare reproductive loss syn-
The pathophysiology of this condition is poorly understood.

Information from a well-established model of placentitis has provided a better understanding of the disease process. Placental infection usually occurs in the last trimester of gestation. In most cases, bacteria invade the caudal reproductive tract and migrate through the cervix to the placenta. Infective organisms colonize the caudal placenta at the cervical star and disrupt the intimate contact between the allantochorion and endometrium. Infection induces an inflammatory cascade with the production of cytokines and prostaglandins. Mares with induced placentitis showed higher interleukin 6 and 8 concentrations in their placentas, elevated concentrations of prostaglandins E₂ and F₂α in allantoic fluid, and increased duration and intensity of uterine contractions compared with uninfected, control mares. Resultant uterine contractions cause premature fetal expulsion. Premature delivery usually precedes fetal exposure to cortisol and maturation processes essential for survival. However, in some cases, inflammation activates the hypothalamic pituitary axis, stimulates cortisol release, and induces maturation of the lungs and gut prior to delivery, thus improving foal survival.

Histopathologic findings from the placentitis model revealed bacteria on the chorionic surface, allantoic and umbilical inflammation, and bacterial colonization in fetal lungs. It is postulated that fetal infection occurs when bacteria passes through fetal membranes and into amniotic fluid that the fetus inhales or swallows. Not all fetuses become infected with placentitis. This scenario is particularly true in mares infected with nocardia-type organisms (actinomycete bacteria such as C. equi and Amycolatopsis spp.), in which there is significant infection and inflammation in the placental tissues but not the fetus.

Clinical Signs

The most common clinical signs of placentitis include premature udder development and purulent vulvar discharge. Vulvar discharge is often the first sign of infection but is frequently undetected if the mare’s perineal region is not monitored daily. Premature mammary gland development can occur in mares with twin pregnancies as well as in mares with placentitis, likely as a secondary response to activation of the hypothalamic pituitary axis. Historical information regarding breeding and identification of mares with twins helps differentiate these mares from mares with placentitis. It is noteworthy that some mares have scant to no vulvar discharge present and/or minimal udder development but have fulminant placental infection. These mares may suffer a subclinical infection that results in death of the fetus. Alternatively, some mares have extensive placental separation as a result of infection but will deliver viable foals. Mares affected with nocardia-form placentitis often have significant placental compromise at the juncture of the uterine body and pregnant uterine horn and produce copious purulent material. Surprisingly, the fetus is not infected. The inflammation present in nocardia-form placentitis induces hypothalamic-pituitary activation, which promotes cortisol release and precocious maturation of the foal. These events can improve foal survival even in the face of early delivery.

Systemic health is rarely compromised in mares with placentitis. Blood counts, serum chemistry values, and blood lactate usually fall within normal ranges. Mares will rarely develop a systemic illness similar to metritis when affected with placental infections.

Transrectal Palpation and Ultrasonographic Examination

Transrectal ultrasonography in late gestation is a useful tool for evaluating placental integrity (at the cervical star), fetal activity, and fetal fluid character. Fetal orbit diameter can be measured to estimate fetal age. The diagnosis of late gestational twins is not reliable from a transrectal ultrasonographic examination.

The caudal aspect of the allantochorion at the cervical star is the most frequently affected area in mares with placentitis because of the ascending route of infection. Thoroughly examining this area using transrectal ultrasonography is critical when diagnosing placentitis. In normal pregnant mares, the combined uterine and placental (chorioallantoic) unit is measured. Values of the combined thickness of the uterus and placenta (CTUP) for mares with normal pregnancies have been established (Table 2). Mares with placental infection or inflammation will have an increase in CTUP measures or separation of the membranes with the presence of purulent material. A 5- or 7.5-MHz linear transducer is placed in the rectum and positioned 5 cm cranial to the cervical-placental junction. The transducer is moved laterally until the large uterine vessel is visible at the ventral aspect of the uterine body. The CTUP is measured between the uterine vessel and allantoic fluid. A minimum of 3 measures should be obtained and averaged. Along with clinical signs, this tool is often the first line of defense for detecting placentitis. In one study, serial transrectal ultrasound examinations were performed in mares that

<table>
<thead>
<tr>
<th>Gestational Day</th>
<th>CTUP (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>271–300</td>
<td>&lt;8</td>
</tr>
<tr>
<td>301–330</td>
<td>&lt;10</td>
</tr>
<tr>
<td>&gt;330</td>
<td>&lt;2</td>
</tr>
</tbody>
</table>

Table 2. Normal Values for Ultrasound Measurement of CTUP

CTUP, combined thickness of the uterus and placenta.
were considered to be at risk for pregnancy loss based on the previous three pregnancies. Treatment was initiated in mares with changes to the caudal placental unit (thickening or separation). There was a 3-fold increase in foal survival from mares after early detection and treatment for placentalitis based on ultrasonographic changes. These data illustrate the usefulness of early disease detection through regular screening of mares with a prior history of pregnancy loss. However, there are limitations to transrectal ultrasonographic evaluations in detecting placentalitis. Changes to the utero-placental unit can be subtle and not readily detectable until the disease has advanced. Operator differences or ultrasound image quality when measuring and interpreting the caudal placentas can also affect an accurate diagnosis of placental infection. Ideally, ultrasonographic changes to the utero-placental unit should be interpreted in combination with other diagnostic tools before initiating treatment.

Transabdominal Ultrasonographic Examination of the Reproductive Tract

Transabdominal ultrasonography is an excellent tool for evaluating fetal well-being, fetal fluid character, and some areas of the placenta. Fetal health can be assessed through transabdominal ultrasonographic measures of fetal heart rate, tone, activity, and size.

Ultrasonographic examination of the equine abdomen should be performed using a 2.5- or 3.5-MHz transducer with a depth setting of 20 to 30 cm. Although a 5-MHz transducer can be used to measure fetal heart rates in late gestational fetuses, the limited tissue penetration of this transducer type precludes its use for the thorough examination required for detecting twins or performing a complete fetal assessment. In most cases, a convex, curvilinear, or sector-scanner transducer is used for optimal depth penetration and image footprint. All four quadrants of the abdomen should be systematically examined: right cranial, right caudal, left cranial, and left caudal. The initial examination usually begins just cranial to the mare’s mammary gland to ensure that a fetus is detected as early as 90 days of gestation. The abdomen is scanned in a sagittal plane through all four quadrants because this is the most common orientation of the fetus (cranial or anterior position, sagittal plane) late in gestation. Subsequent scanning in a transverse plane is necessary for imaging all aspects of the uterus and confirming the number of fetuses. Using this method, both the pregnant and nonpregnant horns can be evaluated in their entirety and the fetal number determined.

Assessing fetal well-being is among the great advantages of transabdominal ultrasound evaluation. The average heart rate in a fetus greater than 300 days of gestation is cited as 75 ± 7 bpm, but significant individual variation in heart rates occurs. Fetal heart rate slows by approximately 10 bpm at greater than 330 days of gestation. Activity level can affect fetal heart rate throughout late gestation. Consistently low (<55 bpm) or high (>120 bpm) fetal heart rates are associated with fetal stress and warrant reexamination and possible intervention.

Transabdominal ultrasonography is the most accurate method for diagnosing twins in late gestation. Confirmation of twins is generally made by identifying two fetal thoraces and/or beating hearts. Measurements of fetal thoraces can be used to confirm the presence of twins if thoracic size differs between fetuses. In addition, the orientation of the thorax can be used to verify the presence of twin fetuses. Care must be taken when attempting to verify late-term twins using transabdominal ultrasonography because imaging through different planes can lead one to believe that twins are present. Systematically examining the maternal abdomen is critical for properly diagnosing fetal twins.

Fetal activity level and tone are easily determined while examining a fetus for heart rate. Fetal activity can vary during the examination period because fetuses have periods of sleep and wakefulness. In response to the ultrasound beam, the normal fetus commonly becomes very active during the examination period. Fetal “tone” is a subjective term that describes the viability of the fetus. A live fetus has an excellent tone in that it is active and flexes and extends its torso, neck, and limbs. An atonic fetus is flaccid and lies passively within the uterus and may be folded in upon itself. Clearly identifying the atonic fetus can be difficult because traditional landmarks, such as the heart, may be obscured by the limbs of the flaccid fetus.

The character of the fetal fluids is also evaluated using transabdominal ultrasonography. Ideally, ultrasonographic examination of pregnancy, including fetal fluids, is performed in the quiet mare and not immediately after transport or exercise. The allantoic fluid is generally anechoic (black) to slightly echogenic in late-term mares. Echogenic particles (snowfall appearance) can be noted after maternal or fetal activity but will resolve over time. The amniotic fluid is mildly to moderately echogenic (light gray) in a normal pregnancy. Fluids that are highly echogenic or consistently have flocculent material present in the resting mare may indicate infection. In mares with ascending bacterial placentitis, echogenicity of the amniotic compartment usually increases before changes in the allantoic compartment. Interestingly, in mares with no-cardia-form placentitis, the amniotic fluid tends to remain normal, but the allantoic fluid becomes highly echogenic.

In normal pregnancies, the chorioallantois is intimately associated with the endometrium and cannot be easily identified as a separate structure. Similar to transrectal evaluation of the utero-placental unit, mares with normal pregnancies should have a minimal CTUP of 7.1 ± 1.6 mm and maximal CTUP of 11.5 ± 2.4 mm. Transabdominal ultrasonogra-
Hormonal Assays and Biomarkers

The limitations in sensitivity of clinical or ultrasonographic parameters to help rapidly diagnose equine placentitis have led to the investigation of laboratory-based tests to improve diagnosis. Several hormonal assays, and more recently blood-based biomarkers, contribute information that can improve the diagnosis of placentitis.

Progesterone Assays

Several progestagens are synthesized by the fetoplacental unit to support pregnancy in the latter two-thirds of gestation. These progestagens are metabolites of progesterone (P4) and pregnenolone (P5). Ousey et al. used gas chromatography–mass spectrometry to measure serial concentrations of progestagens throughout pregnancy in mares with both normal and abnormal pregnancies. All progestagens except P4, which was undetectable at all measurement times, rose gradually in mare serum as pregnancy advanced. Mares with placentitis had higher levels of P4 or P5 compared with normal, pregnant mares. Mild increases in P4 were noted in mares with placential pathology other than placentitis. Mares experiencing stress other than placentitis (coli, laminitis) showed normal, or slightly lower, levels of P4 and P5 metabolites. An accurate measurement of progestagens requires sophisticated mass spectrometry equipment. However, many P4 immunoassays such as radioimmunoassays and enzyme-linked immunosorbent assays cross-react with progestagens produced by the fetoplacental unit. To help detect placental pathology and/or response to therapy, monitoring serial serum progesterone concentrations 3 to 4 times at 1- to 2-day intervals is recommended. These values can be assessed for trends, including a premature, gradual rise (prior to day 300 of gestation) or rapid drop (impending delivery) that may be useful for directing therapies.

Relaxin

Relaxin is a polypeptide hormone produced by the placenta. In mares, placental relaxin production begins around day 80 of pregnancy and continues until expulsion of the placenta. Relaxin concentrations have been advocated as a means of monitoring pregnancy health in mares and other species. Ryan et al. used a homologous radioimmunoassay to measure relaxin concentrations in mares with normal pregnancies and in those with placentitis. They showed a positive relationship between low maternal serum relaxin concentrations and poor pregnancy outcome and noted a high degree of variability in relaxin concentrations in pregnant mares that they felt negatively affected accurate assessment with relaxin. To date, a commercial assay of this hormone is not available.

Biomarkers

Serum biomarkers that reflect the health of the fetoplacental unit are an area of great interest in both human and equine pregnancies. The scope of biomarkers that have been identified in human pregnancy is broad, yet similarities between species are limited. Recently, acute-phase proteins such as se-
rum amyloid A (SAA) and haptoglobin (indicators of inflammation) have been examined in normal and abnormal equine pregnancies. Using enzyme-linked immunosorbent technology, da Silva et al.\textsuperscript{81} measured SAA in normal pregnant mares and in mares with experimentally induced placentitis. SAA concentrations were shown to increase dramatically in the last 36 hours of normal pregnancy. Mares with placentitis demonstrated a premature rise in SAA. When SAA concentrations were monitored in mares that were treated for placentitis, concentrations were shown to decline in some mares, presumably as a response to treatment. In a similar study,\textsuperscript{82} SAA, haptoglobin, fibrinogen, and white blood cell (WBC) counts were monitored in mares with normal pregnancies and in those with experimentally induced placentitis. Similar to the previous study, SAA and haptoglobin increased rapidly after experimental infection and stayed elevated until abortion. However, in contrast to the previous study, SAA (and haptoglobin) did not rise prior to foaling in normal, pregnant mares. Fibrinogen and WBC counts were not affected by infection. Data from these studies demonstrate the usefulness of SAA as a screening tool for mares with placentitis. Serial measurements that can reveal trends in SAA concentrations as a result of infection, or in response to treatment, will likely yield the most useful information. As with previously validated tests for placentitis, changes in SAA concentrations are best used as an adjunct to ultrasonography and clinical signs.

**Treatment Strategies**

Therapies for treating mares with placentitis should be selected for efficacy, known pharmacokinetics of drugs in pregnant mares, and client compliance for administration. Although bacterial infection is thought to initiate placentitis, secondary inflammation and prostaglandin production are likely culprits in the premature delivery of foals. Treatment strategies are aimed at addressing infection, inflammation, and uterine contractility. Several drugs that are commonly used in mares with placentitis are listed in Table 3.

Some therapeutic agents that are used clinically have been tested for placental passage and improved foal viability in normal pregnant and mares affected with placentitis. Drugs tested in pregnant mares for placental passage (with and without placentitis) are compiled in Table 4. Penicillin and trimethoprim sulfamethoxazole (TMS) achieved minimum inhibitory concentrations against \textit{S. equi} subsp. \textit{zooepidemicus} in the allantoic fluid of mares with induced placentitis, whereas gentamicin was detectable at concentrations effective to treat \textit{E. coli} and \textit{K. pneumoniae} (also implicated in placentitis).\textsuperscript{83,84} Pentoxifylline was detected in allantoic

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dosage</th>
<th>Mechanism of Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Potassium penicillin</td>
<td>22,000 U/kg, IV, q 6 h</td>
<td>Antimicrobial</td>
</tr>
<tr>
<td>Procaine penicillin</td>
<td>22,000 U/kg, IM, q 12 h</td>
<td>Antimicrobial</td>
</tr>
<tr>
<td>Gentamicin sulfate</td>
<td>6.6 mg/kg, IV or IM, q 24 h</td>
<td>Antimicrobial</td>
</tr>
<tr>
<td>Trimethoprim sulfa</td>
<td>15–30 mg/kg, PO, q 12 h</td>
<td>Antimicrobial</td>
</tr>
<tr>
<td>Flunixin meglumine</td>
<td>1.1 mg/kg, IV or PO, q 12–24 h</td>
<td>Anti-inflammatory/antiprostaglandin (mixed Cox-1 and 2)</td>
</tr>
<tr>
<td>Phenylbutazone</td>
<td>2.2–4.4 mg/kg, PO, q 12–24 h</td>
<td>Anti-inflammatory</td>
</tr>
<tr>
<td>Firocoxib</td>
<td>Administered on a per body weight basis</td>
<td>Cox-2 selective anti-inflammatory</td>
</tr>
<tr>
<td>Pentoxifylline</td>
<td>8.5 mg/kg, PO, q 12 h</td>
<td>Anti-cytokine/anti-inflammatory</td>
</tr>
<tr>
<td>Albrenogest</td>
<td>0.088 mg/kg, PO, q 24 h</td>
<td>Antiprostaglandin/tocolytic</td>
</tr>
<tr>
<td>Acetylsalicylic acid</td>
<td>50 mg/kg, PO, q 12 h</td>
<td>Anti-inflammatory/antiplatelet</td>
</tr>
</tbody>
</table>

IM, intramuscularly; IV, intravenously; PO, orally.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Mare Plasma</th>
<th>Foal Plasma</th>
<th>Allantoic Fluid</th>
<th>Amniotic Fluid</th>
<th>Colostrum</th>
<th>Placenta</th>
<th>Fetal Tissues</th>
</tr>
</thead>
<tbody>
<tr>
<td>Penicillin</td>
<td>Present</td>
<td>Present</td>
<td>Present</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gentamicin</td>
<td>Present</td>
<td>Present</td>
<td>Present</td>
<td></td>
<td></td>
<td>Present</td>
<td></td>
</tr>
<tr>
<td>Trimethoprim Sulfamethoxazole</td>
<td>Present</td>
<td>Present</td>
<td>Present</td>
<td></td>
<td>Present</td>
<td>Present</td>
<td></td>
</tr>
<tr>
<td>Cefiofur sodium</td>
<td>Present</td>
<td>Present</td>
<td>ND</td>
<td>ND</td>
<td>Present</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>Cefiofur crystalline acid</td>
<td>Present</td>
<td>Present</td>
<td>ND</td>
<td>ND</td>
<td>Present</td>
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</tr>
<tr>
<td>Pentoxifylline</td>
<td>Present</td>
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<td>ND</td>
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<tr>
<td>Flunixin meglumine</td>
<td>Present</td>
<td>Present</td>
<td>ND</td>
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<tr>
<td>Firocoxib</td>
<td>Present</td>
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<td></td>
</tr>
</tbody>
</table>

Open cells indicate areas that were not examined for presence of drug. ND, not detected.

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In contrast, uterine swabs obtained from the uterus when cultured within 3 hours of delivery of TMS had residual bacteria recovered from more than half of the mares that had been administered to normal mares (M. Macpherson, unpublished data, 2014). It is not clear what prevented specific drugs from passing across the fetal membranes, but caution should be exercised when using unproven drugs in pregnant mares, particularly when treating for placentitis.

Delayed delivery and improved foal survival are important endpoints when treating mares with placentitis. Foal viability after mares were treated with some drug combinations has been assessed using a model of ascending placentitis. Long-term TMS and pentoxifylline administration tended (P = 0.07) to extend gestational length in mares with placentitis compared with infected, untreated mares. However, foal survival was not improved in treated animals. When progestins (altrenogest) were added to the TMS and pentoxifylline treatment regimen, 10 of 12 (83%) mares delivered viable foals. All 5 untreated, infected mares aborted or delivered nonviable foals. This treatment regimen has been broadly adopted in clinical practice with varying results. One explanation for variations in efficacy when treating mares for placentitis is the time interval from infection to initiation of treatment. In experimental studies, treatment protocols are implemented immediately after clinical signs are identified in carefully monitored mares. This approach is difficult to adopt in a clinical setting, and subtle changes are often missed. Work by Carrick et al. demonstrated the usefulness of early, aggressive ultrasonographic monitoring of mares in an at-risk population and subsequent early initiation of treatment. Mares were examined starting from days 150 to 180 of gestation and were examined at least every 28 days. Treatment using TMS and altrenogest was begun at the first sign of ultrasonographic or physical abnormalities (vulvar discharge, mammary development). Live foal delivery rate in this clinical population improved from approximately 25% to greater than 80% using this approach.

However, there are limitations to using TMS as a first-line treatment for mares with placentitis. Advantages of the drug are ease of use (oral), inexpensiveness, and moderately broad spectrum of activity. However, studies have shown that TMS is not consistently effective in eradicating S. equi subsp. zooepidemicus in vivo despite in vitro susceptibility of pathogens and high concentrations of TMS at the site of infection.

In a placentalitis model, more than half of the mares that had been administered TMS had residual bacteria recovered from the uterus when cultured within 3 hours of delivery. In contrast, uterine swabs obtained from normal foaling mares were negative. Although being able to orally administer TMS is a clear advantage, the inconsistent in vivo efficacy of this antibiotic against streptococcal organisms undermines its capacity for treating placentitis. Unfortunately, the options for effective antimicrobials that are safe for pregnant mares are easy to administer are limited. Parenterally administered drugs such as penicillin and gentamicin are ideal antimicrobial choices for the typical bacteria in placentitis, but frequent dosing, expense, and/or intravenous administration limit their usefulness. In some cases, it may be prudent to initiate aggressive treatment with intravenously administered drugs for a shorter period of time (i.e., 2–3 weeks) and continue long-term treatment with orally administered drugs. Long-term therapy proved to be beneficial in experimental placentitis.

In summary, equine placentitis is a challenge both diagnostically and therapeutically. Data regarding treatment are sometimes conflicting, and results after treatment in a clinical setting can be disappointing. However, salvaging a pregnancy can be enormously rewarding. Ongoing efforts by several investigators are focusing on earlier diagnostic methods that allow for a more rapid initiation of treatment and, hopefully, more consistent effects of treatment.

3. Retained Fetal Membranes

Usually mares recover uneventfully after foaling and do not require any specific treatment. However, pathologies that occur in the periparturient period can have great consequences for the mare and her future reproductive career. By far, the most common condition that affects postparturient mares is retained fetal membranes. Rapid diagnosis, resolution, and treatment of retained fetal membranes will ensure systemic and reproductive health of the mare.

Etiology

The placenta is normally passed between 1 and 3 hours after foaling in mares. If the membranes are not passed by 3 hours postpartum, the condition is considered to be emergent. Retained fetal membranes, no matter how small the tag of tissue, can result in endotoxemia or septic metritis, both of which are life-threatening to mares. With proper treatment, most mares will pass the retained tissue and not suffer significant systemic illness.

Retention of fetal membranes postpartum in mares occurs at a higher rate than normal depending on the circumstances. Specific examples where fetal membranes may be retained include after cesarean section, after dystocia or prolonged gestation (such as in fescue toxicity), hydropic conditions, uterine inertia, and advancing age. The association of so many different conditions with placental retention is evidence that there are many different pathogeneses related to the failure of chorioallantoic release. The data in the literature about the mech-
anism of placental retention are not consistent and contain many discrepancies.

Some hypotheses that have been proposed include the following: (1) swelling and disruption of the microscopic interface between the endometrium and the chorioallantoic (e.g., inflammation that accompanies endometritis and/or placentitis, altered maternal blood flow to the endometrium, or fetal blood flow to the chorioallantois); (2) abnormal placentation or immaturity of the endometrial/chorionic interface; (3) hormonal imbalances that affect the biology of endometrial epithelial and trophoblast cells; and (4) failure of normal immune/inflammatory components involved in normal induction, delivery, and placental release.

Studies that have compared microscopic changes in fetal and maternal tissues between mares that deliver their placentas normally and those that have retained are very few. Some of the findings are surprising. A study of endometrial biopsies collected on days 3, 6, and 9 days after delivery from both normal deliveries (n = 29) and mares that went on to retain their placentas for at least 2 hours (n = 9) quantitatively determined changes in granulocyte, lymphocyte, macrophage, siderophage, and mast cell populations.

In contrast to the expected assumption that one would likely find differences in the presence of cellular inflammation in endometrial tissues of normal and retaining mares, granulocyte numbers decrease over this time period with no difference between groups; in addition, there were no differences in the observed increase in lymphocytes, macrophages, or siderophages between groups. Surprisingly, the study did find significantly lower numbers of mast cells in the endometria of retaining mares. The authors hypothesized that sufficient numbers of mast cells are required in the endometrium for there to be a normal postnatal involutionary period.

Diagnosis of Retained Fetal Membranes

Examination of the Placenta

In the immediate postpartum period, the placenta should be protected from being torn by the mare so that it can be examined after expulsion. In a normal delivery, the amnion, attached to the umbilical cord, is the first portion of the placenta that is visualized protruding from the mare’s vulva. A common practice is to tie the amnion and any protruding chorioallantois into a knot, which shortens the membrane length and reduces the likelihood of the mare stepping on the placenta causing it to tear. Once the placenta is passed, it must be evaluated carefully. The entire placenta should be weighed after passage because abnormally heavy placentas generally indicate placental infection/inflammation. The fetal membranes should weigh approximately 11% of the body weight of the foal at birth.

Thoroughly examining the placenta after it is passed is probably the most effective means of determining whether a mare has retained fetal membranes. Training farm personnel to perform placental examinations is a simple, effective tool that can prevent serious illness in mares. A systematic examination of the placenta can be achieved by spreading the chorioallantois in the shape of the letter F, which allows for an inspection of completeness (Fig. 3). The amnion is generally attached at the umbilical cord. It is important when examining the placenta to identify two intact and complete uterine horns and to ensure that both tips and an intact uterine body are present. The most common place for membrane retention is the tip of the non-pregnant horn (Fig. 4). The placenta should have ruptured at the cervical star region, and this area should be avillous. Both the chorionic and allantoic sides of the placenta should be inspected for tears, discoloration, thickening, exudate, or plaques, and
the chorionic surface should be examined for avil-
ous areas.

When examining a placenta from a mare sus-
pected of having placentitis, special attention should be
given to abnormalities in the cervical star region
and areas at the base of the gravid horn (nocardia-
form placentitis). The amnion should also be in-
spected for transparency, meconium staining,
plaques, and cysts. The umbilical cord should be
measured.

A common mistake made by farm personnel is to
hastily examine the membranes and deem them in-
tact or to assume that small tears or missing pieces
have been left in the stall or field. As a rule of
thumb, one must assume that there are retained
fetal membranes if any portion of the placenta is
missing upon examination. Again, even a small
portion of retained membranes can lead to serious
illness in the mare.

Examination of the Mare

It is important to examine the overall systemic
health of the postpartum mare to help rapidly diag-
nose periparturient conditions like retained fetal
membranes. In the most straightforward cases, re-
tained fetal membranes will be identified hanging
from the mare, or missing pieces will be identified
during the placental examination. Additional diag-
nostic tools, including routine blood work and trans-
rectal examination of the reproductive tract, can be
useful for determining the cause of postpartum ill-
ness in mares. Routine bloodwork in the mare with
suspected retained fetal membranes should include
the following:

- Complete blood count with differential
- Blood chemistry
- Peripheral serum lactate

Values from these tests are expected to be within
normal laboratory ranges for immediate postpartum
mares. Baseline information can prove to be use-
ful for comparison to subsequent tests if the mare
becomes systemically ill within a few days of foaling.
Additional tests that may be useful in the mare with
more advanced illness include abdominocentesis for
cytological evaluation and possibly peritoneal lact-
tate levels. Samples for microbiology should be col-
clected if placentitis or endometritis is suspected.

Transrectal Examination

Transrectal evaluation of the reproductive tract by
palpation and ultrasound should be routinely per-
formed in postpartum mares. However, this diag-
nostic tool is not foolproof for detecting retained fetal
membranes. The reproductive tract during the im-
mediate postpartum period is significantly enlarged
compared with that found in normal cycling mares.
The uterine horns will be enlarged with the previ-
ously gravid horn generally twice the diameter as
the nongravid horn. The immediate postfoaling
uterus will lack tone. Uterine tone increases over
time with the development of palpable rugae as the
uterus involutes. In the case of retained fetal mem-
branes, uterine tone can be increased or decreased.
Interestingly, in some mares with retained pieces of
membrane, the uterus will have extraordinary tone,
possibly from contraction in an effort to expel the
tissue tag. Conversely, some mares with retained
membranes will have a greatly enlarged, fluid-filled
uterus. Intraluminal fluid accumulation is simi-
larly variable; in the postpartum uterus, 5 to 10 mm
of intraluminal fluid is common. Mares with re-
tained fetal membranes can have scant to exuberant
production of fluid. Furthermore, the ultrasono-
graphic character of uterine fluid can range from
echolucent to moderately echogenic. In some cases,
a tissue piece can be visualized as an echogenic
structure in the uterine body or collapsed lumen of a
uterine horn. If the uterus is distended with fluid,
placental tissue can sometimes be visualized float-
ing in the fluid. Although useful at times, ultra-
sonographic identification of placental tissue is not a
reliable means of diagnosing retained fetal mem-
branes in mares.

Treatment Strategies for Retained Fetal Membranes

If fetal membranes are retained for greater than 3
hours, treatment options will range from conserva-
tive to comprehensive. In any case, the veterinar-
ian is obligated to be proactive with treatment
because it is impossible to predict which mares will
become seriously ill if membranes are retained.
Oxytocin is often the first therapy given for treating
retained fetal membranes. Oxytocin promotes
uterine contractions and facilitates the release of
microvilli from endometrial crypts. The duration of
uterine contractility after oxytocin administration to
postpartum mares is unknown. LeBlanc reported
that 10 IU oxytocin administered intrave-
nously to 4 normal pony mares 12 hours after foaling
resulted in uterine contractions for up to 3 hours
after administration. In some mares, prolonged,
high-amplitude contractions were recorded for 8 to
10 minutes after oxytocin administration. These
data illustrate how little is known about the effect of
oxytocin administration to mares during the post-
foaling period. Practitioners vary greatly in their
use of oxytocin for mares with retained fetal mem-
branes, and delivery methods are largely anecdotal.
Dose and route of oxytocin administration include
(1) frequent boluses of 10 to 20 IU intravenously or
20 to 40 IU intramuscularly every 2 to 4 hours
(usually performed in the first 3 to 6 hours) and (2)
a slow infusion of 60 to 100 IU in 1 L of saline for 30
to 60 minutes or 40 IU/L oxytocin mixed into 5 L of
LRS and administered intravenously at a normal
maintenance fluid replacement rate.

Daily large-volume uterine lavages with 0.9% sa-
line (NaCl) or LRS are a routine part of manage-
ment for mares with retained fetal membranes.
Sterile fluids can be used for uterine lavage but may
be unnecessary because the postpartum uterus houses a mixture of bacteria under normal conditions. For practical purposes, nonsterile saline can be made in large batches by mixing 35 g of table salt with 4 L of tap water, resulting in an approximately 0.9% NaCl solution. For most light-breed postpartum mares, lavage volumes of up to 10 to 12 L are infused into the uterus using a sterile/clean nasogastric tube and stomach pump (Fig. 5). Fluid volume infused into the uterus will vary with mare size and uterine capacity. The fluid is delivered into the uterus using a nasogastric tube guarded by the operator’s hand. Fluid volume is assessed subjectively by the operator, who maintains a hand within the uterus during the delivery. Once the uterus is filled with enough fluid to cause moderate distention, the operator carefully guards the tip of the nasogastric tube to prevent the uterus from being damaged during the siphon process. The tube is placed ventral into the fluid pool, and fluid is siphoned from the uterus with careful attention to bits of tissue that are expelled. The procedure is repeated several times until tissue is recovered or fluid runs clear. Recovering lavage fluid when retained membranes are present is often complicated by tissue occluding the tip of the tube, thus impeding lavage. Carefully guarding the end of the tube with a gloved hand allows lavage of the uterus; however, it may result in the recovery of small volumes of fluid at a time. Although tedious, uterine lavage is essential for treating mares with retained fetal membranes.

Several additional procedures have been advocated to promote mechanical release of the fetal membranes. One technique, termed the “Burn’s Technique” involves distention of the chorioallantois to promote separation of microvilli from the endometrium and subsequent placental release. The chorioallantois must be intact for this procedure to be effective. The chorioallantois is distended with large amounts (up to 12 L) of fluid through a large bore nasogastric tube and tied with umbilical tape or held closed with the operator’s hand. Some have suggested clamping the tube and allowing the mare to exercise for several minutes in a round pen. The technique is thought to stimulate endogenous oxytocin and uterine contractions thus promoting expulsion of the membranes. An additional benefit is thought to be the natural separation of villi from the endometrium resulting in more complete removal of villi after lavage. The method is practical and effective, particularly when used in uncomplicated cases of retained fetal membranes (i.e., those not resulting from dystocia, placentitis, cesarean section etc.). The limitation of this procedure is that it is only effective with intact fetal membranes.

Recently, Meijer et al. demonstrated a variation on umbilical vessel infusion that has been used to successfully remove retained fetal membranes in mares. This type of procedure has been described in cattle and mares using collagenase. The modified technique described by Meijer et al. utilizes a stallion catheter (or tube with a similar diameter) attached to a garden hose fitting with a stopcock (Fig. 6) that is then attached to a simple garden hose. An incision is made into one of the umbilical vessels, and the stallion catheter is threaded into the vessel as far as possible. The hose adapter is turned on, and the vessel is infused with water under low pressure while also occluding the outflow from the vessel. Infusion continues for approximately 5 minutes or until fluid begins to reflux. At the same time, gentle traction is placed on the membranes. The membranes separate from the endometrium and are slowly removed from the uterus.
with continued traction. This procedure works most reliably with a placenta that has not been retained for a long period of time (8–12 hours or less). As with any removal procedure, the membranes should be examined carefully once removed to ensure that pieces were not retained.

In the case of a small tag of placental tissue attached deep in the uterus, removal can be much more challenging. Routine procedures rarely work because the tag is often attached at the tip of a uterine horn where it remains out of reach or ventral in the uterus. The tissue often remains in the uterus until necrosis of the microvilli occurs and the tissue is released. Even after release from the endometrium, the tissue is frequently not expelled because of its dependent location in the uterine lumen. Daily uterine lavage is performed to encourage detachment of the tissue from the endometrium, remove uterine contaminants, and aid in tissue removal. Although somewhat controversial, exteriorizing the tissue tag and placing a small amount of weight on the tissue may facilitate release and removal. One approach is to attach a small plastic bottle with approximately 150 mL of water to the placental tag with suture material or umbilical tape. Alternatively, some will use a rectal sleeve filled with a small amount of lubricant gel (Fig. 7). The goal is to apply gentle but persistent tension on the tissue tag to promote release. Caution must be used with the amount of weight attached to the placental tag so that uterine eversion or prolapse is avoided. Mares with small weights attached to fetal membranes should be observed closely until the tissue is released.

Manual retrieval of fetal membranes is another controversial means of removing retained membranes. Complications cited with manual removal include retention of microvilli and/or tissue tags, hemorrhage, and damage to the endometrium. More recent reports suggest that, in experienced hands, manually removing retained fetal membranes does not impair future fertility of mares. In both reports, the procedure was performed during the immediate (within 2 hours) postfoaling period. In one study, the membranes were removed from the recumbent mare immediately after foaling. The chorioallantoic portion of the placenta was grasped at the distal portion at the vulva or within the caudal vagina. The placenta was exteriorized by pulling gently but firmly while the villi detached from the endometrium. Others describe mild to moderate twisting of the chorioallantois or manually separating the chorion from the endometrium by passing a hand between the two surfaces. In all cases, excessive force or traction are not recommended.

Other treatments that are frequently administered to mares include the following (particularly in cases of dystocia, prolonged retention, or cesarean section):

- Systemic antibiotics to prevent bacterial growth for at least 5 to 7 days
- Anti-inflammatory drugs to prevent endotoxemia and laminitis (flunixin meglumine, firocoxib, pentoxifylline)
- Intravenous fluids spiked with 125 mL of 23% calcium borogluconate added to 5-L fluids
- Icing feet to prevent laminitis (ice bags or boots placed around the feet and changed every 2 hours)

Exercise in mares with retained fetal membranes is essential for promoting uterine contractions and expelling the membranes. Exercise should be controlled and in an area where the mare can be observed for the possibility of uterine prolapse. Exercise is contraindicated in mares at risk for laminitis.

With prompt treatment and resolution, most mares that retain fetal membranes will return to good reproductive health. It has been suggested that mares with retained fetal membranes are not good candidates for breeding on foal heat. However, one study showed no effect of membrane retention on pregnancy rates when mares were bred on foal heat. Each mare should be approached individually when breeding after retained fetal membranes. A reasonable compromise would be to allow the mare to undergo a first ovulation and induce luteolysis with prostaglandin, thus shortening the interval to a second estrus period postpartum.

Acknowledgments

Declaration of Ethics

The Authors declare that they have adhered to the Principles of Veterinary Medical Ethics of the AVMA.
Conflict of Interest

 Portions of this material were generously supported through funding by the Grayson Jockey Club Research Foundation and the University of Florida Research Endowment (ceftiofur and firocoxib studies for placentitis), Zoetis Animal Health (ceftiofur), Merial Animal Health (firocoxib) and Merck Animal Health (altrénogest).

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Why Are Working Equids Still Important to Us and Are We Forgetting Them?

Derek C. Knottenbelt, DVM&S, DECEIM, MRCVS

We need to care about the horses in our own countries but we also have to recognize that there are countless millions of people who rely for their livelihoods, and even for their lives, on the strength and companionship of horses, mules, and donkeys. Author’s address: Philip Leverhulme Equine Hospital School of Veterinary Science–Leahurst Campus, University of Liverpool, Wirral CH64 7TE, United Kingdom; e-mail: knotty@liverpool.ac.uk. © 2015 AAEP.

1. Introduction

There are estimated 100 million working equids in the world. Working equids are a source of financial stability for families and they relieve human burdens, for women and children in particular. Mules and donkeys in particular play a pivotal role in developing rural communities across the world. They are indispensable as farming and working animals and their health status is important to the owners/users.1 They play a fundamental role in individual family prosperity and in the local and national economies. Indeed, it could be said that if the working horse (donkey) were to be removed from society, the economy of the world would collapse. Whereas in our developed societies the horse has become a nonessential, leisure part of society, the working equid remains one of the strongest pillars of society in the developing world. The health and welfare of horses and donkeys relies upon available veterinary support, just as it does in our society. Although enormous resources and attention are focused on ruminant medicine and disease control, very little effort or resource is applied to the working horse. Very few countries and government leaders seem to recognize the value of the working horse.

Almost every developing country has a state veterinary service and almost all have at least one veterinary university (and often several/many). However, it is a matter of enormous regret and concern that in the large majority of cases, neither the state nor the universitie seems to have a significant grasp of the importance and relevance of the working horse in their own societies. There is a massive need for a better “image” of the working equid. Indeed, quite commonly, the owners of equids themselves have scant regard for the needs of the animal. Usually, it is only when things go wrong that the significance of the contribution made by the animal is appreciated by either the society itself or the individual. What, for example, would happen if a devastating fatal disease such as African Horse Sickness were to decimate the working horses and donkeys of Ethiopia? The state economy would surely suffer, but more significantly, the individual owners would suffer.
EQUITARIAN SESSION

What is the Role of the Working Horse/Donkey?

The working equid provides mobility and power and relieves human burden. A working equid requires little maintenance and certainly no spare parts or carbon-based fuel. The working equid in particular relieves the physical burden that is predominately carried by women and children.

2. What Key Veterinary Interventions Are Required?

The health of the working equid is a veterinary matter. Health is dependent upon many different factors including the prevention of epidemic disease, the control of endemic infections, and the provision of emergency care for sick or injured equids.

The truth is that most working equids are owned by people who lack the financial means to pay for, or to access the information needed on harnessing, nutrition, vaccinations and drug treatment. Single-owner farms are often remote from veterinary services, thereby requiring greater emphasis on preventive measures and local remedies.

There is no benefit in gratuitous criticism or failure to recognize the economic, social, cultural, and religious environments in which the owners/users live and prosper. Carefully planned, sympathetically delivered programs for health care and welfare improvement must be undertaken. This means that the needs of the animals and the people must be considered together by people who understand the inevitable constraints that face communities in the developing world.

3. Why Should We Bother to Try to Improve the Welfare of Working Equids?

It is certainly true that a healthy working animal contributes more to its owner and society than an unhealthy/injured one. In some countries many older, injured, or diseased horses and donkeys must work and so they are forced into a compromised welfare position.

The challenges that face individuals and organizations that worry about the welfare of the animals are enormous. It is easy to criticize the welfare status and care of the animals, but gratuitous, ill-informed, and domineering advice based upon little understanding of the circumstances prevailing does no service to the animals or the owners or, indeed, to the critic.

Over the next 50 years, if changes in climate and availability of fuels continue, it is likely that more people, not fewer, will come to depend on working animals. The Equitarian Initiative is the embryo of a philosophy and a working practice that needs to be disseminated much wider than it is now.

4. How Can We Do Better for the Working Animals in the Developing World?

First, individually and corporately, we can recognize the pivotal role the horse/donkey has in local and national economies, and acknowledge that these animals are owned by individuals who depend on these animals for their livelihood. The concept held by much of the developed world that the use of working equids is basically cruel is fundamentally wrong because it takes no account of the particular circumstances. There is personal responsibility and a corporate/national obligation to further the welfare of working animals in particular. Even if it is simply a pragmatic financial decision rather than an emotive/empathetic one, we have to do better.

The veterinary profession must be proactive in education of equine veterinarians and technicians, owners, and ultimately, of course, of the major decision makers and law makers. These are enormous challenges for the veterinary profession. There are, for example, religious and cultural taboos against euthanasia, as well as a vast array of irrational treatments given to sick or injured animals. It is easy to criticize such treatments and taboos, but unless we are willing to devote time, energy, and resources into changing attitudes and behaviors, and we are willing to engage nonjudgementally and positively with societies, it is not productive to simply criticize them.

The level of service and the choice of treatment available to a horse in the United Kingdom or United States are impressive. The array of diagnostic tests, procedures, and evidence-based therapeutic interventions means that equids in these places get the very best care and chance of recovery from disease that is available. However, criticizing people for doing what they have done for centuries is neither helpful nor will it bring any change. One could make a persuasive argument that the best available veterinary attention should be transferred from the multi-million dollar racehorse (upon which lavish attention is applied but which is a plaything without real social value in human terms) to the working donkey (upon which the education and possibly the lives of four small children depend).

To positively affect change, engagement and dialog with practical, positive, and achievable advice is required. Recent intervention studies relating to the perceptions and needs of simple people who rely on working equids have confirmed that appropriate and focused engagement brings welfare, social, and economic rewards. Set against that, there is much evidence that supports the concept that some interventions do not help. Random anthelmintic treatments and the irrational use of multivitamins and antibiotics to horses and donkeys clearly serve no useful purpose in improving either general health or welfare. Indeed, the irrational or irresponsible use of drugs of any sort could be seen to be actually counter to the long-term health of the population.

Simply offering advice will not bring sustainable change. A rural farmer in Mali or Ethiopia or India who has little education and even less understanding of the concepts of animal welfare (in respect of his/her equid) does not want words. He wants action and solid evidence that what is being suggested
is both realistically achievable and beneficial. If welfare organizations fail to provide a “flagship” service that demonstrates the benefit of veterinary intervention, there will be no sustainable response. The provision of “primary first aid” and routine prophylactic treatment for working animals serves many purposes, even though it does itself not bring huge benefits. There would seem to be little point in treating an individual donkey for parasites when his environment is worm ridden and he gets little other help.

However, visible veterinary attention does have many beneficial effects, including improving respect for the animal, elevating the position of veterinarians and in the end, increasing the job satisfaction of the veterinarian. If any animal in the world needs the very best diagnostic and therapeutic interventions it is the working animal, an animal upon which lives and opportunity depend.

Key veterinary interventions will vary widely according to the local circumstances. Broster et al identified the extent of the veterinary welfare issues facing working horses. In some circumstances, vaccination against epidemic disease or against diseases that are locally significant can be critical. For example, historically in Mali, tetanus was a very significant cause of both morbidity and mortality in the working donkey population. A simple vaccination program rapidly resulted in a virtually 100% prevention of this otherwise highly fatal disease. This simple intervention brought significant benefits but required significant logistic organization and some investment. Did it work? Yes it did. Was it worth it? Yes it was.

In Ethiopia, epizootic lymphangitis (histoplasmosis) is a massive issue. It is clear that vaccination would require considerable development involving significant funding, so a policy of euthanasia of severely affected horses has been introduced and is supported by government. This has resulted in significant benefit with fewer new cases being reported. Better awareness has significantly improved the welfare situation for all animals.

In a study by Popescu et al, 697 working horses were assessed by using observation, behavioral tests, clinical examinations, and questionnaires. In working horses, it is clear that the heavier the work they perform, the more important it is for horse owners/breeders/farmers to ensure that their welfare needs are addressed to help them cope with the multiple specific risks imposed by their work. In recognizing this, we raise the standard of animal welfare while reducing human burden at the same time.

There are few studies on the benefits of either education or veterinary input. The need for monitoring has been highlighted by Upjohn et al. Pearson described the outcomes of interventions directed at health and husbandry improvements to working animals in Africa and suggested that interventions were capable of bringing long-term improvement in both the welfare of working animals and the economic circumstances of the users.

The challenges that face all of us who work with working equids in the developing world are enormous. The first priority is to identify the problems (local and regional) and then devise definite, achievable, sensible, and legally binding strategies to address them. Without that fundamental legal support, it is hard to see how progress is going to be made. Continued reliance on nongovernmental organizations that depend on donation income will not bring permanent change. Governments must recognize the need and the obligation to address the broad issues relating to working animal welfare. Education is probably the most basic requirement but education alone does not always overcome habit, prejudice, or antagonism. Education needs to start with children but must involve all levels of society at the same time. It is easier to “teach” a child empathy than it is to get an old farmer to change his donkey’s harness, even if there are financial rewards. Old habits die hard.

5. How Can Veterinary Interventions Be Better Coordinated With Other Intervention Methods?

Given that it is clear that the use of working animals is going to continue into the indefinite future worldwide, we have to make critical decisions on the roles that each of us, individually and corporately, can play in this overall situation. We can individually and corporately put pressure on governments to support sensible preventive medical and veterinary care. We must emphasize the role of the working equid in society and we must try to generate legislative support for the equine species in the economic structure of the countries concerned. Although we can cynically suggest that the development of a vaccine for African Horse Sickness and epizootic lymphangitis would be best driven forward by the fear that these infections would spread to the developed world, it would be far better if the vaccines were developed to improve the societies where the disease is endemic, and so by extrapolation, reduce the risk of spread of the disease.

Burn et al explored environmental factors associated with potential welfare problems in working equids, with the laudable intention of targeting welfare interventions toward the most vulnerable animals. In this study, hoof and limb problems were found in 90% of animals and 85% of working equids were thin. Older, thin animals in poor condition had the most problems. Equids used to transport people or goods by cart, or by pack, or those required to work in brick kilns had more problems than others. Rural horses and donkeys had more problems than urban ones, but urban animals had more obvious skin and other lesions, and were generally less well behaved. All equids were significantly thinner in warmer climates. These results provide significant guidelines for potential interventions leading to improvements in welfare. However, the complexity
of the circumstances and the wide variations in environmental conditions, workload, management, and veterinary care availability make it almost impossible to create standardized measures that would be applicable in every circumstance. The only people qualified to provide individualized advice and support are the local veterinary and paraveterinary professionals. That means they must be properly supported and trained as well.

We understand the balance between a horse and the owner/user. We understand the needs of the injured or sick animal and the consequences of that on the owner. If we help one family, we have made a contribution. If we help hundreds, we have done well. If we help millions, we can stand proud and proclaim that we have made a difference to the world.

Governments and communities must be encouraged to support the working horse. We should be proud of our relationship with horses and we should strive to develop a universally more sympathetic attitude. The drive for this must come from governments and from international organizations such as the United Nations and Organization of African Unity.

Acknowledgments

Declaration of Ethics

The Author declares that he has adhered to the Principles of Veterinary Medical Ethics of the AVMA.

Conflict of Interest

The Author declares no conflicts of interest.

References

The importance of working horses, donkeys, and mules to economic survival and human livelihoods, especially in poor and rural communities around the world, is indisputable. Lifestyle, economics, and the conditions in which people live and work strongly influence their health. When a person's livelihood is dependent on a working equid's contributions to the household, one can understand how working equid health, human livelihoods, and ultimately human health are intimately linked in many communities. Author's address: Mickey Leland International Hunger Fellow, Congressional Hunger Center, Land O'Lakes International Development Division, Lilongwe, Malawi; e-mail: jennielane@gmail.com. © 2015 AAEP.

1. Introduction

A working equid is any horse, donkey, or mule that provides labor for its human counterparts and communities. Working equids and other draft animals remain essential forms of labor, draft, and income in many communities around the developing world. In 2013, the Food and Agriculture Organization reported that there are approximately 112 million working equids in the developing world. Up to 80% of work power on farms in developing countries is provided by draft animals. In some countries, the number of working equids is increasing despite advances in mechanization and urbanization. These animals provide draft power; work in the tourism industry; transport produce and goods to market; and function as the school bus, water tanker, tractor, milk truck, and garbage hauler, among other tasks. Up to 28% of the world's arable land is worked by draft animals; this directly or indirectly serves up to two billion people. To replace this animal labor with mechanized, modern power equipment would be extremely costly, unsustainable in its reliance on mechanical maintenance and fossil fuels, and cause a loss of the traditional methods and values of farming. Many families in disadvantaged, marginalized communities are heavily dependent on the income provided by the power and work of their working equids. An African proverb illustrates the importance of working equids well: "A woman who has no donkey is a donkey." Conversely, dependence on working animals in many countries can carry a stigma for reducing the burden of work for women and children performing daily chores. If the animal is unable to work, then the labor is frequently performed by a woman or child in the family, thereby removing them from other activities such as wage earning or attending school. Researchers from The Brooke have found in some communities that owning a working equid improves social standing and provides opportunities to contribute to the community within certain social circles.

NOTES
difficult to overcome. Media and society around the world often associate animal traction as old-fashioned and associated with poverty and ignorance. In many societies, donkeys are traditionally perceived negatively. This in turn, adversely affects how their owners and users are perceived by society at large, and can explain, at least in part, why this subsection of society could be neglected by policies or social schemes.

2. Social Determinants of Health

Lifestyle and the conditions in which people live and work strongly influences their health. A person’s livelihood may be directly dependent on a working equid’s contributions to their household survival. Working equids may also enable access to health services or social support networks. Considering the social determinants of health as outlined in the World Health Organization document, *The Solid Facts*, a strong case can be made for the relationship between working equids and human health with nine of the 10 social determinants of health, including:

- The social gradient: disadvantaged and marginalized communities have worse outcomes.
- Stress: anxiety, insecurity, social isolation, lack of control over work and more can cause long-term stress with powerful negative effects on health.
- Early life: multiple long-term health effects are a result of poor antenatal and early childhood health.
- Social exclusion: poverty and social exclusion have negative effects on health.
- Work: workplace stress increases the risk of disease.
- Unemployment: higher rates of unemployment are related to increased illness and premature death.
- Social support: strong support networks and friends improve health.
- Addiction: drug, alcohol and tobacco use is influenced by the individual’s environment.
- Food: food security and a nutritious diet are essential for health and wellbeing.
- Transport: healthy forms of transport include walking, biking, and public transit use.

Although working equids are essential to some people’s livelihoods and survival, there is sometimes a stigma faced due to that dependence. Working equid owners are often lower in the social gradient, under social and economic stress, and may be socially excluded. If a working equid is unable to perform, a child or woman may have to take over that duty, which can adversely affect their early life.

Nevertheless, the use(s) of a working equid contribute both to work and employment in the family or community. If a working equid is injured or unable to perform, and if a replacement is not readily available, this may immediately translate into unemployment or lost income and time. Working equids are essential for providing food and for transport in some communities. They have an unquantified but essential contribution to food security on both a local and global level. Working equids can contribute to a household’s social capital and respect within a community. Not forgotten is the human-animal bond that can be particularly powerful between an owner or caretaker and their equid.

3. A Selection of Recent Evidence

*Invisible Helpers, Voices from Women International Report: Women’s views on the contributions of working donkeys, horses and mules to their lives.*

This qualitative research project was based on focus group discussions and interviews with women-only groups in Ethiopia, Kenya, India, and Pakistan. The data revealed the critical nature of working equids in supporting household livelihoods. It also challenged previous assumptions and preconceived ideas about livestock and gender roles in the four countries evaluated. Potential relationships between working equid health and human health could be extrapolated from two of the primary research objectives. These were to understand, from a women’s perspective: “The specific roles that working donkeys, mules and horses play in supporting the lives of women across four countries,” and “How the welfare status of working equids affects the amount and type of support they provide to women.”

Quotations from focus group discussions provided substantial evidence to support associations between working equids, livelihoods and the health of women and children.

“The donkey affects each and every aspect of my life as a woman. On a typical day, the donkey fetches water, which I use to do the laundry, to do the dishes, to clean the house, and for bathing. It also fetches sawdust which I use to cook all meals, then I hire it out and it brings in income on a daily basis that I use to buy flour for the evening meal. In other words, I eat, drink, dress, live off the donkey and moreso as a woman and one not employed, I work hand in hand with the donkey. Basically the donkey is like me but to plainly put it, the donkey is me.” — Lucy Waititu, 23, Kenya

“When you assist your fellow community members with your donkey at no charge, you end up being respected by the community.” — Participant from Tharuni’s Women Group, Kenya

This report concluded with five action points including:

- Making clear links between the relevance of working equids to livelihoods and development
- The importance of recognizing working equids in livestock policies and programs
- Recognizing the unique roles of women in livelihood development
- A need to increase the body of evidence on the roles of working equids in women’s lives
- The importance of improving women’s access to training and extension services.

*Understanding the Association Between Working Equid Health and Human Health in Rural Nicaragua*

A mixed methods (both quantitative and qualitative) research project was performed in two rural regions of Nicaragua in January 2014. Data were collected at an annual mobile veterinary clinic for equids working predominantly as pack animals carrying agricultural goods (coffee, plantains, milk, etc.) in mountainous environments. A cross-sectional, verbal, Spanish-language survey was given to a convenience-sampled population of owners (n = 70) presenting to the clinic. Information collected included basic demographics, standard-of-living markers, access to health services, perceptions of working equid value, and human personal health-related quality of life measures. Anthropometric growth data on children (n = 20) of the families who completed the survey were also measured. A 25-point grading scale for measuring working equid health, based on five separate criteria (including body condition score, integument, lameness, harness/tack sores, and hoof quality) was developed by the author. The criteria were chosen based on previous working equid health assessment research, existing literature, and clinical experience. This grading scheme was applied to animals (n = 132) presenting to the veterinary clinic.

**Results**

A total of 70 surveys were completed. Twenty-two percent of respondents who reported an annual income (n = 31) lived on less than $1.25/day. Seventy-four percent of respondents strongly agreed that the health of their working equids affected the health of their family. The most common human health concerns were upper respiratory tract infection, cough, fever, and diarrhea. Nineteen boys and one girl between 6 and 14 years of age were evaluated for anthropometric growth indicators. Two children (10%) were classified as underweight, with a weight-for-age z score (WAZ) less than −2 standard deviations. WAZ is a measure that indicates either past or present undernutrition.9

One of these children was classified as stunted, with a height-for-age z score (HAZ) less than −2 standard deviations, indicating chronic malnutrition.9 Seven additional children (35%) children had HAZ and WAZ between −2 and −1. One hundred thirty two working equids (81% horses, 14% mules, and 5% donkeys) were scored using the 25-point scale. Mean working equid health score was 19.19 (of 25), with a mean body condition score of 3.8 of 9 (range, 1–7 using the Henneke Scale). The most common equid health concerns reported were hoof problems, ticks, respiratory illness, and lameness. Bivariate analysis between working equid health score and human health score showed a significant association (P = .052). This association remained positive but lost significance after controlling for age and education. There was a strong trend toward a significant association between mean working equid health score and childhood malnutrition (P = .2); however, small sample size precluded rigorous statistical analysis for this aspect of the study. No significant linear association was found between working equid health score and wealth index.

This study was limited by several factors, primarily sample size, sampling technique, the presence of multiple sampling locations, and the use of an unvalidated scale for working equid health. However, based on this author’s clinical experience, the most common health concerns facing the majority of working equids in developing areas are similar, especially in other areas of the world with limited roads and high human dependency on the labor of working equids. The relationships explored in the study are, in the author’s opinion, mediated largely through livelihoods. Research into the relationships between livestock, livelihoods, human health, food security, and nutrition is a growing field within the development community. Needless to say, these are complicated relationships with many variables that are difficult to tease apart from one another; this study provided some insight into these relationships but also opened the door for more questions.

**4. Conclusion**

Growing numbers of veterinary and animal welfare groups are becoming involved with working equids by providing humane treatment, owner education, and policy advocacy in support of working equids and their owners worldwide. These efforts are worthy, essential causes in the fields of animal welfare, poverty alleviation, and sustainable development. Such work should continue to be supported by private donors, as well as garner increased support from governments and international aid agencies. Many of the working equid welfare groups cite not only the obvious animal welfare concerns, but also owner dependency on their horses, donkeys, and mules for household survival, as key reasons to work in these fields.

The complexity of the relationships between working equid health, socioeconomic status, and human health is clear. The body of evidence documenting the importance of working equids to human livelihoods and less directly, human health is growing; however, much more work is needed. Respect for all living beings and a love of working equids and their owners drive those working in this field. This love and respect could inspire additional studies in populations of working equids and their owners.
Application of rigorous public health methodologies, including detailed needs assessments of the entire community, monitoring and evaluation of projects intending to deliver aid to animals and their owners, as well as prospectively designed impact evaluations of equid welfare and veterinary aid projects are indicated. In addition, working equids should be included in the larger research agendas of livelihoods, livestock, and food security.

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Declaration of Ethics

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Conflict of Interest

The Author declares no conflicts of interest.

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Equitarian Projects and Sustainability: Twenty Years of Work in Samana and Why Our Presence Must Be Maintained

Jay G. Merriam DVM, MS

The goal of most relief or humanitarian works or projects on a global scale is to provide immediate aid or support for crises and follow on with support that allows capacity building from within the affected area. The long-term goal is often to empower local or national infrastructure to continue once the short-term support is ended or transferred. And yet for many reasons, this is very rarely the case that a clean end to a project is actually achieved and local providers sustain long-term rebuilding. The author’s experiences in a remote area of rural Republica Dominicana are described and reasons for long-term involvement are discussed. Author’s address: Merriam Equine Consulting, 1800 County Road 109, Glenwood Springs, CO 81601; e-mail: jgmerriam@me.com. © 2015 AAEP.

1. Introduction

The definition of sustainability is: Able to be maintained; maintaining ecological balance. Any project can be sustainable as long as wealthier nations continue to transfer wealth to it. According to one prominent medical charity founder, Dr. Paul Farmer, the concept of sustainability in medical relief projects is in many cases a fantasy.

2. Background

Project Samana was begun in 1993 with the support of the Massachusetts Veterinary Medical Association (MVMA) in response to a request received from a Dominican veterinarian seeking assistance in animal control and humane issues including working equids in distress. The MVMA was actively seeking a partner for some organizational outreach and the call came in at an opportune time. Three members and two technicians went on the first visit to assess and begin the work. Samana is a peninsula well known for its beauty, beaches, mountain waterfalls, and a heavy concentration of agriculture in the form of coconuts, cacao, plantain, coffee, and bananas. These were all moved on the local working equids, primarily horses, a few mules and some donkeys. In addition, tourism was growing as roads became passable and the working animals often did double duty carrying tourists up steep trails to the waterfalls, or on beach rides. There was considerable concern from the government tourism officials about stray dogs scaring tourists, as well as being a pool of endemic rabies. An ecotourism agency in Samana, El Centro para la Conservación y Ecodesarrollo de la Bahía de Samaná y su Entorno (CEBSE), was working on regional water quality. However, CEBSE was mainly concerned with the local marine environment and its effects on whale watching in the Bay of Samana. Exploitation of the waterfalls by overzealous tour operators offering...
horseback rides on chronically malnourished and
overworked animals also was recognized by CEBSE
as a critical problem. Thus, Project Samana was
formed with local support and understanding that
MVMA would provide otherwise-unavailable veter-
inary services and training once and later twice a
year. We agreed to work with and under the direc-
tion of our local veterinarian and the internation-
al nongovernmental organization (NGO) CEBSE.
They provided follow-up and logistical support.

3. Results
As Project Samana matured, it became the largest
and most active committee in MVMA. It was often
oversubscribed with potential veterinary and tech-
nician volunteers and evolved a strong donor base
that allowed equipment purchases and student sup-
port. Project Samana soon overwhelmed the capac-
ity of the original local organizers to absorb all of the
aid. Formation of a local Humane Society, a gov-
ernment-backed rabies immunization program, and
through CEBSE, the formation of a horse tourism
organization, began to help local individual animal
owners to join together to provide healthy animals
for work.

The CEBSE group, with our help, began to keep
records on each animal seen, especially with regards
to body condition scoring, presence of saddle sores,
lameness, hoof problems, and general health. The
team was able to inspect animals at each visit and
prevent those with sores from working until healed
and cleared by a CEBSE volunteer. This alone was
incentive to use proper tack and feeding. Our
teams provided anthelmintics, appropriate care of
sores and other problems, as well as nutritional
advice and support. The team veterinarians were
able to introduce “chop and carry” (Fig. 1) with in-
troduction of appropriate grasses and encourage
owners to purchase and store hay when available.
Other local feedstuffs such as coconut berries, rice
hulls, and some pelleted feeds became available as
demand grew.

Proper farriery was introduced via several work
and training days (Figs. 2 and 3). A skilled farrier
from the show horse region near the capital, Santo
Domingo, was hired to come and work with our
volunteer veterinary podiatrist in several different
locales. Ultimately, one local student was selected
for a 6-month preceptorship with the farrier in the
capital, and supported by team funds. The plan
was for him to then come back to his village and
work for the tourist horses. This was not successful
due to injury, but is still an ongoing effort.

The project partially succeeded in its early goal of
training Dominican veterinarians and students in
clinical skills appropriate to their own levels. There
There are seven veterinary schools in the country, but most are small, poorly funded, and lacking in clinical facilities, especially for large animals. Most graduates are didactically competent, but have little clinical exposure. Many go on to become proprietors of dispensaries that sell antibiotics and supplies and may provide some vaccinations. Others go on to serve long preceptorships with the few established practices or work in regulatory capacity for the government. Only a few of the students who visited had any interest in equine practice. One of the most requested services was castration and to date the team has castrated well over 1200 animals. Team presence in the area two times a year has put the few local (nonveterinary) “castrators” out of business for the most part. Clients are now used to the bloodless Henderson procedure and wait for “la machina” until the team comes again. The U.S. veterinarians have trained several Dominican veterinarians in the Henderson procedures and they are totally competent. There is twice-yearly interaction with the Universidad Pedro Hernandez Uriel veterinary school in the form of clinical skills seminars and lectures (Fig. 4). There are usually one or two vets or vet students present at all our clinical sites.

Over the 23 years of Project Samana, more than 240 veterinarians have come at least one time, many have continued as regular participants. Student numbers have been even greater. Veterinary technician numbers have been fewer, under 200. A parallel team of small-animal veterinarians have performed an average of 250 surgeries per trip. Each trip also entails administration of direct care and some preventive treatments to an average of 225 working equids (based on actual counts in recent years, and doses of anthelmintic in earlier years). They are now widely available, affordable and effective. With the local availability of anthelmintics, this service is no longer offered.

For the long term, the use of working equids in the communities served is slowly changing tourism-based economy, with crop transport occurring as needed seasonally in most areas. Mechanization is unlikely due to terrain and economics. There will be a demand for more veterinary services as the Project Samana team works to transition to more local providers. One local mixed animal practice is currently subsidized. Current investigations and surveys of the population are being conducted following the models of other organizations, to assess future needs and planning.

The leadership of Project Samana is looking at several potential models to create sustainability. Community interviews and needs assessments are ongoing to determine which model or combination of models will be most effective:

- Direct payment to a local veterinarian for each animal operated or treated. (This is being done currently by a German charity for small-animal spay/neuter.)
- Subsidization of an ambulatory unit, including supplies, by a hired or per-diem Dominican veterinarian.
- Continued support by visiting teams, with fees being charged and donated to the local humane society or horsemen's cooperative.

The success of Project Samana presents the question as to why the team veterinarians are still doing this. First, capitalism only works in situations where there is sufficient disposable income to be applied to a desired procedure or action that will allow a direct return on the investment. Deworming a horse and floating its teeth will show some benefits over time, but will not make the animal immediately more attractive or usable for work. Second, local or native veterinarians must be paid sufficiently for the work or they will gravitate toward more lucrative venues. Third, if free or low-cost/high-quality interventions are available twice a year, waiting for them is a desirable option. Fourth, training veterinarians and students is a desirable and achievable goal as they become familiar with the great needs and are motivated to do more throughout their career. Teaching altruism through action is a valid outcome.

Fig. 4. Professionals pass along their skills.
4. Conclusions

The Project Samana veterinarians will continue and perhaps even escalate their presence in the region until the local horsemen can support local, well-trained vets.

The needs of working equids throughout the world are real and veterinarians have an obligation to assist in their care. Opportunities for learning, cultural exchanges, and real, significant changes abound and will continue to attract participants. Like projects and programs should continue as long as there are ways to channel resources and education across borders to benefit working equids.

Acknowledgments

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Conflict of Interest

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Sustainable Health Services for Working Equids on American Indian Reservations

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1. Introduction

The purpose of this presentation is to describe the program of Rural Veterinary Experience Teaching and Service on the Indian Reservations of the Northern Plains, and to suggest how improved veterinary care for reservation horses might become sustainable in the future.

2. Background and History

Over the last 19 years I have been working on Indian Reservations primarily in North and South Dakota, but also in Arizona, New Mexico, Idaho, Washington, Nevada, Utah, Minnesota, Wyoming, and Wisconsin. This began as a combination of public health and companion animal work, but because I am an equine practitioner at heart, the program morphed into our current all-equine operation called Rural Veterinary Experience Teaching and Service. Although the basis of our work is providing veterinary care in these underserved reservation communities, we also found that by incorporating veterinary students in our clinics there is an added bonus of skill acquisition and broadening of horizons for the next generation of veterinarians. Hence, we have developed a curriculum that provides pre-trip preparation, closely supervised technical practice, and daily discussions and rounds.

It goes without saying that horses and plains Indians have had a long history. The *Equus caballus* reintroduction into the Western Hemisphere in the fifteenth century made it possible for the people who inhabited the enormous prairies of central North America to range across the great “sea of grass,” moving, hunting, and warring at will. Although the zenith of the great “horse nations” of the Plains lasted only approximately 200 years, the culture of the Lakota, Oglala, Hidatsa, Arikara, and Mandan communities remain. These traditions are that much more important after years of government mismanagement, and the social problems that come from poverty and marginalization. This is especially true of youth who use horses to participate in rodeo, horse racing, and ultimate warrior challenge competitions, or just find that being able to get out on the prairie “a horseback” gives them connection to their ancestors and a very positive identity. The skilled horsewomen pictured on the Standing Rock Reservation (Fig. 1) are an example.

Many of the Plains Indian communities that we service are at the very bottom of the economic scale. For instance, the median income for a household on...
the Pine Ridge Reservation was $21,089 in 2014. And the per capita income was $6,067. Approximately 61.0% of the population lives below the national poverty line, including 74.6% of those under 18 years of age. Deplorable numbers for life expectancy, incarceration, and infant mortality follow. These truly disturbing statistics mean that residents have few opportunities and resources. What they do have is a lot of open-range and free grazing. Horses thrive in an environment to which they are well adapted and with a people who honor them.

Working equids come in several forms on the reservations. Many serve as recreation and sports horses for youth (rodeo and racing). Several tribal members raise “rough stock” for rodeos. From small contractors who use the low-cost grazing privileges to maintain small bands of bucking horses for local junior and amateur events to individuals with serious breeding programs who aspire to having some of their saddle broncs make the National Finals, these Indian ranchers provide income and jobs to reservation residents. Cattle ranching is another use for horses in the Dakotas, even though much of the work once done by horses is now given to the easier to train and faster all terrain vehicle (ATV). In addition, there are many horses that are bred on tribal lands simply because feed is inexpensive and because extended families have always kept and raised horses. Many of these animals are never trained and some certainly enter the slaughter horse market. A final category are the “ditch horses” who are abandoned on tribal lands, usually near roadways, and have to be herded onto common pastures to prevent traffic accidents and damage to infrastructure. In fact, nobody knows how many horses are actually on the ranges of the Dakota tribes.

Services provided by volunteer veterinarians, registered veterinary technicians, and students include castration, lameness diagnosis and treatment, hernia surgery, dentistry, hoof trimming, acute care for wounds and injuries, and occasionally, humane euthanasia. The approximately 500 castrations and 50 cryptorchids that we do a year in the Dakotas are particularly important. Although many ranch and reservation residents still “cut their own colts,” there are fewer and fewer individuals who can do an unanesthetized castration safely and effectively. We certainly do not condone surgery without anesthesia or analgesia, but several families have been doing their own procedures with a good horse, a rope, and a knife for years. A few feel that this helps maintain their culture. However, most recognize the added safety to all of proper anesthesia, even if they do not necessarily worry about humane concerns. We vaccinate for tetanus and deworm horses on which we do other procedures. However, doing large-scale vaccination and deworming, (something that most tribal members can do themselves) draws resources from other activities that require more skilled veterinary personnel. Parenthetically, most of the dentistry we do is for major abnormalities (fractured teeth, abnormal eruption, extractions in older horses, etc.), with most of the horses presenting for examination having really good mouths, presumably due to a very natural diet of long-stem grass. Given that most of the horses are of the same Quarter Horse breeding seen elsewhere in the country, genetics probably plays a less important part in dental health. On the “rez,” wounds and hoof problems tend to go unattended until they are fairly severe due to a lack of local veterinary service, lack of a basic level of training in the reservation animals, and the fact that many of these horses live on open ranges without frequent human contact. Caring for these injuries can be challenging; however, using the principles of wound healing, techniques such as regional limb perfusion, have resulted in a fair amount of success. Humane euthanasia is occasionally required though, using techniques that do not leave a toxic carcass on the prairie.

Hoof trimming of young horses that are not trained (or never will be) is another important function. Soft spring ground and excellent forage tends to make really serious hoof overgrowth common. Often anesthesia is the only option for dealing with badly overgrown, distorted, or cracked hooves.

All these procedures provide a wealth of experience for veterinary students. To optimize their training and to insure that the horses get the best care possible, it is essential that a teaching program follow the following principles:

- Detailed curriculum which students must study prior to their field experience
- Thorough orientation introducing techniques, protocols, and expectations
- Supervision by staff experienced in field medicine and surgery
- Record keeping on all horses treated
- Followup both in the immediate (daily rounds)
American youth into a veterinary practice. Not only do we find the usual impediments to developing interest in veterinary medicine (cost of education, eventual income, lifestyle, and contact with the profession), but all the very basic problems in science, technology, engineering, and math (STEM) education also apply. These are areas of very active research because they have been identified as serious stumbling blocks to the education of minority ethnic groups and women in STEM subjects. Identified difficulties include lack of role models, perceived lack of relevance to real life in STEM education, and inability to find community in science or professional school. For reservation youth, one must add the fear of severing the attachment to a strong extended family structure at home. Finally, our veterinary profession is recognized as the least diverse of all fields of science, with students identifying as equine emphasis being even more limited to upper middle class socioeconomic status and ethnic Western European descent. Bridging this gap will be the focus of our program in the future. Simply talking to high school assemblies and other youth groups is notoriously unsuccessful in actually bringing marginalized minority students to STEM education, much less professional school. Better organized, more aggressive, and more expensive projects are required. Research experiences for undergraduates have demonstrated efficacy in improving interest in and successful completion of STEM curricula. Perhaps a program patterned after one of the National Science Foundation’s research experiences for undergraduates programs and directed at students from remote rural communities may be a viable approach.

Acknowledgments

Declaration of Ethics
The Author declares that he has adhered to the Principles of Veterinary Medical Ethics of the AVMA.

Conflict of Interest
The Author declares no conflicts of interest.

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Clinical Practice in a Hospital for Working Equids: Morocco’s American Fondouk

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This presentation discusses the unique set up and aims of the Fondouk and looks in detail at some of the most important pathologies that we encounter among our population. Author's address: American Fondouk, B.P. 2048 Fez (V.N.), Morocco; e-mail: gigikayvet@gmail.com. © 2015 AAEP.

1. Introduction
According to recent Food and Agriculture Organization of the United Nations (FAO) estimates there are 15.5 million equids in the developed countries of the world. In comparison, there are more than 100 million equids working in the developing world, 27 million of which are in Africa (FAO statistics, 2000). It has been estimated that working animals are responsible for 75% of traction energy in the developing world and that 50% of the world's population depends on animal power as their main energy source. Recognition of the economic advantages associated with donkey ownership has led both government and nongovernmental organizations (NGOs) to encourage the use of donkey traction in Africa, seeing it as gender friendly, low maintenance, and relatively disease resistant. Despite this, it is still surprising that the use of working equids, far from decreasing with increasing motorization, is actually on the increase. The global donkey population of 44.3 million animals (95% of which are found in the developing world), has increased by 15.6% over the last two decades (FAO statistics 2000).

The threats to the welfare and productivity of this population of equidae are substantial and the economic effects of health challenges can be catastrophic to individual family units. In disenfranchised communities, the wellbeing of an owner and his family is intimately linked to the wellbeing of their animal. Drought, poverty, ignorance, epidemics, disease, increasing motorization, and increasing environmental pollution are all risk factors that cause significant morbidity. Addressing the health needs of these animals and their owners has been an issue largely ignored by governmental and supranational bodies, which have tended to concentrate veterinary resources on improving the health status of production animals. It has fallen to the NGOs to take the lead in defining and implementing strategies of veterinary support for working equids in some of the world’s most disadvantaged areas. The ultimate goal of these programs is to improve the health and welfare of equids in targeted zones, and thereby improve the productivity and longevity of this form of traction. In turn, this should aid in the maintenance of stable and self-sufficient agricultural and periurban populations.

This presentation offers an introduction to the work of the American Fondouk. It highlights some of the challenges faced by our veterinarians in at-
2. Inadequate Husbandry Equates to Morbidity

Much of the equine pathology in the developing world is the direct or indirect result of inadequate husbandry techniques and “traditional” therapeutic practices. As a result, the work of the equine NGOs necessarily involves not only the treatment of affected individuals, but also mass education and information campaigns to try and change established behavior.

Studies from Mexico and Ethiopia confirm that poor husbandry leading to harness sores, wounds, foot problems, and heavy worm burdens are an important cause of compromised welfare. In many countries, these problems are compounded by endemic disease such as babesiosis, trypanosomiasis, and epizootic lymphangitis, which are often difficult or impossible to control or treat.

Inadequate husbandry is not the only culprit that affects the health of the working equid. Horses and mules in the developing world also suffer from all the usual pathologies that equids suffer all over the world, the only difference being that these pathologies have often been allowed to run out of control as access to veterinary care is sporadic and limited. So any owner of a working equid in Morocco may have to deal with a horse that has injured itself in a fall or a spook, a donkey that has sarcoids or melanomas, or a mule that suffers from recurrent airway obstruction or colic.

It is difficult to quantify the magnitude of each of the above threats to the working equid given that discrete geographical populations face different difficulties. However, in Morocco, analysis of our patient population, both inpatients and outpatients, has suggested that approximately 50% of pathology is caused by poor husbandry, and the other half of our patients suffer from disease or pathology that is a direct result of simply being an equid.

The success of any clinical intervention depends on the veterinary skills and the facilities available. This is as true in Kentucky as it is in Timbuktu. It is important to recognize that we cannot make animals better through provision of poor medicine with minimal diagnostic facilities and limited veterinary experience. The author believes that working equids deserve the same standard of care as we would expect for our equids in the West. The difficulty, of course, is in how to achieve this, how to maintain it, and how to pay for it.

3. The American Fondouk

A global network of working equid hospitals exists across the world, most of which were established and run by the three largest working equid charities: The Brooke, Society for the Protection of Animals Abroad (SPANA), and the Donkey Sanctuary. The American Fondouk is the only non-British hospital that exists in Africa and was established by an American tourist to Fes, Morocco in 1927. The American Fondouk is administered by Massachusetts Society for the Prevention of Cruelty to Animals (MSPCA) Angell.

The Mission Statement of the Fondouk is to provide help to the vulnerable sectors of the community who rely on working equids for income generation through the provision of free veterinary care to their animals. Thus, we are as much a humanitarian organization as an animal welfare organization.

The Fondouk aims to provide excellence in veterinary care. This is backed up by a comprehensive range of diagnostic and imaging equipment and is achieved in collaboration with close specialist advice and support from both the United Kingdom and the United States.

The Fondouk works with seven Moroccan veterinarians, of whom five are following an internship program with us. The interns spend 1 year at the Fondouk in Fes and are then sent to a centre of excellence in Europe to carry out another year's internship. After this period, they return to Morocco to work independently. The Fondouk hopes that facilitating access to specialist training for young Moroccan vets will have far-reaching implications for the welfare of equids in Morocco in the future.

In addition to this educational initiative, the Fondouk has established a contract with the University of Glasgow veterinary school whereby we provide a rotation in equine medicine to 50 of their final-year veterinary students each year. Students visit the Fondouk in groups of four and each stay for a 4-week period. This is a formal part of the Glasgow veterinary school curriculum and the income generated from this contract pays a substantial part of the running costs of the hospital. This relationship brings other significant advantages to the Fondouk and its patients. For example, each month a specialist from Glasgow accompanies the students for a week. We also have the support of a team of specialists via weekly Skype ward rounds as well as immediate access to specialist advice as needed.

Telemedicine has become integrated into the daily routine at the Fondouk through the sharing of digital radiographs, ultrasound images, and biochemistry/hematology data. There is no doubt that this has improved therapeutic plans in many cases and optimized the chance of successful outcomes.

This kind of partnership is currently unique in the working equid world and ensures clinical standards and enthusiasm is maintained in what are often demanding circumstances. Unlike many of the
working equid charities, the Fondouk is a single-site organization. For nearly 100 years, we have only had one hospital and that is in Fes. Although the implications of this are that we are able to invest all our resources into building a center of excellence, the flipside of course is that only a relatively small number of animals are able to benefit. The Fondouk hopes that by providing an excellent practical experience for veterinary students from Morocco and elsewhere, and through the provision of high quality continuing professional development in the country, we will be able to widen the circle of influence. The learning experience for all visiting volunteers and students is exceptional, due to the high caseload and extreme pathology that is seen at the Fondouk. Most days we have 15–20 outpatients in the morning, and 30 patients in the hospital that require ongoing and sometimes intensive care.

Many of the animals present with colic, usually caused by severe colon impactions, often complicated by plastic bags, rope, and other foreign bodies that the animals have scavenged. Volunteers and students at the Fondouk get extensive hands-on practice at passing nasogastric tubes, performing rectal examinations, and belly taps, which makes it a unique learning experience. Another area where visitors are able to gain experience is in lameness diagnosis. Studies carried out by the Brooke have shown that more than 80% of working equids have limb pathology and many have multiple limb lameness. Catastrophic farriery techniques such as toe dumping lead to chronic pastern arthritis in huge numbers of working mules. Diagnosing these multiple limb problems can be challenging, particularly so because mules usually object violently to nerve-blocking attempts. The Fondouk is well equipped with digital radiography and ultrasonography and this facilitates accurate diagnosis, but therapy remains challenging. Very few owners can afford the luxury of “box rest,” and horses are obliged to keep on working even in the face of severe orthopedic issues. The American Fondouk owns a number of working mules that we loan to owners in an attempt to buy time for them to leave their lame animal at the hospital to recover. Sometimes we even cover an owner’s daily earnings for a week to persuade them to leave their lame animal at the hospital to recover.

The Fondouk staffers are real advocates for the working equid and we try all strategies to ensure that we can deliver care where it is needed.

4. The Way Forward
Ensuring an adequate standard of welfare in the global population of working equines is a daunting task. It requires coordinated efforts, not only of the veterinary NGOs, but also all stakeholders with an interest in agricultural development in Africa and Asia. These include local and national governments, supranational organizations, veterinary and agricultural institutions, and the agricultural communities themselves. It is time that microtransport took its place alongside microfinance and microbusiness development as a priority issue on the development agenda.

Primary objectives should be to improve accessibility to both preventive husbandry education and to essential care for the common causes of morbidity and mortality within zones of heavy equid utilization. Many countries within Africa and Asia still have no access to such support. Secondary objectives should focus on ensuring delivery of appropriate and effective care, including choice of safe and effective drugs, appropriate equipment scale, sufficient veterinary and para-veterinary training, and sufficient funding for the clinical population. The gold standard for any working equid organization is to try and insure a sustainable provision of care that improves the overall welfare of the population into the future.

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References and Footnote

Impact of Mandatory Social Service on Professional Veterinary Development in Mexico

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Mandatory social service allows veterinary undergraduates to put into practice their knowledge of equine medicine, surgery, and management for the betterment of society. It provides an opportunity for learning about communities that rely on working equids and how they relate to people and to other animals, preparing professionals to perform in a wide range of roles, from private equine practice to international programs for sustainable development. Author’s address: Department of Equine Medicine, Surgery, and Science and the Donkey Sanctuary–National Autonomous University of Mexico (UNAM) Program, College of Veterinary Medicine and Animal Science, UNAM, 04510 Coyoacan, Mexico; e-mail: mhg_fmvz@unam.mx. © 2015 AAEP.

1. Introduction

An original social service initiative of the National University of Mexico (UNAM) was adopted and maintained by all public universities in Mexico. Social service is a requisite for obtaining any bachelor's degree. All undergraduate students complete a 6-month period of community service that reciprocates society’s support of their university studies.

There are more than 50 veterinary schools in Mexico, most of which belong to state universities that created a veterinary program to primarily address needs in animal production to uphold food security. Currently, the importance of animal welfare and health is also recognized, as well as the opportunities future veterinarians have for earning a living in veterinary practice.

The College of Veterinary Medicine and Animal Science of UNAM runs a project with the Donkey Sanctuary that, in collaboration with World Horse Welfare, assists equids in Mexico. The original approach involved visiting communities to implement treatments and preventive medicine with some extension work. The current approach focuses on establishing community partnerships for sharing experiences and disseminating knowledge while finding areas of opportunity for veterinarians in their own communities. These veterinarians eventually will facilitate welfare outreach to more equids.

Inspired by the model of Catley et al.¹ and taking advantage of the universities’ programs of social service, the Donkey Sanctuary–UNAM teams have been involving social service students (SSS) in their projects since 2008, encouraging them to live in a village to play the role of community-based promoters of animal welfare in areas where human livelihoods rely on equids.

The strategy has been successful in strengthening the link between the community and teams but has mostly represented a milestone for future veterinarians because some have envisaged a specific direction to take in regard to their professional development, whereas others have simply developed the confidence...
required for establishing a practice of their own in their particular area of interest.

This article presents the experience of the Donkey Sanctuary–UNAM joint program on how the mandatory social service program may positively affect the professional development of veterinarians in Mexico to either become involved in equine practice or succeed in organizational work.

2. Materials and Methods

SSS are recruited twice a year. One group starts between February and March and finishes between August and September; the second group starts between August and September and finishes between February and March of the following year.

The Donkey Sanctuary–UNAM joint program has projects in more than 250 communities in 13 provinces throughout Mexico. Students are assigned back to their communities (i.e., university or geographical) to an area of their particular interest (e.g., equids in rural or peri-urban areas or those working in small-scale dairy production systems or agriculture systems for subsistence), or to an area of the Donkey Sanctuary–UNAM program’s own needs (e.g., investigating socioeconomic contribution of equids, reducing disease occurrence, improving harnessing, etc.). Once the area is defined and a work plan is produced, the SSS get involved and are integrated into three stages: induction, training, and in-community experience.

3. First Stage: Induction

Induction is a 2-week period during which students are introduced to equine welfare concepts and assessment. Because managing project cycles is central, the SSS are trained in needs assessment, planning, implementation, evaluation, and monitoring. Appropriate extension methods, participatory research methodology, community partnership techniques, training of trainers practice, and how to identify main actors and to build relationships and networks are all taught during this first stage.

4. Second Stage: Training

This is a 6- to 8-week period during which students work with the team of field services, learning from veterinarians, farriers, harness makers, and behaviorists on topics such as medicine, surgery, and management for equine practice and welfare. Every training lesson is delivered in the context of the whole life of the equid, the local culture, and the agricultural and working practices of that culture, all of which are in reference to the 5 animal-based indicators of welfare per the Donkey Sanctuary’s “Hands-On Donkey Welfare Assessment” framework: (1) equine behavior and equid–human interaction, (2) nutritional state and feeding practices, (3) physical integrity and basics of harnessing, (4) lameness management and foot care, and (5) disease occurrence and management. It is also during this time that the student interacts with the community team, which includes social scientists. In summary, this time empowers the student to be effective in the community.

5. Third Stage: In-Community Experience

This is a 4-month period during which the student lives in the community and plays the role of veterinarian for equids, a role that very well may include care of any species in need. Assisted by the guidance of the team leader in charge of the project, the student performs basic procedures of diagnosis and therapy. When more complex cases are faced, such as those requiring surgery, the team veterinarian is called in for support. As part of providing help in health care and management, the student uses the tools provided to obtain information necessary to complete the study of the region.

6. Results and Discussion

The social service program is a key step in the professional development of veterinarians in Mexico. This particular program provides the opportunity for many future veterinarians to become involved in both rural development and equine practice. It has proven to be effective in facilitating the involvement of those future veterinarians in their own communities—now as professionals with the experience and competence required for dealing with conditions that put equid welfare at risk. Students develop the necessary skills for understanding welfare, putting into practice knowledge of clinical medicine and surgery, expertise in equine management issues, experience in extension methods, and the ability to interact with people.

For the Donkey Sanctuary–UNAM program, the involvement of SSS has been helpful in (1) providing elements for understanding the socioeconomic contributions of working equines, (2) finding areas of opportunity for the professional development of veterinarians, and (3) raising the quality of life in the field of working equines.

In Mexico, like in other countries, there are many human livelihoods that rely on equids. Equids are involved in supporting the livelihoods of the human population—most for subsistence, some that foster self-employment, and a small percentage that generate income. Types of livelihoods vary from small-holder units in remote, poor rural areas, such as in countries with similar ecological and economic conditions, to entities in forsaken, underprivileged peri-urban areas of cities, such as in countries with challenging social and economic circumstances. Equids bring complementary benefits to agriculture and livestock, generating cash when sold or rented and becoming a way of keeping assets by reducing expenses. Depending on the geographic characteristics and type of system, workloads can increase during a specific time of year, thereby reducing people’s vulnerability but increasing that of equids. The effect of this may be addressed by owners who seek the services of veterinarians, who in turn aim
to promote all aspects of welfare—a fact that demands that the institutions that produce professionals to address national needs reinforce their vision regarding the balance between the productivity of a system and the welfare of the animals. This is becoming an exigency of society.

Therefore, a program of social service that supports the professional development of future veterinarians interested in equids allows students to receive training that enables them to develop skills in veterinary medicine and animal science for equids and other animals. Students learn appropriate extension methods for equine owners and community partnership methods for introducing knowledge, both of which enable them to recognize needs and to develop strategies in systems that include equids.

7. Conclusion

The social service experience has a positive impact on both the professional and personal development of future veterinarians. On the professional side, veterinary students who have been involved in this program agree that their level of competency increases by facing real cases in a sector of the equine population with demanding needs. These equids belong to a sector of human population in need, where owners must stay vigilant to ensure equid quality of life. In the particular case of veterinarians, their level of competency in approaching conditions that affect the health and welfare of equids with a medical, surgical, or management solution considerably increases to the extent that they can establish relationships between those conditions and the root causes that lead to their occurrence and severity. On the level of personal development, the students highlight the satisfaction they get from providing rural and peri-urban communities with skills and knowledge, as well as finding how rewarding humanitarian work is not only in regard to people but also to equids.

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References
Value of Welfare Partners and Simultaneous Education of In-Country Veterinary Students: Equitarian Honduras Model

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Collaboration with established nonprofit organizations and veterinary colleges in developing countries accelerated the growth of a new veterinary nonprofit organization. This collaboration subsequently enabled new veterinary projects for the health care of working equids, equine veterinary education, and new opportunities for veterinary students; it also increased the sustainable impact of these interventions. Authors’ addresses: Equitarian Initiative, 10777 110th Street North, Stillwater, MN 55082 (Wilson); Merriam Equine Consulting, 1800 County Road 109, Glenwood Springs, CO 81601 (Merriam); 3281 Luneman Road, Placerville, CA 95667 (Turoff); Turner Wilson Equine Consulting, 10777 110th Street N, Stillwater, MN 55082 (Turner); World Horse Welfare, Honduras, Anne Colvin House, Snetterton, Norfolk, NR16 2LR, UK (Warboys); and Colegio de Medicos Veterinarios de Honduras, Universidad Nacional de Agricultura, Nombre de Culmi, Kilometro 215, Barrio El Espino, Catacamas, Olancho, Honduras (Reyes); e-mail: wilso011@umn.edu. *Corresponding and presenting author. © 2015 AAEP.

1. Introduction

The health care needs of the estimated 90 million working equids are largely unmet because of economics, a lack of training of owners and veterinarians in situ, cultural differences, and government disinterest. Nonprofit welfare organizations dedicated to working equids internationally have been well-established in Great Britain for decades. These include the Society for the Protection of Animals Abroad (1923); World Horse Welfare (WHW, 1927; formerly known as the International League for the Protection of Horses), the Brooke (1934), and the Donkey Sanctuary (1969). All have programs with a veterinary component but provide little opportunity for veterinary volunteers who wish to donate their time and expertise for short periods. In contrast, the American Fondouk in Morocco (1927) provides a permanent veterinary care facility that also serves as a teaching venue for veterinary students from multiple countries. The Donkey Sanctuary in Mexico chose to augment its program by incorporating veterinary students with obligatory social service requirements. Through this collaboration, teams of veterinary students and veterinarians provide working equid health care to enrolled communities every 6 months. In the United States, Humane Society Veterinary Medical Association’s Rural Animal Veterinary Service (HSVMA/RAVS), Christian Veterinary Mission, and Project Samana
have supported small groups of equine veterinarians and American veterinary students in providing working equid health care internationally. However, no comprehensive program to expressly train equine veterinarians to improve working equid welfare and health has existed.

2. Partnership Development

A group of American equine veterinarians joined together in 2009 as the Equitarian Initiative (EI) to serve the common purpose of improving health care for working equids. The American Association of Equine Practitioners (AAEP) encouraged the group’s formation. Mentorship was immediately provided by 3 organizations: the Donkey Sanctuary, Universidad Nacional Autónoma de Mexico (UNAM), and WHW. Working together, the Equitarian Workshop was developed to train veterinarians and other equine professionals to understand the needs of working equids and learn best practices for health care delivery. Utilizing the community service model of the Donkey Sanctuary in Mexico, workshop participants learned through a combination of didactic presentations, discussions, and hands-on health care delivery to working equids in poor communities. American veterinarians with experience in international equid work served as instructors alongside faculty from UNAM and the Asociación Mexicana de Medicos Veterinarios Especialistas en Equinos. WHW provided saddlers and farriers as part of their program of training local individuals to take up these professions and subsequently invited EI veterinarians to partner with them in Central America and Haiti. The resultant project in Honduras included that country’s new and first veterinary college at the Universidad Nacional de Agricultura (UNA).

3. Results

The growth of the fledgling EI has been rapid and catalyzed by mentorships and partnerships with like-minded organizations and universities in developing countries. Five annual equitarian workshops have been held to date: 4 in Mexico in conjunction with the Donkey Sanctuary and UNAM and 1 in Nicaragua led by American equitarians. These workshops have been financially supported by registration fees from participants and grants from the American Association of Equine Practitioners Foundation. Participants have included veterinarians from the United States, Canada, Mexico, Panama, Nicaragua, Costa Rica, the United Kingdom, Portugal, and India. Equine science professionals, veterinary students, veterinary technicians, a humane agent, and other supporters have also attended.

The Donkey Sanctuary Mexico taught the Equitarians three key points about executing programs to improve working equid health:

- Concurrent participation by in-country veterinary students is mutually beneficial: the workshop provided unique learning opportunities for the students, who in turn served as translators for non-Spanish speakers to more effectively communicate with owners and who were indispensable in several of the roles typically delegated to veterinary technicians.
- Cultural differences and owner expectations need to be recognized to effectively deliver both health care and targeted education. In Mexico, this critical component is led by social anthropologists who spend additional time in the communities. These same personnel also spend time educating children and some adults at the work sites. Veterinarians experienced in equine behavior are very important in demonstrating much needed, gentle retraining of resistant and aggressive horses. They also address the owners who believe misbehavior and aggressive correction are acceptable.
- Effective organization of a community work-site enhances workflow. The site should include specified zones to address the multiple needs of the animals. Grassy athletic fields are optimal, but shade should be considered in site selection. Zones or stations that are commonly included are registration, vaccination, deworming, dentistry, surgery, internal medicine, reproduction, farriery, saddlery, and an owner education station.

The strong bonds that have emerged among collaborating welfare organizations were evident at the 5th Equitarian Workshop, where 8 nonprofit organizations discussed best practices for effective health care delivery, education, and sustainability. These included the EI, WHW, the Donkey Sanctuary, HSVMA/RAVS, RVETS, Sustainable Vets International, Christian Veterinary Mission, and World Vets. Leaders from these organizations contributed to the curriculum and field work in communities. The final partner in this workshop was the veterinary college at Universidad de Ciencias Comerciales (UCC), 1 of 7 veterinary colleges in Nicaragua. UCC provided lecture facilities for part of the workshop and 2 faculty members and 20 students who participated in both lectures and community work. The caliber of the teaching at the workshop and value to veterinarians interested in becoming skilled in equitarian work was recognized with Registry of Approved Continuing Education (RACE) approval for continuing education hours.

The equine science professionals who have participated in equitarian workshops have undertaken several small research projects that evaluated workshop behavior and body condition scoring. One such participant subsequently created a 4-H project to make a coloring book to increase children’s awareness of equid health needs. This coloring book of health lessons is now widely used in equitarian proj-
Mentors and collaborators have supported 5 new international veterinary programs launched by participants in the equitarian workshops: Costa Rica, Honduras, Mexico, Guatemala, and Haiti. Costa Rica is a weeklong annual project that was started in 2011 and is supported by the participating veterinarians and grants from the AAEP Foundation. Similarly, Honduras is a weeklong annual project that was started in 2012 and is partially financed by private donations. Mexico’s project is typically a 5-day annual project that began in 2012 and is partially funded by donations from the leader’s clients. Guatemala’s project began 3 years ago and includes 2 annual work weeks. It is supported by Full Bucket, members of the Texas Equine Veterinary Association, MWI, and the AAEP Foundation. The Haiti project began 2 years ago and continues with 2 weekend visits a year. It is primarily funded by donations and fundraisers supported by the leader’s clients.

Veterinarians from Project Samana and HSVMA/RAVS, now also equitarian leaders, have mentored new project leaders, helping each develop supply lists and plans for negotiating permits and passage through customs. WHW has been an invaluable partner in Honduras and Guatemala and an increasingly important one in Costa Rica and Haiti. Individual workshop attendees have gone on to gain additional experience by volunteering for other working equid health care projects sponsored by HSVMA/RAVS in Peru, Nicaragua, and Guatemala; Project Samana in the Dominican Republic; and Sustainable Veterinarians International in Nicaragua. Several Mexican veterinarians from the preceding workshops have joined a number of these projects. Two Americans have been inspired to change their career path, seeking advanced training in international public health to better understand the link to the welfare of working equids. One past participant now works full time for the Brooke in the United Kingdom. Equine health programs on American Indian reservations have benefitted from participant training, including a new one that was initiated after the workshop.

With the support of WHW’s infrastructure, the equitarian project in Honduras has become the model for effective in-country engagement to both benefit working equids and provide valuable clinical training to future veterinarians. WHW initially invited a team of equitarian veterinarians to join their farrier and saddler training program for 4 days in San Pedro Sula, Honduras, in 2012. Concurrently, the new UNA veterinary college faculty were invited to bring their charter class of students (n = 10) to join the US veterinarians to gain clinical experience. This new veterinary college is in a very poor country and has no teaching hospital, so clinical skills must be gained by external rotations. Local veterinarians (n = 8) also joined in and actively discussed the health problems observed and new veterinary techniques. Health care services included vaccination against tetanus and rabies, deworming, harness sore and wound care, dentistry, castrations, lameness evaluations, and disease diagnosis. Evening seminars on dentistry, parasites, colic, and lameness were provided to the veterinary students and local veterinarians. Very positive feedback on the value of the learning experience was received from the veterinary college, encouraging further collaboration.

The evolution of the WHW program model has shaped EI’s perceptions of program components to augment impact and sustainability. In 2013, WHW chose to move its Honduran program to Choluteca based on strong support of the mayor, high number of working equids, and need for greater safety of the participants as a result of rising crime. The new model is based on a 3-year plan to support more than 20 communities that rely on working equids to haul firewood, building materials, trash, and agricultural products. A subset of equids works as personal transportation and/or herding cattle. This model, in addition to training farriers and saddlers, includes training a person to become a community-based equine advisor in each community. These individuals become a resource for working equid owners on subjects of animal health and welfare.

WHW’s annual invitation of UNA and the equitarian team to work together for a week has had a significant impact on UNA’s veterinary students. UNA leaders have chosen to bring the entire 3rd-year class each year: 23 students in 2013 and 27 in 2014. At the start of the week, several veterinary students were frightened by horses, and most lacked physical examination skills. By the end of the week, however, the students had participated in the treatment of hundreds of working equids, developed physical examination skills equivalent to US veterinary students at the beginning of their senior year, and experienced an effective form of community service. Equine veterinary topics were taught in classroom lectures and wet labs at WHW’s office. For better efficiency, the lectures were provided on the first 2 days of the week in 2014, and the wet labs were held in the mornings before traveling to the communities. Community-based equine advisors, farrier and saddler trainees, staff, and local veterinarians were also invited to these educational activities.

The equitarian veterinarians were inspired by the veterinary students’ eagerness to advance their knowledge and skills. The equitarians learned the challenges of working in extreme tropical heat, the impact of cultural differences in horse handling, and the limits of availability and affordability of medications. American veterinarians new to the team quickly became familiar with pervasive issues such as harness sores, seasonal recurrent airway obstruc-
tion, vampire bat bites, and vesicular stomatitis. The Americans’ dedication to volunteer community education and female gender modeling by the project leaders was commented upon by the veterinary students and somewhat of a cultural surprise for the working equid owners.

The strong focus on Honduran veterinary student education led to an invitation of a subset of Honduran veterinary students to join subsequent equitarian projects in Nicaragua and Guatemala. These students integrated readily into these teams and were immediately able to provide support to veterinarians while continuing to learn from them. Veterinary students from other Central American countries also participated, resulting in a friendly, enthusiastic network of students interested in horses. This network may serve as a future focus for mutual support and learning after graduation. Externships in the United States are planned for some of the most dedicated students.

The success of the Honduran model of equitarian work has led to an increased effort to collaborate with in-country veterinary colleges. As a result, more students have become involved in equitarian work in Nicaragua, Guatemala, and Costa Rica. In Costa Rica, animal science students have also been included. Greater effort to find funding for American veterinary student participation in these projects is anticipated.

4. Discussion
Cornerstones for sustainable international development have emerged from the decades of work and research of the British nonprofit organizations that focus on international working equid welfare. These foci must become components of sustainable equitarian work. Recent research by the British groups focuses on their role in human livelihoods. In areas of poverty, donkeys, horses, and mules have been shown to be the most important livestock for assuring stable family income. Economic studies have estimated their contribution to total family income to be 14% in Ethiopia, 57% of total productive assets in Guatemala, and nearly 80% of the annual income for purchasing food in India.1–3 Similarly, the Brooke has clearly shown that community-led actions are more effective for lasting change than short-term foreign assistance.4 Emerging work suggests that these communities may be most effective with a matrix of support beyond the health care of working equids, including microfinance, public health, social services, and training to enhance their economic productivity.5 Future and existing equitarian projects should consider linking their veterinary care to other organizations and governments that can sustain infrastructure to help provide these services continuously. Building capacity within the community is also critical for sustainability, as exemplified by the training of farriers, saddlers, and community-based equine advisors in WHW’s project in Honduras and the augmentation of the training of in-country veterinarians in most existing programs. Mentorship and collaboration have enabled rapid growth and success of the EI. Veterinarians from developed countries have had much to learn in regard to sustainable development and the impact cultural differences and varied owner expectations can have. The opportunity for equine veterinarians to train for and provide this level of support to working equids is unprecedented. For veterinarians who work full time and want to volunteer their expertise and vacation time, the challenges of trying to plan and execute a successful veterinary aid program are daunting. However, these challenges can be surmounted by partnering with larger organizations that provide infrastructure and a much more in-depth program that will benefit by veterinary team support.

The health of hundreds of working equids can be improved by simple interventions, demonstrating the value of routine health care to their owners. Many equid owners lack basic husbandry skills, especially in urban areas. This deficit can be addressed by owner education and by providing training in critical skills such as farriery and saddlery in the local community. Equine health lessons for children should not be overlooked because they are often the daily caretakers and may be more open to positive change.

Working equids are a mainstay for the survival of many small farmers who struggle to afford any veterinary care—if, that is, care is even available. Veterinary colleges in developing countries are poorly funded and typically weak in curricular support of equine medicine and surgery. Provision of equine-specific education for local veterinarians, veterinary students, and working equid owners is one tool that is essential for sustainability. Although a veterinarian in these countries cannot profit significantly from working equid care, some may choose to support working equid health by volunteering their time or offering low-cost services. Government or private sector financial support is rare but should nonetheless be encouraged. Many of the basic skills emphasized in programs such as the one in Honduras can be extrapolated to other species and will be useful to the veterinary students upon graduation. Long-term measures of impact still need to be developed to further refine both the equitarian workshop and the Honduran model.

5. Conclusions
Long-established nonprofit organizations such as WHW and the Donkey Sanctuary have enabled American equine veterinarians to rapidly and effectively develop both a training program and diverse country projects. The preexisting and growing infrastructure of these nonprofits in areas of great need of working equid welfare improvement greatly facilitates team planning and community work. For sustainable impact, veterinarians must simultaneously provide educational opportunities for in-country veterinary students, veterinarians, and
horse owners to care for the working equids when the equitarian team cannot be there. International efforts of collaboration strengthen all of those involved and should be encouraged.

The model of partnership between EI, WHW, and UNA in Honduras met shared goals to synergistically create a program to more sustainably improve working equid health. The strengths of this collaborative model should be emulated in other regions of need.

Acknowledgments

The authors recognize WHW’s Des Bridges for the invitation to initiate the Honduras and Guatemala equitarian projects. Roly Owers of WHW has strongly encouraged project growth and further interorganizational collaboration through the 7th International Colloquium on Working Equids in 2014. Stephen Blakeway and Mariano Hernández Gil of the Donkey Sanctuary were instrumental in the creation and growth of the equitarian workshop.

Declaration of Ethics

The Authors declare that they have adhered to the Principles of Veterinary Medical Ethics of the AVMA.

Conflict of Interest

The Authors have volunteered their time for the work and results reported herein. Drs. Wilson and Turoff have received a portion of AAEP Foundation grants to the workshops as organizers. Dr. Warboys is an employee of WHW.

References

Patients Without Borders: Collaborative Telemedicine Among University, Private, and Pro Bono Practices

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1. Introduction

Telemedicine is defined by the American Telemedicine Association as “the use of medical information exchanged from one site to another via electronic communications to improve a patient’s clinical health status.” Telemedicine can be as simple as a landline consultation or as complex as computer-based real-time videoconferencing but at all levels, the goal is to allow patient data to be reviewed and examined by experts far away from the patient. The human medical world has been quick to implement telemedicine to help close the socioeconomic gap in medical care. But veterinary telemedicine is still used mainly to bring expert consultation to veterinary clinics within the industrial nations rather than extending the reach of specialists to the developing world.

Working equids remain important to the economy even in rapidly developing countries such as Morocco, where agriculture continues to employ a large part of the population. Despite the cost of these animals, their owners often lack the financial means to provide even basic veterinary needs. In addition to their value in delivering basic and preventive care, with the advent of increasingly sophisticated equipment and telecommunications technology, pro bono hospitals also strive to deliver sophisticated diagnostics and treatment. We report collaboration and experiences with 137 patients shared among specialists at Cummings School of Veterinary Medicine at Tufts University and the Equitarian Initiative in conjunction with the Director of the American Fondouk, termed Patients Without Borders. This collaboration allows timely intervention in complex cases and gives working equids and their owners access to specialist care that would otherwise be out of their reach both spatially and financially. We describe the types of cases that most
commonly require consultation, and the type of electronic communication that is seen to be most beneficial in these cases, as well as the educational benefit to students in the United States.

2. Materials and Methods
The American Fondouk, a pro bono hospital for working equids in Fes, Morocco, was founded in 1927 by Emily Bend Bishop, and is affiliated with the Massachusetts Society for the Prevention of Cruelty to Animals. This busy hospital is operated by a limited number of qualified veterinarians working long hours. The Fondouk serves both the local urban population of equids working in the Medina, as well as those from the rural outskirts that travel miles to access the excellent veterinary care available. The Fondouk has an onsite laboratory and a small surgical facility as well as hospital accommodations. Diagnostic equipment includes digital radiography, endoscopy, and ultrasound.

3. Origin of Patients Without Borders
Patients Without Borders originated with email consults done on an ad-hoc basis, but our group quickly realized that regularly scheduled, real-time rounds using free videoconferencing services would be of great benefit to the Fondouk patients, and a twice-monthly video-rounds was established. E-mail consults continue as needed in between the rounds.

Several obstacles were recognized and largely remediated in these long-distance rounds, such as the need for high-speed Internet access and allocation of all available bandwidth to the rounds. We had to be cognizant of important local events that might use up available bandwidth. Creativity in acquiring data are important, and forethought and timing is important. Although most recommendations are made during video conferencing, when cases fall outside the purview of general internal medicine or general surgery, Cummings faculty participants then query other veterinary specialists at the University including radiologists, ophthalmologists, neurologists, and surgeons. There is never a charge for these services, rather, Cummings faculty provide consultation on a pro-bono basis.

4. Data Analysis
Cases seen over a period of 1.5 years were organized according to body system and broad causes, such as trauma, infectious, neoplastic, etc. The type of digital information that was given in different types of cases was also delineated. It is important to remember that these cases represent only a small fraction of the cases seen at the Fondouk, and thus cannot be taken as a representation of the most common pathologies of working equids in Morocco, rather, it is a representation of cases that require consultation.

The body system that was most commonly involved was musculoskeletal, followed by gastrointestinal, integument, respiratory, neurologic, hematologic and genitourinary. The most common cause of referral within each category was as follows: musculoskeletal trauma with secondary infection; neurologic-infectious; respiratory-infectious; gastrointestinal-infectious; integument-neoplastic, genitourinary-miscellaneous; hematologic-infectious.

Cases seen, in order of frequency, were as follows:

- **Musculoskeletal**: septic arthritis secondary to trauma including street nail, fracture, abscess, hoof abnormality, wound, abdominal hernia
- **Gastrointestinal**: colitis, impaction, neoplasia, dysphagia
- **Integument**: neoplasia (sarcoïd, melanoma, spindle cell tumor), fungal or bacterial infection, parasitism
- **Respiratory**: iatrogenic pneumonia, bacterial or viral pneumonia, upper airway abnormality
- **Neurologic**: infection, trauma, unknown
- **Hematologic**: babesia, neoplasia
- **Genitourinary**: omphalophlebitis, infectious, neoplasia

The interventions made as a result of telemedicine rounds were divided into four broad categories: Further diagnostics, surgical advice, modification of fluid therapy, or modification of drug treatments, which in turn encompassed recommendations for further imaging, surgical techniques and guidelines, analysis of fluid therapy outcomes, and antibiotic, chemotherapy, and anthelmintic use.

5. Discussion
The vast majority of equids lives in the developing world, and those animals are critical to the traction needs of both rural and urban populations. Few if any specialists are employed at the pro bono hospitals in these regions. Although telemedicine has been used increasingly as a tool to advance the quality of medicine, especially expert consultation, for humans in the developing world it is an underdeveloped resource in veterinary medicine. The authors herein offer their experiences to others wishing to develop veterinary telemedicine for the developing world.

Personal initiative and contacts were important in this case for initiating our veterinary telemedicine project. Two of the authors (J.G.M., one of the founders of the Equitarian Initiative, http://www.equitarianinitiative.org, and G.K.) worked together in person at the Fondouk and first established mutual interest, trust, and enthusiasm for the mission of the Fondouk. This project then extended to Cummings faculty. On the Cummings side, there was both the desire to make a difference to the developing world, and there was a perceived benefit to students given that they were able to participate in rounds and learn through further reading and guided discussions. Students were also able to participate by developing electronic
cases for use by other students. On the Fondouk side, there was the benefit of collegiality and the perceived benefit of acquiring opinions from multiple experts on many varying cases. Above all, there must be an intrinsic, self-motivated desire to continue any such work that is outside the scope of an ordinary working day.

In order for telemedicine, or e-medicine, to be effective, there must be an available, solidly established information communications technology (ICT) infrastructure. This may be as simple as email accessibility or as sophisticated as use of videoconferencing. Lack of bandwidth is a persistent problem that affects real-time telemedicine more than it does transmission of images or written information, which can be sent at times when Internet demand is lower.

Although the healthcare needs of working equids in the developing world have been described as primarily those associated with poor husbandry or routine preventive care, for the nongovernmental organizations that provide the majority of veterinary care for the working equid population in the developing world there is both pressure and merit to tending the needs of the individual animal. This presents a need for intensive care and sophisticated case management. The effectiveness of telemedicine can really only be as good as the information that is delivered; the absence of a strong basic healthcare system is the most important limiting capacity for collaboration with specialists in human medicine in developing countries. The American Fondouk is thus an ideal partner because it has unusually good diagnostic capability and strong veterinary capacity.

In summary, long-distance veterinary medical collaboration, or veterinary telemedicine, provides the benefits of expert consultation for hospitals in the developing world. A sense of collegiality and partnership arises when videoconferencing is pursued regularly, and learning becomes very much a shared enterprise as those of us in the developed countries learn about disease and ingenuity from our partners in the developing world, and indeed, the world grows small when the problems are shared.

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Declaration of Ethics
The Authors declare that they have adhered to the Principles of Veterinary Medical Ethics of the AVMA.

Conflict of Interest
The Authors declare no conflicts of interest.

References and Footnote

a [http://www.americantelemed.org/about-telemedicine/what-is-telemedicine#.U7bJuxbQr20](http://www.americantelemed.org/about-telemedicine/what-is-telemedicine#.U7bJuxbQr20).
1. Introduction
The purpose of this presentation is to provide aspiring equitarians with the material and regulatory requirements for successfully accomplishing an international project to benefit working equid health care. Strong leadership and detailed advance planning are critical.

2. Materials and Methods
The information presented herein represents conclusions drawn from 14 years of trial and error equitarian work in Guatemala, Nicaragua, Honduras, Mexico, Costa Rica, and two distinct regions of Peru. It may not be as applicable in settings outside Latin America. Some of the guidelines simply reflect personal opinion and preference. Each country provides unique challenges, particularly as a new project begins, but these are easier to overcome with the assistance of experienced project organizers.

3. Results
Essential supplies include anthelmintics; ectoparasiticides; tranquilizers; general and local anesthetic agents; anti-inflammatory drugs; injectable and oral antibiotics; topical and ophthalmic medications; a variety of needles, syringes, intravenous catheters, and drip sets; general supplies; gloves; record-keeping forms; and tetanus toxoid. Everything possible (available) should be sourced in the host country (availability varies by country), with exceptions only for donated supplies and prohibitive host country cost (e.g., tetanus toxoid cost in Nicaragua is $5/dose vs. $1.40/dose in the United States). Care should be taken to ensure that supplies both donated and obtained in the host country have not passed their expiration date and that the concentration of the medication is specified. As programs recur, eventually inventory will accumulate in the host country for use in subsequent years. Gloves and suture, in particular, do not stand up well to storage in the tropics, but all other accumulated inventory is useful in case there are import delays because of a failure to obtain permits, permits not honored at the point of entry, or customs impediments.

Permits should be sought for the importation of all supplies brought from the United States, although they may not be attainable in some countries and at some times. It must be recognized that absent permits (and sometimes even with permits), import attempts may fail. The government agency from which the import permits must be sought is the host country’s rough equivalent of the U.S. Department of Agriculture. Examples are Ministerio Agropecuario y Forestal (MAGFOR) in Nicaragua and Servicio Nacional de Sanidad Agraria (SENASA) in Peru and Honduras. Successful application for permits is only possible with the help of diligent and
persistent local contacts, preferably veterinarians. This is important because the government agencies involved may not respond promptly (or at all) to communications by telephone or e-mail. The permits should be sought at least a month in advance of the start of the equitarian work, with the exception of Nicaragua, which will not accept permit applications more than 30 days before the import date. Everything to be imported should be undamaged, unopened, and within the expiration date. The precise quantity and concentration of each import should also be specified. The same information should be included in packing lists that can be presented to customs officials by suitcase or box.

For several reasons, legal export of controlled substances from the United States is not practical and should never be done illegally. Ketamine can be obtained in all Latin American countries. All other controlled substances are not essential, and other options are available for euthanasia, including transrectal laceration of the abdominal aorta, rapid intravenous administration of a saturated solution of magnesium sulfate (Epsom salt) or potassium chloride, and intrathecal injection of lidocaine through the foramen magnum. All three of these methods should only be used following deep general anesthesia with xylazine and ketamine, and all three will also leave the carcass nontoxic to scavenging dogs and wildlife.

Essential equipment components are as follows: basic surgical instruments, including the Equitwister for castration; basic farrier tools; battery-operated dental equipment; oral speculum; molar extractors; and a 400-W inverter to charge equipment batteries from vehicle batteries. All supplies and equipment must be organized and packed to conform to airline size and weight constraints. Rubbermaid’s 24-gallon “Action Packers” are just within the size limits imposed by airlines, are waterproof, and can be modified with drawers and compartments to organize supplies for field deployment. One of the basic challenges of equitarian work is simply keeping equipment and supplies organized, dry, clean, and safe from theft, insects, and rodents.

Logistical details will vary by country and location. In all cases, arranging transportation, housing, communication, advance work in communities, and liaison with host country government agencies will absolutely require one or more local contacts, with whom telephone and e-mail communications are possible. The local contacts should be known personally by the trip leader as a result of a brief pilot trip that precedes the first fully engaged intervention. Ideally, local logistics are best left to collaborating local, nongovernment organizations (NGOs) and universities. Examples of this collaboration working well are the NGO Yanapana Peru and Universidad Nacional de Agricultura in Honduras. Absent such support, the trip leader must take responsibility to arrange rental vehicles, lodging, and meals with the assistance of local contacts. This may involve, for example, finding unused churches and school rooms where participants can be allowed to spend the night on the floor in sleeping bags or in hammocks.

Medical treatment facilities may be distant and rudimentary in many areas where equitarian work is indicated. Illness and injury do occasionally occur, so emergency planning must take place in advance of the project. Participants may need to be hospitalized for fluid therapy for dysentery, contract Dengue fever, or be injured by kicks or vehicle accidents, for example. Therefore, all participants should be required to have medical evacuation insurance policies for the duration of the trip that can be purchased short term and at reasonable costs from companies such as International SOS,1 World Nomads,2 and Medijets.3 Such policies are available from other companies as well. The trip leader should maintain a file on each participant with that information, color copy of the passport picture page, information about ongoing or recurrent medical problems, and emergency contacts at home. A legal liability release form should be signed by each member of the team. Similarly, all participants must consult their physician to determine whether malaria prevention or vaccines are warranted before departure. A management plan should also be discussed for diarrhea prevention and treatment.

The trip leader must assume the responsibility of becoming and remaining informed of possible sources of danger or dysfunction in the host country in the months leading up to the trip. The leader must do whatever is necessary to keep participants safe, many of whom may be traveling outside of developed economies for the first time. On past trips, this has at times not been an issue and has been as simple, for example, as staying off the roads at night; at other times, hiring off-duty police officers as armed police guards was necessary. Participants should register for the Smart Traveler Enrollment Program4 through the U.S. Department of State to receive travel advisory alerts. Occasionally, it will also be prudent to notify the U.S. embassy in the host country of the equitarian team’s presence and itinerary.

4. Discussion
Equitarian work in Latin America is important both for the equine and human populations of the host countries. This form of international community service has many benefits for program participants as well but presents a myriad of organizational challenges. These challenges can effectively be met with a systematic approach and attention to detail. Laying the correct groundwork with participants and the host country in the initial stages of a program will maximize potential for effective longevity and minimize it for disruptive and dangerous error.

Acknowledgments
Declaration of Ethics
The Author declares that he has adhered to the Principles of Veterinary Medical Ethics of the AVMA.
Conflict of Interest

The author has worked in the equitarian context solely as a volunteer and has no relevant financial conflicts.

References
Simplified Castration With the Equitwister

Tracy A. Turner, DVM, MS, DACVS, DACVSMR*; David R. Turoff, DVM; and John C. Haffner, DVM, DABVP (Eq)

The Equitwister is an ideal instrument for use in Equitarian work. The instrument is inexpensive and easy to use. Thus far there have been no complications to castration using the Equitwister. Students have found this an easier technique to master than either emasculators or the Henderson castration device. Authors’ addresses: 16445 70th Street Northeast, Elk River, MN 55330 (Turner); 3281 Luneman Rd., Placerville, CA 95667 (Turoff); and Horse Science Center, Middle Tennessee State University, Murfreesboro, TN 37167 (Haffner); e-mail: turner@unokaequine.com. *Corresponding and presenting author. © 2015 AAEP.

1. Introduction

In delivering health care to working equids, castration is the most common surgical procedure performed. In Equitarian work, castration is typically done on a single day in a community during a yearly visit. Due to the lack of ability to follow up on these patients, it is imperative that reliable techniques with few complications be used. In addition, these techniques are often simultaneously taught to local veterinarians or veterinary students. Ideally, the techniques should be simple, easily repeatable, and use equipment that is affordable.

The most common perioperative complication of castration is hemorrhage. Other post castration complications are swelling, infection, and evisceration. Swelling and infection are directly related to postoperative care, but hemorrhage and evisceration are directly related to the procedure. Interestingly, closed castration techniques have fewer complications than other techniques. However, the size and contents of the vaginal tunics prevent the use of closed castration techniques in some instances. This is usually due to the fact that with large vaginal tunics, emasculators cannot achieve sufficient crush to prevent excess hemorrhage. The Henderson tool castration method is a solution to this problem. The technique uses a clamp that can accommodate the entire cord. The instrument is then attached to a battery-powered drill and the testicle is twisted off. The twisting technique is not new and has been used on swine for years, not only to reduce hemorrhage, but also to reduce the possibility of evisceration (a common problem in swine). The twisting of vessels to stop hemorrhage was the preferred surgical technique for hemostasis until the advent of absorbable suture for ligatures. The Henderson tool has become a popular method to use for equine castrations, particularly in Equitarian work because of its reliability.

Sustainability is an important goal of Equitarian work. In this context, sustainability may be defined as the ability to continue the spectrum of veterinary work after the Equitarians leave. In this case, the local veterinarians or animal health workers should be able to continue to perform excellent quality castrations. This can be difficult for no
other reason than equipment is unaffordable, as good-quality emasculators will cost several hundred dollars. Although cheaper ones can be obtained, it has been the author’s (Turner) experience that cheap surgical tools fail to function properly much more often than expensive instruments made of good materials. In the case of the Henderson technique, the Henderson clamp is expensive, the power drill is expensive, and castrations may be performed in areas without access to recharge batteries, making the tool unusable.

The purpose of this paper is to describe how the Equitarian Initiative and Christian Veterinary Mission developed the Equitwister, the technique for using the instrument, and its successful results.

Instrumentation
The authors developed and tested an instrument that would twist the spermatic cord like the Henderson castration tool but required only manual effort and cost only a few dollars to make. An 18-in., 5/16th stainless steel rod is bent at two right angles so that there is a 9.5-in. shaft, 3.5-in. Crank, and 5-in. handle. A 6-in. piece of the 5/16th-in. steel is bent at an acute angle and welded to the end of the shaft. One-inch PVC is used for the sleeves, which are placed over the shaft and handle that prevent surgical gloves from being entrapped in the instrument as it is turned (Fig. 1). Total cost of materials to make one Equitwister is $4.71 and a couple of hours of time.

2. Materials and Methods
Before performing a castration in the equine patient, a preoperative examination is performed. The scrotum must be palpated (under sedation, if necessary) to confirm the presence of one or both testes as well as to detect any indication of inguinal herniation. The presence or history of inguinal herniation is a vital consideration to help avoid evisceration postoperatively. Evidence of inguinal herniation or the absence of one or both testes requires a different surgical technique. Other preoperative considerations include tetanus prophylaxis, antimicrobial administration, and preoperative administration of nonsteroidal anti-inflammatory drugs.

The authors prefer to use general anesthesia when castrating in the developing countries because surgical exposure is improved. In addition, it carries less risk for the surgeon and patient. Various drugs and combinations of drugs can be used. Drug combination familiarity is essential for proper patient monitoring and safety. In addition, one should use only drugs that are locally obtainable. Most commonly, a combination of xylazine and ketamine hydrochloride is used to induce and maintain anesthesia. After induction, the patient is positioned in lateral recumbency. The upper hindlimb is secured with a rope around the pastern and hock. The limb can be secured in any number of ways to protect the surgeons. After recumbency, an antimicrobial scrub is performed on the scrotum and surrounding area. Local anesthetic (10–20 mL lidocaine hydrochloride) is injected into each spermatic cord, the testicular parenchyma, or both. This reduces the need for additional doses of anesthetics as well as retraction of the cord by the cremaster muscle. Standing over the dorsum of the patient, the surgeon has good access to the surgical site and is in a safe position. The midline of the scrotum is cross clamped with two large hemostats or Carmalt clamps, and a 5-cm diameter section of skin is excised with scissors. Minimal skin bleeders result. Both testes can be exteriorized from this opening.

The scrotal fascia is stripped from one exteriorized testicle using a dry piece of gauze. The fascia is stripped from the spermatic cord as far proximally as possible. The same procedure is performed on the second testicle. After testicular exteriorization and stripping of the spermatic cords, the most ventral testicle’s cord is wrapped between the forks of the sterile Equitwister castrating tool with the testicle preventing the cord from sliding free (Fig. 2).
One hand holds the Equitwister and mild tension is applied to the instrument so that the spermatic cord remains straight. The other hand turns the Equitwister like a crank. As the Equitwister is turning, the spermatic cord will retract into the scrotum as it initially shortens when it begins to twist. Light tension is maintained on the cord to prevent the cord from twisting upon itself. The cord will continue to twist until it fatigues and separates with only minimal tension by the operator, typically in 15–25 turns. The testicle is removed from the castrating instrument, leaving behind a tightly coiled and sealed segment of the closed spermatic cord. The second testicle is removed in the same fashion. The horse is then recovered.

Horses are observed for 1 hour after surgery for potential complications. Further followup is by phone call assessment via local animal health care individuals. Complications were noted that consisted of continued bleeding after recovery, any tissue hanging from the scrotum or excessive swelling during the first 3 days after surgery.

4. Discussion
The authors have successfully castrated more than 100 horses this past year using the described technique without any complications. The average weight of the horses was approximately 300–370 kg and they included a wide range of ages. We have used the technique for standing castration but have not yet tried to use it for removing a retained testicle.

As previously stated, in Equitarian work, followup of horses can be difficult. Therefore, it is imperative to use reliable techniques with as few potential complications as possible. It is also important that the technique be simple so as to teach others, and inexpensive so that the service is sustainable. In addition to the safety of the patient and surgeon, the primary advantage of the Equitwister technique is the perception of significant reduction of intraoperative and postoperative complications. After 100 horses, we have not had a complication. There is minimal, if any, bleeding. Like the Henderson tool, the Equitwister instrument coils and seals the testicular artery and spermatic cord. Clamping the scrotal skin eliminates most scrotal skin bleeder. The sealing of the spermatic cord seems to reduce the possibility of evisceration. The large scrotal opening allows for excellent drainage. Castrated horses are allowed to return to light work the day following surgery, thus reducing postoperative swelling. The Equitwister costs just a few dollars to make compared with hundreds of dollars to purchase a Henderson tool or a good emasculator. More than 50 veterinary students have been trained to use the tool and when asked they have found the tool easier to use than either the emasculator or Henderson tool.

In the authors’ opinion, the slow twist of Equitwister is superior to the power twist of the Henderson tool because the spermatic cord is less likely to double on itself and the slower speed provides a tighter twist. Experience with this instrument in developing countries suggests this may be a superior technique for routine use, and the authors encourage others to try the instrument.

Acknowledgments
Declaration of Ethics
The Authors declare that they have adhered to the Principles of Veterinary Medical Ethics of the AVMA.
Conflict of Interest
The Authors declare no conflicts of interest.

References
More Than One Way to Trim a Hoof in Developing Countries

Stephen E. O’Grady, DVM, MRCVS

1. Introduction
Equitarian Initiative or projects of this nature were designed to target working equids in developing or impoverished countries throughout the world. A working equid could be defined as an animal (horse, mule, or donkey) that performs physical work that provides income for a family and in some cases sustains an entire family. The duties performed by these animals are varied dependent on the location but could range from family transportation, tourism, all aspects of agriculture in poor countries, and industry such as the brick kilns in Egypt and commercial taxi horses in Ethiopia. It is obvious that animals performing physical work require routine hoof care and it is just as obvious that the lack of such care can and will cause immeasurable hoof problems and lameness in this population of working equids. Therefore, the importance of farriery in all these countries must be realized. The approach to improving foot care is difficult as it not only involves care for the animals while there, but instruction of the local populace in the importance of good farriery, and also how to implement it in a very basic manner. The approach to accomplish this will vary from country to country but the general theme will always revolve around teaching.

2. The Approach
Poor countries have not been introduced to good farriery or the benefits of such farriery. Owners generally perform it (if at all) in a primal manner with primitive or makeshift tools (Fig. 1). For one to perform and teach farriery in these countries, the veterinarian must be adept at farriery or have a farrier join the group. With the language barrier that often occurs, even with an interpreter, these people must learn by observing. A 1-day-or-longer farrier course designed for the locals has been rewarding and has yielded long-term benefits (this will be described later). Appropriate farrier tools are important and usually not available in the various projects. We have addressed this in many instances by having farrier supply companies donate shoes, nails, and tools. Lately, we have even asked farriers to donate used tools that could be used by the local farriers while we are there and then left for them to continue what they have been taught.

Finally, a very basic, understandable approach to basic trimming and placing a shoe (when necessary) must be taught in a very limited amount of time. Bringing these three concepts together creates the challenge.
3. The Basic Technique

The author has been involved in Equitarian projects with the Sioux Indians in South Dakota, the Havasupai Indians in Supai at the bottom of the Grand Canyon, and citizens of the Dominican Republic, Costa Rica, Mexico, and Ethiopia. The technique used will be based upon what type of work the animal performs, the amount of work performed, and the surface upon which the animal works. Therefore, a generalized statement can be made that not only applies to working equids, but to all equines, and that is: to remain comfortable, when wear on the bottom of the foot exceeds growth at the coronet, some form of protection is necessary. In this category are equids that do strenuous hauling, tourism, and provide taxi services. All of these equids are asked to perform on hard surfaces. In contrast, there is a large population of horses that are able to exist without shoes. However, the lack of foot care or the lack of knowledge and tools to perform said farriery care, leads to severe hoof distortions. The overgrown horn that causes the hoof distortions often results in abscesses, separations, and/or cracks and places abnormal forces on the digit above the hoof.

The basic technique begins with a very simplified version of the trim. The trim can be further modified depending on whether it is an extremely distorted and overgrown foot or whether it will be a foot that will receive some type of protection. The rudimentary trim used with the working equid is based on proportionality of the foot rather than teaching them how to do a step-by-step trim. For example, the frog is first located, the hoof wall next to the frog is trimmed (by whatever means) until all structures are on the same plane. A line is then drawn across the apex of the frog (or this area is just pointed out) and then the toe is reduced (from the outside wall or the bottom of the foot) until there are approximate proportions on either side of the line or point of the frog. It must be remembered at all times that you are generally teaching an owner or caregiver with perhaps little interest and no farrier skills. I will use Equitarian projects in two totally different countries to expand on this concept.

4. The Dominican Republic

There has always been a multitude of foot problems encountered during Project Samana in the Dominican Republic. Our farriery efforts here have been rewarding as there have been observable positive results over the past few years. In 2009, we decided to do a very basic farrier course for the horse owners and caregivers. We started by having two major farrier product distributors donate shoes and nails. This material was shipped to a container in Florida and sent on to the Dominican Republic to await our visit. We generally set aside 1 day of our schedule during the week to teach this course, which consists of demonstrations in the morning and then hands-on training in the afternoon for the participants. A Dominican veterinary student acts as the interpreter, which is ideal because they understand both the foot anatomy and the farrier terms.

During this course, instruction was given and repeated constantly on how to use the hoof knife, the nippers, and a rasp. With feet that are markedly overgrown, the heels grow tall and the frog recedes down below the hoof wall at the heels. Our approach is always to find, identify, and lightly trim the frog as the frog will always show the amount of hoof wall that has grown forward. Using the nippers and a rasp, the heels are taken down to the point where the hoof wall at the heels and the frog are on the same plane, which makes this section of the foot load sharing. Something as simple as a straight piece of wood is laid across the heel area to demonstrate all structures on the same plane (Fig. 2). Next, the same piece of wood or the edge of a rasp is placed across the foot at the point of the frog (Fig. 3). This shows the student how much excessive hoof wall or toe is present in front of this line. Modestly using the nippers, the hoof wall at the toe is reduced and the entire foot is rasped as level as possible. Dividing the foot into approximate halves
seems to demonstrate how to perform an acceptable trim in a relatively brief period of time.

The application of shoes was the second part of the demonstration. The selection of shoes here is very limited, almost to the point of one size fits all. Many of the lamenesses we saw in shod horses were from misplaced shoes. Shoes were too small for the foot so when shoes were nailed on, the nails were placed well behind the sole wall junction, invading sensitive tissue and causing infection (Fig. 4, A and B). So when applying shoes, I emphasized choosing a shoe that was large enough to cover the surface of the trimmed foot. I was not as emphatic about the fit of the shoe as I was about selecting the appropriate size (Fig. 5, A and B). The other important part of nailing was to teach that the design on the head of the horseshoe nail must be facing toward the frog so the nail would go in the proper direction and exit through the hoof wall when the nail was hammered into the foot. Finally, when the nails were all placed in the foot, the students were taught to give three taps on each nail head when drawing the nails tight so as not to overtighten the nails. In subsequent years, when we returned and watched the same individuals shoeing their horses, every one counted “one, two, three” out loud as they tightened the nails. It is amazing how a few simple fun maneuvers keep the technique fresh in their mind and they adhere to the few important steps laid out in the initial course. It is remarkable how much the quality of hoof care has improved on this island.

5. Ethiopia

Ethiopia is a very poor country and animal suffering is rampant. The people do not have enough to eat let alone the means to feed their horses properly. I traveled to this country on behalf of the Society for the Protection of Animals Abroad (SPANA), a charitable organization based in the United Kingdom. The mission was to spend a week there to improve the foot care by teaching the farriers. The horses provide the sole income for their owners and are used to haul cargo or as taxi horses. This project was a real challenge as the tools and materials used in this country could not be changed (Fig. 1). In this country, pieces of tires are nailed to the horse’s feet for protection instead of steel horseshoes. As little in the methodology or equipment could be changed, I set out as usual to teach farriery principles. Principles were how to evaluate the foot, guidelines for trimming (with their tools) and how to fit (proper size) and place their shoes (tires).1,2 Ten farriers and five technicians (who teach the farriers) from the university attended the farriery seminar, which was held in a classroom at a little school house in the middle of a town called Debere Berhan. There were 2 hours of lectures in the morning, which consisted of mostly pictures but the language was translated very effectively by Dr.
Nigatu Aklilu, an Ethiopian veterinarian who is affiliated with the veterinary school in Addis Abba. After lectures, the group had lunch and then went out into the field to shoe horses.

When being shod, the animal is never taken out of the cart. The owner holds the horse’s foot and the farrier kneels down behind it. The entire foot is trimmed with a hammer and toeing knife. The farriers were taught the bare basics of trimming and how to remove flares from the outer hoof wall using the toeing knife (Fig. 6). Their method of nailing was so different as they place nails in the foot in a vertical direction far from the perimeter of the foot (actually on the inside of the white line); they then slant the nails and drive them partially through the sole and then exit out the hoof wall (Fig. 7). This method of nailing is not always successful, so obviously, foot abscesses are epidemic. Another major complication is that the round carpenter nails used to attach the shoes cannot be imbedded in the outer hoof wall or ‘clinched’. So when the animal trots, the nail sticking out will often strike the opposite limb causing a severe laceration. This was further complicated by the fact that these horses were so malnourished, which caused a very narrow chest, which made the animal more prone to strike the other limb.

By the second day, we started to see results. The farriers would look at the foot, start to trim the heels/remove flares, and cut out shoes that actually fit the foot. The larger pieces of tire actually seemed to help the nailing process and keep the nails in the insensitive horn. The farriers complained that the small shoes were necessary because they had to get so many shoes out of a given tire for what they paid for the tire – so we bought some tires (20 Bir each which is equal to $2) and they were happy to cut out the proper size.
Each day we went to a different taxi station and by the end of the week, I could readily see that we made a difference (Fig. 8, A and B). Two steps here that would help would be some type of funding or outlet for a cheap source of tires and the importation of some type of cheap horseshoe nails (possibly from China) that could be used in the rubber shoes. The head of the horseshoe nail could be imbedded in the tire better and the flat thin shank of the nail could be placed closer against the hoof wall and would not be sticking out.

6. Conclusion
Farriery in developing countries is such an important aspect of not only relieving pain, suffering, and lameness, but also increasing the productive life of working equids. The challenges here are immense because the local culture, education of the caregivers, language, tools, and equipment must be taken into consideration. Teaching the local owners some very simple, understandable principles seems to be the most successful approach to improve farriery. The instruction must be given in a very understandable manner by either a veterinarian who understands farriery or a farrier versed in this type of work and who understands the principles. The last point is to provide some equipment and shoes so what has been taught can be continued. On a personal note, participating in these projects allows one to provide quality veterinary and farriery care to these animals, the personal satisfaction is huge and it is a way of giving back to one’s profession.

Acknowledgments

Declaration of Ethics
The Author declares that he has adhered to the Principles of Veterinary Medical Ethics of the AVMA.

Conflict of Interest
The Author declares no conflicts of interest.

References and Footnote

Deciphering Potential Causes of Poor Body Condition in Working Horses in Nicaragua

Sara Gomez-Ibañez, DVM

A preliminary survey of working horses from four communities in central and southwestern Nicaragua documented high prevalence rates of anemia, internal parasitism, and equine piroplasmosis; equine infectious anemia was found to have a low but variable prevalence. Although no single factor correlated directly with body condition score, the study provided a more complete picture of the health problems facing this population of horses. In the context of Equitarian work, even basic diagnostic testing and epidemiological investigations have great potential to inform clinical decision making and help prioritize allocation of resources. Author’s address: Sustainable Veterinarians International, 10249 6th Street SW, PO Box 749, Kildeer, ND 58640-0749. Author’s current address: 2402 East Washington Road, Washington, NH 03280; e-mail: fulleclipse28@gmail.com. © 2015 AAEP.

1. Introduction
Thin or emaciated body condition is one of the most visible welfare problems of horses used for pulling, plowing, or carrying heavy loads. It is upsetting to observers, and may serve as a trigger for Equitarian intervention. A commonly identified cause is insufficient caloric intake for the amount of work that is required of these animals. In addition to malnutrition, other major contributing factors include infectious diseases, parasitism, and severe dental abnormalities. This paper summarizes a pilot project undertaken to better understand the infectious and parasitic causes of poor body condition among working horses in Nicaragua. The specific objective of this project was to estimate the local prevalence of several regionally important infectious and parasitic conditions, and then compare these findings to physical examination parameters such as body condition score.

This investigation was undertaken after the author had participated in various equitarian projects in Nicaragua and other Central American countries. Frequently, owners would voice nonspecific complaints such as poor appetite, lethargy, and ill thrift. An absence of diagnostic capabilities, coupled with little or no evidence-based knowledge of local diseases made it difficult to offer appropriate treatment to these animals and advice to their owners. Diseases of interest were chosen based on conversations with local veterinarians, reports from other countries,¹,² and the availability of serological testing.

2. Materials and Methods
Cases were selected at random from among the horses presented for routine and preventive veterinary care during the Equitarian Workshop in October 2014. At each of four clinic sites (Granada, Managua, La Paz Centro, and Ometepe) the horses were numbered on admission; every other horse was sampled until the number reached approximately 10% of the caseload. Each animal underwent a full physical examination by one of five United States-
trained veterinarians with the help of Nicaraguan veterinary students. At a minimum, the following information was collected: the horse’s age in years, body condition score (BCS) from 1–9 on the Henneke Scale, temperature (in Fahrenheit), pulse, respiration, mucous membrane color (MM), and estimated numbers of external parasites (ticks) were recorded. Whenever possible, photos of the horses were taken to help ensure that body condition scoring was consistent across the different veterinarians. Blood and fecal samples were taken to be processed or submitted for the following:

- Packed cell volume (PCV)
- Plasma total solids (TS) via refractometer
- Blood smear evaluation
- Quantitative fecal flotation via McMaster chamber
- Equine infectious anemia (EIA) via agar gel immuno-diffusion (AGID)
- Babesia caballi via cELISA with polymerase chain reaction (PCR) followup
- Theileria equi via cELISA with PCR followup

Samples were processed the same day they were collected. Serum was separated and refrigerated, and whole blood was used to make a blood smear and determine PCV and TS. All readings for PCV and TS were performed by the author. Blood smears were later stained and evaluated by the author for presence of hemoparasites. Differential counts were not performed. All serology was performed the following week, at the diagnostic laboratory of the Universidad Nacional de Costa Rica in Heredia, due to lack of diagnostic capabilities in Nicaragua and difficulty in obtaining permits to bring samples back to the United States. Fecal samples were refrigerated during storage and processed by the author using standard technique for quantitative fecal flotation using a McMaster counting chamber.

3. Results

The sample set of 35 horses included 10 mares, 10 stallions and 15 geldings (Table 1). Ages ranged from 1 to 19 years with an average of 8.4 years. Body condition ranged from 1.5 to 5 of 9 on the Henneke scale, with an average of 2.9/9. The average body condition varied slightly by community: Granada averaged 2.9/9, Managua 3/9, La Paz Centro 2.6/9, and Ometepe 3.1/9.

The majority of horses (33/35 or 94%) were classified as anemic with a PCV less than 32%, and the average PCV was 26.6%. The average value of total plasma solids was 7.0 g/dL with a range of 6.4 to 9.3 g/dL. No animals were found to be hypoproteinemic. The subjective classification of mucous membrane color as “pink” or “pale” was a weak predictor of actual PCV. For the 20 horses classified as “pink”, the PCV value ranged from 20 to 37% with an average of 26.8%. For the 13 horses classified as “pale”, the PCV ranged from 15–30% with an average of 26.1%. BCS did not correlate to PCV (Fig. 1). However, the three horses with the highest PCV (all >30%) also had body condition scores of 4/9, which is higher than the average of 2.9/9.

The AGID test for EIA was positive in four of 35 samples, for an overall prevalence rate of 11%. However, three of four positives were found in Granada (38% local prevalence rate) and no positives were recorded in Managua or Ometepe. The average PCV for the four positive horses was 22%, lower than the overall average of 26.6% and the average BCS was also lower than average at 2.4/9.

Evaluation of the blood smears did not reveal any hemoparasites, but various indicators of anemia were frequently observed, such as anisocytosis, marked rouleaux formation, and increased central pallor of red blood cells.

Almost all horses (34/35, or 97%) were positive on the cELISA screening test for one or both of the organisms that cause equine piroplasmosis; coinfections were common. The predominant organism varied by community, as shown in Fig. 2. In Granada, 63% were positive for B. caballi and 100% were positive for T. equi; in Managua, 60% were positive for B. caballi and 80% were positive for T. equi; in La Paz Centro, 83% were positive for B. caballi and 58% were positive for T. equi; and in Ometepe, 100% were positive for B. caballi and 20% were positive for T. equi. When the horses were grouped by cELISA status, there were modest differences in body condition and PCV value. The six animals that tested positive for only T. equi showed a lower-than-average PCV (24.5%) but a higher-than-average BCS (3.1/9); the 13 animals positive for only B. caballi had a higher-than-average PCV (28.5%) and an average BCS (2.8/9); the 15 animals positive for both organisms were close to the general averages at 26.2% and 2.9/9, respectively. The one horse that was negative for both T. equi and B. caballi was also negative for EIA, and yet was still moderately anemic with a PCV of 23% and a BCS of 2.5/9.

Due to financial limitations, only a subset of positive samples were selected for follow-up PCR to investigate what percentage of positive animals either had active piroplasmosis or were serving as reservoirs for the organism. For B. caballi, 13 samples with positive cELISA were also submitted for follow-up PCR, and only one tested positive (7.7%). For T. equi, 12 samples with positive cELISA were submitted for follow-up PCR and seven were found to be positive (58.3%). Of the seven animals with positive cELISA and PCR results, the average BCS was 2.6/9; the average BCS for the five horses that were cELISA positive and PCR negative was 3.6/9.

Fecal flotation revealed strongyle eggs in 24 of 29 samples (83%). Of the 24 samples with strongyles, five samples had less than 500 eggs per gram (EPG) and were classified as low shedders; 14 samples had from 500-1150 EPG and were classified as moderate...
shidders; the remaining five samples had 1200 or greater EPG and were classified as high shedders. No additional testing was performed to distinguish large strongyles (S. vulgaris) from cyathostomes. One sample was positive for both strongyles and pinworms (Oxyuris equi). The distribution of parasite burden is summarized in Fig. 3.

4. Discussion

The purpose of this preliminary study was to investigate which, if any, of several infectious and parasitic conditions might correlate with low body condition score. Unfortunately, the ability to draw conclusions was limited by the small sample size, as well as the uniformly low body condition scores, which made comparisons within the data set difficult. For example, comparisons between animals with BCS >5 or <5 was not possible because no animals had a body condition score greater than 5/9. Similarly, animals that were seronegative and sero-positive for piroplasmosis could not be compared due to the lack of seronegative animals. BCS also did not clearly correlate with either the level of anemia, or the number of strongyle eggs shed by these horses. Due to the small sample size and the preliminary nature of the study, statistical analysis was not performed on the data. In addition to small sample size, major weaknesses of this study included potential variation in body condition scoring as performed by several different veterinarians as well as multiple confounding variables that were not accounted for such as diet and workload.

Two factors did suggest a possible correlation with low BCS: seropositivity for EIA and a positive PCR result for T. equi. A larger number of horses would be needed to confirm these observations, which were based on only four and seven animals, respectively. Although these results cannot be used to draw conclusions, they do suggest future areas of investigation. In the case of EIA, confirming this correlation

### Table 1. Compiled Results

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<th>Age, y</th>
<th>BCS (1–9)</th>
<th>PCV, %</th>
<th>TS, mg/dL</th>
<th>EPG Strongyles</th>
<th>Mucous Membrane</th>
<th>EIA AGID</th>
<th>B. cab cELISA</th>
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*Mixed infection with Strongyles and Oxyuris equi.*
would provide more evidence in favor of enhanced control and surveillance programs (see below the discussion regarding anemia). In the case of *T. equi*, confirming this correlation could in turn generate two different hypotheses: 1) that the active form of the disease causes poor body condition, and/or 2) that the horse’s ability to control *T. equi* parasitemia is dependent upon having adequate overall body condition.

The second hypothesis leads to the topic of nutrition, which was not addressed in this study. Common sense suggests that malnutrition is likely to be the single most significant factor influencing body condition score in this population. Malnutrition may also directly contribute to anemia. Barriers to improving nutrition for these horses include lack of resources, lack of owner knowledge, and the dry-tropical climate. Methods of feeding horses using local, rather than imported feedstuffs, especially during the dry season, is a topic that has great potential to improve the welfare of these animals; it is unfortunately beyond the scope of this paper.

A high incidence of anemia (94%) was demonstrated in all four locations. EIA, whereas only found in 11% of samples, is likely to have significant effect on infected horses, as suggested by the lower-than-average PCV and BCS of the four positive horses. A larger sample size would be required to confirm this correlation. The local variation in EIA prevalence rates should encourage locally specific, evidence-based recommendations. In Granada, which had the highest incidence of EIA in this study, additional testing is essential. If Granada is confirmed to have an EIA prevalence rate greater than 30%, it provides solid evidence justifying the implementation of control measures, which could range from tabanid traps to an enhanced surveillance plan sponsored by the local government. Officially, Nicaraguan policy requires the humane destruction of horses confirmed to be infected, but in practice there is no mandatory testing or enforcement of this rule. The AGID test is available only in Managua, and it is prohibitively expensive for owners of working horses, even if they were motivated to test their
animals. Potential ways to address this problem could include local implementation of vector (tanna) control, or a program to promote and subsidize testing sponsored by the local government. Any successful program would need to include a mechanism to facilitate the replacement or compensation for positive horses so that owners of positive horses do not lose what is often their sole source of income.

Equine piroplasmosis is endemic with 97% of horses testing positive on cELISA for one or both causative organisms. Blood smear evaluation was confirmed to have a very low sensitivity for detecting infection. Follow-up PCR testing confirmed that a positive titer to *T. equi* is more likely to represent active hemoparasitemia than a positive titer to *B. caballi*. This is consistent with previous findings from Brazil and Venezuela.\(^1,2\) In general, the clinical course of *B. caballi* is often less severe than that of *T. equi*\(^3\) although both infections can be fatal. The high prevalence of piroplasmosis found in this study raises the issue of whether to treat the disease when it is suspected. Ometepe, for example, had 100% seroprevalence for *B. caballi*; veterinarians working in that area can confidently recommend improved tick control, and the select use of imidocarb dipropionate may be justified for horses showing acute clinical signs of anemia. The goal in treating these cases would not be to clear the organism, but to improve clinical signs and prevent serious complications, potentially enabling the horse to mount an improved immune response and control the parasitemia.

It is the author’s hope to encourage more Equitarians to perform PCV and blood smear evaluation in the field. Blood smear evaluation was confirmed to have a very low sensitivity for detecting hemoparasites, but it is simple and inexpensive to perform, and can potentially yield a definitive diagnosis if hemoparasites are confirmed. An unexpected observation in this study was the low correlation between the subjective observation of mucous membrane color and the objective measurement of PCV. This should encourage more Equitarians to test for PCV in the field on suspect cases, even if the mucous membranes look pink.

Moderate to high strongyle burdens were found in 66% of horses, but fecal egg counts did not correlate with BCS or PCV. Although this sample size is very small (29 animals), the results are consistent with a similar, larger study from Mexico,\(^3\) which also failed to correlate EPG to BCS or PCV. The high prevalence of strongyles does provide some evidence to justify the administration of anthelmintic drugs to horses in the context of equitarian work, even when fecal results are not immediately available. Given that anthelmintic medications are readily available and widely used in Nicaragua; it is likely that anthelmintic resistance may already be commonplace. In the future it would be beneficial to include follow-up fecal testing whenever possible to monitor for anthelmintic resistance.

Finally, other infectious diseases that are known or suspected to be present in this population of horses include anaplasmosis, ehrlichiosis, vesicular stomatitis, leptospirosis, rabies, West Nile virus, and Venezuelan equine encephalitis, among others. Lack of resources and/or adequate diagnostic testing capabilities prevented these diseases from being included in this study. Additional investigation is warranted, especially considering the zoonotic potential of several of these diseases.

5. Summary

Poor body condition is multifactorial. In the context of Equitarian work, it is almost impossible to separate “uncomplicated” cases of malnutrition from other pathological problems. Although this study failed to correlate body condition score to any single infectious disease, it did succeed in providing a more complete picture of the overall health of this particular population of working horses. Epidemiological investigations can and should be included more frequently in the context of Equitarian work. Even simple investigations have the potential to elevate the standard of veterinary care provided locally, and to better direct resources and intervention efforts.

Acknowledgments

The project was made possible by the support of the organizers of the 2014 Equitarian Workshop, including Dr. Dave Turoff, Dr. Julia Wilson, and Dr. Jay Merriam, as well as all the participants who helped with sample collection and processing. Special thanks to Dr. Kimberly Thabault who coordinated the permits and transportation of samples from Nicaragua to Costa Rica.

Financial support for this work was provided by Sustainable Veterinarians International based in Killdeer, ND.
Declaration of Ethics
The Author declares that she has adhered to the Principles of Veterinary Medical Ethics of the AVMA.

Conflict of Interest
The Author declares no conflicts of interest.

References and Footnotes


"cELISA, VMRD, Inc., Pullman, WA 99163.
"Dip-Quick stain, Jorgensen Laboratories, Loveland, CO 80538.
Creative Fundraising for Working Equids in Haiti

Judy Batker, DVM* and Kelly Crowdis, DVM

Sponsorship is a viable way to raise money for ongoing equitarian projects. Authors’ addresses: Country View Veterinary Service, 1350 S. Fish Hatchery Rd., Oregon, WI 53575 (Batker); Animal Care Center Haiti, Rte. Nat’l 1, #9 Impasse Senat, Bon Repos, Haiti (Crowdis); e-mail: jbatker@frontier.com. *Corresponding and presenting author. © 2015 AAEP.

1. Introduction

Funding is one of the biggest challenges that faces equitarian projects in improving working equid health. An important goal of the Equitarian Initiative is to form relationships and develop projects that are sustainable long term. This means fundraising needs to be an ongoing activity. A fundraiser that creates repetitive donations is ideal and is possible while practicing veterinary medicine and balancing work and family. Veterinarians strive to develop relationships in equitarian projects with local veterinarians, students, and owners. Sponsorship will help donors develop a similar relationship because it is not practical or logistically feasible to have all donors travel to project sites. Donors can become more engaged in the work through interactive fundraising events and ongoing education.

The equitarian project in Haiti focuses on a group of working equids in Milot, which is also the National Historical site of the Citadel (built in 1804 to protect Haiti from invasion). The Citadel is located at almost 3,000 feet above sea level and is accessible only on foot or horseback. In early 2014, before the project began, the working animals in this area received limited veterinary care and no preventative care. Because the government is currently investing in boosting tourism in Haiti, equine owners have become increasingly concerned that tourists will not want to ride their horses because the horses are thin and have poor saddles. The owners embraced the project after seeing positive results. Results included improved body scores and diminished saddle sores. However, impressed with the initial results, we saw the need for ongoing support and education to produce long-term transformation. After assessing the situation and brainstorming, we decided to create a sponsorship program for the working animals. Sponsorship by a monthly donation for sending children to school and providing humanitarian needs is a successful method of fundraising recognized worldwide. In Haiti, there are many well-established programs by large organizations such as Compassion International, local organizations, and churches. This method of fundraising is something both donors and recipients can identify with.

2. Materials and Methods

In the equitarian project in Haiti, sponsorship is the method used for creating a personal relationship among the donor, the working equid, and the owner in a remote location. The donors are mainly clients in our experience. The program has three increasing sponsorship levels: walk, trot, and gallop. Each level represents a dollar amount that identifies what annual sponsorship will provide. For example, the
ing, the body conditions had decreased again. The cold and there is considerably less vegetation growing.

ary 2015, when the air is routinely dry and severely severe. In May, only 20% had saddle sores and 2 on every horse. Seventy percent of the horses in body condition score had increased by a score of 1 to 344. As determined by the Henneke scoring system, the year of work with these horses were dramatic.

nies and distributors. The changes seen in the first generous contributions from pharmaceutical companies, permanent identification of the horses, and supplemental feed. Medical supply costs are offset by generous contributions from pharmaceutical companies and distributors. The changes seen in the first year of work with these horses were dramatic. As determined by the Henneke scoring system, the average body condition score in February 2014 was 2/9. None was above a 5/9. In May 2014, the body condition score had increased by a score of 1 to 2 on every horse. Seventy percent of the horses in February had saddle sores graded as moderate to severe. In May, only 20% had saddle sores and were graded as mild to moderate. In early February 2015, when the air is routinely dry and severely cold and there is considerably less vegetation growing, the body conditions had decreased again.

4. Discussion

There are several benefits to a sponsor-a-horse–type fundraiser apart from the obvious benefit of raising money. It is a practice builder in many ways. Clients ask about and look forward to correspondence about the Haitian horses. Events that draw people in showcase the clinic and its staff and services. Many clients are proud of their association with a project they see as charitable and benevolent, especially a project so genuinely committed to the welfare of horses. Many feel this is a way they can give back to an animal to which they are devoted. It is appealing for people to know where their money is going and to see results. This type of charitable giving is interactive and will help prolong the project’s longevity.

For the veterinarians involved in equitarian work, the personal advantages are tremendous and create a new perspective from which we view practice. Many of the challenges of our profession such as burnout, managing work and family, and waning interest in equine practice can be overcome. Veterinarians who participate in this work regain a passion for their profession and are inspired to providing more community service and education. They also show a renewed interest in learning about new techniques and diseases.

Engaging people who are not involved in the horse industry can also be achieved with this method of fundraising; seeing the tangible impact veterinary care can have on an individual horse is incredibly important. Through education and progress reports, one can learn how the owner and family depend on one animal as their sole source of income. This working equid, in many cases, has multiple uses. Its use can include being a “tourist horse” used for transporting goods to market and children to school. It becomes clear that equine health and family health are deeply intertwined.

One of the unfortunate disadvantages of using a sponsor-a-horse fundraiser is the added time it takes to collect and disburse information and keep records. Although this team approach helps to spread out tasks to each individual at the worksite, it may be difficult to implement in situations where the horse population is less controlled and where different horses may receive veterinary care during each visit.

Another reality that could be seen as a disadvantage is the shortened lifespan of these horses, especially mares. Sponsors may “lose” their horses. However painful, this experience provides yet another educational opportunity on the causes of early death and,
unfortunately, the resulting grief and anguish the horse’s owner’s family must face in its aftermath.

In summary, the sponsor-a-horse method of fundraising is well-suited and helpful for funding equitarian projects long term. Together with fundraising events centered around the veterinary practice, client engagement is strengthened, which can have a positive impact on practice growth.

Acknowledgments
Boehringer Ingleheim, Merial, Merck, Zoetis, and Midwest Vet Supply have all donated to this specific project.

Declaration of Ethics
The Authors declare they have adhered to the Principles of Veterinary Medical Ethics of the AVMA.

Conflict of Interest
The Authors declare no conflicts of interest.

Reference
Ultrasonographic Evaluation of the Coxofemoral Joint

Mary Beth Whitcomb, DVM, MBA, ECVDI (LA Associate)*; and
Betsy Vaughan, DVM

Ultrasound of the coxofemoral joint can be very rewarding given adequate training and experience. Although it remains a challenging structure to evaluate, the use of the “clock” technique should help motivated practitioners improve their comfort level in evaluating the coxofemoral joint. Ultrasound can diagnose many types of coxofemoral abnormalities, although accurate configuration of fractures should not be expected in most cases. Authors’ addresses: Department of Surgical and Radiological Sciences, School of Veterinary Medicine, University of California, Davis, CA 95616; e-mail: mbwhitcomb@ucdavis.edu. *Corresponding and presenting author. © 2015 AAEP.

1. Introduction

Pelvic ultrasound has become more widely accepted since it was introduced in the 1990s to document ilial wing fractures in Thoroughbred racehorses.1–2 Ultrasound has since been used to diagnose many types of pelvic fractures and coxofemoral injuries, including acetabular fractures, subluxation, luxation and osteoarthritis.3–12 Ultrasound can potentially yield a rapid diagnosis stall-side in the ambulatory or hospital setting and is less expensive than nuclear scintigraphy or radiography. Based on strong agreement with the latter, ultrasound has recently been recommended as the initial imaging modality in horses suspected of having a pelvic fracture.8

Pelvic radiography under general anesthesia is generally considered the antemortem gold standard in diagnosing coxofemoral injuries, but it can subject horses to further fracture displacement during anesthetic recovery.13–17 Radiographic techniques in the standing horse have been described but provide limited information in adult horses, especially in regard to the coxofemoral joint.18,19 Standing views, specifically ventrodorsal projections, may not be possible with all radiography equipment, even in the hospital setting.

With appropriate training and experience, an ultrasonographic examination of the ilium, tuber coxae, tuber sacrale, and tuber ischii is relatively straightforward. In contrast, evaluating the coxofemoral joint is generally considered much more challenging, and even intimidating, by equine practitioners. The coxofemoral joint is admittedly the most difficult pelvic structure to evaluate and requires the most skill for image acquisition and interpretation. Equipment availability is becoming less of a limitation. Driven by the desire to perform abdominal ultrasounds and/or sacroiliac injections, increasing numbers of equine veterinarians own the low-frequency (2–5 MHz) curvilinear transducer that is required to evaluate the coxofemoral joint.
The purpose of this article is to describe a unique instructional approach to facilitate ultrasonographic examinations of the coxofemoral joint and to describe the ultrasonographic features of various coxofemoral injuries from a referral hospital with a large imaging caseload. To facilitate and enhance the viewer experience, 3D video simulations will be paired with corresponding ultrasound videoclips or images of the normal and abnormal coxofemoral joint.

2. Ultrasonographic Technique

Indications for ultrasound of the coxofemoral joint and pelvis include horses with severe acute lameness, especially with a known or suspected history of trauma such as a fall, getting a limb caught in a fence or gate, or hearing an audible pop. Perineural anesthesia is often avoided in acute cases. The absence of distal limb swelling or synovial effusion may help to localize the lameness to the upper limb. Palpable or audible crepitus may also direct the exam to the pelvic region; however, crepitus is known to be absent in approximately two-thirds of horses with pelvic fractures. Pelvic asymmetry is a less common finding but is useful to direct the examiner to the pelvis in some horses. Gluteal and quadriceps atrophy may be found but typically is limited to horses with chronic coxofemoral injury. Rectal palpation findings of swelling or asymmetry may be present but are also inconsistent. In horses with less severe lameness, pelvic ultrasound is usually performed after distal limb perineural or intra-articular anesthesia fails to improve the lameness. Ultrasound may also be requested based on positive nuclear scintigraphy findings of increased radiopharmaceutical uptake in horses with moderate to severe lameness.

Ultrasound of the pelvis and coxofemoral joint is possible with alcohol saturation in short-coated or thin horses; however, clipping the hair with #40 blades is necessary in large, overweight, or thick-coated horses. A complete transcutaneous ultrasound exam of the pelvis should include evaluation of the deep structures of the pelvis (iliac wing, ilial body, and coxofemoral joint) with a 2- to 5-MHz curvilinear transducer and superficial structures (tuber sacrale, tuber coxae, tuber ischii, and third trochanter of the femur) with a mid- to high-frequency linear transducer (rectal or tendon probe). Transrectal ultrasound is recommended in all adult horses, regardless of rectal palpation findings. This should include evaluation of the ischium, pubis, and axial surfaces of the acetabulum and ilial body.4–6,10 Transcutaneous evaluation of the coxofemoral joint requires a scanning depth of 15 to 25 cm depending on the size of the horse. The coxofemoral joint is located beyond the scanning depth of a tendon, rectal, or microconvex transducer in adult horses, although imaging is possible with these transducers in neonates. Tips to improve understanding of coxofemoral ultrasound include recalling that the coxofemoral joint is merely a simple ball and socket joint. The use of bony specimens, when available, will help to improve both client and practitioner understanding of ultrasound images. A general description of the ultrasonographic approach to the cranial, craniodorsal, and dorsal joint surfaces will be described, after which the “clock” technique will be presented as a method of obtaining these same views. Although the clock technique is admittedly simplistic, it has been extremely effective in teaching veterinary students and graduate veterinarians to reliably obtain diagnostic images of the coxofemoral joint.

To locate the coxofemoral joint, the path along the ilial body to the joint is first defined by palpating the tuber coxae and greater trochanter of the femur. The transducer is placed to obtain a longitudinal view of the ilia along this path and slid caudally until the articular surface of the acetabulum and femoral head are seen. This will produce a transverse view of the cranial joint margins (Fig. 1). The transducer should be moved more caudally from this location because the greater trochanter will obscure visualization of the joint. The craniodorsal and dorsal surfaces are next evaluated by sliding the transducer slightly dorsally and caudally while simultaneously rotating the transducer in a...
clockwise direction when scanning the right coxofemoral joint and counterclockwise when scanning the left coxofemoral joint. The transducer is directed ventrally to visualize the craniodorsal and dorsal joint surfaces and avoid interference by the greater trochanter of the femur. This imaging sequence is best accomplished through the use of the clock technique.

To employ the clock technique, it is beneficial to actually draw a clock in the gel or on the hair with the center of the clock at the greater trochanter. A finger is adequate to make tracks in the gel or hair rather than actually drawing with ink or another marking product. The clock should be approximately 12 inches in diameter. Clock numbers will then be used for transducer placement to visualize the cranial, craniodorsal, and dorsal surfaces of the joint. To evaluate the right coxofemoral joint (Fig. 2), the transducer is located at approximately 3:00 to visualize the cranial joint margins. To evaluate the craniodorsal joint margins, the transducer is placed at 2:00 and then at 1:00. To evaluate the dorsal joint margins, the transducer is placed at 12:00 and at 11:00 in some horses. Beginners should look back and forth between the ultrasound machine and the horse to pick up and place the transducers during position changes. With experience, imagers should eventually be able to make a continuous sweep to view all joint surfaces, but this should not be attempted until images are reliably obtained with all transducer placements. For the left coxofemoral joint, transducer positions are at 9 to 10:00 for the cranial margins, 10 to 11:00 for the craniodorsal margins, and 11 to 12:00 (to 1:00 in some horses) for the dorsal joint margins.

In normal horses, the acetabular rim and femoral head should be tightly articulated with only a slight incongruity visible at the joint articulation. Beginners are often underwhelmed by the appearance of the joint. Joint effusion is typically not visible in normal horses. Bony surfaces should be smooth. Along the cranial margins, the acetabulum slopes toward the transducer slightly to produce a slight ski-jump appearance. The acetabulum shows a more flattened appearance along its craniodorsal and dorsal margins.

3. Ultrasonographic Abnormalities

Evidence of acetabular rim fractures is the most common ultrasonographic finding in horses evaluated with pelvic ultrasonography at our hospital. Small fragments appear as hyperechoic structures displaced slightly from the underlying parent bone, similar to fracture fragments in other joints. Step defects may also be seen at the joint margins (Fig. 3). Joint effusion may be present, but massive effusion is not expected. Rim fractures can be challenging to detect radiographically as a result of summation—even with ventrodorsal projections—unless the fragment is located at the cranial joint margin. Affected horses often show lameness at the walk but are typically willing to move forward once past the initial injury phase. Horses with lone rim fractures have been known to progressively improve over time to become pasture-sound and even to be ridden at low levels.

Coxofemoral subluxation and luxation resulting from complete or partial disruption of the round ligaments may also be diagnosed with ultrasound. Horses with subluxation can show a relatively normal joint configuration when the affected limb is resting or partially weight-bearing; however, upon full weight-bearing, the femoral head can be seen to displace dorsally (Fig. 4). The femoral head will return to the acetabulum once the horse resumes resting the limb. In horses with complete luxation, the femoral head remains displaced regardless of weight-bearing status. Acetabular rim fractures and severe joint effusion typically accompany both subluxation and luxation. In longstanding cases, severe effusion of the cranioventral recess of the joint is often evident and should not be mistaken for muscle tearing. Affected horses are lame at the walk but seem less lame than complete acetabular fractures and more lame than sole acetabular rim fractures. Gradual to acute deterioration in lameness can be expected; however, some horses have shown an improved comfort level with therapeutic
coxofemoral injections and have remained pasture-comfortable for 1 to 3 years after diagnosis.

Horses with mild osteoarthritis may show slight irregularity of joint margins. Mild effusion may also be seen. Horses with moderate to severe osteoarthritis will show more prominently irregular joint margins and can be difficult to differentiate from horses with acetabular rim fractures. Historical features and clinical presentation are often helpful in differentiating between acute and chronic injury in such cases. Horses with chronic acetabular rim fractures may progress to show evidence of severe osteoarthritis with prominently irregular joint margins throughout all imaging windows. In longstanding cases, it is often challenging to determine whether all ultrasonographic changes are caused by osteoarthritis or whether previous fractures precipitated the degenerative changes.

Complete or comminuted acetabular fractures (Fig. 5) can also be identified but often require longitudinal imaging of the acetabulum to evaluate for discontinuity of acetabular surfaces, including gaps or large step defects that may occur as a result of overriding fragments. Longitudinal views are generally more challenging than transverse views of the acetabulum/joint and are best performed once transverse views are mastered. Although ultrasonographic evidence of fracture is typically apparent in horses with complete or complex acetabular fractures, accurate configuration is nearly impossible to assess ultrasonographically. It should also be mentioned that horses with significantly displaced or overriding ilial body, pubic, and/or ischial fractures may create large step defects in the acetabular region that could be mistaken for acetabular fracture. Horses with complete or comminuted acetabular fractures are often severely painful and reluctant to move. Stall-side exams are often necessary. Prognosis is poor to grave.

Transrectal ultrasound of the axial surface of the acetabulum is useful in helping to identify complete acetabular fractures (acetabular rim fractures will not be visible during transrectal ultrasound). A standard rectal transducer is ideal for axial acetabular imaging, but a microconvex transducer can also be used because its small size allows for easy manipulation. Immediately upon entry into the rectum, the ischium is visualized by directing the transducer ventrally to show its smooth bony surface. The axial surface of the acetabulum is found by following the lateral extent of the ischium cranially until the transducer is located at the level of the coxofemoral joint (as estimated from external landmarks). Alternatively, the axial surface of the acetabulum can be found by sweeping the transducer laterally from an image of the pubis. The pubis is first identified by advancing the transducer over the obturator foramen from the ischium until the convex bony surface of the pubis is seen at approximately mid-forearm’s length. Fractures are identified by the presence of step defects with varying degrees of

Fig. 3. Transducer position to produce a transverse ultrasound image of an acetabular rim fracture in a yearling with a 30-day history of lameness after poorly navigating a gate.

Fig. 4. Ultrasonographic diagnosis of coxofemoral subluxation. A, B, With the horse resting the limb, the femoral head is seen in a relatively normal location within the acetabulum. Acetabular fragments are also visible (arrows). C, D, Upon full weight-bearing, the femoral head is seen to displace dorsally and assume a subluxed position.
gaping. Fracture movement may be noticed with limb movement or shifting of the horse’s weight. In questionable cases, comparison to the contralateral limb should be performed. Callus formation may be also seen in horses with healing fractures. Callus will produce a tufted or somewhat proliferative appearance to the bone. Hematoma formation may be present but is a surprisingly uncommon finding in horses with coxofemoral fracture. The absence of visible or palpable hematomas should not dissuade the examiner from the possibility of fracture.

Proximal femoral fractures are relatively uncommon but can be identified during coxofemoral ultrasound. Capital physeal fractures may be found in foals and are best seen on transverse views of the coxofemoral joint (Fig. 6a). A gap between the femoral head and neck may be visible with varying degrees of displacement. Care should be taken not to overinterpret the appearance of cartilage and physes in the immature horse (Fig. 6B). Comparison to the contralateral limb should be performed to differentiate between fracture and the normal physeal appearance.

4. Results
Sixty-eight horses with ultrasonographic evidence of coxofemoral injury were identified from January 1, 2000 through December 31, 2014. Ages ranged from 2 weeks to 29 years (median age = 11 years).

Fig. 6. A, Capital physeal fracture in a 9-month-old Paint filly. The femoral neck is dorsally displaced relative to the femoral head, which is seen within the acetabulum (A). B, Normal appearance of the coxofemoral joint in a 2-month-old foal showing the normal capital physis and overlying cartilage. IB = ilial body.
There were 10 foals <1-year-old, 10 yearlings, 11 horses from 2 to 5 years, 6 horses from 6 to 10 years, 19 horses from 11 to 15 years, and 15 horses aged 15 years and older. Multiple breeds and uses were represented. Many horses were presented with a history of trauma. All but 6 horses were lame at the walk (Grade 4–5/5, AAEP Lameness Scale).

Primary ultrasound diagnoses included acetabular rim fractures (22), subluxation (15), complete/complex acetabular fracture (7), lone osteoarthritis (7), lone effusion (4), luxation (4), femoral fracture (4), and evidence of joint widening or slight subluxation (2). Three horses had negative ultrasound exams but underwent ultrasound-guided injections based on other positive imaging findings. Nearly half (47%) had corroborative imaging (nuclear scintigraphy [10]; radiography [8]), postmortem findings (11), and/or a positive response to intra-articular coxofemoral anesthesia (6).

Ultrasonographic evidence of acetabular rim fractures was the most frequently identified abnormality during coxofemoral ultrasound and was visible in a total of 38 horses. This included horses with primary acetabular rim fractures and the majority of horses with evidence of subluxation and luxation. Complete or complex acetabular fractures generally showed multiple step defects throughout the joint upon transcutaneous exam. Transrectal ultrasound was helpful to confirm complete fractures via detection of fracture displacement along the axial surface of the acetabulum (Fig. 7). Affected horses were in severe pain, unstable, and reluctant to move. Ultrasound exams were generally performed in the stall. Four horses were euthanized soon after diagnosis, and necropsy confirmed ultrasonographic findings in 3/3 necropsied horses. One horse recovered to pasture soundness. The outcome of the remaining two horses is unknown.

Horses with subluxation and luxation generally presented with a chronic history of lameness of varying degrees at the walk. Affected horses included 4 foals/yearlings and 15 adults aged 6 to 29 years (median = 15 years). Gluteal and quadriceps atrophy was a common feature in many of these horses (Fig. 8). Most showed evidence of severe osteoarthritis, acetabular rim fractures, and severe effusion and synovitis, especially of the cranioventral recess. Two additional horses showed evidence of mild subluxation and joint widening. This was interpreted as joint laxity caused by severe atrophy of the affected limb in one undiagnosed horse that presented for severe leaning and, in another horse, to a large lumbar squamous cell carcinoma mass that interfered with femoral nerve function.

Ultrasonographic evidence of osteoarthritis was identified in a total of 24 horses, including the 7 horses with lone osteoarthritis. Osteoarthritic changes were subjectively graded as mild in 6 horses with focal osteophytes of the femoral head or acetabulum, moderate in 3 horses with multiple small osteophytes, and severe in 15 horses with diffuse proliferative changes visible throughout the coxofemoral joint (Fig. 9). Horses with severe osteoarthritis included horses with subluxation (8), rim fractures (4), luxation (2), and primary osteoarthritis (1).

Effusion and/or synovitis (Fig. 10) was visible in 43 horses and subjectively graded as mild (15), moderate (14), or severe (14). Mild effusion was generally only visible as a small anechoic accumulation along dorsal joint margins. Moderate effusion appeared similarly but with larger amounts of fluid visible dorsally and occasionally ventral to the cranial joint margins. Horses with severe effusion often had large accumulations within the cranioventral recess and showed varying degrees of synovial thickening. A total of 18 horses showed cranioventral effusion.

Nineteen horses underwent ultrasound-guided injections for diagnostic and/or therapeutic purposes. Most injections were performed using a dorsal approach to the joint with a 16-gauge spinal needle with a length of 6 to 8 inches (Fig. 11); however, a cranioventral approach was used in the majority of injected horses with severe cranioventral effusion. A few underwent multiple therapeutic injections to improve comfort level. Injections were generally not anticipated to abate lameness.

Fig. 7. Acetabular fragments (arrows) in 3 horses with coxofemoral lameness. The foal in A showed evidence of subluxation upon limb manipulation. A large fragment is seen in Horse B. The horse in C showed subluxation upon weight-bearing. Acet = acetabulum; F = femur.
5. Discussion
Coxofemoral injury can occur in a wide range of ages, breeds, and uses. Similar to previous reports, injuries were often the result of trauma, such as a fall or becoming cast in a stall. We have found that in the acute injury phase, horses with coxofemoral or other pelvic fractures are often severely lame regardless of the fracture site, and it is difficult to predict fracture location based on the degree of lameness. Many of our horses lacked pelvic asymmetry and/or crepitus, and many were negative on rectal palpation. In horses with positive findings on rectal palpation, palpable changes were often subtle. The absence of these clinical signs should not reduce the suspicion for fracture.

It is important to consider that horses with suspect coxofemoral or pelvic injury and horses with sacroiliac disorders seldom present similarly in the clinical setting. Horses with coxofemoral or pelvic injury typically present with acute-onset severe
lameness rather than mild intermittent lameness, performance or behavioral issues that typify sacroiliac disorders. Evaluation of the sacroiliac region has received much attention in recent years and may overshadow the consideration of pelvic disorders as a cause of lameness. In fact, some horses reported herein presented to our clinic with a previous diagnosis of sacroiliac injury.

Over the past 15 years, ultrasound has become the primary imaging modality in horses suspected of having a pelvic fracture or coxofemoral injury at our hospital. This is consistent with a recent report that compared ultrasound and radiography of the femoro-pelvic region. We previously relied heavily on nuclear scintigraphy to identify areas of increased radiopharmaceutical uptake within the pelvis, and such findings would then be used to direct further imaging, either ultrasound or radiography. We now begin with ultrasound, and if the ultrasonographic findings do not support the clinical suspicion of pelvic or coxofemoral injury, scintigraphy is performed to evaluate for nondisplaced or stress fractures that are unlikely to be apparent ultrasonographically.

Most horses with severe coxofemoral injury were treated with confinement. Options include a tie stall, a tie line in a stall, or loose in a stall. Regardless of confinement type, many horses may eventually lay down and can become cast, especially in a narrow tie stall. Such horses are at risk for further injury and fracture displacement when struggling to rise. The use of a tie line can also restrict a horse’s ability to rise. Many horses with coxofemoral injury at our clinic are therefore managed with stall confinement alone, without additional restriction.

We have found that ultrasonographic reevaluation of the coxofemoral joint is generally unrewarding because acetabular rim fractures usually remain displaced. In general, prognosis for fractures of the coxofemoral joint is difficult to predict in the early stages.
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stages, but many horses with acetabular rim fractures can become quite comfortable and pasture-sound. Many horses with severe lameness in the acute phase of injury will show gradual improvement in their degree of lameness. It can be tempting to base the decision for euthanasia on the initial severity of lameness. Euthanasia should be considered if the degree of pain or lameness becomes unmanageable. In the authors’ experience, this is most often seen in horses with a complete fracture through the acetabulum but may also be seen in horses with proximal femoral fractures. Sacral fracture should also be considered because it can produce severe pain and neurologic signs.

Several horses were managed with ultrasound-guided therapeutic injections. Some treated horses with acetabular rim fractures were able to eventually become sound enough to ride. Injections were typically performed 6 to 12 months after diagnosis. Although horses seldom became completely sound afterward, some owners reported that they were serviceably sound to be ridden at low levels after a long convalescence. Multiple horses with subluxation were also managed with single or repeated therapeutic injections to improve their comfort level while at pasture. Although these horses never became rideable, owners were often pleased with the results, and some horses were maintained for 1 to 3 years after diagnosis. Therapeutic effect was often reduced with each injection.

In summary, ultrasound of the coxofemoral joint can be very rewarding given adequate training and experience. Although it remains a challenging structure to evaluate, the use of the clock technique should help motivated practitioners improve their comfort level in evaluating the coxofemoral joint. Ultrasound can diagnose many types of coxofemoral abnormalities, although accurate configuration of fractures should not be expected in most cases.

Acknowledgments

Declaration of Ethics

The Authors declare that they have adhered to the Principles of Veterinary Medical Ethics of the AVMA.

Conflict of Interest

The Authors declare no conflicts of interest.

References


Alternative Ultrasound-Guided Approach to the Coxofemoral Joint in Horses With Severe Coxofemoral Pathology

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Cranioventral distention occurs in horses with severe chronic coxofemoral injury. Ultrasound-guided access to this recess provides an alternative approach in affected horses that may benefit from diagnostic or therapeutic injections. Authors’ addresses: Department of Surgical & Radiological Sciences, School of Veterinary Medicine, University of California, Davis, CA 95616 (Whitcomb, Vaughan, Katzman); and Animal Imaging, 6112 Riverside Drive, Irving, TX 75039 (Hersman); email: mbwhitcomb@ucdavis.edu. *Corresponding and presenting author. © 2015 AAEP.

1. Introduction
Intra-synovial access to the coxofemoral joint is notoriously challenging. Blind injections rely upon inconsistently palpable landmarks, and ultrasound-guidance requires expertise for accurate needle placement. Aspiration of synovial fluid is recommended to avoid sciatic nerve anesthesia but is inconsistent. Cranioventral synovial distention in horses with coxofemoral disease may provide an alternative injection site.

2. Materials and Methods
Horses with cranioventral distention identified during pelvic ultrasonography from 2009 to 2014 were considered for injection. The cranioventral recess was identified adjacent to the proximal femur and ventral to the cranial joint margins with a low-frequency transducer. Using aseptic technique, spinal needles were placed cranial to the transducer and advanced caudomedially into the recess.

3. Results
Nine injections were performed in six horses for therapeutic (n = 6), diagnostic (n = 1), and post-mortem (n = 2) validation purposes. Except for one foal, all were aged horses (15–29 y) with prominent lameness due to subluxation (n = 3), luxation (n = 2), or osteoarthritis (n = 1). Synovial fluid was retrieved in all cases with one needle placement. Another seven horses had cranioventral distention but were not injected or underwent ultrasound-guided injection using dorsal approaches. All but one had severe coxofemoral pathology.

Research Abstract—for more information, contact the corresponding author

NOTES
4. Discussion

When distended, ultrasound-guided access to the cranioventral recess is straightforward and may reduce extra-synovial placement of injectants. Distention is often accompanied by severe pathology that may respond to therapeutic injections.

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Conflict of Interest

The Authors declare no conflicts of interest.
Associations Between Thoroughbred Yearling Sesamoiditis, Subclinical Ultrasonographic Suspensory Ligament Branch Change, and Subsequent Clinical Injury

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Untrained yearlings with significant sesamoiditis are more likely to have corresponding significant subclinical suspensory ligament branch change (SBC). This combination increases the risk of subsequent clinical suspensory ligament branch injury (SLBI). Authors’ address: Florida Equine Veterinary Associates, 10195 N Hwy 27, Ocala, FL 34482; e-mail: mpvets@hotmail.com. *Corresponding and presenting author. © 2015 AAEP.

1. Introduction
The objective was to report for the first time the prevalence of sesamoiditis and SBC in untrained yearling Thoroughbreds, to determine the significance of any association between the two, and to determine whether the presence of concurrent sesamoiditis and SBC increases the risk of subsequent clinical SLBI.

2. Materials and Methods
Radiographic and ultrasonographic examination of bilateral forelimb proximal sesamoid bone and suspensory ligament branch pairs was performed on yearling Thoroughbred horses at the commencement of their training careers. Subsequently, horses were monitored for clinical signs of SLBI.

3. Results
Fifty horses were eligible, resulting in 200 forelimb sesamoid/suspensory ligament branch pairs. A significant relationship existed between possibly significant (PS) sesamoiditis grades and PS suspensory ligament branch change (P = 0.0001). The odds ratio of sesamoids with PS sesamoiditis also demonstrating concurrent PS SBC was 5.1.

A significant relationship also existed between the concurrent presence of PS sesamoiditis and PS SBC and the subsequent development of clinical signs of SLBI (P = .0001; odds ratio, 11.7; 95% confidence interval, 4.1–33.4).

4. Discussion
The associations identified in this study highlight the importance of ultrasonographic examination of suspensory ligament branches in horses with PS sesamoiditis grades. This information should not only enable more accurate prognostic advice regarding potential SLBI development but also provide opportunities for intervention and prevention of clinical SLBI.

Acknowledgments

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Conflict of Interest
The Authors declare no conflicts of interest.
Diagnosis, Treatment, and Outcome of Hindlimb Proximal Suspensory Desmopathy in Sport Horses: 75 Cases (2008–2014)

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Surgical treatment and extracorporeal shockwave therapy (ESWT) of hindlimb proximal suspensory desmopathy (PSD) have a similar rate of return to previous level of athletic function. Horses treated with ESWT return to their previous level of work sooner. Some horses require multiple treatment modalities and extended rehabilitation. Authors’ addresses: Virginia Equine Imaging, 2716 Landmark School Road, The Plains, VA 20198 (Norvall, Allen, Johns); and Department of Veterinary Biosciences & Diagnostic Imaging and Large Animal Medicine, University of Georgia, College of Veterinary Medicine, Athens, GA 30602-7338 (Giguère, Selberg); e-mail: amynorvalldvm@gmail.com. © 2015 AAEP.

1. Introduction
Hindlimb PSD is a common sport horse problem with reported medical or surgical treatment successes ranging from 41 to 87%. The purpose of this study is to describe the rate of return to athletic function in 75 sport horses with hindlimb PSD treated either surgically, with ESWT, or with a combination of the two modalities.

2. Materials and Methods
Inclusion criteria included: improvement after diagnostic analgesia, ultrasound findings consistent with PSD, treatment with only surgery, a series of three ESWT treatments or a combination of the two modalities, and a similar rehabilitation protocol.

3. Results
Forty-one horses underwent surgery with 24 returning to their previous level of work. Average time to return was 10.1 months. One returned to a lower level.

Thirty-four horses received ESWT with 20 returning to their previous level of work. Average time to return was 7.4 months. Four returned to a lower level.

Fifteen horses remained lame after the primary treatment and were treated with the other modality. Seven returned to their previous level after both treatments.

Thirty-five of 75 horses had a unilateral injury.

4. Discussion
Surgery or ESWT for hindlimb PSD results in similar rates of return to previous level of athletic function. ESWT returns horses to their previous level significantly sooner.

Acknowledgments
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Conflict of Interest
The Authors declare no conflicts of interest.
Proximal Suspensory Desmopathy in Hindlimbs: A Correlative Clinical, Ultrasonographic, Gross Post Mortem and Histological Study

Sue Dyson, MA, VetMB, PhD*; and Maria Jose Pinilla, PDVM

Ultrasonography is reasonably reliable for the detection of proximal suspensory desmopathy (PSD) based on histology as gold standard, but the ability to detect gross adhesions is limited. Authors’ addresses: Centre for Equine Studies (Dyson) and Centre for Preventative Medicine (Pinilla), Animal Health Trust, Lanwades Park, Kentford, Newmarket, Suffolk, CB8 7UU, United Kingdom; e-mail: sue.dyson@aht.org. *Corresponding and presenting author. Current address for Dr. Pinilla: Finn Pathologists, The Veterinary Laboratory, Hoxne Road, Diss, IP21 5TT, United Kingdom. © 2015 AAEP.

1. Introduction
It has been suggested that ultrasonography is unreliable for the detection of hindlimb PSD based on comparison between ultrasonographic and magnetic resonance images.

2. Materials and Methods
Nineteen horses with hindlimb PSD diagnosed based on the response to local anaesthesia and ultrasonography were humanely destroyed. The ultrasonographic abnormalities were graded prospectively as mild, moderate, or severe based on predefined criteria. Thirty-seven lame limbs were examined grossly and 36 suspensory ligaments (SLs) were examined histologically. Images were graded blindly based on predefined criteria (0–3 for each tissue type; 0 = normal, 3 = severe abnormality).

3. Results
Ultrasonographic lesions were graded moderate in 31/38 (81.6%) and severe in 7/38 (18.4%) limbs; in 4/36 (11.1%) limbs adhesion formation between the proximal aspect of the SL and adjacent soft tissues was predicted. Gross post-mortem examination revealed substantial adhesions between the proximal aspect of the SL and adjacent soft tissues in 10/37 (27.0%) limbs; in 10/37 (27.0%) limbs there were adhesions between the body of the SL and the mid plantar aspect of the third metatarsal bone. Histology revealed abnormalities (grades 1–3) of the collagenous tissue in 25/36 (69.4%) limbs, muscle in 35/36 (97.2%) limbs, adipose tissue in 16/36 (44.4%) limbs, and nerves in 23/36 (63.9%) limbs. In 1/36 limbs no abnormality was detected.

4. Discussion
Ultrasonography may predict PSD, but not necessarily adhesion formation.

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Conflict of Interest
The Authors declare no conflicts of interest.
How to Interpret a Distal Limb Magnetic Resonance Imaging Report

Sarah J. Gold, DVM

1. Introduction
Magnetic resonance imaging (MRI) has grown more prevalent as a means to diagnose causes for lameness in the distal limb.\(^1\) Although many resources exist to try to understand the complex images generated by this diagnostic modality, many practitioners rely on reports generated by board-certified radiologists, surgeons, and other veterinarians experienced in this modality for interpretation of the images. These reports can often be complex and difficult to understand for new graduates and practitioners who do not have a great deal of experience with this technology. The purpose of this paper is to clarify what a veterinarian can gain from the magnetic resonance (MR) report, to best correlate that report with the clinical scenario. With a greater understanding of the information provided in the report, clinicians should gain a greater satisfaction with the information provided by the MR examination and be encouraged to incorporate this modality in their lameness diagnostic workup.

2. Materials and Methods
The structure of the MR report varies greatly depending on the facility and the person creating the report. However, all radiology reports should contain patient identifier information, region imaged, technical information about the exam, a list of pertinent findings, and varying degrees of interpretive comments.

Patient Information
The patient information section is self explanatory, and could be as simple as the patient and owner name, or may contain signalment or historical information. The completeness of this section is generally reliant on the history provided by the owner or referring veterinarian. It is important as a referring veterinarian to provide as much information as possible because this generally aids insuring that the correct regions are imaged, and what, to some degree, can be expected to be seen. For example, a young, lightly worked Quarter Horse will have a different looking foot than an Upper Level Warmblood Jumper of middle age, and what might be reasonable to see on the MR image in the seasoned competitor and not considered a cause for lameness could be significant in the younger Quarter Horse.

Study Description
There is typically a section that includes the region of interest or body part that was scanned and can also include a list of obtained sequences (i.e., technical information about the scan). It is important

NOTES
to be familiar with the MR system that the facility elected to perform the study will be using. The standing low-field system has a smaller field of view than the larger high-field systems that require general anesthesia. Therefore, if the MR request is for the “left front foot” for a standing imaging system, only the foot is will be imaged and the pastern and fetlock must be obtained as separate studies (Fig. 1). Each facility also varies in whether comparison views of the contralateral limb will be obtained, so this should be clarified prior to the patient’s arrival.

Although usually brief, this section of the MR report can look rather complicated to the practitioner unfamiliar with MR language. First, there may be an indication of the planes obtained in the study, such as sagittal, transverse, and frontal or dorsal planes (Fig. 2). Multiplanar imaging is important to allow for a three-dimensional (3D) understanding of lesion location. There may also be a list of the sequences obtained, which vary between magnets. For example, a low-field magnet sequence list might include T1-weighted (T1W) gradient echo (GRE), T2-W GRE, proton density weighted (PDW), T2-weighted (T2W) and Short T1-inversion recovery (STIR) fast-spin echo (FSE) scans. The choice of sequences is determined by the radiologist or veterinarian in charge of the MR program and what is important for the general practitioner to understand is that each sequence provides different anatomic and physiologic information. The names of the sequences stem from the principles of MR physics and references exist that fully explain the generation of the scan titles.

In brief, MR images are acquired through the detection of proton resonance; that is, living tissue contains protons (hydrogen nuclei) that oscillate at different frequencies. When these protons are exposed to the magnetic field of the magnet, they align along the axis of the field. A radiofrequency coil is then applied to the region of question, which emits a pulse to change the orientation of the protons to different degrees. Following the pulse, the protons release energy as they return to the axis generated by the magnet. This energy is measured by the coil and transmitted to a software program that translates this signal into an image.

The most common sequences used in musculoskeletal imaging are FSE and gradient echo (GE) sequences, and these are weighted based on the length of the radiofrequency pulse and the time when the radiofrequency is released and detected. This

Fig. 1. T1W sagittal (A) and frontal (B) images of the digit from a low-field standing system. Note the field of view is smaller than in the high-field system foot image (E). In this case, the horse had significant lameness which improved with abaxial sesamoid analgesia. C and D, As the MR images of the foot did not demonstrate significant pathology, the margins of the study were expanded to include the fetlock, where osseous cyst-like lesions in the proximal phalanx and injury to the third metatarsal bone were identified.
weighting is referred to as T1 and T2 weighting. Depending on the particular tissue and the physiologic state of the tissue (inflamed or scarred, for example), the tissue will demonstrate high (white), intermediate (varying shades of gray), or low (black) signal on the MR image. The same tissue will have high or low signal depending on the particular sequence being viewed. STIR or fat-suppressed imaging inverts the high signal generated by fatty tissue as is found in bone marrow, which allows for fluid to be detected in the bone as bone carries a high signal in other sequences.

MR Findings
The MR findings are usually reported in paragraph or list form. This is generally the section that is the most descriptive, referring to the particular structure involved and how it is abnormal. For example, size, shape, and margins of a ligament may be defined as abnormal, or a joint space may be described as having increased fluid content. Although some reports describe the nature of the pathology in terms of the degree of injury, this section can also contain terminology characterizing the MR signal of a structure. Bone inflammation accompanying a fracture line may be reported as a region of increased signal of the bone on STIR imaging, and decreased signal on T1 and T2* W GRE imaging (Fig. 3).

Some reports do list the findings in order of clinical relevance, and some also include reference images or refer to specific image slices. This can be helpful in becoming more familiar with the various pathologies, as well as understanding the 3D anatomy of the structure.

Conclusions
Depending on the consulting radiologist or interpreter, this section may be a summary list of all the MR findings, or a short list of the clinically relevant findings that the interpreter believes to be relevant based on the signalment and provided history.

Interpretive Comments
A radiology report does not necessarily include a statement indicating which pathologies listed on the MR report are clinically relevant. If a pathology is striking enough, a statement is usually made indicating the most significant finding on the study. If a thorough history has been provided, most radiologists do offer comments about what are likely the most significant findings on the MR study. Reports generated by surgeons or veterinarians familiar with their MR systems will typically include treatment recommendations, prognoses, and summaries of the case if the history has been provided.

3. Results
The author has performed MR examinations on more than 1700 patients from July 2005 to March 2015 between two imaging facilities. Greater than 90 percent of these cases were interpreted by radiologists and formal reports were obtained. In the earlier years of MR reporting, the MR reports were primarily a list of findings. This was regarded as somewhat unsatisfactory to some of the veterinarians in charge of the patients because the cause of lameness was not always clear from the reports. In most cases, the MR examinations would be ordered by the referring veterinarian, usually accompanied by specific questions that concerned the origin of lameness. When the reports were received by the veterinarians, dialogues

Fig. 2. Sagittal (A), transverse (B), and frontal (C) views of a flexor surface erosion of the navicular bone in the standing low-field system.
between the radiologist, author, and referring veterinarian would ensue as the question as to what the primary cause of lameness was in a particular case, and in some instances, what the next steps should be in terms of treatment and management. Often, veterinarians would inquire as to how the lesions appeared on the MR examination as well.

Therefore, a more comprehensive MR report was created to address the referring veterinarian’s concerns, which included reference images and allowed the information to be more readily correlated with the clinical scenario (Appendix). As the understanding of the significance of MR findings has evolved, this MR report has been refined to offer more clinical information to the referring veterinarian; namely the most likely cause for lameness. With this information, the veterinarian could more readily correlate the MR findings with the clinical history, and offer a more tailored approach to treatment and management of the patient. This has resulted in a higher degree of satisfaction for the referring veterinarian and horse owner.

4. Discussion
The final imaging report offers the referring veterinarian an exciting opportunity to broaden their knowledge of anatomy and musculoskeletal imaging. MR imaging, just like radiographs, or ultrasound, is highly dependent on understanding anatomy, especially in more than one dimension. Therefore, if one can understand a given pathology in three dimensions, one can also further understand how to find the injury with other modalities in certain cases, which can be very useful for monitoring. The author found it to be most helpful to review the MRIs with a good anatomy reference at hand,4 and the radiology report. Identifying the various pathologies trains the interpreter to start to see the signal patterns that help define the physiology of the structures, which can also help in remembering these pathologies when presented with a similar case, as well as improving client communication. For example, seeing a bone contusion, rather than just reading that a patient has suffered one, is easier to explain to a client than reporting a bone has increased osseous fluid. Knowing exactly where the fluid is can also allow for radiographs to be obtained with attention to the affected area, so for this example, if a lameness becomes worse or fails to improve, one can look for further degenerative bony changes over time.

It can be difficult in many cases to include a definitive statement for a cause for lameness unless the pathology is quite severe for several reasons. If the lameness is multifactorial in origin, or a thorough history was not provided with the imaging request, offering a statement that indicates which finding is most significant is not always appropriate. In cases of horses with longer performance careers, there can be many factors contributing to the lameness and sometimes it takes a conversation between the attending clinician and MR interpreter to determine what the next step for the patient should be. Establishing good communication with the interpreter will also aid in understanding future reports, as once one becomes familiar with the language of a report, it becomes easier to rank the findings.

Perhaps the most important to the practitioner is to relate the MR report to the patient to determine prognosis and treatment plan, and be able to communicate this information to the client. First, the owner typically asks, “How did this happen?” It is difficult to present to such an owner or case of a
middle-aged horse that has been performing well with an acute onset of lameness with a list of findings, many of which could be considered chronic, and not necessarily the cause for lameness. In such cases, it must be clear to the attending doctor precisely what the current cause of lameness is, and how to go about treating it to reach client understanding and compliance. As the attending becomes more familiar with MR diagnoses, and what sort of abnormalities that can be intrinsic to a particular breed or discipline on an MR study but are not necessarily pathologic, it becomes easier to sift through the sometimes overwhelming amount of information generated by an MR examination and focus in on the lesions that are clinically significant.

From an interpretation standpoint, one must remember that the purpose of the MR report is to report on the findings. How those findings correlate with the clinical scenario is a collaborative effort between the referring veterinarian and the MR interpreter. Therefore, if the diagnoses on the MR report do not fit the clinical picture, or there is question of what are the most relevant findings, following up with the MR interpreter is imperative and encouraged. MR cases can be complicated; indeed, if they were straightforward, often the horses would not need to have had the MRI examination in the first place. Therefore, a clinician should not feel discouraged if the MR study results seem confusing, or the answer is not entirely what was expected. Horses are sent for MR examination for many different reasons, but it is important to remember that the goal is definitive diagnosis to come up with an appropriate treatment plan for the patient.

Understanding the location and nature of the pathology noted on the MR examination can allow for monitoring of the pathology by more readily accessible means by the attending practitioner. For example, a patient may have a lesion localized to the fetlock region that is not readily apparent on routine ultrasound and radiographic examination and is referred for MR evaluation (Fig. 4). In this instance, the MR study demonstrates a lesion that is located at the level of the ergot, or a lesion that collapses when the horse is standing. Follow-up ultrasound with attention paid to the location of the lesion can now be performed, and the injury can be monitored by the referring veterinarian, allowing for a more tailored rest and rehabilitation program.

Another example is acute bone contusion. In the acute phases of injury, there may be no obvious radiographic signs. Osseous fluid is readily identified on MR examination as increased signal on STIR or fat-suppressed imaging and if located across a joint space, raises concern that the cartilage could be affected (Fig. 5). Monitoring the affected region with radiographs and ultrasound of the cartilage surface (if accessible) can be performed in the field when the location of the region is understood to monitor for degenerative change of the joint. This also applies to occult or nondisplaced bone fractures, where knowing the precise nature of the fracture line can allow for special radiographic views to identify the injury and monitor the patient for osseous union or periosteal changes.

Lesions of the deep digital flexor tendon in the foot are one of the more frequent diagnoses found on MR examination, and the prognosis and treatment options can vary depending on the degree and size of injury of the lesion as well as the location. The MR report may indicate a mild degree of degenerative injury to the tendon, but this is not necessarily the cause for lameness in the aged sporthorse (Fig. 6). Lesion location and size also becomes signifi-
cant in cases where palmar digital neurectomy is being considered. Understanding the nature of the deep digital flexor pathology is paramount in terms of case selection for this procedure given that certain lesions do not respond well to neurectomy, resulting in residual lameness or early recurrence of lameness post-operatively. 

A frustrating aspect of MR imaging is when there are no demonstrable abnormalities found on the examination. This scenario can certainly result in dissatisfaction and discouragement on the part of the referring clinician and owner. In these cases, it is imperative to re-examine the clinical history and lameness workup. With the current understanding

Fig. 5. A horse was referred for standing MR examination following acute onset severe right fore lameness localized to the foot following a jumping competition. A, Sagittal STIR image of the right fore demonstrates contusion (increased signal) of the navicular bone and the second and third phalanx. B, The left fore foot of the same horse is shown for comparison, which demonstrates normal STIR signal in healthy pedal bones (the bones are uniformly of decreased signal). C, A lateral radiograph taken at the time of injury, within normal limits. D and E, The horse remained lame, and radiographs were taken 6 months later. The radiographs demonstrated periarticular and articular degenerative bone disease and degenerative change of the navicular bone. These findings were confirmed on follow-up MR examination.
of the migration of diagnostic analgesia, it is possible that the region ordered for the MR evaluation did not include potential other areas that could be affected by perineural or intra-articular blocks. In these cases, the margins of the studies must be expanded during MR imaging. Subtle cartilage defects can also be overlooked in certain systems and should be considered in cases where persistent synovitis is present and diagnostic analgesia has been accurately and repeatedly performed in a manner that localizes the lameness to the joint. Lameness without structural injury to the live tissues of the foot can result in a negative MR study (hoof wall or solar pain). In cases where the appropriate region has been imaged, a negative report may be the best result for the patient from a prognostic standpoint, as no major structural lesions were identified, and a relatively short course of rest may be all that is required to return to work. A negative result on MRI may also indicate that the pathologic condition may need to be imaged with another modality such as nuclear scintigraphy. Understanding these potential situations can help manage client expectations before the MR examination is performed so the client understands the value of the examination, regardless of the results.

The cornerstone of lameness evaluation is still the clinical examination; advanced diagnostic imaging
serves to complement a complete history and thorough clinical evaluation. The MR examination is not typically used for screening purposes: the study should be ordered when the lameness has been well localized to a specific anatomic region. In other instances, such as pre-purchase examination, the region to be imaged can be ordered with a specific clinical question in mind. Communication between the MR interpreter, referring veterinarian, and horse owner must be maximized to gain the most from the examination. The primary care clinician must therefore be well versed enough in the distal limb anatomy and pathophysiology of lameness to convey the need for the MR examination, in the same manner that radiographs or ultrasound are offered. Upon receipt of the report, the referring veterinarian must then decide whether they can determine the most significant pathology (or pathologies in multi-factorial lameness cases), and if it agrees with the clinical presentation to reach a final diagnosis and treatment plan. With increasing familiarity of the language of the MR report, this imaging modality becomes an exciting tool in the evaluation of lameness.

Acknowledgments

Declaration of Ethics
The Author declares that she has adhered to the Principles of Veterinary Medical Ethics of the AVMA.

Conflict of Interest
The Author declares no conflicts of interest.

Appendix. Sample MRI Report

<table>
<thead>
<tr>
<th>Signalment</th>
<th>2003 WB Hunter Gelding</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pertinent history</td>
<td>As per RDVM. Acute onset RF lameness after spooking in a lesson a few weeks ago. RF Grade 2+/5, worse on circle right. Initially blocked to ASNB, on recheck blocked to PDNB.</td>
</tr>
<tr>
<td>Imaging evaluation</td>
<td>MRI, right front foot and pastern</td>
</tr>
<tr>
<td>Radiologist</td>
<td>NAME</td>
</tr>
<tr>
<td>Impressions</td>
<td>● The cause for acute onset right fore lameness is related to extensive marked injury to the deep digital flexor tendon. There is associated navicular bursitis which may be contributing to the current lameness presentation. The navicular bone abnormalities are not associated with significant fluid in the bone, and therefore may only be making a mild contribution to the current clinical presentation.</td>
</tr>
<tr>
<td>MR findings</td>
<td>● Recheck MR examination is recommended in approximately 4–6 months to monitor the deep digital flexor tendon, with the exact timeframe depending on the case progression and treatment plan as prescribed by the RDVM. Ultrasound guided intra-lesional regenerative therapies could be considered in this case, with stall rest immediately following the procedure. Within a few weeks post-procedure, a controlled walking program should be prescribed to prevent further adhesion formation of the soft tissues of the navicular apparatus. Soundness re-evaluations should be performed at 30 day intervals to insure the lameness has not become substantially worse, and is continuing on an appropriate course of improvement.</td>
</tr>
<tr>
<td>Conclusions</td>
<td>● This injury can take up to one year to heal and carries a guarded prognosis for long term soundness at high levels of jumping work. It is our experience that the majority of horses with this sort of injury return to work for about a year before lameness returns if they continue to jump at moderate to significant levels, with some horses performing longer, and some not returning to a level of serviceable soundness for athletic work.</td>
</tr>
<tr>
<td></td>
<td>● There is distal margin fragmentation of the navicular bone with defects in the distal medial and distal lateral margins (7–9 mm in width). There is injury to the medial lobe of the deep digital flexor tendon beginning at the mid aspect of the proximal phalanx extending to the level of the navicular bone. This injury is characterized by focal core lesion measuring between 3 and 5 mm in diameter that becomes a dorsal margin tear at the level the navicular bone. Distal to the navicular bone, there is moderate degenerative injury in the deep digital flexor tendon. There is moderate chronic navicular bursitis characterized primarily by synovial membrane thickening and synovial proliferation. The synovial proliferation extends to the dorsal margin of the deep digital flexor tendon and may represent adhesion formation. There are moderately enlarged synovial invaginations in the distal aspect of the navicular bone. There is mild to moderate thickening and scarring of the collateral sesamoidean ligament. The appearance of the lateral collateral ligament of the distal interphalangeal joint is most consistent with magic angle effect. However, degenerative injury cannot be ruled out for this appearance.</td>
</tr>
<tr>
<td></td>
<td>● Distal margin fragmentation and moderately enlarged synovial invaginations, navicular bone, right fore</td>
</tr>
<tr>
<td></td>
<td>● Extensive deep digital flexor tendon injury, right fore</td>
</tr>
<tr>
<td></td>
<td>● Moderate chronic navicular bursitis, right fore</td>
</tr>
<tr>
<td></td>
<td>● Mild to moderate thickening and scarring, collateral sesamoidean ligament, right fore</td>
</tr>
<tr>
<td></td>
<td>● Magic angle effect, lateral collateral ligament of the DIP joint, right fore</td>
</tr>
</tbody>
</table>
References and Footnote

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"EQ2, Hallmarq Veterinary Imaging Inc, Acton, MA 01720.
Pathologic Changes Found With Magnetic Resonance Imaging of the Distal Tarsus in 43 Horses

Myra F. Barrett, DVM, MS, DAVCR*; Kurt T. Selberg, DVM, MS, DACVR; Sherry Johnson, DVM; and Jake Hersman, DVM

Multiple types of osseous and soft tissue injuries can affect the distal tarsus and lead to lameness. Many horses with pain localized to the proximal suspensory ligament had more severe changes in the distal tarsus. Magnetic resonance imaging (MRI) helps to characterize these lesions. Authors’ addresses: Department of Environmental and Radiological Health Sciences (Barrett), Department of Clinical Sciences (Johnson), Colorado State University, Fort Collins, CO 80523; Department of Veterinary Biosciences and Diagnostic Imaging, University of Georgia, Athens, GA 30605 (Selberg); and Animal Imaging, Irving, TX 75039 (Hersman); e-mail: barrettdvm@gmail.com. *Corresponding and presenting author. © 2015 AAEP.

1. Introduction
Pathologic changes of the distal tarsus commonly cause lameness. Due to superimposition and the joint complexity, radiographs are thought to underestimate these changes. MRI allows for more complete assessment of the joint and identification of lesions that may otherwise go undetected.

2. Materials and Methods
High-field MRIs of the distal tarsus and proximal metatarsus of 57 limbs of 43 horses were retrospectively evaluated by two board-certified veterinary radiologists. Pathologic changes were characterized and graded by degree of severity. The patients’ breed, age, and results of diagnostic analgesia were also recorded.

3. Results
Osteoarthritic changes of the centrodistal and tar-sometarsal joints were the most common findings. Other findings included bone marrow lesions, degenerative changes of the small cuboidal bones, subchondral cystic lesions, and intertarsal desmopathy. Limbs that responded to analgesia of the proximal suspensory ligament (35.7%) had more severe changes in the distal tarsus.

4. Discussion
A range of pathologic changes can occur in the distal tarsus that result in lameness. The use of MRI has revealed types of lesions that can be difficult to impossible to identify with radiographs. Understanding the range of pathologic processes in the tarsus can help the practitioner identify when advanced imaging may be warranted in cases refractory to standard treatment protocols.

Acknowledgments

Declaration of Ethics
The Authors declare that they have adhered to the Principles of Veterinary Medical Ethics of the AVMA.

Conflict of Interest
The Authors declare no conflicts of interest.
Stifle Injuries Treated With Regenerative Therapy With or Without Arthroscopic Surgery Fare Better Than With Arthroscopic Surgery Alone: A Study of 98 Horses

F. Ross Rich, DVM, PA-C†; Elaine M. Carpenter, DVM, MS, DACVS; and Martin Vidal, BVSc, MS, PhD, DACVS, DACVSMR*

Regenerative medicine with or without arthroscopic surgery for stifle injuries provides better results than arthroscopy alone. Authors’ address: Cave Creek Equine Surgical and Diagnostic Imaging Center, 34705 N 14th Street, Phoenix, AZ 85086; e-mail: frossrich@icloud.com. *Corresponding author; †presenting author. © 2015 AAEP.

1. Introduction
Stifle injuries are common in performance horses and can have long recovery times that end the career of an equine athlete. To date there are no reports that describe the use of adipose tissue–derived stromal vascular fraction and interleukin-1 receptor antagonist protein for equine stifle injuries.

2. Materials and Methods
Medical records of 98 horses (189 stifle injuries) were evaluated. These horses were treated with regenerative medicine (stromal vascular fraction and interleukin-1 receptor antagonist protein) (group 1, n = 21 horses) or arthroscopy and regenerative medicine (group 2, n = 38 horses) or arthroscopy (group 3, n = 39 horses). All stifle injuries were diagnosed through lameness examination, diagnostic anesthesia, and radiographs. All horses completed a standardized, 6-month rehabilitation program. Horses that returned to full work (RFW) for greater than 1 year without recurrent lameness were considered a success.

3. Results and Discussion
Horses in group 1, 2, and 3 RFW at prior or higher level of performance for >1 year at 66.7, 68.4, and 59%, respectively. Approximately 26 to 28% of horses RFW at a lower level, and 5% of horses in groups 1 and 2, and 13% of horses in group 3 were retired. Preoperative use of corticosteroids and lameness score negatively affected return to prior level of performance. Lameness and chondromalacia reduced the chance of healing within 270 days. Treatment with regenerative therapy and arthroscopy or just regenerative therapy improved the chance of healing in less than 270 days compared with arthroscopy alone.

Acknowledgments

Declaration of Ethics
The Authors declare that they have adhered to the Principles of Veterinary Medical Ethics of the AVMA.

Conflict of Interest
The Authors declare no conflicts of interest.
How to Perform a Facilitated Ankylosis of the Carpometacarpal Joint for Treatment of Carpometacarpal Osteoarthritis

Spencer M. Barber, DVM, DACVS*; Hayley M. Lang, DVM, DACVS; Erinn T. Hillberry, DVM; Angela MacKay, DVM; and Luca Panizzi, Med Vet, Med Vet Sc, DACVS

1. Introduction
Carpometacarpal osteoarthritis (CMA OA) was a term first used to describe a specific clinical condition in horses with osteoarthritis (OA) of the carpometacarpal joint (CMC jt), which was considered a condition exclusively of Arabian horses. However, a subsequent case series was published that also included Quarter Horses and Thoroughbreds. In fact, CMC OA may indeed not be a new condition as older textbooks describe horses with “high medial splints” that develop lameness and have OA of the CMC jt. An additional case series identified CMA OA in several breeds, with the highest incidence in Arabians and Quarter Horses.

CMA OA is a lameness that occurs predominately in older horses, and normally has a characteristic swelling on the medial aspect of the carpus (Fig. 1) over the metacarpal 2 (MC2)/second carpal bone (C2) articulation (MC2/C2). Radiographic changes consist of periosteal new bone formation, primarily on MC2, at the MC2/C2 articulation (Fig. 2A), narrowing of the MC2/C2 joint space, and often subchondral lysis of MC2 and C2 (Fig. 2B). These horses normally are quite lame (grade 3–4), and the severity is progressive and debilitating. Lameness can initially be insidious; however, rapid progression to a crippling lameness has been seen. Although the CMC jt is a low motion joint, spontaneous fusion seems to be rare, and horses left untreated or treated with conservative methods had a poor prognosis and most were euthanized for humane reasons within 4 years of diagnosis.

Because the CMC jt is a low motion joint, similar to the distal tarsal joints, bony fusion should be possible without affecting a horse’s normal gait. Bony fusion of the CMC jt has been reported after a facilitated ankylosis technique was used that included inserting a drill bit into the joint space, and fanning of the drill bit horizontally and vertically to promote cartilage and subchondral bone damage in
an attempt to stimulate bony fusion. All horses had reduced severity of lameness, 83% were considered sound, 67% returned to their original activity, and all owners considered the treatment successful. The technique was very successful; however, the case numbers reported were low, and because the amount fanning with the drill bit could not be quantitated and requires judgment by the surgeon, this technique might not be as consistently performed and successful for other surgeons. Also, fracture of C2 and middle carpal joint subluxation have been reported. A less-invasive three-drill tract technique was compared with the fanning technique in an ex-vivo study and their results suggested it may be superior to the fanning technique. But the three-drill technique was subsequently shown to be less successful in producing ankylosis when used in research horses, with only 33% of horses developing ankylosis and considered free of lameness. When the three-drill-tract technique was used to treat clinical cases of CMC OA it was less successful than the fanning technique because only 17% were considered lameness free, and 50% of owners considered the treatment a failure.

Fortunately, CMA OA is relatively uncommon because it is a crippling condition that requires joint fusion. Currently, a facilitated ankylosis using the fanning technique is the most successful reported treatment. The purpose of this paper is to familiarize practitioners with how to perform a facilitated ankylosis using the fanning technique.

2. Materials and Methods

Case Selection

Because of the severe progressive nature of CMA OA, the lack of spontaneous ankylosis, and the very poor prognosis with no or conservative treatment, the authors recommend surgical treatment in most cases. Horses with severe varus deformity, rare in some clinical case, likely are poor candidates as are those with marked degenerative radiographic changes of the middle carpal joint.

Fig. 1. A horse with unilateral (right) CMC OA and the typical carpal swelling. Compare the appearance of the affected limb (double arrows) with that of the normal limb (single arrow).

Fig. 2. Radiographic views of changes seen with CMC OA. A, Dorsopalmar view. Note the proliferative new bone primarily of proximal MC2 but also of C2 (medially), and narrowing of the MC2/C2 articulation. B, Dorso-medial-palmarolateral (DM-PL) oblique view. Note the slight proliferative new bone on proximal MC2, the very narrow MC2/C2 articulation and subchondral bone lysis of both MC2 and C2.
Preoperative Medication
The authors administered preoperative antibiotics (22,000 U/kg sodium penicillin\textsuperscript{b} intravenously) and phenylbutazone\textsuperscript{c} (4.4 mg/kg intravenously).

Restraint
All horses are operated under a general anesthetic for accurate drill placement, and fanning without risk of drill bit breakage.

Equipment Needed
A pneumatic drill and basic Association for the Study of Internal Fixation (ASIF) equipment is required. Because intraoperative imaging is required for accurate drill alignment within the joint, and to determine the depth of drill insertion, either intraoperative radiographs or fluoroscopy are required.

Positioning
The horse is anesthetized, placed in lateral recumbency with the medial aspect of the limb upper most, and the carpus clipped, prepped, and draped for orthopedic surgery.

Sites of Drill Bit Insertion
Usually the drill bit is inserted into the CMC jt at three sites, but as many as five sites have been described.\textsuperscript{5} The three common sites are: 1) medial-lateral at the C2/MC2 articulation, 2) dorsomedial-palmarolateral (DM-PL) between third carpal bone (C3) and third metacarpal (MC3), and 3) dorsolateral-palmaromedial (DL-PM) between C3 and MC3 (Fig. 3A). If the radiographic changes show severe narrowing of the MC2/MC2 articulation, and marked modeling of the subchondral bone of C2 and MC2, this portion of the joint may fuse spontaneously without drilling and may not be drilled at the surgeon’s discretion (Fig. 2B). The other two sites are always drilled because radiographic evidence of degenerative changes here are minimal, and cartilage and subchondral bone damage is required to promote ankylosis. Because the craniomedial swelling characteristic of CMC OA makes identification of the CMC jt by palpation difficult, the horse is positioned in lateral recumbency with the medial side upper most.

Order of Drilling Sites
The authors prefer to drill the MC2/C2 articulation first because it is the hardest site to identify the joint space, followed by the DM-PL site, and then the DL-PM site (Fig. 3A). To drill the DL-PM site, the surgical table is elevated to get better access to the lateral aspect of the carpus. Although drilling this last site is a bit awkward with the horse in lateral recumbency we prefer it to dorsal recumbency because we feel we are better oriented to the anatomic landmarks for all three drill sites.

Drilling Technique/Identification of Joint Space
At all three sites the technique followed is the same. Three needles (18 or 20 gauge) are used to identify the joint space and proper angle for drill bit insertion. They are placed parallel to each other in a vertical line, with the middle needle placed over the estimated joint space, and all needles perpendicular to CMC joint articulation. A fluoroscopic image or digital radiograph is taken perpendicular to the needles to check their placement and angulation to the joint. Proper placement of the needles on the first attempt is difficult medially because the soft tissue and bony swelling make palpation of normal bony landmarks difficult (Fig. 4). Repositioning of the needles or alterations of their angle relative to the joint may be required and additional imaging views taken. When a needle identifies the location of the

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\textsuperscript{a} sodium penicillin
\textsuperscript{b} phenylbutazone
\textsuperscript{c} intraoperatively
joint space and is positioned with the proper angulation, the other needles are removed.

Drilling Technique/Insertion of the Drill Bit
A stab through the soft tissues down to the bone is made with a #10 scalpel blade immediately adjacent to and at the same angle as the needle. The authors usually use a 5.5-mm drill bit. The drill bit, with its tissue protector, is inserted into the stab wound and inserted approximately 1 cm into the joint space with a pneumatic drill. A radiograph or fluoroscopic image is taken to verify proper positioning of the drill bit between and parallel to the articular surfaces (Fig. 5). If adjustment of position or angle is not required, the drill is inserted to a depth of approximately 25–30 mm into the joint, the position and depth checked by imaging (Fig. 6).

Drilling Technique/Fanning of the Drill Bit
After determining that the drill bit is properly placed, it is fanned multiple times in a horizontal plane. At the MC2/C2 drill site, the bit is moved cranially and caudally to fan an area with an ~30° arc, whereas it is fanned approximately 50° at the other two sites (Fig. 3B). Then the drill bit is fanned vertically, but because of the subchondral bone the drill bit movement is much less, approximately 5–10°. It is important not to press too hard on the drill as it could result in possible breakage of other structures.
NEW INNOVATIONS IN THE DIAGNOSIS AND TREATMENT OF LAMENESS

the drill bit or unequal damage to the articular surfaces,7 with the latter resulting in greater destruction on the upper vs the lower articular surface.7 The amount of cartilage and subchondral bone damage created cannot be determined but is important for producing fusion, so the authors are quite aggressive with this fanning technique.

Closure/Bandaging
After all sites are drilled, the stab incisions of the skin are sutured with nonabsorbable sutures in either a simple interrupted or cruciate pattern. A nonadhesive dressing is placed over the stab incisions and a light wrap applied over the carpus.

Postoperative Radiographs
The authors routinely take postoperative radiographs prior to discharge from the hospital to serve as a baseline in case we have a chance to get followup radiographs. Often the immediate postoperative views show little change, with only some drill holes or tracts being visible.

Postoperative Care
Crystalline sodium penicillinb (22,000 IU/kg) is continued every 6 hours for 24 hours. Horses often are not much lamer after surgery than before. Horses with advanced CMC OA are usually quite sore prior to surgery and require oral phenylbutazone postoperatively for an extended time period to make them comfortable. It is administered intermittently (e.g., 4 days on, 4 days off) at a dosage adjusted to the needs of the horse (2–4 mg/kg daily) to minimize the risk of toxicity. Horses with early CMC OA that have less severe clinical signs require little postoperative phenylbutazone initially, but sometimes they become lamer after a couple months as the OA gets worse as a result of the drilling, prior to bony fusion occurring. The surgical sites are kept covered with a dressing for 14 days and then the sutures are removed. The horse’s activity is markedly restricted for 2–3 months by either confinement to a box stall or more commonly a very small corral. By 3 months there is usually significant clinical improvement and confinement is limited to a larger corral for another 3 months, at which time clinical improvement is usually marked and the horse can either be left in the larger corral, turned out to pasture, or returned to light work, depending on the degree of improvement.

3. Results
The authors have treated 20 horses (23 limbs) with this fanning technique, which includes those previously reported.5 Although follow-up clinical and radiographic evaluation would be ideal, this is often not possible due to problems of client noncompliance, expense, and distances required for the client to travel. Our evaluations were predominantly based on a telephone questionnaire of the client’s assessment of their horse. Although this is not ideal clients can certainly tell a horse that is grade 3–4 lame and unusable from one that has little or no visible lameness that can return to work. So we believe our evaluations are valuable.

Of the 20 horses treated, one was lost to followup, one developed a septic joint immediately postoperatively and was euthanized, one was improved dramatically but had only been operated 3 months previously, and one only had a followup of 1 month. So long-term followup was available on 16 horses. Of these 16 horses, one horse (6.25%) was a failure because it fractured C2 and subluxated its middle carpal joint and was euthanized. Of the remaining 15 horses, the severity of their lameness was reduced in all of them (93.75%), and 11 (68.75%) were considered “sound” by the owners. Of the four horses that were improved but still lame two had a lameness barely noticeable with hard work, and two had a lameness easily noticeable to the owner regardless of gait, but less severe than prior to surgery. The surgery was deemed “successful” by 13 of the 16 owners (81.25%)

4. Discussion
The goal of a facilitated ankylosis is to achieve bone fusion between the articular surfaces. With a drilling technique this is dependent on the destruction of some of the articular cartilage and subchondral bone and the production of bridging bone during healing. Bony fusion over the entire articular surface is not possible, and not required for success.

However, the percentage of the joint surface that must be damaged to achieve fusion is unknown. Earlier papers regarding facilitated ankylosis of the distal tarsal joints suggested that less than 60%9 was needed; however, later work with a three-drill tract technique in distal tarsal joints reported good success with less than 60%.10 An in-vitro study evaluating the fanning technique and the three-drill technique in the CMC jt reported damage to only approximately 25–33% of the total articular cartilage surface.7 They did not find a significant difference between the fanning technique performed with a 4.5-mm drill bit and a three-drill tract technique performed with a 5.5-mm drill bit. The fanning technique has been proven successful in treating clinical cases of CMC OA,5 although the three-drill tract technique has not been as successful in use in either research horses8 or for treatment of cases of CMC OA.5 With the fanning technique there is a tendency for the surgeon to press down on the drill directing the bit proximally, resulting in less damage to the distal articular surface.7 Given that damage to both surfaces are required to get bony fusion, it is important to avoid this during surgery. The authors prefer a 5.5-mm drill because its great width over a 4.5-mm drill bit produces greater surface damage,7 and we fan quite aggressively to maximize damage to the articular surface. However, we try to avoid damage to the caudal aspect of the articular surfaces and to the caudal soft tissues of
the joint by limiting the depth of drilling. We have previously reported fracture of C2 and subluxation of the middle carpal joint, which might be associated with inappropriate drilling. So the depth the drill bit is inserted into the joint is adjusted based on the size of the horse’s articular surface.

Although we have successfully operated both limbs of a bilateral case at one time, we prefer to stage the procedures 3–6 months apart. Postoperative pain is variable, and although often not marked, staging the procedures increases the likelihood that postoperative pain is easily managed. Some horses with early CMC OA and minimal lameness are initially comfortable postoperatively but become more uncomfortable before eventually the lameness resolves. We suspect the drilling causes greater arthritic changes and pain before bony fusion can occur. Although the carpometacarpal and middle carpal joint normally communicate postoperatively, OA of the middle carpal joint secondary to the release of inflammatory mediators from the drilling of the CMC joint does not seem to be a problem.

The authors’ success with this procedure has been very comparable with that reported for facilitated ankylosis of other joints. To maximize success we recommend 1) staging surgical treatment of bilateral cases, 2) warning owners that horses with early CMC OA and mild lameness sometimes will get worse before getting better, 3) using a 5.5-mm drill bit, 4) making sure there is accurate placement of the drill bit within the joint space, 5) fanning aggressively in a horizontal direction, 6) not pushing down excessively on the drill when fanning horizontally, 7) avoiding damage to the caudal joint surface and soft tissues, and 8) not operating on horses with marked OA of the middle carpal joint.

The success rate with the fanning technique has been shown to be reasonable, and we consider it the treatment of choice for CMC OA.

Acknowledgments

Declaration of Ethics

The Authors declare that they have adhered to the Principles of Veterinary Medical Ethics of the AVMA.

Conflict of Interest

There were no funding sources for this work: it involved clinical cases treated at a university vet school. There are no financial interests or potential conflicts for any of the author. No materials were supplied from companies for this project. No one other than the authors reviewed this submission to assure full disclosure.

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Buzone Injectable, Vetaquinol, Lavaltrie, QB J5T 3S5, Canada.
Butequine, Bioniche, Belleville, ON K8N 5J2, Canada.
Clinical Study Evaluating the Success of Injection of the Distal Hock Joints in the Horse

Kathryn A. Seabaugh, DVM, MS, DACVS, DAVCSMR*; Kurt T. Selberg, DVM, MS, DACVR; P.O. Eric Mueller, DVM, PhD, DACVS; Randall B. Eggleston, DVM, DACVS; John F. Peroni, DVM, MS, DACVS; Kevin M. Claunch, DVM, DACVS; Harry J. Markwell, BVSc; and Gary M. Baxter, VMD, MS, DACVS

The accuracy of injecting the tarsometatarsal (TMT) and distal intertarsal (DIT) joints of sedated horses was 96% and 42%, respectively. This data supports the clinical impression of the difficulty of injecting the DIT joint and suggests that practitioners should use adjunctive methods, such as radiographs, to ensure proper needle placement. Authors’ address: University of Georgia Veterinary Medical Center, Department of Large Animal Medicine and Surgery, 2200 College Station Drive, Athens, GA 30602; e-mail: seabaugh@uga.edu. *Corresponding and presenting author. © 2015 AAEP.

1. Introduction
Intra-articular diagnostic anesthesia and/or therapeutic injection are relied upon to help diagnose and treat osteoarthritis of the DIT and TMT joints. The objective of this study was to determine the accuracy of arthrocentesis of the TMT and DIT joints using a sample population of equine surgeons and surgery residents.

2. Materials and Methods
Six operators each injected two TMTs and two DITs. The joints were injected with a 4-mL solution of 50:50 contrast medium. A minimum of two radiographs of each joint was obtained to evaluate the presence of contrast medium within the target joint. Clinical experience of the operators was included in the statistical evaluation.

3. Results
The TMT and DIT joints were successfully injected in 23/24 joints (96% accuracy) and 10/24 joints (42% accuracy), respectively. Communication between the TMT and DIT joints was visible in only 26% of successful TMT injections. Experience did not significantly affect the accuracy of injection.

4. Discussion
The TMT joint was injected successfully nearly 100% of the time, whereas the DIT joint was frequently missed with contrast medium being placed extra-articularly. It is recommended that radiographs be used to ensure proper needle placement for DIT arthrocentesis.

Acknowledgments

Declaration of Ethics
The Authors declare that they have adhered to the Principles of Veterinary Medical Ethics of the AVMA.

Conflict of Interest
The Authors declare no conflicts of interest.

Ky Mortensen, JD, MBA

This presentation explains the concept of strategy in simple terms and explores why strategy is important, how it can foster great leadership, and how it can be applied to your practice and customized to your needs. Author’s address: Inova Partners, LLC, 193 Coy Road, Weatherford, TX 76087; e-mail: ky.mortensen@inovapartners.com. © 2015 AAEP.

1. Introduction

Running a business without a strategic direction that is well understood by everyone on your team is like commanding a ship with no navigational equipment and no knowledge of where you are going. You can still get up, put your oars in the water, and go somewhere—you just don’t really know where you’re headed and have no idea if you’re even remotely close to your destination. The same is true for business. Even if you seem to be profitable, you must understand why, both in terms of the reasons for your success and what you intend to do with the fruits of your labor so that you are perpetuating a positive cycle.

Strategy is essentially an organized approach to getting what you want. It is a plan. It is an understanding of what it is that you do and what you want to be and focuses on how you plan to get there. In veterinary medicine, there are countless ways of focusing the efficiencies of your practice and a myriad of approaches you can take to become the practice you have envisioned. Defining success and building a roadmap to get there, however, is often the barrier to entry that prohibits many practices from achieving the growth they could potentially have realized. Equally as important in defining what you want is knowing what you don’t want or, rather, what you don’t intend on achieving so that you aren’t sidetracked easily in your quest for your given objective. Beyond identifying the goal, strategy is the act of knowing, as a business, how to ask for what you want from your team and from the marketplace at the appropriate time and in the appropriate manner.

2. Leadership

Two of the most important attributes of effective leaders are the ability to predict and to delegate. Mastering the art of strategy and designing and implementing a strategic plan in your practice are essential in supporting these attributes. You plan so that you can identify an objective and measure your progress against that objective. Measurements are data, and data drive the power to predict.

Consider the importance of prediction in your current environment. To manage cash, you need predictions to set an annual budget. To set an annual budget you need to be able to predict revenues. Predicting revenues requires an understanding of costs, caseload, and the amount of business you think you can generate. To support that caseload
you have to plan on the appropriate level of service you can offer, the number of veterinarians you need to satisfy a flow of business, and the number of technicians they'll need to support the workload. Then there is the matter of space and inventory. In short, there is no end to the need of accurately predicting revenues, and your level of understanding of where you currently are and identifying and describing where you want to be are essential in supporting this capability.

The second prong of leadership is delegation. This is essentially the art of describing your end goal to someone else and helping them to understand their role and level of accountability in reaching this final goal. Great delegation is creating enthusiasm for the task and harnessing the synergy of your team and its capacity to work as a group toward a common vision.

3. Knowing What You Want
A mere 7% of employees today fully understand their company’s business strategies and what’s expected of them to help achieve company goals. In short, it is likely that your team has little knowledge of the ultimate goals for your practice, how they fit in to the picture, and how they can assist with the overall effort. Even of greater concern is whether you as a practice owner can adequately describe the goals to your team to get their buy-in, create enthusiasm for the process, and engage them in moving the practice forward in a focused direction.

Easily one of the most challenging questions you will face in life and in business is identifying what you want. You know the answer of course: more. More clients, more caseload, more money, more time, more support—more. Right? Or is it less? Less accounts receivable, less stress, less turnover—less. Or perhaps it is a mix of the two: less of the bad, more of the good, and always as soon as possible. Whatever the case may be, it is the “knowing” that will likely present the greatest challenge, and that is where strategy begins.

If you interview each of your employees and ask them what they want it is likely that few will have a definitive answer. The reality is that when you fail to identify what you want someone else will decide for you. If you allow this to happen in your practice you give up the ability to manage your own destiny. As a business owner or manager, it is essential to understand what success means from the viewpoint of all stakeholders in your organization. When the definition of success is clearly understood, the plan for achieving the desired outcome can begin.

Understanding what you want personally from your practice and what is desired by your team as a whole can be explored by conducting a SWOT analysis. SWOT simply stands for strengths, weaknesses, opportunities, and threats. Strengths and weaknesses are exercises in evaluating the internal organization, and opportunities and threats are external forces that exist regardless of the internal situation.

By conducting this exercise and evaluating these areas, you reveal truths about your organization in terms of issues that need to be addressed and competitive advantages that need to be exploited. You’ll also unveil areas of business that deserve more focus and represent your greatest opportunities for success.

4. Understanding Your Competitive Advantage
Your success as a business is inextricably linked to your competitive advantage—knowing what it is and honing in on your strengths to capture more market share and revenues. Conducting a SWOT analysis will give you the foundation for understanding your current situation and evidentiary support for why you should pursue a given set of objectives. As you consider your position relative to your SWOT analysis, it is imperative that an understanding of your competition and marketplace are thoroughly explored.

Your ability to succeed depends largely on the arena in which you are prepared to compete and how well you can establish your strengths within that defined arena to give you a competitive advantage. In other words, what is it that sets you apart from your competition in the minds of your customers that drives home the value of doing business with your organization?

To assist us in understanding the competitive arena, we utilize Michael E. Porter’s Five Forces of Competitive Position model, which was described in the Harvard Business Review article “How Competitive Forces Shape Strategy.” Porter identified in this article the following forces that govern industry competition:

1. Threat of new entrants: Consider how easy it would be for a new practice to enter your market.
2. Bargaining power of suppliers: How advantageous is the position of your suppliers?
3. Jockeying for position among current competitors: How loyal are your current clients? Do you consistently make adjustments to try and win them over from competing practices? Have you introduced new services or specialties?
4. Bargaining power of customers: Are your customers in a position to demand a lower price or particular service? Are they dictating in any way the manner in which you do business?
5. Threat of substitute products or services: Are there alternatives in the marketplace that your customers could pursue that would take the place of the services you offer?

Ultimately, these areas are designed to assist you in truly evaluating the competition in your marketplace. Consider the amount of time you currently think about your customers. Do you ask them for feedback? What are the outcomes of the services you provide? Is the patient experience being assessed on any level? Are you evaluating the level of
The fundamental principles that run a business are the fundamentals by which that business is operated. In Verne Harnish's book *Mastering the Rockefeller Habits*, the fundamentals that John D. Rockefeller followed in business are ex-

Although these three fundamentals make running a business easier to understand, each stands for a deeper level of strategic thinking:

- **Priorities:** Does the organization have 5 key objectives defined as the goals for the year and the top 5 goals for the quarter that feed into the yearly objectives? More importantly, of the top 5 goals identified, is there one that stands out above the rest as the most important? Consider this for your business. Are there goals at all? Does anyone know what they are? Have they been discussed?

- **Data:** In support of the power to predict, does your organization have the necessary data on a timely basis to determine how well the organization is running? Consider the services you offer and how well you can track profitability in each area. How can you manage your inventory in such a way that you are taking full advantage of rebates and discounts, and how does this factor into your annual bud-

- **Rhythm:** Does your organization have an effective rhythm of meaningful daily, weekly, monthly, quarterly, and annual meetings? Consider the daily huddles and weekly meetings. Are they effective? Are they with the appropriate personnel?

In many practices I have consulted with, the two most difficult areas of strategic planning are found in the implementation stage. Most have success in the planning process and can identify goals that they believe will drive the practice forward; however, a clear understanding of the goals is often an obstacle that is revealed later in the process, as is developing the continued rhythm and enthusiasm required to see the process through to fruition. Understanding the goals is an area I emphasize when approaching strategy. Too often I see practices set goals with somewhat unrealistic expectations based on the rationale that simply hiring an additional person or getting a new piece of equipment will largely solve a current issue in the practice and thus help it meet those expectations. The training time for new personnel to get up to speed or the proficiency and the level of usage of a new piece of equipment are frequently overlooked, however, and can thus lead to frustrations later on in the process.

In terms of rhythm, many practices experience an initial surge of enthusiasm that quickly wanes as the process evolves. This typically results from unrealized expectations, which means in essence that the practice outlined the goals, pursued the goals, and when the goals evolved, no longer were necessary, or took longer than expected to achieve, the practice perceived the endeavor as a failure, when the real failure was setting the expectation in the first place.

In short, managing the team’s expectation is key, both in terms of goal setting and in the ongoing implementation process.
6. Conclusion

When you consider the individuals that make up your practice, yourself, and your partners, associates, and support staff, there are many lines of thought as to what each of these people want out of their affiliation with your business. Whether the goals for your practice are to be more profitable, to be positioned to be bought or sold, or to grow in a given service or particular geographic area, you must focus to achieve the desired outcome. By understanding what strategy is and the tools that can help you define it for your practice, you can make decisions to better position you and your team today for greater success tomorrow.

Acknowledgments

Declaration of Ethics

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References

Developing and Implementing a Strategic Plan for Your Practice

Ky Mortensen, JD, MBA

This presentation focuses on a seven-step, one-page strategic plan and how to design and implement it in your practice. The process helps reveal truths about your organization and your market, promotes alignment, and brings focus to your everyday work ultimately boosting profitability and increasing team morale. Author’s address: Inova Partners, LLC, 193 Coy Road, Weatherford, TX 76087; e-mail: ky.mortensen@inovapartners.com. © 2015 AAEP.

1. Introduction

The underlying purpose of strategic planning is to develop a focused effort toward achieving a given level of success for your company and to describe it in enough detail that it becomes a reality that you and your team can work toward. Without specific goals, you have no idea whether you ever achieve anything relevant, and if you do somehow reach a degree of success, you lack the understanding of how you got there, which makes the likelihood of repeating your success very slim.

Many practice owners begin the process with good intentions and set out to write a mission statement for their practice without understanding how it fits into an actionable plan. A mission statement is really an exercise in defining what you do and why you do it. However, the activity of crafting the company mantra can be a black hole for hours spent wordsmithing a meaningless collection of words when not properly tied in to an actionable plan.

A vision statement is a long-term idea, a conceptualized statement that spells out where you want to go as an organization. In sum, the idea of going through a strategic plan in your practice can sound overwhelming. The development of mission statements, vision statements, and goals is a cumbersome task and can be intimidating and futile. Without substantive action items that describe how you intend to live up to your mission, along with responsible parties, measurements, and reporting structures, all of your efforts can amount to little more than theory that has no practical application in your business.

However, with the right framework, the process of strategic planning can be approached and implemented by adhering to a system that everyone understands. Missions and visions are really just fodder for getting the fire started and helping to keep it lit. But alone, they are not enough. To truly harness the power of your team, you must understand what success means to you and to each of your employees and how everyone plays an active role in writing the story that is the success of your practice.

By following a seven-step process, you can implement a strategic plan in your practice:

1. Understanding your purpose
2. Defining your core values
3. Selecting your business strategy
4. Understanding the market and your place within it
5. Bringing focus to your efforts
6. Getting your team aligned
7. Keeping the engine tuned

Getting Started

First, you need some background work to define the issues you are currently facing. This requires some survey data from your internal team, your key staff members, your Board of Directors (if you have one) and it might even benefit from some insight from your customer base. This is an opportunity for your people to talk about their personal view of the issues facing the organization: that’s the reward they get for participating and also a chance for you to uncover some key weaknesses that probably need to be addressed. Like it or not, whatever your staff, associates, and partners say, they’re right.

The next part of the data-gathering process is to give everyone the task of describing solutions to the issues and to think about the broad scope success of the organization. You will be surprised at the vision that your own people have been harboring. This might be the most eye-opening piece of the exercise. But you have to be trustworthy enough to handle the information. You need open, honest data. People need to feel that there will be no backlash for their honest opinions, which is why a neutral facilitator is often a good solution, but not absolutely necessary. The data can literally be collected in a survey or questionnaire, or it can happen in a series of interviews with the key stakeholders in the organization. The point is, you need input, and it needs to be honest and forthcoming. My suggestion for this phase, and really every phase, but especially this one, set the expectation so that the team understands what you need, and why. By that I mean you must let your people know the process you are going to go through as a group, and whether their responses will be confidential, shared with a group, etc. Tell them what you are after, why you need it, and how you intend to use it. And then keep your word.

Second, spend some time with key members defining the reasons they came to the party in the first place. By this I am talking about partners, shareholders, and key members of your executive team. Remember, your employees, your partners, your team as a whole, they are all engaged in your business by choice; their choice. They can choose otherwise. Guard against taking their talent and creative energy for granted. They are in your life and part of your business for a reason, and it is time you found out why. Focus on long-term objectives so that you can appreciate why your people are involved in your business, and where they want to be in the next year, 5 years, and even 10 years.

2. Understanding Your Purpose

You will need to spend some time looking at what your organization is really all about. What is your purpose and what are your strengths? By this, I mean, what is it that you really do? What are you really good at? And what do your clients actually value you for? It sounds like a repeat question, but these can be very different things and identifying the real purpose of your organization and your real strengths is key in focusing those strengths where there is potential within your market.

A great tool to get to the heart of the matter in terms of what your organization does, is to ask yourself the “five whys.” Here you describe what your organization does. For example, you state that you make a certain product or offer a certain service. Then ask, “Why is that important?” After a few whys you’ll begin to uncover the true purpose that your organization even exists. Again, do not just say what you do, but define what your business is uniquely good at, what is different or better than your competition, what can be valued by your customers. Once you’ve got the answer, ask why. That’s it. Just ask why. And then repeat the question five times. By the time you’ve answered “why” five times, you’ve likely drilled down to the core of what you are really all about as a business and why your customers need you.

In sum, your core purpose is the fundamental reason that your organization exists. The realization of this purpose can be a springboard to making significant changes within your organization and the way that you have been approaching your business.

3. Defining Your Core Values

Values get at the core essence of who you are as an individual. Do you value hard work? Do you value your staff? Do you value profitability, and if so, at what cost? A good way to get down to what it is you really value is to pay attention to the themes and phrases that you like to say and to those that are spoken by your partners, associates, and staff. Most people, and most businesses have a few common “phrases” that are used periodically, and inevitably become the culture of the person or the business. For example, if someone is fond of saying “no free rides,” and they say it frequently, it is likely that this person values commitment, hard work, and earning your way through life. That is a value. And values, when added up, define an individual. The collective sum of your individual influences will define your corporate culture.

In essence, core values are the guiding principles that guide the course of your company. Being true to those values and how they relate to your purpose will create the framework for your organizational focus.
4. Selecting Your Business Strategy
Next, evaluate your business model in terms of the type of strategy you want to employ. There are four basic strategy types described in detail in the book, *Strategy*, published by the Harvard Business Review Press. The strategies are: low-cost leadership, product/service differentiation, customer relationship, and network effect.  

**Low-Cost Leadership.** This is the Wal-Mart model where you compete on price … period. Are you the most affordable option your customer base can find? Do you want to be?

**The Differentiation Model.** This is the type of business model you often see in the automobile industry. You put three full-size pickups side by side, representing different brands. Each will try and show you the difference in their automobile: the engine, the towing capacity, the strength of the body, the number of miles you should get, the interior space and amenities, and so forth. Maybe they are really quite different, maybe not, the point is, they focus on this as their business strategy and they stick to it.

**The Customer Care Strategy.** Here the focus is on taking care of the customer, building that relationship and fostering the ongoing dialog between the organization and its market. This is where product differentiation is not such a big deal, price is never a bargaining tool, but the experience is forefront of everything the organization does.

**Network Effect.** Lastly, in this model we seek as a business to leverage our relationships and create the one-stop shop experience so that our customers can get all they need in one spot—our spot.

The point of the exercise is that you must decide who you are in terms of your business model strategy, and stick to that strategy. Although you will likely end up covering ground in other areas, the focus must only be on one. Wal-Mart happens to be pretty good at being the “one-stop-shop,” but that is not their focus. Price is their focus and they put their strengths into winning that game. Trying to be too many things to too many people will simply leave your customer base confused and will create fragmentation in your operational approach. Regardless of the strategy type you select as the core direction for your practice, always look for alignment between the strategy and your client base.

5. The Market and Your Place Within it
The next step is to understand and evaluate the market that is your current customer/client base. Discuss who they are, their demographics, income brackets, locations, their interests outside of horses. Drill down here and really try and identify who your current customers are and what category they represent in terms of the marketplace.

Now the important part of the customer equation: are they really the customers that you want? Do they use your practice for the strengths that you want to build on? Do they refer additional business? Do they pay at time of service? Do they represent your greatest opportunity for growth as a company? Essentially, are you playing in the right field of the market? And if so, are you winning? If not, you need to find out why and make the necessary changes to bring realistic positive change to your practice by going after the right group of customers.

Remember, many organizations feel that there is power in going after a broader market. They perceive that the greater the slice of the pie, the more opportunity there is to be had. The opposite, however, could create greater opportunity. By focusing on a smaller segment of the market, and essentially a smaller piece of the pie, your competitive advantage increases, allowing you to succeed on a larger scale in that section of the marketplace.

6. Bringing Focus to Your Efforts
With the above elements defined, you are now ready to go to work. Action brings a sense of tangibility to the forefront and creates enthusiasm for the process. A body in motion stays in motion, and the same is true for a business. The trick is to move in a common direction.

Now that you have given focus to your values, your purpose as an organization, the type of business strategy you would like to hone in on, and evaluated your market, you will undoubtedly start to see some trends. There will be overlapping themes and reoccurring thought processes as the essence of your business, or what you wish it could be, will begin to take shape.

Next, you take the sum of that data and compare it to your current issues and solutions to those issues that your team unveiled to you during the initial interview stage at the beginning of the process. With this data in hand, you are ready to start setting some goals.

This is a particularly challenging piece of the process. It can be difficult to inhale all of the data above and then churn out one or two action items that is going to solve your problems. Think of it in time increments and the process becomes more manageable.

A quick exercise I like to use is to think about a special holiday that is going to come along in approximately 1 year. If it is around the end or beginning of a new year, you can think about next Christmas. Picture yourself at the end of that special day, during a quiet evening, at home in front of the fireplace in a relaxed atmosphere. As you picture the moment, you are reflecting on what a great year it has been for you professionally. You are feeling great about it personally, it has been financially rewarding and you feel valued and prosperous. You feel as if you truly accomplished something this year and you’re looking forward to the next. Now, describe in as much detail as you can, what it is that has happened during this past year that has led to so
many good feelings. Do you feel valued? Describe the things that have happened that have led to you feeling this way. Do you feel financially rewarded? Describe what that looks like and why that money came through. Most importantly, describe what that money is for. Do you feel like you accomplished something? Describe what it is that you will have accomplished? And are you enthusiastic about the coming year? Describe in great detail why you would be so fondly looking forward to the coming year.

Now write all of this down. It may be a paragraph. It may only be a sentence or two. It may just be a few words. Whatever it is to you, be very honest, and describe it.

Weigh your vision against the initial data gathered in your planning process to make sure it is in alignment with what you have decided for your organization. What you have just described is your 1-year plan. It may be a very personal 1-year plan, but it fits into the overall workings of the organization. It has to, because that is where your professional life exists.

When everyone on the decision-making team has had a chance to describe that world, you have a better idea of what a year’s success truly means to your organization on an individual basis. Now you have to make it work for the organization as a whole.

From that simple exercise we can then project a few more years down the road. For example, let’s say that your 1-year goal was to have less anxiety in your workplace and you think that could come from having a great front office team that is well educated on your software, great with customer service, and really efficient at scheduling and getting everyone what they need. If you look out a few years at what that will do, it essentially creates a better customer experience for both your outside customers and your internal employee experience as well. The 1-year goal then is to improve front office operations. The 3–5-year goal is to create a stress-free environment to increase employee morale and enhance the client experience.

Now jump backward in your planning and describe what you can do about this 1-year objective in the next 90 days. Could you hire someone? Could you get more training? Could you invest in a better software program, or get more training in the software that you have? What could you do in the next 90 days to get you headed in that direction? Once you answer that, you have the makings of a real plan. You have your 3–5-year goals, you have the 1-year measurable that tells you if you are getting there, and you have a 90-day plan to start moving in that direction. Why 90 days? Because that is enough time to actually make some headway, yet not so much time that you lose sight and enthusiasm of the effort. Plus it gives you the opportunity to reassess things in the next quarter, adjust your objectives to better align with your long-term goals and head out again with renewed vigor for your overall purpose.

As you work through this exercise, you’ll find that several of the one year success stories have overlapping themes. They will likely be about operational efficiencies and day to day struggles. They may address specifics like space or personnel inadequacies, or they may be larger issues such as cash flow, or caseload numbers. Either way, the yearly objectives will likely feed into a larger, 3–5-year goal that can be identified from the yearly objective.

On an individual basis, you will also have to challenge yourself and your team to work daily toward their own personal 90-day (quarterly) objectives that feed into and support the quarterly objectives of the organization. The team should ask themselves every day, “What can I do today to move in the direction of my quarterly objective?” This daily challenge and the resulting activity will add up to weekly progress and quarterly success, which turns into the realization of annual goals. Sounds simple, but it works.

7. Getting Your Team Aligned

Finally, you must make sure the quarterly objectives really make sense. A great tool for ensuring the accuracy of your quarterly goal setting is to adhere to the practice of setting SMART goals. Developed through Peter Drucker’s *Management by Objectives*, this is an approach in which a balance is sought between the objectives of employees and the objectives of an organization. The essence of Peter Drucker’s approach is to determine whether the goals are aligned and to provide feedback on results.1

To make the concept simple and easy to understand, Drucker coined the mnemonic, SMART:

- S—Specific
- M—Measurable
- A—Attainable
- R—Relevant
- T—Timely

Each quarterly objective set by an employee, which subsequently feeds into the organizational quarterly objectives, should adhere to this concept to ensure that the task is in concert with the annual organizational goals. Quite simply, the SMART inquiry is to determine whether the goals you have set really make sense for the employee and the organization. Do they add value? Can you actually accomplish them in the time allotted? Is it necessary that you do? Will you be able to tell if you were successful? If you can answer yes to the above, then it is likely that you’re on the right track.

8. Keeping the Engine Tuned

Equally as important as developing the goal in the first place is the problem of actually tackling the goal and taking strides to see its completion.
Again, managing the expectation is the key. Set goals that are realistic yet challenging enough to keep the team honest. Be fair in your assignments in terms of the weight of the task, even distribution of workload among employees, and the time allotted to bring the goal to fruition. A common mistake is for too many objectives to be piled onto one person or department, or to create too high of an expectation in terms of immediate results or the magnitude of the initial quarterly goals that are set. This is a strategic plan for a business and a business is made up of everyone who works there. It is important that the strategy is well spread out among the team and that the accountabilities are equally shared. It has taken time for your organization to arrive where it is currently and it will take time to get it back on track.

9. Conclusion

Through careful analysis of the issues and potential solutions from the individuals that make up your team, you come to better understand what is important on individual levels and collectively as a group. This understanding, expanded upon with the influence of your overriding core purpose and organizational values will function as a guideline in assessing your market, your strategy type and ultimately developing yearly objectives. Once these are defined, it is a matter of breaking them down into manageable tasks, assigning them to accountable parties with realistic timelines, and maintaining a rhythm of reporting so that everyone stays on task.

Only through careful monitoring of the objectives themselves, the way they are measured and the involvement of the entire team will synergy truly be created, a common vision be developed, and a collective effort be employed to getting you and your employees what you truly desire as an organization.

Acknowledgments

Declaration of Ethics

The Author declares that he has adhered to the Principles of Veterinary Medical Ethics of the AVMA.

Conflict of Interest

The Author declares no conflicts of interest.

References

Promoting Your Business Strategy

Mike Pownall, DVM, MBA

Strategy for a veterinary business is the guiding light that shows whether a business is going to have a sustainable competitive advantage in the marketplace. By definition, a successful strategy is unique to a business because if it were the same as another business, there would not be a compelling reason for consumers to pick one business over another, so neither would have a competitive advantage. Once a strategy is crafted, it is essential to create awareness of the value being offered to the end client. Promoting a strategy should consider 4 key components of a strategy: goals, value proposition, target client(s), and core business strengths. This presentation will guide the veterinary business in the best way to use these strategic elements to increase the effectiveness of business promotion using common examples in equine veterinary businesses.

A business goal could be general or specific. For example, a veterinary practice could aim to have the best reputation as a lameness referral center, or it could want to increase radiology sales by 10% in the coming year. Whereas the latter is specific, the former is general because it is hard to precisely measure the outcome. Similar examples can be used over a variety of veterinary practice types, with the common difference being that one should promote the overall business reputation while the other should focus primarily on radiology services rather than the lameness skills of the veterinarians. Regardless of the goal, specific metrics must be established to help measure the success of promotional efforts over regular intervals. Much like monitoring anesthesia to ensure proper anesthetic depth, regularly assessing a marketing campaign is essential for properly responding to current efforts.

A key component of a strategy is the value proposition, or the reason why a client picks one business over another. It is a simple articulation of the value a business offers to its clients. Examples of successful value propositions would be Walmart’s “everyday low prices” or BMW’s “ultimate driving machine.” In a veterinary practice, a value proposition could illustrate how the practice is “always there for emergencies” or “can handle every veterinary need” in the case of a referral hospital to “1-stop lameness diagnosis and treatments” for a sports medicine specialist. Defining a value proposition can be the hardest part of creating a strategy and marketing plan. It is easy for a veterinary business to state that they care or are compassionate. Those statements are no different from any other vet practice—they are a given. Instead, promote what sets you apart from the competition. Having a clear focus on your service offerings and ideal client is vital for effective promotion. Nordstrom has a different target client and quality offering than does Walmart; veterinary businesses should have a similarly clear idea of their target...
clients and offerings. If you are aiming to be a high-end English sport horse practice, you shouldn’t spend a lot of your time promoting your services to backyard horse owners. If your service focus is on reproduction, then targeting horse shows in your area might be a wasted effort unless there is an emphasis on weanling or breed classes. If you are a specialist, your target client might be referring vets from within a wide area. They are going to need a different approach than horse owners. If your business deals with large barns or training centers, your target client might be the barn owner or trainer because they control access to their clients. This is not to suggest that the horse owners should be ignored; rather, focusing on creating awareness of your services to the trainer or barn manager may yield a bigger and quicker return. Knowing the type of target client helps keep the focus on the appropriate tools to use to reach them; rural clients might lack broadband Internet access, so print media might be necessary, whereas urban-based dressage riders might be looking for information on social media.

Finally, every business has core capabilities that are unique to them. It could be as obvious as the ability to see horses on the farm by an ambulatory vet when compared to a referral hospital. It could also reflect the ability of staff and vets to engage in social media or client education seminars. If you are a single-vet ambulatory practice, you probably don’t have time to create and post social media content, so you may focus on creating excellent client impressions that lead to strong word-of-mouth recommendations. Any business should either focus on their existing promotional capabilities or create new ones to fit the current strategy.

Ensuring that a business has a clear grasp of their goals, value proposition, target client, and core capabilities before promoting a business strategy is essential for the ultimate success of the strategic plan. As veterinarians, we wouldn’t attempt a castration without the right tools, assistance, and knowledge, and we shouldn’t tamper with our business reputation and ultimate success without careful preparation. Too many small businesses attempt to promote their services by advertising aimlessly without taking into consideration what they are promoting and what value they offer—and to which clients. A differentiating strategy is so rare in business in general that a veterinary practice that spends the time to craft a competitive edge and promotes it well is positioned for success at the expense of their competition.

Acknowledgments

Declaration of Ethics

The Author declares that he has adhered to the Principles of Veterinary Medical Ethics of the AVMA.

Conflict of Interest

The Author declares no conflicts of interest.
1. Introduction

Have you ever stepped outside to embark on your day, excited and maybe a bit hurried, when right as you closed your door you realized your keys were sitting on the coffee table? The wave of dread rushing over you as you thought about the time and effort it was going to take to extract those keys from your home to get on with your day? This is a relatively simple problem with a relatively simple solution—place a “hide-a-key” in your side yard so that you can easily unlock the door when needed and boom! Problem solved.

But in business we don’t have hide-a-keys; we must think strategically to set ourselves up for success. Strategic planning is the process of identifying organizational objectives and determining what actions are required to reach those objectives.1 It is far easier to stay out of trouble than to get out of trouble. So, in business, we must proverbially check for our keys before walking out the door. All decision-making should support your goals, and you should be proactive rather than reactive in achieving those goals.

So what guides our strategic thinking? How do we determine what is best for the company when making decisions large and small? Well, we must begin with the end in mind. After all, we’ve got to know where we are going before we get there, don’t we?

Why are you here? The answer to this question will give you purpose and motivation to continue on. Who do you serve? The answer here will establish a basis for the type of services or products you provide within your industry. What do you stand for? These guiding principles will keep you from veering off course. What are your goals? You must begin with the end in mind to create clear, productive action steps. This is the first set of strategic questions you must ask yourself.

Three to five well-defined company goals and strong core values, along with a clear mission statement, should answer each of these questions. Your goals, core values, and mission statement lay the foundation for your company’s culture, and with a strong culture you will have the ability to confidently delegate decision-making to others. More on this later.

It should be obvious that large, macro-level decisions (i.e., business locations, services/products provided, etc.) should be made methodically. These types of decisions have the highest probability of
resulting in overall success or failure. But what about the mundane? What about the decisions that seem inconsequential and banal? In reality, every decision that is made should support the strategic position and vision of the company. Once you have clearly defined and communicated your strategic position to your associates, the decisions they make become easier for them and better for you.

So the question then becomes how a company defines and communicates its strategic position to its associates. This presentation will cover the human resources practices that have brought success to Nugget Markets, a small grocery chain with 15 stores and 1,500 associates in Northern California. Since 1926, Nugget Markets has advocated for and implemented the highest of workplace standards in the grocery industry. *Fortune* has ranked Nugget Markets as high as fifth in the nation on their list of the “100 Best Companies to Work For,” and we have made the list 10 years running (2006–2015).

2. Defining Your Strategic Position

There are no shortcuts to defining your strategic position within an industry. It requires deliberate thought and reflection. There are no catch phrases or key words that should be universally woven into an organization’s strategic visions. To be uniquely successful, your definition of success and your vision must be uniquely derived. Any attempts to implement strategic thinking must be intrinsically and organically motivated rather than “cut and pasted.”

A traditional business approach to determining your strategic position is to conduct a strengths, weaknesses, opportunities, and threats (SWOT) analysis. When conducting a SWOT analysis, you are looking at your company’s internal strengths and weaknesses while assessing the external opportunities and threats present in the environment. Some textbooks say to plot these 4 attributes on a matrix, but I find that to be unnecessary in most cases. On the other hand, collaboration is extremely helpful when conducting a SWOT analysis, but only when trust among the team is high; brutal honesty and robust conversation are critical to this exercise. So what form does a SWOT analysis actually take? Let’s break it down into even simpler terms.

Look Inward

To begin defining your strategic position, you must start by analyzing your own organization. Look inward at your organization to determine your strengths and weaknesses. Look at everything. Where does your staff excel and where are they deficient? In turn, where do you excel, and where are you deficient? How is your website? Is your facility in the optimum location? What does the front desk look like from your customer’s perspective? Every small detail adds up, and over time, it is these details that create great outcomes. Everything matters.

I’m reminded of one of the most influential 30-minute meetings I had back when I was a kitchen manager at one of our stores in Northern California. I had scheduled weekly meetings with one of our vice presidents to look at what was working and what wasn’t. Some of these meetings were more valuable than others, but one in particular stands out above the rest. It was a rather peculiar meeting, I might add. As the meeting began, we exchanged pleasantries and then headed straight onto the sales floor. As we walked up to the first area to be inspected—my “grab ‘n’ go” wall—he asked me 1 simple question: “Are you proud of that?” I answered with a sort of absentminded “sure,” and then we moved on to the next display—this time the soda fountain machine. Again, “Are you proud of that?” This time I responded with a less confident “I guess.” Then again at the entreé case, and again at the handwashing sink, and then again and again each time in more rapid succession: Are you proud of that? Are you proud of that? Are you proud of that? For nearly 30 minutes the vice president continued asking the same question. The message was loud and clear. Every single detail in your business matters. Every single decision you make, no matter how big or small, is important. When looking inward to determine strengths and opportunities, look at everything and remember the important question: Are you proud of that?

Look Outward

After analyzing your company, it’s important to look at others in your industry to see where you stand against them. After all, this is what your customers are doing. This is also what newcomers to the workforce are doing. With the availability of information in this technology-driven age, everyone surveys the field before making their decisions. Shouldn’t you? If you only look inward, your picture is severely incomplete, and any assessment you make will be critically uninformed.

When looking outward, it is important to identify opportunities and threats. Opportunities are sectors of your industry that have been untapped. Are there any populations being underserved in your area? Look at your competitors and see whether they are missing the mark anywhere. Their missed marks are your opportunities. Are there emerging technologies that can be adopted early so that you become the authority in that specific area? Look at your industry to see where the overall trends are headed. These new trends are your opportunities.

You must also assess your threats when looking outward. If your organization is deficient in a specific area, this can become a threat. If your competition excels in a certain service or product, this too can become a threat. The key here is that these discrepancies can only become threats when you try to compete in these areas. That doesn’t mean that you can’t compete, but if you want to beat someone...
else that is more talented in a specific area, you better have a really good plan for adapting and evolving. Maybe your plan involves hiring staff that have more talent in that area. Maybe your plan is to get more training in that area. These are both fine plans that, if executed properly, can lead to favorable results in defeating the threat, but there is another option.

Differentiate. What makes you uniquely suited to provide a service or product over the other guys? If you have analyzed your own organization, taken a look at what the competition does, and recognized the trends of the industry, then you can identify the areas where you naturally stand out. Focus on the positives from your SWOT analysis (the strengths and opportunities), and go in that direction.

Once you have determined your strategic position, your company’s true competitive advantage, it is time to define this position in concrete terms so that it can be understood and followed by everyone in the organization. It is time to circle back to your core values, company goals, and mission statement. Are they still a good reflection of your company’s trajectory? Do they still make sense with regard to where you stand among the competition? Although things like core values, company goals, and mission statements should remain relatively static, it should be understood that these items can, and probably should, change over time. If your organization has values, goals, and mission or vision statements, when was the last time they were evaluated? Are they leading you where you want to go? Remember, everything matters.

3. Communicating Your Strategic Position

Communicating your strategic position really comes down to creating systems for disseminating your message and holding people accountable to your vision. Most of the time when discussing communication, we focus on the free-flowing nature of face-to-face meetings or think about the advantages and disadvantages of an e-mail versus a phone call. That is not the sort of communication we are focusing on here. I want to focus on systems. It starts internally with your associates, and it starts right at the moment a prospective employee reaches out to your organization.

Communicating Your Position

When a job candidate initially makes contact with your organization, what does it look like? What does it feel like for them? It is wise to create a warm, welcoming environment for prospective hires because they are likely testing the market. Do you feel like the best choice for them? Put together a system for responding to every application that is received. It can be something as simple as an automated message that reads, “Thank you for inquiring about employment within our organization. We carefully review every application received and will contact you within 7 days if your application meets the current criteria we are searching for. Thank you for your interest in our organization, and we wish you the best of luck in your job hunt.” A message like this will set a clear expectation for your applicants with regard to timing, and it also gives them the impression that you are really on top of things.

During the interview process, consistency in your system is key. Whether you implement a quantitative, subjective, or other selection system, your process and criteria for selection must be consistent to ensure that your results are valid. There are times when you can tell that a prospective associate would be a bad fit within the first moments of an interview, but it is important that you stick to your process and see it through. This will not only further validate your decision but will also help to develop a favorable reputation throughout your business community. People will appreciate the opportunity to practice interviewing, and even if they are not offered the job they will feel like they were heard.

Once an applicant is interviewed and hired, it is critical that systems are in place to communicate your company’s goals and expectations. Job tasks and descriptions should be given here. What is expected from your new hires in the day-to-day operations of the business unit and specifically their role within that unit are critical. Do you have a written job description for every position within your organization? If you haven’t done so, try writing down the actual expectations of every position in your organization. After writing them down, determine the best way to effectively communicate these expectations to your associates (simply handing out the written expectations will likely be inadequate).

Communicating to your newest associates goes far beyond positional responsibilities and expectations. You have to give them some context about the strategic position of the company. Harken back to your mission, goals, and values at this point. This doesn’t mean you have to conduct a 3-week orientation or spend valuable resources on expensive videos and presentations (although those types of things tend to help keep your message consistent, particularly in large organizations). What you do need, however, is a method for conveying the things that are most important to your organization to your newest associates, as well as a process for answering all of their questions. Whether written, verbal, or by some other method, it is critical that you tell your story and that you also meet your new associates’ needs and expectations.

When it comes to communicating with your staff, and particularly your newest hires, always remember that the best communication is going to be clear, comprehensive, and consistent. Clear communicators use the simplest language possible to convey their message. By cutting through excess verbiage, you can cut right to the core of the message you
would like to convey. Comprehensive communicators anticipate the questions that may arise before they are asked and make a concerted effort to answer those questions before they are asked. Consistent communicators ensure that the same message gets through to everyone. A written aid or guidebook can prove to be extremely valuable here.

Accountability
Disseminating your message is only the beginning of the communication process. Simply communicating your message is not enough; good communicators ensure that their message gets through. When I visualize effective communication, I think of the loading symbol on my iPhone or web browser, constantly rotating and looping over onto itself until the application is loaded. This is how we should think of communication—a loop of information that is constantly reinforcing itself until everyone is on the same page.

Once everyone is on the same page, we can begin to hold associates accountable, but not a moment sooner. So many workplace issues, from associate job performance to driving business results, guest loyalty, and profitability, could be easily resolved if more managers engaged in holding themselves and their teams accountable. Accountability is about setting the expectation, clearly communicating it, and then holding yourself and everyone on your team responsible for consistently meeting the established goals. Holding associates accountable to the expectations that have been set is a cyclical process. It is not about telling people quickly what you expect them to do and then moving on to the next thing. It takes time, but stick to your guns.

Regular performance reviews are important to this process. I’ll never forget when a superior of mine told me, “You must inspect what you expect.” Performance reviews allow for focused time to discuss where associates are and where they need to go; keep in mind, however, that the best performance reviews should allow for communication to flow both ways. In many organizations, associates fill out a self-evaluation that is compared with the evaluation that their manager has completed.6 When we take the time to truly evaluate ourselves, it opens up opportunities for growth. It amazes me how often the reviewer and the reviewee have nearly identical views of a particular situation, but the exercise of the reviewee first writing his or her own thoughts down allows for the discussion to be had in a non-confrontational, more constructive way.

Communication is just as much about listening as it is about speaking. So we must listen to our associates as well. Explore the idea of conducting reverse or 360-degree7 reviews where associates are given the opportunity to provide feedback to their superiors and/or peers in an anonymous and confidential platform (for instance, anonymous surveys and/or digital apps). In smaller organizations or business units, anonymity can be a challenge, but the process of receiving honest feedback from your direct reports and/or peers is invaluable. After all, the people who work for you are going to be the best judge of your ability to lead, how well you communicate, and how committed you are to holding everyone accountable. They are the ones that deal with all of your strengths and deficiencies every day.

Performance reviews are only one important facet of holding your team accountable. Reviews provide written documentation of our coaching sessions, but everyday coaching is in many ways far more important. Consistent, frequent coaching of associates allows for forward progress and movement.8 When coaching is infrequent, it necessarily forces the dynamic of the conversation into the past. You will be reviewing what went wrong and what went right but leaving much less time to focus on the current situation and plan of action moving forward. Frequent coaching allows for more progressive, productive, and—dare I say—strategic thinking.

Coach the expectation—not the person. This philosophy says that we should place more focus on the action and less focus on the individual. When you have clearly defined and well-written expectations, they are easy to reference when holding associates accountable. Rather than solely focusing on the deficiencies of the individual, coaching the expectation is about acknowledging those deficiencies—getting them out in the open—and then moving forward to the solution (the expectation). This allows us to continue moving forward as we endeavor to better our business.

4. In Closing
When a company sets out to change their culture and define their competitive advantages, it can be a daunting task. It takes time. A lot of time. But by following a clear plan with clear intent, it can be done effectively.

It starts by taking the time to reflect on what is important to you and your operation. Why are you here? Who do you serve? What do you stand for? What are your goals? Then look inward at your organization to identify strengths and weaknesses and outward at your industry’s landscape to identify opportunities and threats. Define your company’s goals, core values, and mission and then communicate that strategic position to your associates through whatever means it takes to make that message sink in. Finally, hold your associates accountable to meeting your expectations and monitor your position in relation to your goals. Make adjustments as needed. Before stepping outside to start our day, we must first grab our keys.

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Declaration of Ethics
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The Author declares no conflicts of interest.

References
Taking the Pulse of the Equine Veterinary Industry: Results From the 2014 American Association of Equine Practitioners Listserv Members Survey

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The 2014 American Association of Equine Practitioners (AAEP) listserv survey examined several different factors that affect the lives of equine practitioners. Several broad themes emerged from the results: the importance of practice culture, the importance of control and choice, and the degree of stress in the profession. Strategies for mitigating challenges need to address these key components.

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1. Introduction
The equine veterinary industry currently faces an unusual collision of forces that have made the environment challenging for future operation. A combination of the recession of 2008, changing demographics, and emerging sociocultural factors have caused the equine industry to contract. There are almost 50% fewer horses in the US than there were just 10 years ago.1 At the same time, increasing numbers of new US veterinary graduates are projected over the next 20 years as a result of increased enrollment at existing veterinary schools and the opening of 3 new institutions. It was estimated in 2013 that of the approximately 3,775 new veterinarians likely to graduate in 2014, approximately 6% (226) would be focused on equines.2 This growing workforce comes into a field where research projects a 23% national surplus of equine veterinarians through 2025.3 The number of veterinarians graduating with significant educational debt has increased concurrently, and a negative future value of more than $500,000 for equine practitioners compared with those with a bachelor’s degree was calculated by the American Veterinary Medical Association (AVMA) economic team in 2014.4 These new environmental factors exacerbate the traditional challenges of veterinary practice (e.g., the physical dangers inherent in the profession, the mundane human resources issues faced by any small business, etc.).

To explore the perceptions equine veterinarians have regarding their work environment, an online

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survey was conducted among practitioners to identify the factors that have been driving a recent reported trend of increase in solo equine veterinary practices, the most pressing concerns of practitioners with regard to their business and professional life, and the business education needs of veterinarians.

2. Materials and Methods

An online survey of equine veterinarians was conducted in the fall of 2014. A link to the survey was posted on the listservs of the AAEP and Equine Clinicians Network (ECN). The initial survey was limited to those practitioners whose practice is >75% equine and was closed after a week of responses. Upon reviewing the raw results, however, we discovered a problem in how the data were collected and discovered. Because of defects in the functionality of the survey, some respondents were not offered the full complement of survey questions. Consequently, we posted a repaired version of the survey that included the missing questions to both listservs in late November 2014 with an explanation and invitation to the affected respondent segments. In addition, because of requests from listserv members, those providing <75% equine services were invited to participate in the second survey. To have valid data, previous (redundant) responses from duplicate Internet protocol addresses were discarded. Responses from both surveys were then compiled additively.

The survey research design included the collection of both quantitative and qualitative data. The target group for the survey initially was equine veterinary practitioners with a >75% equine case load and limited through the use of two qualifying questions; however, because of listserv member feedback, the target group was expanded in the second survey to include all respondents who perform equine services.

3. Results

A total of 516 veterinarians responded to the survey. At the time there were 1,520 AAEP members on the general listserv. It is likely—that although not certain—that there was a significant overlap with members of the ECN. If considering only the membership of the AAEP general listserv, this would equate to an approximately 33% response rate and yield roughly a 96% (±4%) confidence interval. When considering the results of the survey, it is important to remember that the population surveyed were those members that were active on the listserv, which by necessity excluded the members not using this mode of communication. Important differences may be present between the nonlistserv practitioners and those surveyed.

Survey respondents differed in some demographic characteristics from the AAEP membership reported in the 2014 AAEP annual report. Respondents were made up of 38.4% solo practitioners (AAEP 39%), and 61.6% group practice members (AAEP 19.8% associates; 15.3% practice–owner partnership; 6.4% no response). Of the group practices, 50.6% respondents worked in practices with 2 to 6 veterinarians, and 11% worked in practices with 7 or more veterinarians. There were 60.4% female (47% AAEP) and 39.6% male (53% AAEP) respondents; 44.9% of survey respondents graduated between 2004 and 2014, and 55.1% graduated in or before 2003. The percentages of respondents by region were as follows: 22% Northeast, 12% Midwest, 14% Southeast, 5% South Central, 12% Northwest, 21% Midwest, and 14% Southwest.

Solo Practitioners

Of the 185 solo practitioners who responded, 75.4% had previously worked in a group practice (excluding internships); of these, 77.8% were past associates, and 22.2% had been owners or partners in group practice. The survey asked these practitioners what their primary reasons were for now being in solo practice. Dissatisfaction with practice culture was the most chosen response among respondents that were formerly associates. Many of the previous associate respondents chose “other” and gave written responses; these responses were focused on dissatisfaction with compensation, time constraints, practice management, and lack of ownership opportunity. Of the respondents previously employed as partners or shareholders in a group practice, 26.7% cited partner/shareholder discord as the primary reason for wanting to be in solo practice. Many also cited the dissolution of their group practices, primarily because of economic factors.

Solo practitioners were also asked how important certain factors were in their decision to open their own equine veterinary practice or how important certain factors were in their decision to leave group practice and practice as a solo practitioner. The two factors most cited as “very important” or “important” in decisions to be in solo practice were the desire to make their own decisions about how to practice medicine and the desire to have control over their work schedule and life balance. Of veterinarians that had always been solo practitioners, 77.1% cited the first statement (making own decisions) as being most important and 68.6% the second (setting own work schedule), which corresponded similarly to those who made a decision to leave group practice to practice solo (83% and 76.6%, respectively; Figs. 1 and 2).

When asked whether there were any other factors in their decision to be a solo equine practitioner, 72 veterinarians gave individual responses. Common themes emerged, including the lack of ownership opportunities, lack of effective business management practices, and disagreements about ethics and philosophy of practice.

There were several aspects of practice that solo practitioners found stressful. Respondents were given the following choices for response: “not stressful,”...
“somewhat stressful,” “stressful,” “very stressful,” or “extremely stressful.” “Negative client interactions” received the most (26) “extremely stressful” scores, followed by “clients who disrespect personal boundaries” (23), but neither of these stressors was the top contender when scores for all degrees of stressful responses were considered additively (Fig. 3). Those graduating between 2004 and 2014 were twice as likely to choose “extremely stressful” for these two aspects as those graduating in or before 2003. Pairing only “extremely stressful” and “very stressful” responses yielded a high of 62 veterinarians who cited...
“negative client interactions,” followed by 55 who cited “emergency on-call duty” and “workload too much or not enough.” When adding all stressful scores, “workload too much or not enough” was the most frequent cause of stress (88%), followed by a virtual tie between “negative client interactions” (82.4%) and “emergency on-call duty” (82%).

When considering the business aspects of running a solo practice, respondents felt the least stress with the following aspects: “inventory management (e.g., how to order; tracking),” “choosing professional advisors,” “controlled substances compliance,” “accounts payable,” and “state and federal regulations.

The most challenging 3 aspects of being a solo practitioner reported by respondents were, in order, “fee setting,” “accounts receivable/collections,” and “cash flow” (Fig. 4). It is important to note that the many aspects of managing a small business are a significant cause of stress for the solo practitioner; indeed, most respondents reported some level of stress (“somewhat stressful” to “extremely stressful” responses considered additively) in all components with the exception of “choosing professional advisors.” About half of solo practitioners (47.2%) employ staff, and according to the survey responses, the most difficult aspects of employing staff are recruiting, training, and affording appropriate compensation for their employees.
Group Practices

Survey responses were obtained from 297 equine veterinarians working in group practices. Of this group, 82.2% practiced in groups with 2 to 6 veterinarians (hereafter called the “small group”), and 17.6% practiced in groups of 7 or more veterinarians (hereafter called the “large group”). Associates and partners/shareholders made up 57.5% and 41.4% of the respondents, respectively, with the remainder consisting of interns/residents.

Associates

When asked about the importance of certain factors related to their satisfaction with their position in the group practice, practice culture and compensation were the top factors among associates, with 90.1% responding that both of these were “important” or “very important” to their job satisfaction. “Making your own decisions about how to practice medicine” (86.6%), “opportunity to learn new skills” (84.8%), and “challenge of caseload at group practice” (83.3%) were the next in frequency of response. Least significant to job satisfaction of associates was the option to work part-time hours, with only 21.3% considering this “important” or “very important.”

Associates were asked what their satisfaction was with their current position based on the same factors. Responses of both small- and large-group associates were similar. Associates from both groups stated that they were “extremely satisfied” or “very satisfied” within their positions. The 2 aspects of their positions they liked most were, respectively, “making [their] own decisions about how to practice medicine” (small group: 72.7%; large group: 70.8%) and “location of practice geographically (desire to live in area)” (small group: 70.9%; large group: 70.8%).

When associates’ responses on what factors were the most important to them for job satisfaction were compared with their actual current level of satisfaction, dissonance emerged. Looking at the 3 aspects reported as most important, “compensation” was “very important” or “important” to 90.2% of associates, and only 31% reported being “extremely satisfied” or “very satisfied.” “Culture of practice” was “very important” or “important” to 90.1% of associates, and just 49% reported being “extremely satisfied” or “very satisfied.” However, “making [their] own decisions about how to practice” was “very important” or “important” to 86.6% of associates, and 72.5% reported being “extremely satisfied” or “very satisfied.” Areas with the least amount of congruity between what associates reported they wanted versus what they experienced were “compensation” (90.2% vs. 31%) and “life balance” (77.7% vs. 33.2%) (Fig. 5).

Associates who cited being “somewhat dissatisfied” to “very dissatisfied” with certain aspects of their current positions most commonly reported being unhappy with “life balance” (29.6%), “time re-
requirements of position” (26.3%), “work schedule flexibility for family responsibilities” (22.6%), and “culture of practice” (20.9%). When segmented into small- and large-group practices, the large-practice associates’ responses (of which there were 30) differed; the most commonly reported dissatisfactions were with “life balance” (37.5%), “culture of practice” (29.2%), and “time requirements of position” (25%), with “work schedule flexibility for family responsibilities” and “competition between veterinarians within the practice” tied for fourth (20.8% each). Small-practice associates, perhaps because of their higher numbers (173), aligned with the group findings.

When considering responses from “stressful” to “extremely stressful,” the aspects of practicing as an associate that were considered the most stressful or challenging were “negative client interactions” (81.2%), “negative patient outcomes” (72.5%), and “workload too much or not enough” (71%). However, when examining only “extremely stressful” responses, “governance of practice (management decisions)” was the aspect of practice most frequently cited as causing extreme stress, with 25% of respondents choosing this response (Fig. 6).

Practice Owners (Partners/Shareholders)
Practice owners were asked about their level of satisfaction with their position at their group practice. Factors that provided the highest level of satisfaction included “making [their] own decisions about how to practice medicine” (94.2% were “extremely satisfied” or “very satisfied”), “location of practice geographically” (80.8%), “culture of practice” (69.2%), and “benefits” (69.2%). The lowest percentage of practice owners reported satisfaction with the “option to work part-time hours” (30.8%) and “time requirements of position” (34.7%) (Fig. 7).

Practice owners were also asked what aspects of practice were the most challenging or stressful to them. Aspects that were challenging (additive responses of “stressful,” “very stressful,” and “extremely stressful”) for most respondents included “negotiating/conflict resolution” (69.2%), “human resources” (65.4%), “cash flow” (65.4%), and “accounts receivable/collections” (61.5%). The smallest number of respondents were challenged by “theft or embezzlement” (25%) and “controlled substance compliance” (30.8%). All aspects of practice management listed were the source of some degree of stress in at least 50% of respondents when those that responded “somewhat stressful” were added (Fig. 8). Many aspects of managing employees were also found to be stressful by the respondents, led by “recruiting/hiring” (75%) and “compensation costs” (59.6%) (Fig. 9).

Generational and Gender Differences
Respondents were also asked about their educational debt at the time of graduation. When comparing those veterinarians who graduated between 2004 and 2014 with those who graduated in or before 2003, only 15.6% of the former graduated with $25,000 or less in debt, whereas 52.3% of the latter were in this sector. Similarly, 27.1% of those who graduated between 2004 and 2014 had an educational debt that exceeded $150,000 compared with only 2.9% of those who graduated before 2004 (Fig. 10).

When comparing those veterinarians who graduated between 2004 and 2014 with those who graduated in or before 2003 or before regarding the question of why they began practicing solo, the responses were quite similar. The largest discrepancy of named reasons was “job loss,” with a greater percentage of younger veterinarians (13.8%) having
switched to solo practice for this reason versus older veterinarians (5.9%) (Fig. 11).

Some differences between generations emerged in responses to the relative importance of factors in the decision to practice solo. Of those graduating between 2004 and 2014, 53.3% responded that the “desire to have flexibility for family responsibilities” was “very important” or “important” in their decision; in contrast, only 29.4% of those graduating in or before 2003 cited this type of flexibility as a factor. In addition, males (33.3%) were less likely than females (48.4%) to attach importance to this factor.
Because of demographic changes in the veterinary industry, this may be partly a result of generational differences.

Of those factors considered most important, there was little difference between the generations: the “desire to make [their] own decisions about how to practice medicine” was considered “important” or “very important” to 80.4% of those graduating between 2004 and 2014 and to 81.2% of those graduating in or before 2003; the “desire to have control over work schedule and life balance” was similar as well (78.2% and 70.6%, respectively).

For those associates in group practice, the factors affecting satisfaction that had the least congruity were “life balance” and “compensation”. Those graduating between 2004 and 2014 were less likely than those graduating in or before 2003 to be satisfied with life balance (25% vs. 46%), and female respondents were less likely to report being satisfied than males (28.6% vs. 45.3%). Interestingly, no generational differences were observed in satisfaction with compensation, and just 30% of both graduating groups reported satisfaction. Only 20.8% of females were “extremely satisfied” or “very satisfied,” in contrast to 33.3% of males.

4. Discussion
The AVMA estimated that there were 3,816 equine veterinarians in the US in 2014, representing 5.9% of the total number of veterinarians. These equine practitioners were 51.2% male and 48.8% female, but with a student ratio of 85% female to 15% male, it is predicted that within 5 years most equine veterinarians will be women. Currently, 50% of equine practitioners are over the age of 50. Fewer young veterinarians can afford to purchase practices from retiring practitioners because of rising student debt partnered with low salaries, forcing many equine practitioners to continue working because they cannot sell the assets needed to fund their retirement. In 2012, veterinary school graduates in equine practice had an average educational debt of $151,672 and average starting salaries of $37,143. Increasing numbers of veterinarians graduate each year, and the AVMA projects a 23% overcapacity of equine veterinarians from 2012 to 2025. This perfect storm of recessionary pressures, slackened demand for veterinary services as a result of decreased equine industry activity, an increased supply of equine practitioners with dimin-
ished capacity for practice purchase, and an aged cohort of equine veterinarians unable to harvest retirement assets from their practices currently makes equine veterinary medicine a difficult environment.

One impetus for the creation of this survey was the suggestion from preliminary data of the 2012 Merck-Henry Schein National Equine Economic Study that the percentage of solo equine veterinary practices was increasing. Given the oversupply of equine veterinarians and the high debt load of new graduates, one hypothesis was that new graduates were unable to find employment or could not afford to purchase an existing practice and were therefore opening solo practices. Of the 2,995 solo practitioner members of the AAEP in 2014, 221 were only 5 years or so removed from graduation. Alternatively, it was hypothesized that associate veterinarians were dissatisfied with their employment experiences and thus sought self-employment. The Millennial generation is more comfortable with self-employment than previous generations; in fact, 70% of this global demographic stated in a recent survey that they wanted to launch their own companies. Finally, the economic pressures from a recessionary economy and shrinking horse industry were thought to possibly be driving this trend, with formerly 2 to 3 veterinarian practices shrinking to solo practices. This survey aimed in part to determine which of these factors were significant in the suggested increase.

However, an examination of 6 AAEP annual reports revealed that the percentage of solo practices has stayed fairly constant over the last 7 years (37%–39%), reliably making up about 71% of all practice owners. Thus, although solo practice clearly makes up most private practices, the data do not indicate a change in prevalence (Fig. 12).

In view of the difficult environment, this survey also sought to identify the areas of practice that contributed most to the stress experienced by all equine veterinarians. By examining many different factors that are a part of life as an equine practitioner, strategies for mitigating stress could potentially be identified from the results of the survey. Several broad themes emerged from the results: the importance of practice culture, the importance of control and choice, and the degree of stress in the profession.

Practice Culture
Because there are clear advantages to group practice such as economies of scale, shared responsibilities, and professional support, this survey sought to understand the reasons that so many equine veterinarians choose to practice alone. Many veterinarians made that choice at the beginning of their careers; 24.6% of survey respondents had never worked in any practice other than their own, except for an internship. According to survey results, they made this choice to ensure they could be in control of
their work environment—to be able to make their own clinical choices, manage their own work schedule, and essentially create their own culture. The remaining solo practitioners who responded to the survey had left a group practice, and practice culture was the primary reason given that drove these doctors to depart. Associate respondents employed at both small- and large-group practices valued practice culture as highly as compensation in determining their satisfaction with their position. Unfortunately, many of the practices where they worked did not provide a culture that satisfied them. Practice owners may not have sufficient insight into this deficit; 69.2% of partner/shareholder respondents indicated they were “extremely satisfied” or “very satisfied” with the culture of their practices.

Practice culture is extraordinarily important, an idea that is supported by the responses to this survey. According to the HR Insights blog, “Culture is the character and personality of your organization. It’s what makes your organization unique and is the sum of its values, traditions, beliefs, interactions, behaviors, and attitudes.” Because equine veterinarians spend so much more of their time working than they do with their families, they need that time to be meaningful, enjoyable, and productive. This is often more important than the money they earn. “Workplace culture drives employee loyalty. It is easier to attract and retain the best employees when an organization has a dynamic, thriving culture where employees feel valued and enjoy their work. In addition, their perceptions of the workplace may influence the public reputation of the organization.”

Leadership is the essential foundation of culture. Practice owners, for better or worse, are the leaders of the practice, and the behaviors they model become the practice culture. If owners communicate poorly or avoid conflict, culture suffers. If long hours and revenue production are the only traits valued highly but have no connection to profitability and client satisfaction, the culture will reflect this. “Culture in the workplace involves a system of behaviors, beliefs, attitudes and values. It develops over time and is passed on from one generation of workers to the next. It is also a reflection of what organizational leadership values.”

When leaders cannot make decisions or are frequently absent, the culture is often driven by bureaucracy and detailed procedures or conversely lurches reactively from crisis to crisis because employees have little or no understanding of how their work fits into the overall goals of the organization. In practices like this, team members tend to work in isolated silos, and there is little collaboration or cooperation. When a practice’s team understands the shared objectives, the leaders are decisive and inclusive, and employees understand their roles and are engaged in the work, the organization has a strong culture. These firms typically reach their strategic objectives, retain staff, and enjoy greater satisfaction in the work.

Control and Choice
It is no secret that many equine veterinarians are considered as being Type As, workaholics, and control freaks. However, although many personality traits are innate, most researchers now believe that Type A personality characteristics are more of a reaction to environmental factors and are influenced by culture and job structure. These include jobs that put heavy demands on time and penalties on mistakes, create stress, or attract people with a natural tendency toward being more intense and achievement-oriented.

Each different cohort in this survey favored responses that maximized their control of their practice experience. Having the ability to make their own decisions about how to practice clinically was valued highly by solos, associates, and owners. Most respondents were satisfied by their actual experience in this regard. It seems that most veterinary practices have wide leeway in allowing their doctors to practice as they see fit. Although this drives satisfaction, it may detract from forming a strong practice brand equity if a wide diversity of approaches to clinical problems exists among staff doctors.

According to a study published in Psychological Science, both having power over others and having choices in your own life are about the basic need for control. The study found that people are willing to trade one source of control for the other. For example, if people lack power, they clamor for choice, and if they have an abundance of choice they do not strive as much for power. Equally, if people are denied choice, they hunger more for power. In summary, while people can accept their lot if they have either power or choice, they become discontent when they have neither.

Management decisions were cited by associate survey respondents as the source of extreme stress more than any other stressor. Allowing associates as much choice as possible, and including them in decision making, can help them feel more positive about their job and responsibilities.

Younger veterinarian respondents expressed a higher importance on the need for flexibility for family responsibilities in the survey than older-genera
tion veterinarians, possibly because of their life stage. Although certainly more females graduated than males between 2004 and 2014, possibly skewing the results, when looking at the responses of males versus females across all graduation years, just a third of all men attached strong importance to family time compared with almost half of all women. This gender difference will increasingly affect the profession.

The top regret cited by veterinarians in a survey published by dvm360 magazine in May 2015 was not having enough time for personal life and family.
Having the control to make choices about how to spend their time is a key factor in the happiness of most people, and the responses to this survey indicated that many respondents felt dissatisfied in this regard. These findings are in alignment with other broader studies. For example, a global generations survey of nearly 10,000 workers worldwide conducted by Ernst & Young revealed that close to 80% of Millennial and 73% of Generation X workers surveyed were part of dual-income couples in which both work full-time. In contrast, just 47% of the baby-boomer respondents have a full-time working spouse. The study points out the following:

More than a quarter of baby-boomer workers have a spouse at home, or one who works part time or with flexible hours and is responsible for taking care of all home-front duties. Millennial workers, the group that companies say they are scrambling to attract and retain, are the most dissatisfied. Survey after survey, including the EY one, show that what millennials most want is flexibility in where, when and how they work. Millennials were most likely in the survey to say that they would take a pay cut, forgo a promotion or be willing to move to manage work-life demands better.17

The lives of equine veterinarians often lack balance. AAEP listserv member survey respondents working in both small- and large-group practices cited life balance as the aspect of their position that made them the most dissatisfied. Small-practice associates next cited the time requirements of practice, whereas large-practice associates next cited practice culture. Difficulty in achieving balance often relates to the demands of equine practice with its seasonality, the need to provide emergency services, and the pervasive culture of not establishing boundaries on work hours or days. Horse owners or others in the equine industry have responsibility for horses 24 hours a day, 7 days a week, 365 days a year, and they expect no less of a commitment from others in the equine industry. More than half of veterinarians own or work as part of an equine practice,18 and the majority of these practices are owned or managed by one or two doctors.19

A persistent lack of control in a person’s life often leads to depression and anxiety. Anything that makes us feel helpless and lacking fundamental control over our surroundings can have a lasting impact. Gaining more control of our surroundings, on the other hand, makes us more content and less at risk. Our need for control may help us to protect ourselves, operating as a survival mechanism. A great deal of our technological progress might be thought of as an expression of our need to gain more control over our world and our lives.18

Veterinarians are often seriously affected by the lack of control in their lives. More than 10% of practitioners reported having serious psychological distress in a recent study, and 1 in 6 veterinarians have considered suicide since graduating from veterinary school.19,20 The Center for Disease Control and Prevention’s Weekly Morbidity and Mortality Report caused a stir in February 2015 when it reported the following:

Since graduating from veterinary school, 24.5% and 36.7% of male and female respondents reported experiencing depressive episodes, respectively, 14.4% and 19.1% suicidal ideation, and 1.1% and 1.4% suicide attempts. In comparison, male and female U.S. adults had a lower lifetime prevalence of depressive episodes (15.1% and 22.9%, respectively) and suicidal ideation (5.1% and 7.1%) but a higher prevalence of suicide attempts (1.6% and 3%).20

Mitigating the stress caused by the oftenthwarted need for control lies in having choices. Leading by example, owners should engage in collaborative decision making whenever possible, provide flexible scheduling, minimize competition among doctors, and foster a culture that embraces a balanced life.

Stress

There are many pressing concerns and sources of stress for practitioners related to their business and professional lives. Of the multiple stressors listed in the survey questions, it is notable that only choosing professional advisors and professional interactions had less than 50% of respondents reporting associated stress. Most of the other aspects of practicing and operating a business were considered stressful by robust majorities. Some of the factors reported to be most stressful were those related to lack of control and choice or to practice culture. A strong linkage exists among these themes.

With new graduates’ substantial loans, financial stress is inevitable. When asked about how satisfied they were with their compensation, only 20.8% of female survey respondents were satisfied, compared with 33.3% of males. This is not unexpected for several reasons. The AVMA 2015 report on veterinary markets revealed that female graduates have lower starting salaries and higher debt than male graduates.21 This may result from endemic pay disparities between males and females. The Institutes for Women’s Policy Research reports the following:

Women are almost half of the workforce. They are the equal, if not main, breadwinner in four out of ten families. They receive more college and graduate degrees than men. Yet, on average, women continue to earn considerably less than men. In 2013, female full-time workers made only 78 cents for every dollar earned by men, a gender wage gap of 22 percent. Women, on average, earn less than
In equine veterinary medicine, data show that most women desire to work 10 fewer hours a week, whereas men are equally divided between wanting to work 10 fewer hours or 10 more, which may be a factor in salaries.23

Most solo and group practice owners who responded to the survey indicated they experienced stress in most aspects of running their business, with financial aspects concerning nearly all respondents. In addition, communication, negotiation, and conflict resolution were important sources of ongoing worry. Solo practitioners were more likely to struggle with client communication, whereas group practice owners had more difficulty with aspects of managing staff. Learning more about effective business practices and developing new communication skills could mitigate some of the stress that practice owners experience.

5. Conclusion

The information gained by this study is important because all stakeholders in the equine veterinary industry are affected by the current environment. Sole proprietors may benefit from collaborative efforts with other local solo practices to share emergency coverage as well as provide relief coverage for illness, family time, vacation, and maternity leave. Existing group practices will compete for market share with emerging solo practices and would be wise to determine how to fill unmet needs of horse owners who are outside of the offerings of solo practices (e.g., emergency services or services that require a significant investment in technology). Aging practice owners may be unable to harvest value from their practices for retirement if they are unable to attract and retain associates willing and able to purchase ownership shares. To address the dissatisfaction with employment experiences that emerged in the survey, practice owners will need to alter their approach to managing associate veterinarians to retain them. Industry partners (providers of pharmaceuticals, medical supplies, and equipment) have a vested interest in the sustainability of healthy practices to retain customers to purchase their product lines; these companies are increasingly investing in collaborative efforts with business consultants to educate practice owners with the goal of boosting profitability (and long-term success) of practices.

Because of the unique challenges facing the equine industry, emotions surrounding the issues can be strong. New graduates with high educational debt and limited employment opportunities experience high stress, as do older practitioners who are unable to sell their largest asset on which they had depended for retirement funds. Stress can spawn bitterness and strife among generations. It is in the best interest of all practitioners to work together toward the common goal of a strong equine veterinary profession. This survey has elucidated common themes that, if addressed, can significantly improve the experience of equine practice.

Acknowledgments

Declaration of Ethics

The Authors declare that they have adhered to the Principles of Veterinary Medical Ethics of the AVMA.

Conflict of Interest

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References and Footnotes


aDwyer AE. Scottsville, NY (personal communication) 2013.
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1. Introduction

Abnormalities of the upper airway (UA) are frequently implicated as a cause of poor performance, exercise intolerance, or abnormal respiratory noise in the equine.¹⁻³ Common abnormalities involving the function of the arytenoid cartilages include recurrent laryngeal neuropathy, arytenoid chondritis, and laryngeal dysplasia. Dorsal displacement of the soft palate (DDSP) and pharyngeal collapse affect the pharyngeal region. Congenital abnormalities and dynamic larynx collapse associated with poll flexion can affect multiple regions of the larynx and pharynx.

Resting UA endoscopy is widely available and is often the first diagnostic tool used in evaluation of UA abnormalities. In many cases, resting endoscopy is sufficient for diagnosis. However, dynamic evaluation, either using a treadmill or a portable over-ground videoendoscopy system, is considered to provide a more accurate diagnosis than does resting endoscopy.⁴⁻⁶ Unfortunately, dynamic UA examination requires specialized equipment that may not be available locally, may be cost prohibitive for some clients, and may not be appropriate for some horses. In addition, some UA abnormalities cannot be fully assessed using UA endoscopy.

Laryngeal ultrasonography, first described by Chalmers et al,⁷ is a relatively new addition to the diagnostic repertoire for UA disorders. It has proven to be useful clinically in the diagnosis of many conditions of the UA, especially for disorders resulting in abnormal arytenoid movement, including recurrent laryngeal neuropathy, arytenoid chondritis, and laryngeal dysplasia, a congenital malformation of the larynx.⁷⁻¹⁰ Laryngeal ultrasonography can be especially helpful in cases where a dynamic examination cannot be performed.

An appreciation of the pathologic changes that occur in diseases of the UA is helpful in understanding the ultrasonographic changes that accompany each disease. Recurrent laryngeal neuropathy results in denervation atrophy and subsequent loss of function of the intrinsic laryngeal muscles innervated by the recurrent laryngeal nerve, including the cricoarytenoideus dorsalis (CADM), cricoarytenoideus lateralis (CALM), transversus arytenoideus, vocalis, and ventricularis muscles.¹¹,¹² Clinically, this condition manifests itself as decreased or absent arytenoid abduction and affects the left arytenoid more commonly than the right arytenoid.⁴ Denervated muscle has a characteristic ultrasonographic appearance; the muscle becomes hyperechoic to normal muscle and has a more homogeneous appearance with a loss of the normal striated pattern.¹³⁻¹⁵ These changes have been observed ultrasonographically in the CALM and CADM of horses with treadmill UA endoscopy-confirmed recurrent laryngeal neuropathy.⁸,¹⁰
The precise etiology of arytenoid chondritis remains unclear, but likely results from local trauma to and subsequent bacterial infection of the arytenoid cartilage. Some horses may have granulomas only on the axial surface of the arytenoid cartilage, whereas the pathology may progress to involve the entire arytenoid cartilage in other horses. In more severe cases, the arytenoid becomes thickened with irregular margins and has impaired movement, which may be appreciated endoscopically. The presence of arytenoid cartilage thickening, irregular margination, and abnormal echogenicity typical of this disease has been imaged using ultrasonography.

A congenital defect known as laryngeal dysplasia (also known as fourth branchial arch defect) may cause abnormal arytenoid movement of either the left or right arytenoid cartilage. Horses with this condition may also have constant or intermittent rostral displacement of the palatopharyngeal arch during UA endoscopy or dorsal displacement of the soft palate. Horses with laryngeal dysplasia have characteristic anatomic abnormalities, including lack of a cricothyroid articulation and extension of the thyroid lamina dorsal to the muscular process of the arytenoid cartilage and they may have pharyngeal muscle abnormalities as well. The ultrasonographic appearance of these anatomic features has been described.

The pathophysiology of DDSP has not been fully elucidated, but may involve an inability to maintain a rostral position of the larynx. Interestingly, a change in the position of the basihyoid bone after surgical treatment (laryngeal tie-forward) is associated with an increased likelihood of racing postoperatively. This finding prompted initial investigation into an ultrasonographic marker for DDSP which has shown that horses with DDSP had a smaller average distance between the skin and the basihyoid bone than did horses without DDSP. However, the difference in this measurement between horses with and without DDSP was less than 2 mm, limiting its use in clinical situations.

The etiology of other UA disorders, including pharyngeal collapse, billowing of the soft palate, epiglottic retroversion, laryngeal collapse associated with flexed head position, and axial deviation of the aryepiglottic folds, remains unclear. No characteristic ultrasonographic findings of these disorders have been described.

The purpose of this paper is to provide the practitioner with instruction on how to perform a laryngeal ultrasonographic examination and incorporate this tool into his or her evaluation of the UA.

2. Materials and Methods

Horses are prepared for the examination by sedation with xylazine hydrochloride (0.4 mg/kg IV). Most horses do not need to have the laryngeal region clipped prior to examination, but if the coat is coarse or thick, clipping will improve image quality. The head is held in an extended position by a handler or a stand to move the laryngeal region caudally in relation to the mandible. A linear or curvilinear transducer can be used for the examination. A frequency of 8 to 10 MHz typically provides adequate penetration while preserving resolution.

Examination of the lateral portion of the larynx can be performed in the dorsal and transverse planes. In the dorsal plane, an initial image of the superficially positioned thyroid cartilage, the cricoid cartilage caudal to the thyroid cartilage, and the arytenoid cartilage deep to the thyroid cartilage can be obtained (Fig. 1).
The CALM and vocalis muscle are imaged between the thyroid and arytenoid cartilages and the cricothyroidus muscle is imaged between the thyroid and cricoid cartilages. By moving the transducer slightly dorsally, one can image the cricothyroid articulation, formed by the caudal cornu of the thyroid cartilage and the articular process of the cricoid cartilage (Fig. 2). Mineralization of the caudal cornu of the thyroid cartilage is common, but this does not seem to be clinically significant. From the cricothyroid articulation, the transducer is moved dorsally and angled slightly ventrally. In this location, one can image the cricoaryte- noid articulation between the muscular process of the arytenoid cartilage and the dorsal cricoid cartilage. The lateral portion of the CADM may be evaluated as well (Fig. 3).

The lateral aspect of the larynx is also evaluated in the transverse plane. The initial image obtained is the superficially positioned thyroid lamina with the arytenoid cartilage deep to the thyroid lamina and the CALM and vocalis muscle between the thyroid and cricoid cartilages (Fig. 4). In some horses, the vocalis muscle is imaged distinctly from the CALM, but in others, the distinction between the two muscles cannot be defined. If the transducer is moved caudally, the caudal cornu of the thyroid cartilage, the cricothyroid articulation, and the cricot cartilage are imaged.

The ventral portion of the larynx can be examined in transverse and median planes. In a transverse plane, the tracheal rings can serve as a reference point. From the tracheal rings, as the transducer is moved rostrally, the ventral aspect of the cricoid cartilage is identified (Fig. 5), followed by the thyroid cartilage. Deep to the thyroid cartilage, the vocal folds may be imaged and their movements can be observed (Fig. 6). The mineralized rostral aspect of the ventral thyroid cartilage is encountered next. Between the thyroid cartilage and the basihyoid bone, the thyrohyoid bones may be imaged laterally. The basihyoid bone appears as a horizontal line (Fig. 7), and if the transducer is angled rostrally from this position, the ceratohyoid bones are imaged. By moving the transducer rostrally, the lingual process of the basihyoid bone is imaged. The depth of the basihyoid bone can be measured at the junction between the lingual process and the body of the basihyoid bone. In the median plane,
the relationship between the lingual process and the mineralized rostral aspect of the thyroid cartilage can be evaluated. The ventral aspect of the cricoid and tracheal rings can also be imaged.

In general, the overall symmetry of the laryngeal cartilages and associated musculature should be evaluated. The cartilages should be smoothly marginated with homogeneous echogenicity, although mineralization of the thyroid and arytenoid cartilages is fairly common (Fig. 8), especially in older horses. The muscles normally have heterogeneous echogenicity and have a striated pattern in a longitudinal view.

3. Results

Uses of laryngeal ultrasonography in cases of recurrent laryngeal neuropathy, arytenoid chondritis, and laryngeal dysplasia, have been documented in the recent veterinary literature.

Comparison of the relative echogenicity of the left and right CALM, vocalis muscle, and CADM enables the practitioner to assess whether the musculature has the hyperechogenicity characteristic of denervation atrophy of recurrent laryngeal neuropathy. Ultrasound machine settings should be kept constant between left and right sides of the larynx, and images should be evaluated in dorsal and transverse planes. Side-by-side comparison is often useful (Fig. 9). It should be borne in mind that many factors will contribute to the ultrasonographic appearance of the muscles, so comparison within a horse is preferable to comparison between horses. In one study comparing subjective laryngeal ultrasonography to results of dynamic endoscopy, ultrasound had a sensitivity of 90% and a specificity of 98% for an overall accuracy of 96% at predicting which horses would have abnormal arytenoid cartilage movement during exercise. Another study yielded similar results with a sensitivity and specificity of 95% for subjective comparison of musculature echogenicity and additionally showed that quantitative comparison of muscle echogenicity differed between different grades of arytenoid movement.
The contour of the arytenoid cartilages should be assessed critically. The arytenoid cartilages should have a trumpet bell shape with smooth margins. Horses with chondritis have irregularity of the axial and abaxial margins with thickening of the cartilage and abnormal echogenicity within the arytenoid cartilage compared with normal arytenoid cartilages (Fig. 10). Horses may have only a granuloma or chondroma on the axial surface of the arytenoid cartilage without diffuse arytenoid chondritis. Ultrasonographically, this manifests as focal irregularity of the axial margin and a smooth abaxial margin and normal arytenoid cartilage width.

The anatomic abnormalities characteristic of laryngeal dysplasia can also be observed. The extension of the thyroid cartilage dorsal to the muscular process of the arytenoid cartilage can best be imaged in the transverse plane (Fig. 11), whereas the lack of a cricothyroid articulation is best imaged in the dor...
sal plane (Fig. 12). In these horses, the relationship between the thyroid cartilage, CALM, and arytenoid cartilage is abnormal; the CALM is often positioned caudal to the thyroid cartilage in the gap between the thyroid cartilage and the cricoid cartilage.9

Other more unusual anatomic malformations have been diagnosed in individual cases. One foal with abnormal UA noise and exercise intolerance had cyst-like structures in the thyroid, arytenoid, and cricoid cartilages as well as the first tracheal ring.28 An adult horse with DDSP was found to have a basihyoid bone malformation. A Paint horse heterozygous (N/H) hyperkalemic periodic paralysis (HYPP) had hypertrophy of the left vocalis muscle.

4. Discussion

Ultrasonography of the laryngeal region can be performed easily in an ambulatory or hospital setting, is a noninvasive and safe procedure, and requires no specialized equipment beyond an ultrasound machine with a linear or curvilinear transducer. Clipping of the hair is not required and light sedation is generally sufficient to ensure patient compliance, so client and patient acceptance of the procedure have been excellent.

Currently, ultrasonography of the larynx is an excellent tool for investigation of the reason for abnormal arytenoid movement, as the cause may be difficult to determine with resting UA endoscopy alone. Evidence of hyperechogenicity of the left CALM, vocalis muscle, and CADM would support a diagnosis of left recurrent laryngeal neuropathy as a cause of poor performance or abnormal UA noise. In cases of arytenoid chondritis, ultrasonography permits imaging of nearly the entire arytenoid cartilage (arrows) is severely thickened with irregular margins and increased echogenicity in its interior. Dorsal is to the left of the image and ventral is to the right of the image.

Fig. 9. Comparison of echogenicity of the CALM and vocalis (arrows) and CADM (arrowheads) musculature. Horses with recurrent laryngeal neuropathy have increased echogenicity and more homogeneous echogenicity of the CALM and CADM. Dorsal plane ultrasound images of the CALM muscle of a horse with recurrent laryngeal neuropathy (A) and a normal horse (B). Transverse plane ultrasound images of the CALM and vocalis muscles of a horse with recurrent laryngeal neuropathy (C) and a normal horse (D). Dorsal plane ultrasound images of the CADM of a horse with recurrent laryngeal neuropathy (E) and a normal horse (F). In the dorsal plane images, rostral is to the left and caudal is to the right and in the transverse plane images, dorsal is to the left of the image and ventral is to the right of the image.

Fig. 10. Transverse plane ultrasound image of the lateral aspect of the larynx of a horse with arytenoid chondritis. The arytenoid cartilage (arrows) is severely thickened with irregular margins and increased echogenicity in its interior. Dorsal is to the left of the image and ventral is to the right of the image.
This has allowed us to assess the extent of disease in the cartilage, diagnose any associated abscesses or perilaryngeal inflammation, and monitor response to treatment. In some of the cases in this report presented for evaluation of suspected arytenoid chondritis, the ultrasonographic findings did not support a diagnosis of arytenoid chondritis, leading to additional investigation and revision of the diagnosis. Congenital abnormalities involving the laryngeal cartilages can be imaged directly, instead of being inferred from UA endoscopic abnormalities.

In the author’s practice, ultrasonography of the larynx has been incorporated as a routine procedure for investigation of UA disorders, as it contributes to a more thorough evaluation of the UA. Endoscopy is an excellent tool for assessment of laryngeal function and abnormalities visible at the luminal aspect of the larynx, but ultrasonography allows more complete examination of the laryngeal cartilages and associated musculature, structures which were previously difficult to evaluate. In addition, ultrasonography can be useful in evaluation of poor performance or abnormal UA noise in cases where a dynamic UA examination is not feasible (due to availability, financial or liability concerns, concurrent lameness, or other factors). Hopefully, future work will expand the uses of laryngeal ultrasonography for a variety of UA conditions.

Acknowledgments

Declaration of Ethics
The Author declares that she has adhered to the Principles of Veterinary Medical Ethics of the AVMA.

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The Author declares no conflicts of interest.

References


How to Understand Differences Between High- and Low-Field Standing Magnetic Resonance Imaging

Katherine S. Garrett, DVM, DACVS

1. Introduction

The availability of magnetic resonance imaging (MRI) has increased greatly over the past 10 years. This has led to tremendous advances in our ability to make more accurate and/or specific diagnoses in a variety of body regions. For the practitioner, it is important to understand different types of MRI scanners to appropriately refer cases.

Multiple types of MRI scanners are currently on the market—all with advantages and disadvantages. High-field (1.5 T) scanners were the first to come into clinical use for horses. High-field scanners require general anesthesia and specialized nonferrous anesthetic equipment. However, they allow for higher-resolution images, thinner slices, larger fields of view, and faster scan times. Low-field (typically 0.3 T) scanners are available in both recumbent and standing designs. The recumbent design requires general anesthesia and specialized nonferrous anesthetic equipment. However, they allow for higher-resolution images, thinner slices, larger fields of view, and faster scan times. The standing design is prone to artifacts from motion that worsen as the scan region moves proximally from the foot. It is important to keep all of these factors in mind when choosing where to refer a case, as some cases are better served with one design over another.

Image quality is generally superior using high-field MRI. Increased magnet strength permits the use of thinner slices and higher resolution compared with low-field MRI, which aids in identifying cartilage pathology.1 Oftentimes, short tau inversion recovery sequences can be extremely useful in diagnosing bony lesions, but these sequences are very sensitive to motion and may be nondiagnostic in some standing horses. Other work has shown that even in cadaver limbs, high-field images are superior at detecting pathology compared with low-field images.2

Because MRI scans of a unilateral region typically take approximately 45 to 60 minutes, it is very important to attempt to localize the area of lameness as specifically as possible. This generally involves perineural and intra-articular anesthesia. Unfortunately, especially in the case of perineural anesthesia, we have learned that anesthetic agents can migrate over a great distance, leading to a lack of specificity. For example, a palmar digital nerve block can migrate as far proximally as the fetlock region.3 For this reason, when MRI operators scan a horse that has blocked to a palmar digital nerve block, they always include at least one sequence that...
includes the pastern and the fetlock to the level of the proximal sesamoid bones. In some cases, the foot is relatively normal, but the primary lesion involves the pastern region. The field of view and coil design of a high-field scanner permit imaging the pastern region without repositioning the horse or the coil or performing additional localizer sequences, making inclusion of this region convenient for the operator. Unfortunately, the standing MRI design requires that the coil and magnet be repositioned with new localizer sequences to obtain images of the pastern region, and these tasks can be time-consuming. The superior image quality of the high-field images also allows the interpreter of the images to be more confident of what the primary source of lameness may be.

It has been shown that significant diffusion of contrast material occurs after instillation at the base of the proximal sesamoid bones. In the author’s opinion, if a horse has blocked to an abaxial sesamoid nerve block without a peritoneal dialysis performed previously, the lesion could be located in the foot, pastern, fetlock, or distal metacarpal region. This is a large area to image in a practical sense, so in this case the author encourages reblocking the horse more specifically. If repeating the lameness examination with different blocks is not possible, high-field scanners can accommodate imaging the foot, pastern, and fetlock regions with much less difficulty than in a standing design, where multiple repositionings of equipment are necessary. Additionally, in a standing design, horse compliance may become an issue with a three-region scan, especially if bilateral images are obtained. It has also been shown that anesthesia of the proximal metacarpal/tarsal region can anesthetize the distal carpal and tarsal regions and vice versa. For that reason, when operators scan a carpus or tarsus, they include the proximal metacarpal/metatarsal region; when scanning a proximal metacarpal/tarsal region, they include the carpometacarpal and middle carpal joints or the tarsometatarsal and distal intertarsal joints. Proximal regions of the limb are very prone to motion in a standing design, and the field of view often does not permit including both regions in the same sequence without repositioning.

We have found high-field MRI to be extremely useful in cases of orthopedic sepsis that are not responding to treatment as would be expected. Areas of osteomyelitis, physisis, or bony necrosis are readily identified. In smaller foals, areas as proximal as the pelvis and axial spine can be imaged using recumbent designs, which is helpful because radiography and ultrasonography of these areas are more challenging. Standing MRI is generally not feasible in these cases, because it may be too painful for the horse to bear weight and remain motionless for the time required to obtain the images.

Although high-field MRI is not always an option or indicated for every case, there are many situations in which it provides valuable or vital information beyond what can be obtained from standing low-field MRI.

Acknowledgments

Declaration of Ethics

The Author declares that she has adhered to the Principles of Veterinary Medical Ethics of the AVMA.

Conflict of Interest

The Author declares no conflicts of interest.

References

How to Use Standing Magnetic Resonance Imaging to Substantiate or Disprove a Provisional Lameness Diagnosis

Stacey W. Kent, VMD

Standing magnetic resonance imaging is an important tool in the diagnosis of lameness in the equine distal limb. Author's address: EQUIGEN, LLC, 125 White Horse Road, Cochranville, PA 19330; e-mail: staceywk@verizon.net. © 2015 AAEP.

1. Introduction
The first standing magnetic resonance imaging (MRI) unit was installed in 2002 at the Bell Equine Veterinary Clinic in Kent, England. Since that time, some additional 70+ units have been installed throughout the world, with 22 units currently operational in the United States and Canada. MRI offers the unique benefit of simultaneous imaging of both soft tissue and bony structures in a single comprehensive evaluation. The usefulness of MRI in achieving a diagnosis in equine lameness cases is well documented and has been presented at multiple multinational professional veterinary conferences. Prior to being referred for a standing MRI evaluation, horses will usually have been thoroughly worked up with multiple comprehensive lameness evaluations with peripheral nerve diagnostic anesthesia and/or intra-articular anesthesia and radiographs, ultrasounds, and/or nuclear scintigraphies. As a result of the information gathered from these procedures, it is not uncommon for a horse to be referred with a provisional diagnosis. In these situations, the provisional diagnosis has been relayed, and the intention of the standing MRI evaluation is to confirm this diagnosis and rule out other less obvious areas of potential injury. This article presents three cases in which standing MRI was intended to confirm a working diagnosis but instead gave altogether unexpected results.

2. Materials and Methods
For all cases outlined in the following sections, MRI evaluation was performed using a 0.27 Tesla standing unit. Additional imaging, including radiography and ultrasonography, was completed using various units by the referring veterinarians. The isocenter of the magnet for imaging the equine foot was focused over the distal dorsal edge of the navicular bone (Fig. 1), and a standard “foot protocol” was used that consisted of the following sequence types and associated slice positioning: T1-weighted (W) 3-dimensional (3D) gradient echo (GE) sequences (imaging planes: frontal, sagittal, and transverse at an angle perpendicular to the deep digital flexor tendon (DDFT) proximal to the navicular bone; Fig. 2); T2W 3D GE sequences (imaging planes: frontal, sagittal, and transverse at an angle perpendicular to the DDFT proximal to the navicular bone; Fig. 2); T2W fast spin echo sequences (FSEs) (imag-
ing planes: sagittal and transverse at angles parallel to the ground, perpendicular to the DDFT proximal to the navicular bone, and perpendicular to the DDFT at the insertion on the distal phalanx; Fig. 3); short tau inversion recovery (STIR) FSE sequences (imaging planes: sagittal and transverse at angles parallel to the ground, perpendicular to the DDFT proximal to the navicular bone, and perpendicular to the DDFT at the insertion on the distal phalanx; Fig. 3); and proton dense-weighted (PDW) spin echo (SE) sequences (imaging plane: transverse at an angle perpendicular to the DDFT proximal to the navicular bone; Fig. 2).

The isocenter of the magnet for imaging all metacarpo/metatarsophalangeal joints was focused over the middle of the metacarpo/metatarsophalangeal joint (Fig. 4), and a standard “fetlock protocol” was used that consisted of the following sequence types and associated slice positioning: T1W GE sequences (imaging planes: sagittal, frontal, and transverse at angles parallel to the ground and perpendicular to the distal sesamoidean ligaments; Fig. 5); T2*W GE sequences (imaging plane: sagittal); T2W FSE sequences (imaging planes: transverse...
at angles parallel to the ground and perpendicular to the distal sesamoidean ligaments; Fig. 5); and PDW FSE sequences (imaging planes: transverse at angles parallel to the ground and perpendicular to the distal sesamoidean ligaments; Fig. 5).

All horses were sedated using an initial loading dose of detomidine hydrochloride (HDL; 3–5 mg IV). Once an adequate level of sedation had been achieved, a consistent sedation level was maintained using a smaller dose of detomidine HCL (1–2 mg IV) every 10 minutes until all sequences were obtained (approximately 60–75 minutes). All MRIs were reviewed by Dr. Natasha Werpy, DACVR, Equine Diagnostic Imaging, Gainesville, Florida.

Case 1

A 2011 Thoroughbred gelding used as a flat racehorse presented with an acute right hind (RH) lameness (3.5/5 in accordance with the American Association of Equine Practitioners [AAEP] guidelines for grading lameness). At the time of initial evaluation, the lameness was localized to the distal limb using abaxial sesamoid peripheral nerve anesthesia. Radiographs of the RH foot were taken, and there appeared to be a fracture of the lateral plantar process of the distal phalanx (Fig. 6). The horse was seen 3 days later and was noted to be non-weight-bearing lame on the RH. Anesthesia of the lateral plantar digital nerve improved the lameness to the extent that the horse would weight-bear on the RH at the trot. The horse was referred for a standing MRI of the RH to confirm the diagnosis of a fracture of the lateral plantar process of the distal phalanx and to identify any associated soft tissue injury.

Per standard protocol, a complete MR evaluation of the RH was completed as previously described. In addition to the standard sequences and imaging planes, the magnet was also lowered and moved dorsally so that the isocenter was positioned over the distal aspect of the extensor process of the distal phalanx. The change in the magnet position was done to achieve appropriate fat suppression in the distal phalanx in STIR sequences and therefore to identify areas with potential abnormal fluid, as would be expected with a fracture of the lateral plantar process of the distal phalanx. There were no appreciable abnormalities identified in either the distal phalanx or the associated soft tissue structures. Given the initial blocking pattern and lack of any appreciable abnormalities in the distal and middle phalanges, the magnet was again repositioned with the isocenter over the metatarsophalangeal joint. An incomplete, midline sagittal fracture in the proximal phalanx was immediately identifiable (Figs. 7 and 8). After the MRI evaluation, radiographs were taken by the referring veterinarian, and the fracture was evident radiographically (Fig. 9). As a side note, there was no appreciable metatarsophalangeal joint effusion appreciable upon physical examination before, during, or after the MRI evaluation.

Case 2

A 2005 Connemara gelding used for dressage had been lame in the left front for approximately 1 year. Over the course of that year the horse was seen by multiple veterinarians and received a multitude of treatments, including but not limited to intra-articular therapy of the front distal interphalangeal, front proximal interphalangeal, and lower hock joints and regional limb perfusion of the distal limb with tiludronate disodium and corrective shoeing. These different therapies had varying degrees of success in resolving the left front lameness. Before being referred for a standing MRI evaluation, a thor-
ough soundness examination, which included peripheral nerve diagnostic anesthesia, was completed by the referring veterinarian. This horse was noted to be sound when trotted in hand but 1/5 lame on the left front under tack when trotted to the left. There was also some lameness noted in the right front when trotted under tack to the right, but this lameness improved readily. Anesthesia of the lateral and medial palmar digital nerves of the left front partially improved the lameness to the left and exacerbated the right front lameness on a circle to the right. A mid-pastern ring block of the left front abolished the left front lameness. No further blocking was done of the right front limb to further characterize that lameness.

Radiographs of the right front foot and pastern were unremarkable, and radiographs of the left front foot and pastern indicated mild remodeling of the dorsolateral aspect of the left front middle phalanx at the joint capsule insertion. Radiographs of the left metacarpophalangeal joint were unremarkable; those of the right indicated a small osteochondral fragment associated with the dorsomedial aspect of the proximal phalanx. An ultrasound evaluation of the left front pastern indicated an incomplete, midline sagittal fracture in the proximal phalanx.

Radiographs of the right front foot and pastern were unremarkable, and radiographs of the left front foot and pastern indicated mild remodeling of the dorsolateral aspect of the left front middle phalanx at the joint capsule insertion. Radiographs of the left metacarpophalangeal joint were unremarkable; those of the right indicated a small osteochondral fragment associated with the dorsomedial aspect of the proximal phalanx. An ultrasound evaluation of the left front pastern indicated a subtle enlargement of the proximal and lateral aspects of the left front straight distal sesamoidean ligament with subtle fiber disruption proximally (Fig. 10). An MRI evaluation was requested to confirm desmitis of the left front straight distal sesamoidean ligament.
ament and to rule out additional pathology in the foot.

Per standard protocol, a complete MRI evaluation of the left front foot was completed as previously described. In addition, given the blocking pattern and ultrasound findings, the magnet was raised to position the isocenter over the left metacarpophalangeal joint. An MRI of the distal limb indicated multiple significant findings, including but not limited to mild-to-moderate fluid in the navicular bone and asymmetry in the shape of the navicular bone flexor surface. The straight distal sesamoidean ligament was noted to appear mildly abnormal in the study, with no additional areas of abnormality detected outside of those already identified with ultrasonography. However, this mild abnormality was not indicated as the most significant finding at the level of the metacarpophalangeal joint. Instead, the most significant finding at this level was noted to be metacarpophalangeal joint arthrosis characterized by mild enthesiophyte formation associated with the joint capsule attachment (Fig. 11) and focal sclerosis of the distal medial third metacarpal bone and proximal phalanx (Fig. 12). As mentioned previously, radiographs of the left front metacarpophalangeal joint were unremarkable (Fig. 13).

Case 3

A 2009 Hanoverian gelding was noted to be acutely unsound (2/5 in accordance with the AAEP guidelines for grading lameness) on the left front limb. The lameness was localized to the distal limb using abaxial sesamoid peripheral nerve anesthesia. Radiographs of the left front foot were judged to be within normal limits. At the time of the initial evaluation, the referring veterinarian discussed with the owner the merits of returning for additional peripheral nerve diagnostic anesthesia to further localize the lameness and that a standing MRI evaluation may be necessary because a soft tissue injury of the foot was likely given the negative radiographic findings. Believing that the lameness resulted from soft tissue injury, the owner declined any further workup and after some independent Internet research elected to place the horse on strict stall rest with minimal hand grazing. Approximately 6 weeks later, the owner then elected to send the horse in for a standing MRI evaluation of the left front foot without consulting the veterinarian that initially evaluated the horse. Because the owner was convinced the horse had sustained a soft tissue injury in its foot, the MRI evaluation was requested.
to characterize this presumptive soft tissue injury and to determine whether the horse was ready to begin a controlled return-to-work program.

Per standard protocol, a complete MRI evaluation of the left front foot was completed as previously described. A complete short transverse fracture at the junction between the ossified ungual (collateral) cartilage and the medial palmar process of the distal phalanx and extending into the palmar process was identified (Fig. 14). Ossification of the ungual cartilages is not an uncommon finding in routine foot radiographs; however, it is often impossible to discern whether the mineralization is normal or abnormal. As has been discussed in the literature, ossification of the ungual cartilages can lead to a fracture of the palmar process of the distal phalanx, as demonstrated in this case. Given the location of this fracture, it is in the author’s opinion very easily missed in standard radiographic views of the distal phalanx. A review of the radiographs taken by the veterinarian who initially evaluated this horse did not give any indication of a fracture. Given the information from the MRI evaluation, adjusting the dorsal ventral radiographic angle of the distal phalanx from 60° to approximately 75° dorsal ventral was effective in highlighting the fracture (Fig. 15).

3. Discussion
As previously stated, most equine lameness cases that are referred for standing MR evaluation have been worked up extensively, and there is often a presumptive or working diagnosis at the time of referral. Case 1 is an example whereby the working diagnosis was disproven and a different pathology was identified and shown to be the underlying cause of the lameness. The blocking pattern and suspicious radiographs led in this case to a working diagnosis of a fracture of the lateral plantar process of the distal phalanx when in fact there was an incomplete midsagittal fracture of the proximal phalanx. Additionally, this case demonstrates the potential for perineural anesthesia to eliminate pain associated with structures well proximal to the area intended to be desensitized.

Case 2 is an example often experienced by many veterinarians when they refer cases for MRI evaluation. In this case, there were additional pathologies identified in the MRI study that were outside of the working diagnosis and that could have contributed to the lameness. As such, these additional pathologies will often require additional clinical correlation such as additional perineural or intra-articular therapy and/or response to therapeutic intervention to determine their clinical relevance. In case 2, the working diagnosis was substantiated in that mild abnormalities in the straight distal sesamoidean ligament initially identified with ultrasonography were also identified with MR but additional areas of pathology were also identified that suggested the need for further clinical correlation.

In case 3, there was no working diagnosis to substantiate or disprove because of the owner’s noncompliance, which is not entirely uncommon in equine practice because owners often insist on going outside of their veterinarian’s recommendations for additional diagnostics or therapies. And although further workup may have provided additional information at the time of referral, the use of MRI in case 3, as suggested at the time of initial evaluation by the attending veterinarian, was extremely important in reaching the ultimate diagnosis of a short, complete transverse fracture of the ossified ungual
(collateral) cartilage and the medial palmar process of the distal phalanx. Although advanced diagnostic imaging is not always necessary in achieving an accurate diagnosis, standing MRI evaluation should be considered as an important additional diagnostic imaging modality in cases where a working diagnosis has been established, as well as in cases where the working diagnosis is unclear and in cases such as those presented herein where the lameness evaluation and diagnostic anesthesia are either inconclusive or inconsistent with the clinical picture.

Acknowledgments

Declaration of Ethics
The Author declares that she has adhered to the Principles of Veterinary Medical Ethics of the AVMA.

Conflict of Interest
The Author declares no conflicts of interest.

References and Footnote

*Hallmarq Veterinary Imaging, Acton, MA 01720.
How to Perform Dynamic Respiratory Endoscopy

Jennifer J. Smith, DVM, DACVS

1. Introduction
Equine upper airway obstructions are a common cause of impaired performance in horses of all breeds and disciplines. Dynamic obstructions of the upper respiratory tract typically occur during high-speed or peak exercise and are often influenced by certain head positions (poll flexion). As a result, these transient events are likely underdiagnosed or misdiagnosed by resting endoscopic examination alone.1,2 In the past, exercising endoscopic examination of the upper respiratory tract in horses was only available via high-speed treadmill examination. Dynamic respiratory endoscopy (DRE), or overground endoscopy, is a recent technology that has substantially improved our ability to accurately diagnose upper respiratory disorders in horses that exercise under their normal environmental conditions.3,4 DRE is indicated in horses with known or suspected disorders of the upper airway, abnormal noise production during exercise, exercise intolerance, and/or poor performance.

2. Materials and Methods
Several exercising endoscopic systems are now available on the equine veterinary market and vary somewhat in their components and attachments to the horse. However, the basic components of each system consist of an endoscope and light source, a recording/transmitting device, a receiver unit, and power sources. The detailed description that follows will refer to the dynamic respiratory endoscopic system the author has used.

Equipment

Endoscope
The endoscope consists of a 1-meter, 9-millimeter diameter, malleable tube with six light-emitting diodes at the tip. The endoscope possesses a down-tip deflection system (a manual dial that permits adjustments in the vertical positioning of the endoscope) to ensure appropriate positioning of the endoscope and optimal viewing of upper airway structures. The endoscope is secured to the horse via a special-purpose noseband that fits over the horse’s bridle (Fig. 1).

Mounted Module
The endoscope connects to a transmitter/recorder unit that contains a light source, an automatic wash system for the lens, and a supporting battery power source. The transmitter/recorder unit is secured to the horse via one of the following means depending on the discipline of the horse: (1) a specially designed saddle pad (sport horses or Thoroughbred racehorses) (Fig. 2) or (2) a specially designed pack (Fig. 3) that attaches to the shaft of a racing bike or sulky (Standardbred racehorses or miniature horses). Newer
versions of the system have replaced the sulky bag with a specially designed saddle pad for harness horses as well as a saddle pad designed for yearlings.

The endoscopic video images are recorded continuously in a digital format onto a removable secure digital (SD) card and are transmitted in real time to a wireless monitor (Fig. 4). A remote control initiates the recording start and stop functions. The automatic wash system cleans the lens at regular intervals (every 30 seconds) with an adjustable volume of water. The battery source provides sufficient power for recording times of at least 45 to 90 minutes, with newer systems capable of recording for more than 120 minutes.

**Receiver Unit**

The receiver unit consists of a handheld, wireless monitor that receives the video signal from the transmitter unit and allows real-time viewing of the video images from up to 600 meters away (Fig. 4). The receiver unit also houses its own supporting battery power source.

**Software System**

A video-processing and exam-reporting software facilitates review of the recorded examinations. The software allows one to view the recordings at regular speed or in slow motion and to create still images or shortened video clips. The still pictures and video clips can be archived into a medical record, incorporated and printed in an exam report, or e-mailed to owners, trainers, and consulting and/or referring veterinarians.

**Instrumentation of the Horse**

Instrumentation of the horse with the aforementioned endoscopic system can be safely and efficiently performed with two people (a horse handler and the veterinarian performing the DRE examination). The horse is first equipped with its normal tack and equipment in a box stall or barn aisle. The special-purpose noseband is fitted and fastened to the horse’s head. For riding horses or Thoroughbred racehorses, the dedicated saddle pad is positioned securely under the saddle. For Standardbred or harness horses, the sulky or racing bike is fastened to the horse, and then...
the dedicated pack is placed over the shaft of the sulky or bike and tightly secured to the surcingle.

The viewing monitor and recording/transmitting units are turned on, and the recording is started by means of the handheld remote control. A nose twitch is applied to the horse’s nose and held in place by the horse handler. The endoscope is passed up either nostril of the horse by the DRE veterinarian while the positioning of the endoscope in the upper respiratory tract is observed on the monitor. Proper positioning of the endoscope is imperative for achieving a high-quality diagnostic examination. The endoscope is secured to the horse’s head via the dedicated nose band using elastic bands or cable ties. The up/down deflection of the endoscope can be adjusted manually by the dial on the endoscope. Once proper positioning of the endoscope rostral to the epiglottis is established, the nose twitch is removed, and the rider/driver may commence the exercising portion of the examination.

Exercising Examination

The DRE exam is best performed under the exercising conditions in which the horse typically exhibits impaired performance and/or abnormal noise production. Because of the variance in exercising conditions for horses of differing disciplines, a standardized exercise test for DRE exams for all horses has not yet been established as it has for treadmill examinations. For Standardbred racehorses, a 2-minute mile (or faster) with the horse trotting or pacing by itself or in the company of others is performed. For Thoroughbred racehorses, the horse is breezed at least 3/8 of a mile (or preferably 5/8 or more) individually or in company to obtain a diagnostic examination. For sport horses, exercise regimes are more variable but should be designed to replicate the conditions under which the respiratory abnormality is typically noted (head flexion, tack/equipment modifications, rider influences). For horses that exhibit clinical signs prior to race training, DRE examination can be performed at nonracing speeds as long as the conditions under which the abnormality is typically noted are achieved.

Completion of the Examination

After the exercise test is completed, the recording is stopped, and the endoscope and equipment are removed from the horse.

Review of the Examination

The recorded examination is downloaded from the removable SD card to a computer. The software
system \(^b\) allows for a review of the DRE video from start to finish and gives one the ability to replay the video in slow motion. Creating still images and video clips that may be shared with all interested parties (owners, trainers, referring veterinarians) is also possible.

3. Results

More than 250 DRE examinations were successfully performed in Thoroughbred and Standardbred racehorses, sport horses (dressage [10], 3-day eventing [7], hunter/jumper [3]), and a miniature driving horse (1). No DRE examinations were unsuccessful because of horse compliance issues, and most horses seemed to tolerate the DRE equipment well. Some horses exhibited transient sneezing or snorting, however, upon initial placement of the endoscope in the nasal passage, and some horses made occasional attempts to rub the endoscope on their limbs. No horse, rider, or driver injuries were encountered, and the equipment did not impede the horse, rider, or driver’s normal exercise routine. Mild mucosal irritation adjacent to the tip of the endoscope upon completion of examination and self-limiting epistaxis during examinations performed in cold weather were noted infrequently.

Nondiagnostic DRE examinations were encountered during initial experiences with the equipment and were the result of operator error (poor or improper positioning of the endoscope in the upper airway, improper shutdown, loss of video), technological failures (water pump malfunction, worn or broken video cables, corrupt video file), and rider interference (power loss, displacement of endoscope). Minor modifications to the DRE system and improvements in technique resolved these early issues.

Definitive diagnoses were obtained using DRE in 94% of horses having a history of abnormal noise production and poor performance. In horses having a history of poor performance without abnormal noise production, definitive diagnoses were obtained in only 44% of the horses. Concurrent (more than 1) respiratory abnormalities were observed in 22% of the horses examined. The two most common upper airway abnormalities observed independently were intermittent dorsal displacement of the soft palate (20%) and left arytenoid collapse with unilateral or bilateral vocal cord collapse (12%). Other disorders observed independently or concurrently included axial deviation of the aryepiglottic folds, intermittent epiglottic entrapment, palatal instability, pharyngeal collapse, vocal cord collapse, arytenoid chondritis, right arytenoid collapse, ventroaxial luxation of the apex of the corniculate process of the arytenoid cartilage, exercise-induced pulmonary hemorrhage, excessive mucus, and proximal tracheal collapse.

4. Discussion

Important factors to consider when choosing which type of exercising endoscopic exam to perform on a horse with a suspected upper airway disorder include the ability to replicate the conditions under which the horse typically exhibits the abnormality to arrive at a definitive diagnosis, the safety of the procedure and risk of injury to the horse, the financial investment to the owner, the quality of the video images obtained, and the ability to perform the exam in a timely manner. DRE offers a convenient, safe, affordable alternative to high-speed treadmill endoscopy while also providing high-quality images that accurately assess a horse’s upper airway function during its normal exercising conditions. The equipment seems to be well-tolerated by horses, and the impact of external influences (tack, head position, rider/driver interference, footing, weather conditions, other horses) can be evaluated during the examination.

The accuracy of diagnosing an upper airway abnormality using DRE is greater for horses with a history of abnormal noise rather than poor performance alone.\(^3\) Therefore, in horses exhibiting poor performance alone or when a lower airway disorder is suspected, high-speed treadmill endoscopy may offer a comprehensive advantage over DRE as both cardiac and lower respiratory function can be evaluated during the examination.

Standardized testing for DRE examinations has not yet been established for horses of various breeds and disciplines in the same way it has for treadmill endoscopy. Discrepancies between the diagnostic accuracy of DRE versus high-speed treadmill endoscopy have been documented previously, but exercise regimes (distance, duration, speed, incline) were not directly comparable.\(^5\)\(^,\)\(^6\)

To obtain a definitive diagnosis with DRE, the importance of replicating the conditions under which the horse typically exhibits its abnormality cannot be emphasized enough. The diagnostic and imaging capabilities of the DRE system and the real-time viewing of the examination on the monitor are selling features of the technology, as is the ability to share images from the examination with all interested parties in a timely fashion. Owners and trainers commonly respond that DRE allowed them to make sound fiscal decisions regarding their horse. Additionally, DRE offers a unique opportunity for performing preoperative and postoperative evaluations of horses undergoing surgical procedures to correct identified upper airway disorders. DRE can provide objective documentation that the surgical procedure corrected or failed to correct the previously diagnosed airway disorder.

The ability to make these direct, horse-to-horse comparisons offers a new means of assessing the outcome of surgical treatments and should help redefine the true success or failure of a procedure as well as the prognoses for various upper airway disorders.

In summary, DRE offers a safe and convenient alternative to high-speed treadmill endoscopy for...
horses with suspected upper airway disorders, abnormal noise production during exercise, and/or poor performance. The likelihood of establishing a definitive diagnosis using DRE is greater for horses that exhibit abnormal noise production during exercise than poor performance alone. The lack of standardized field exercise tests for DRE and the variation in exercise demands for horses of differing disciplines highlight the importance of replicating the conditions under which the horse manifests the abnormality as closely as possible to establish a definitive diagnosis.

Acknowledgments

Declaration of Ethics

The Author declares that she has adhered to the Principles of Veterinary Medical Ethics of the AVMA.

Conflict of Interest

The Author declares no conflicts of interest.

References and Footnotes


aDynamic Respiratory Scope, Optomed, Les Ulis, France.
bSICRE, Optomed, Les Ulis, France.
How to Perform Standing Cervical Centesis in Horses Using a Lateral Approach Between C1 and C2

Anthony P. Pease, DVM, MS, DACVR

1. Introduction
Diagnosing the cause of equine ataxia can be quite difficult for the practitioner. Often the patient is hard to assess and has vague clinical signs. One key component in identifying the cause of the neurologic signs is obtaining cerebrospinal fluid (CSF). The standard method for obtaining the fluid is through a lumbosacral centesis. However, obtaining CSF from the lumbosacral cistern can be difficult because it is approximately 10 cm under the skin, and the opening is approximately 1 cm in size. Ultrasound has been used to help guide the procedure, but even with ultrasound guidance, the needle extends into the vertebral canal and risks either penetrating a nerve root, which is painful, or the vertebral vessels, which can lead to blood contamination.

The cervical region also has windows that allow for CSF collection. The most frequently used window is the cerebellomedullary cistern at the atlanto-occipital joint. This method is routinely used to acquire CSF from horses under general anesthesia and has recently been reported in standing horses using ultrasound guidance. The concern with this procedure is that if the horse’s head moves, there is a risk that the needle could penetrate the spinal cord.

A second window is in the lateral aspect of the atlantoaxial joint (C1-C2). Using ultrasound guidance, centesis can be performed using a lateral approach. This approach minimizes the risk of damage to the spinal cord and is dorsal enough to avoid the nerve roots and vertebral vessels. This method results in minimal blood contamination, can acquire large volumes of CSF if needed (>20 mL), and can be performed in approximately 2 minutes from when the needle is first introduced to when the fluid is removed.

2. Materials and Methods
To perform centesis through the lateral aspect of the atlantoaxial joint, horses are placed in stocks, although the procedure can be performed in the stall or with recumbent horses. The site for C1-C2 CSF collection is identified using ultrasound. Although either side of the neck can be used, most horses are prepared on the left side to allow for the needle to be held in the right hand. The centesis site is located just caudal to the caudal aspect of the subarachnoid space where the needle is placed.
The dura mater, spinal cord, central canal, and vertebrae are identified. An approximately 15 × 15-cm area is shaved and aseptically prepared. The horse is then sedated using 0.01 to 0.02 mg/kg detomidine hydrochloride intravenously; this is followed approximately 3 minutes later by 0.06 mg/kg morphine sulfate intravenously. The morphine sulfate is usually dosed at 30 mg for horses weighing more than 500 kg and 15 mg for those weighing less than 500 kg. Forty milligrams (2 mL) of 2% mepivacaine hydrochloride is administered subcutaneously prior to the final aseptic preparation of the skin. A nose twitch can be used prior to placing the needle but generally is not needed if sedation is adequate and someone is holding the head. Using a sterile glove placed over the ultrasound probe, an 18- or 20-gauge, 8.4-cm (3.5-in) spinal needle with a stylet is introduced ventrally to the ultrasound transducer and advanced medially to the level of the dura mater (Fig. 1). The stylet is left in place as the needle is advanced through the dura mater and into the subarachnoid space. With ultrasound guidance, the needle should always be seen as it goes through the neck muscles, through the dura mater, and into the subarachnoid space.4 Upon removing the stylet, CSF generally does not flow freely. Depending on head position, it may be necessary to place a gloved finger over the needle hub to prevent excessive air from entering the subarachnoid space. A higher head position yields more air influx, and a lower head position typically produces CSF spontaneously through the needle. Next, a 5-mL syringe is attached to the needle, and CSF is collected using gentle suction. After collecting approximately 3 to 5 mL of CSF, the first syringe is discarded, and a second 5-mL syringe is attached to obtain a sample for evaluation. The needle is then removed, and the horse is returned to its stall. The CSF obtained can be analyzed for red blood cell contamination, microprotein content, nucleated cells, specific gravity, and packed cell volume, as well as serologic testing for equine protozoal myeloencephalitis.

3. Results
To date, approximately 50 horses have undergone this procedure without adverse effects. One horse had a small subarachnoid space from which only 3 mL of CSF could be obtained. In 19 of the 50 cases, serology was obtained on the CSF (for the remaining cases, the CSF was simply submitted for analysis). Of those 19 cases, 2 (11%) had greater than 50 red blood cells per microliter (RBC/μL) in the CSF (585 RBC/μL and 53 RBC/μL), 3 (16%) had acceptable levels of red blood cell contamination (47 RBC/μL, 38 RBC/μL, and 27 RBC/μL), and the remaining 14 horses (74%) had less than 10 RBC/μL. Of the 14 horses with less than 10 RBC/μL, 11 (58% of the total) had fewer than 4 RBC/μL.

In 4 (21%) of the 19 horses, the initial and second samples were both submitted for cytology. The first horse’s initial sample contained 2,575 RBC/μL; there were only 38 RBC/μL in the second sample. The second horse had 245 RBC/μL in the initial sample and 27 RBC/μL in the second sample. The third horse had 37 RBC/μL in the first sample and 12 RBC/μL in the second sample. The fourth horse had less than 2 RBC/μL in both samples.

No horses had adverse reactions to the procedure (such as falling down or seizures). One horse was reported to have slight neck pain for 5 days after the procedure but responded well to phenylbutazone. One potential difficulty with the procedures is that a reverberation artifact can occur at the level of the dura mater if it is not penetrated; this can obscure the image of the procedure. If this occurs, and an image of the spinal cord is not obtained, the procedure can be immediately performed on the opposite side of the horse’s neck.

4. Discussion
Standing cervical centesis in horses from the lateral aspect of the atlantoaxial joint using ultrasound guidance is a rapid method for obtaining CSF. Compared with other methods of obtaining CSF, this method is safer for both the horse and the veterinarian. Morphine anesthetizes the dura mater so the horse does not react to needle puncture. The veterinarian is positioned near the head rather than the hind end of the horse, thus minimizing the risk of being kicked. Although other sedative-analgescic drugs have been used, The author has found that morphine allows for routine anesthesia of the horse’s central nervous system and helps keep the horse’s stance solid.

Morphine cannot be given to a horse as a stand-alone drug because it tends to cause excitability.5 Thus, detomidine is initially administered to provide sedation. The benefit of this combination is that it is relatively long-lasting; the duration of morphine’s effect can last approximately 2 hours, and detomidine can provide sedation for 30 to 45 minutes. This usually provides adequate time to perform the procedure; however, if necessary, additional detomidine can be administered. Colleagues have reported success using mixed opioid agonists/antagonists with a combination of xylazine hydrochloride for performing the procedure, but when first attempting this technique, numer-
ous combinations of drugs were used on normal horses and none provided the analgesia seen with the morphine and detomidine combination.

With practice and coordination, cervical centesis from the lateral aspect of the atlantoaxial joint can be performed in the field and has been done in stalls as well as in 1 down, grade 4 ataxic horse. The ability to obtain CSF in the field to help evaluate a neurologic disorder is extremely valuable, but care should be taken to practice the procedure on euthanized horses prior to trying it on a standing one.

Acknowledgments

Declaration of Ethics

The Author declares that he has adhered to the Principles of Veterinary Medical Ethics of the AVMA.

Conflict of Interest

The Author declares no conflicts of interest.

References

How to Use Radiography and Local Anesthesia to Confirm Pain Associated With Dorsal Spinous Processes

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1. Introduction

In the athletic horse, back pain is a common cause of poor performance. Diagnosis is difficult because the signs are vague and horses that are sensitive to palpation or pressure over the back may not necessarily have pain in this area. This situation is complicated by the fact that many affected horses present for ill-defined gait or performance abnormalities rather than overt thoracolumbar pain. Adding to the complexity of defining and diagnosing back pain is the paucity of large-scale scientific studies in horses.

Many lesions associated with back pain involve the structures along the thoracolumbar spine and radiographic evaluation is the most common imaging modality performed. High-output x-ray equipment and digital radiographic systems have facilitated the acquisition of diagnostic images. Post-processing algorithms further enhance the bony detail; however, good high-quality exposures and accurate position remains essential.

In most horses, the dorsal spinous processes (DSPs) of the thoracolumbar vertebral bodies are readily visible on radiographic evaluation. The broad width of the back in some Warmbloods, Draft horses, and obese horses can hinder adequate imaging, especially with lower-output portable x-ray equipment. Conversely, horses with a “weak topline” are often easy to image due to decreased epaxial muscle mass.

 Radiographic abnormalities may include impinging and overriding of the DSPs (aka, “kissing spines”), fractured DSPs, and/or enthesiophyte formation along the origin/insertion of the interspinous ligaments. A wide variety of abnormal radiographic findings of the DSPs can be present in both clinically normal horses and horses with back pain, and previous studies have shown that the severity of radiographic changes do not always correlate with clinical signs. Therefore, the clinical significance of overriding DSPs or other bony lesions in horses with back pain cannot be made on radiographic signs alone.

Diagnostic analgesia of the back can be used to determine the clinical significance of impinging and/or overriding DSPs. In horses with suspected back pain, local anesthetic solution can be infiltrated in or near the interspinous spaces between the DSPs. After injection, the gait of the horse can be assessed. In a horse with authentic
back pain, there will be marked improvement or resolution of abnormal clinical signs.

The objective of this presentation is to familiarize practitioners with radiographic technique for imaging the back and how to obtain good image quality. How to anesthetize interspinous space(s) will also be presented. Both of these techniques can enhance the clinical examination of horses with poor performance and authenticate abnormal physical and/or radiographic abnormalities as a cause of back pain.

2. Methods

Radiographic Evaluation of the DSPs

Prior to radiographic evaluation, the horse’s back should be brushed over to ensure there is no mud or other substances that might lead to radiographic artifacts. Sedation should be performed using detomidine hydrochloride (0.005–0.01 mg/kg) or xylazine hydrochloride (0.25–0.5 mg/kg) intravenously. The dose is at the discretion of the attending veterinarian and should be aimed at providing adequate sedation so the horse will not move or raise its head during radiographic evaluation. Given that high doses of sedation can cause truncal swaying, it is preferred to use lower doses and resedate if necessary rather than giving high doses initially. The horse should stand squarely, taking the weight evenly on all four limbs. The head and neck position should be in alignment with or slightly ventral to the horse’s topline. High head and neck position should be avoided because it decreases the intervertebral distance between the DSPs.

Adequate imaging requires radiographic equipment capable of producing outputs of 75–120 kV and 100–250 mAs. Ideally, the x-ray tube is mounted on an overhead gantry with a linked cassette holder to ensure horizontal alignment of the x-ray beam and the sensor panel (Fig. 1). The sensor panel should be oriented perpendicular to the ground and the x-ray beam should be aligned with the center of the sensor panel (Fig. 2). At the author’s institution, an 800-mAs x-ray tube and a 14 × 17-in. wireless panel with a 10:1 grid is used. The distance between the x-ray generator and the sensor panel is 40 in.

Typically five to six overlapping lateral radiographic images (Fig. 3) are acquired. For the first radiographic image, the sensor panel is positioned just above the highest point of the withers and placed as close to the horse as possible. The second image is positioned just caudal to the first, overlapping the DSPs in the withers. Moving caudally, remaining images are obtained in the thoracic and lumbar regions. In the mid-thoracic region, the x-ray beam is centered just above the vertebral bodies, 10–15 cm below the dorsum (Fig. 2). To image the first (T1) DSP, a lateral radiographic image at the base of the neck is obtained. Imaging of the DSP of T1 is not included in standard back radiographs.

Diagnostic radiographic images can also be obtained using a mobile unit but only the summits of the DSPs are consistently imaged. Collimation should be used to reduce the amount of scatter radiation generated to improve image quality. Accurate imaging requires a horizontal x-ray beam in alignment with the sensor panel, which is difficult to achieve using portable equipment. When the x-ray
generator is handheld to the level of the vertebral bodies, there is a tendency for the x-ray beam to be angled in a proximal–ventral to distal–dorsal oblique direction (Fig. 4). This upward beam angle

Fig. 4. The position of a handheld x-ray tube head and sensor panel. Note the x-ray beam (arrow) travels upward from the generator to the sensor panel.

is even more evident when the clinician is short and the horse is tall. Due to angulation of x-ray beam and scatter, distortion of DSPs is produced when using portable x-ray equipment (Figs. 5 and 6). Standing on a step ladder can improve the alignment between portable x-ray generator and sensor panel; however, obliquity and equipment limitations hinder proper image acquisition.

A lead marker can be placed along the dorsal midline to serve as a point of reference (Figs. 2 and 3C). This marker is positioned at the location of back soreness or a radiographically abnormal DSP. If additional diagnostics or treatment of the back is forthcoming, the site of the lead marker is then identified on the dorsal skin with whiteout (Fig. 7) or by removing a small amount of hair. Minimal clipping should be performed to insure accurate location. For example, removing a very narrow band of hair using a No. 40 clipper blade oriented perpendicular to midline can be used.

Back Injections: Local Infiltration of the Interspinous Space(s) of the DSPs

The specific site for injection can be determined by palpation. Counting cranially from the eighteenth rib, the corresponding DSP is identified. Or, the site of injection can be determined by previously marked location obtained during radiographic evaluation. Once the site is identified, the horse should stand square and the area is aseptically prepared.

Adequate restraint and application of a nose twitch should be performed to prevent horse movement during needle placement and injection. In the refractory horse, sedation is performed using 0.25–0.3 mg/kg xylazine hydrochloride intravenously.

The injections are performed on dorsal midline into the interspinous ligamentous space. A shallow, thumb-sized depression is appreciated between two DSPs (Fig. 8). A 20-guage × 3.5-in. spinal needle is inserted in the caudal aspect of the depression to avoid contact with the caudally protruding summit of the cranial DSP. The needle is then advanced ventrally to an approximate depth of 5 to 7 cm. When advancing, the angle of the needle should mirror the angle of the DSPs at the site of injection. From T1–T14, the DSPs are angled in the dorsocaudal direction, then upright at T15 (the antecrinal vertebra) and are angled dorsocranial from T17–L6 (Fig. 9). Accordingly, needles in the cranial back are advanced in a cranoventral direction, vertical upright direction at T14–T16 and ad-
advanced in a caudoventral direction in the caudal back. In horses with overriding DSPs, placement of the spinal needle into the interspinous space using a center midline approach is not possible. For these horses, injection is performed approximately 2 cm to the right or left of midline, abaxial to the affected DSPs. Once the needle is positioned, 2% mepivicaine hydrochloride in a disposable slip tip syringe (8–10 mL) is injected. Anesthetic solution (3–5 mL) is injected at the ventral depth and the remainder of the anesthetic solution is injected as the needle is withdrawn dorsally. Injection of solution should not be performed within the supraspinous ligament, which is situated superficially between the skin and the dorsal rim of the dorsal spinous processes. If there is significant resistance, the needle should be slightly redirected until there is less resistance.

If more than one site is injected, a cranial or caudal interspinous space can be injected by palpating the subcutaneous depression in front or behind, retrospectively, the first clearly marked site. Alternatively, multiple spinal needles are inserted through the skin and positioned prior to injection. After injection, the horse stands quietly until effectiveness of the block is evaluated. The area is covered with a clean, dry, thin towel before the saddle pad is applied.

3. Results

For horses admitted to the hospital, radiographic evaluation of the dorsal spinous process is performed using a high-output 800-mAs x-ray generator and 14 × 17-in. wireless cassette. Usually five to six lateral images of the DSPs from the withers to the lumbar region are obtained. Due to the extensive soft tissue mass, different exposures are used for the summits of the DSPs and the vertebral bodies (higher exposures for vertebral bodies). Radiopaque markers are used routinely to correlate physical examination findings and/or areas of injection with radiographic images.

Fig. 6. Radiographic images of the mid-thoracic region. A, Image obtained using technique in Fig. 1. B, Image of the same horse obtained using technique in Fig. 4. Note the narrowing of the interspinous space between T14–T15 due to radiographic technique.

Fig. 7. Local anesthetic is deposited between DSPs. An additional caudal interspinous space is marked with whiteout (arrow).

Fig. 8. Spinal needles positioned for back injections. The needles are positioned along the caudal aspect of the depression (arrows) created between the dorsal summits of two DSPs.
creased radiopharmaceutical uptake during nuclear scintigraphic evaluation with radiographic abnormalities or vice versa.

Impingement and overriding DSPs is a fairly common radiographic observation, even in clinically sound horses. Cortical sclerosis and subcortial radiolucencies are frequently noted and often in horses with “kissing spines.” Fractures of the DSPs usually occur in the withers. Displacement and malformation of the DSPs are infrequently observed. Equally informative is the absence of bony abnormalities in the horses that block to the back. Additional imaging such as ultrasound and/or nuclear scintigraphic evaluation can be useful in these horses.

Local infiltration of the interspinous space(s) of the DSPs is performed on an as-needed basis but most typically performed in horses with gait and/or behavioral abnormalities. The procedure is also frequently performed to discern the clinical significance of radiographic abnormalities, usually “kissing spines,” and/or increased radiopharmaceutical uptake of the DSPs. In either situation, lameness evaluation including exercise with a rider is the preferred method of assessing the effect of the block. For safety reasons, application of a weighted surcingle during gait assessment can also be used. Provided the horse exhibits performance problems with the apparatus (some will only do so with a rider), exercise with the surcingle before and after blocking the DSPs is another viable method of testing. One or two interspinous spaces are commonly anesthetized, most frequently in the caudal thoracic saddle region (T14–T18). If the horse’s gait is not altered, an additional site along the back is selected. Less commonly, selective infiltration of interspinous space(s) of the DSPs is performed to determine surgical site(s) prior to interspinous ligament desmotomy. Complications are exceedingly rare and include transient swelling along the dorsal midline. Application of a clean, dry towel prior to post-block ridden evaluation may decrease the potential of occurrence.

4. Discussion
The diagnosis of back pain in the horse is challenging. High-quality radiographic evaluation of the DSPs is essential for identification of underlying bony abnormalities that can cause thoracolumbar pain. However, radiographic abnormalities of the DSPs are common findings in horses with and without back pain. Therefore, the diagnosis of back pain should not be made on radiographic evaluation alone. Local infiltration of the interspinous space(s) of the DSPs is an effective diagnostic tool and is used to authenticate the thoracolumbar region at a bona fide source of pain in horses with suspected back problem. Both of these tools are extremely helpful in assisting the clinician with the accurate diagnosis of back pain in horses.

Acknowledgments

Declaration of Ethics
The Author declares that she has adhered to the Principles of Veterinary Medical Ethics of the AVMA.

Conflict of Interest
The Author declares no conflicts of interest.

References and Footnotes

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1. Introduction

Lameness associated with carpal sheath effusion results from a variety of underlying factors. Pathologic changes associated with the carpal canal include septic tenosynovitis, superficial digital flexor (SDF) tendinopathies, and desmopathies of the accessory ligament of the superficial (AL-SDFT) and deep digital flexor tendons.1–8 A recent report identified several cases with intrathecal tears of the accessory ligament of the superficial digital flexor tendon with associated hemorrhage in the carpal sheath.9 Although many equine practitioners exhibit proficiency with the ultrasonographic evaluation of the metacarpal structures, the more proximal carpal canal and antebrachium can be a source of frustration and intimidation. Previous reports have described the ultrasonographic anatomy and technique for evaluation of the AL-SDFT.2,10,11 This paper expands on these studies with a thorough anatomic review. The proposed technique describes the evaluation of each structure individually in transverse and longitudinal planes, in contrast with the more historic zone approach.11 By improving ultrasonographic proficiency and anatomic competency, the practitioner will be better equipped to diagnose various conditions of the equine carpal canal.

2. Materials and Methods

Anatomic Study

Three forelimbs from horses euthanized for unrelated reasons were obtained for the anatomic portion of this study. Ultrasound examination and magnetic resonance images (MRI) of the limbs from the level of mid radius to mid metacarpus were performed postmortem on two of the limbs. Proton density (PD), proton dense fat-saturated, and T1-weighted gradient echo images were obtained in the transverse and sagittal planes. One of these limbs was frozen, and transverse, 2-mm cut sections were made to correspond with previously acquired ultrasound and MRIs. Figure 1 is used as a reference for corresponding anatomic locations of the transverse sections used in the subsequent figures. In a third cadaver limb, used solely to demonstrate the margins of the carpal canal, noniodinated contrast medium (30 mL iohexol® [350 mgI/mL], diluted to a total volume of 60 mL) was injected into the carpal
canal and lateral and dorsolateral to palmaromedial oblique radiographs were obtained (Fig. 2).

Ultrasound Study
The caudomedial aspect of the limb is clipped with a No. 40 to No. 50 blade from 4 cm proximal to the chestnut to the mid metacarpus. The limb is washed with warm water and soap, dried, and ultrasound-coupling gel is applied. A high-frequency (8–18 mHz) linear transducer is used.

A total of 10 veterinary students and veterinarians were recruited to perform the technique as described on a standing horse. There were a total of seven veterinary students with minimal ultrasound experience and three veterinarians with novice to moderate ultrasound knowledge and skills and with minimal to no experience imaging the carpal canal. The written technique was provided to participants and followed in a step-by-step manner.

Carpal Sheath
The carpal canal contains the superficial and digital flexor tendons. Its proximomedial extent is bordered by the AL-SDFT or superior check ligament. The distal margin is bordered by the accessory ligament of the deep digital flexor tendon (AL-DDFT). The palmar carpal ligament borders the dorsal aspect of the carpal sheath and the palmar and medial margins are delineated by the palmar carpal retinaculum. In Fig. 2, intrathecal contrast medium shows the proximal extent (distal third of the radius) and distal extent of the carpal sheath, which extends to the mid-metacarpal region in most horses. The flexor retinaculum forms a band on the caudal aspect of the carpus, and spans from the accessory carpal bone to the medial collateral ligament and proximal aspect of the second metacarpal bone (Fig. 3). The tension of the flexor retinaculum results in restriction of the fluid within the carpal sheath at this level, which can be seen in Fig. 2 at the level of the accessory carpal bone. A larger accumulation of contrast is visible proximal to the flexor retinaculum.

Ultrasound examination of the metacarpal structures can identify effusion contained within the carpal canal, which is usually seen between the deep digital flexor tendon (DDFT) and the AL-DDFT. With more severe effusion, it is visible surrounding, or lateral and medial to the SDFT and DDFT. Proximal to the carpus, the largest accumulation of fluid is visible laterally between the ulnaris lateralis and the lateral digital extensor and medially between the flexor carpi ulnaris and flexor carpi radialis. In the authors’ experience, inadvertent introduction of gas can be present in the carpal canal after a low 4-point diagnostic block is performed. Many veterinary students and practitioners may be surprised by the distal extent of the carpal canal.

Identifying Home Base
The starting location is just distal to the chestnut on the medial aspect of the radius. This area will herein be referred to as “home base” and serves as a reference point should the ultrasonographer become...
disoriented. Depth can be set between 4 and 5 cm, depending on the size of the horse, and focal zones are positioned in the near field at approximately 1 to 2 cm deep. When the transducer is placed on the palmar aspect of the limb, the marker is positioned laterally, corresponding with the left side of the screen. For imaging the structures as described, the transducer is held in the same orientation so that when it is placed directly on the medial side of the radius, the marker will essentially be directed caudally. (Note: Although this is not traditional radiology protocol, as structures are followed distally, the transducer indicator will end up in a normal lateral location.) The AL-SDFT can be identified at this level by placing the transducer in a transverse (short axis) plane, directly medial on the limb, just below the chestnut (Figs. 4 and 6).

Accessory Ligament of the Superficial Digital Flexor Tendon

The AL-SDFT will appear as the most echogenic structure adjacent to the caudal surface of the radius and is surrounded by the caudal antebrachial structures that are still predominantly muscle at this level (and will be described individually later) (Fig. 5). The vessels overlying it, including the median artery and cephalic vein (more superficial and cranial) are also used as landmarks. Cranial to the AL-SDFT, partially visible in the superficial portion of the image, is the curvilinear echogenic caudal surface of the radius. The flexor carpi radialis is visible directly superficial to the AL-SDFT. All descriptions will be given from this location at home base, with the probe in a transverse plane (Fig. 6).

Once identified, the ligament can be followed toward the SDF muscle in the transverse plane. As the ligament courses from the origin on the caudal cortex of the radius toward its insertion on the SDF, it can be visualized passing deep to the median artery. The curved walls of the median artery, as well as the flexor carpi radialis, create linear, anechoic edge artifacts in the deeper AL-SDFT. As the probe moves distally it must also be moved in a slightly caudal direction. The shape of the AL-SDFT changes from square proximally to oblong as it inserts into the SDF (Fig. 7). When the AL-SDFT inserts into the SDF near the level of the musculotendinous junction, the SDF may contain some muscle fibers at this level.

To evaluate the fiber pattern of the AL-SDFT in long axis, the probe is placed in transverse, directly under the chestnut, and rotated in a proximodorsal to distocaudal orientation (counterclockwise), to elongate the fibers. The marker of the transducer is oriented in a dorsal or proximal direction in long axis. As the probe is rotated, it must be pivoted on the central axis of the AL-SDFT; keeping the AL-SDFT in the center of the image will ensure that the probe remains on the AL-SDFT. The distinct double walls of the median artery can be visible in long...
axis when the fibers of the AL-SDFT have been elongated to their fullest extent.

Note: Evaluation of the entire AL-SDFT can be performed in the transverse plane from the origin on the radius, but imaging in the proximal region is made difficult due to poor contact by the chestnut. Due to the orientation of the AL-SDFT at its origin, obtaining a “true” longitudinal view of the origin has been difficult in the authors’ experience and relatively unrewarding.

Superficial Digital Flexor Tendon
From home base, the SDF muscle is located just caudal to the AL-SDFT. Depth setting of 3 to 4 cm with focal zones in the mid zone of the image is used. At the level of the chestnut, the SDF is composed

Fig. 5. A. Gross image. B. Proton dense fat-saturated MR image in a transverse plane at the level of the origin of the AL-SDFT (red line, Fig. 1). a, radius; b, AL-SDFT; c, SDF; d, DDF radial head; e, DDF ulnar and humeral heads; f, flexor carpi radialis (FCR); g, flexor carpi ulnaris (FCU); h, ulnaris lateralis (UL); i, median artery.

Fig. 6. Transverse images at the level of home base (orange line, Fig. 1). A. Gross image. B. Proton dense fat-saturated MR image. C. Ultrasound. The dashed blue line shows the relative position of the transducer on the limb, with the corresponding structures labeled in the ultrasound image. The transducer is positioned so that cranial is on the right and caudal is on the left of the ultrasound image. At this level, the AL-SDFT is the most echogenic structure identified in the image, just deep or medial to the FCR. The median artery (i) is also a useful landmark for identifying the AL-SDFT. a, radius; b, AL-SDFT; c, SDF; d, DDF radial head; e, DDF ulnar and humeral heads; f, FCR; g, FCU; h, UL; i, median artery.
predominantly of muscle. Delineating the exact borders of the SDF muscle from the deep digital flexor (DDF) muscle is difficult at the level of the mid to distal radius. Determining the borders of the SDF from the DDF muscles in the distal radius can be performed with a retrograde approach. By identifying the borders of the SDFT in the proximal metacarpus and following it proximally through the carpal canal, the dorsal border of the SDF and caudal border of the DDF are more apparent at the level of the distal radius.

Imaging of the SDF muscle and tendon in transverse is accomplished by sliding the transducer slightly caudally along the caudomedial aspect of the distal radius. As it courses distally, the musculotendinous junctions are visible as well-defined hypoechoic to anechoic areas surrounded by the echogenic tendon fibers (Fig. 8). In long axis, these muscle units are visible as triangle-shaped hypoechoic areas that blend into hyperechoic tendon fibers (Fig. 9). Evaluation of the SDFT at the level of the carpus is performed with the ultrasound beam directed in a slight caudomedial to craniolateral direction. The lateral location of the accessory carpal bone prevents imaging the SDFT from a palmar/caudal approach through the carpal region, and thus the transducer is maintained in a more medial position. Distal to the accessory carpal bone, the remainder of the SDFT can be viewed from a palmar approach. The musculotendinous junctions should not be mistaken for tearing and/or lesions. If a hypoechoic area is found, it should be followed proximally to determine whether the hypoechoic area blends normally into muscle at the level of the distal radius. The contralateral limb should also be evaluated for comparison.

Deep Digital Flexor Tendon
From home base, the DDF muscle is visible deep to the AL-SDFT. Depth setting should be increased up to 6 to 7 cm and the focal zones lowered to the far field of the image to evaluate the entire DDF muscle in the distal radius region. Frequency may be lowered or a microconvex, mid-range frequency trans-

Fig. 7. Transverse images at the level of the distal radius (green line, Fig. 1). A. Gross image. B. Proton dense fat-saturated MR image. C. Ultrasound. The fibers of the AL-SDFT have almost completely blended into the SDFT at this level (arrowheads). The shape of the AL-SDFT is oblong. Note the remaining muscle within the DDFT at this level, that is also visible on the magnetic resonance image as the bright, hyperintense tissue centrally within the DDFT (arrow). The dashed blue box shows the corresponding position of the transducer on the limb. a, radius; c, SDF; g, FCU; h, UL.

Fig. 8. A. Transverse proton dense fat-saturated MR image. B. Ultrasound images at the level of the distal radius (yellow line, Fig. 1). Cranial and medial is on the right, caudal and lateral is on the left of the image. The increased signal intensity of the remaining muscle fibers in the SDF and DDF tendons are visible as well-defined hypoechoic areas in the corresponding ultrasound image (arrow).
ducer, can also be used to evaluate the deeper muscle of the DDFT. Using a trapezoidal view option helps include more structures at this level of depth when using a linear probe.

The radial head of the DDF muscle is visible deep to the AL-SDFT and just caudal to the radius. It is more echogenic than the adjacent muscle and has a smooth echotexture (Fig. 10). The ulnar and humeral muscle bellies of the DDF are caudal to the radial head, and are hypoechoic, with normal hyperechoic muscle striations. To evaluate the entire DDF muscle, the probe must be moved caudally and laterally to image the more laterally located ulnar and humeral heads, which may be indistinct from the adjacent SDF muscle. A similar medial approach for imaging the DDF can be applied as was used to image the SDF through the carpal region, due to the accessory carpal bone.

Flexor Carpi Radialis

From home base, the flexor carpi radialis (FCR) is the most superficial structure, adjacent or just caudal to the radius, and superficial to the previously identified AL-SDFT. Imaging of the remaining structures is performed at approximately 3 to 4 cm of depth, with the focal zones moved to the near field (1 cm deep). The FCR is a relatively smaller structure in relation to the other caudal antebrachial structures. Proximal to the chestnut, it is predominantly muscle. As it courses distally along the medial aspect of the radius, the muscle fibers condense into tendon fibers, at the level of the chestnut, and can appear bi-layered, with a mix of hypoechoic muscle and hyperechoic tendon fibers (Fig. 11). The FCR can be followed distally on the medial aspect of the carpus to its insertion on the proximal aspect of the second metacarpal bone (head of the medial splint bone). The FCR has a tendon sheath at the level of the carpus, which is contained within the flexor retinaculum.

To evaluate the tendon in a longitudinal imaging plane, the tendon can first be identified in transverse and the transducer rotated counterclockwise to elongate the fibers. The linear hyperechoic surface of the radius is identified just cranial to the FCR and as the transducer is slid caudally, the first visible structure is the FCR. Similar to the SDF, the triangular, hypoechoic termination of the muscle is visible, as it blends into the linear, hyperechoic tendon fibers (Fig. 11).

Flexor Carpi Ulnaris

The cranial margin of the flexor carpi ulnaris (FCU) muscle is partially visible from home base; it is located directly caudal to the FCR. The remainder of the muscle is visible by sliding the probe caudally. As the FCU courses distally, it can be imaged from a more caudal approach. Similar to the FCR, it has a bi-layered appearance (Fig. 12). As the muscle condenses into the tendon fibers, it is located superficial and just caudal to the SDFT, and can be followed to its insertion on the palmar/caudomedial aspect of the accessory carpal bone. To evaluate the muscle and tendon in long axis, it can be identified in transverse and the transducer rotated counterclockwise. Alternatively, from the radius, it can be identified by

Fig. 9. A. Sagittal proton dense fat-saturated MR image showing the musculotendinous junction of the SDF and DDF. The hyperintense muscle fibers blend into the hypointense tendon fibers. B. On the corresponding, long axis ultrasound image, the triangular-shaped end of the hypoechoic muscles of the SDF and DDF are visible blending into the hyperechoic linear tendon fibers (arrows).

Fig. 10. Transverse ultrasound image obtained just distal to the chestnut. The smooth echotexture of the radial head of the DDF muscle is visible in the center of the image (d), adjacent to the hypoechoic muscle of the SDF and DDF (ulnar and humeral heads). a, radius; b, AL-SDFT; d, DDF radial head; i, median artery.
sliding caudally from the fibers of the FCR to the FCU.

Ulnaris Lateralis

From home base, as the probe is moved from medial to lateral the muscles can be identified in order: FCR, FCU, ulnaris lateralis (UL). Depth is further decreased to approximately 3 cm and focal zones are maintained in the near field. The UL is located laterally on the antebrachium, caudal and lateral to the FCU. It is a superficial and relatively slender muscle belly, with a bi-layered appearance similar to previously described structures (Fig. 12). As it becomes tendinous just proximal to the accessory carpal bone, it is located more caudolaterally. It briefly forms a distinct tendon before splitting into a cranial and caudal component. The caudal component inserts proximolaterally on the caudal aspect of the accessory carpal bone. The cranial component is less distinct and courses in an oblique, dorsodistal direction to insert proximally on the fourth metacarpal bone. As it courses over the carpus, maintaining contact with the protuberances of the carpal bones may be aided by the use of a standoff pad. To find the muscle/tendon in long axis, the previously described approach of either rotating the transducer from transverse into longitudinal or sliding the probe around the limb, and following the structures from medial to lateral (FCR, FCU, UL) can be used.

3. Results

Ultrasound images corresponded well with MR and gross images, validating the description of the anatomic and ultrasonographic appearance. This technique was used by a total of 10 individuals inexperienced in evaluation of the carpal canal and caudal antebrachium. Following this technique, exams were performed successfully and all structures were identified. All scans were directly observed by one of the authors to confirm the correct structures were identified and proper technique was used. There were no observable time differences to successfully image the structure based on the ultrasonographer’s experience. Total examination time varied between 30 and 60 minutes. Most ultrasonographers became comfortable with readily identifying home base by the time they reached the DDFT section of the exam. Using the retrograde approach for delineating the borders of the SDF and DDF muscles in the distal antebrachium was helpful for most people. Following the UL over the car-

Fig. 11. A. Transverse and B. Long-axis ultrasound images of flexor carpi radialis (between the arrows) at the level of “home base.” A. Cranial is to the right and caudal to the left of the transverse image. B. Proximal is on the left of the long-axis image. The FCR appears bilayered, with hypoechoic muscle fibers and the more superficial hyperechoic tendon fibers. The triangular-shaped musculotendinous junction is visible in the long axis image. The AL-SDFT is visible deep to the FCR. The fibers of the AL-SDFT are elongated in the long axis image. b, AL-SDFT.

Fig. 12. Transverse images at the level of the distal radius showing the bilayered appearance, created by the musculotendinous junction, of the FCR and UL. A. Gross image. B. Proton dense fat-saturated MR image. C. Ultrasound. B. The intermediate signal intensity muscle fibers in the MR image correspond with C, the hypoechoic muscle fibers in the ultrasound image. B. The hypointense tendon fibers in the MR image correspond with C, the hyperechoic tendon fibers in the ultrasound image. g, FCR; h, UL.
pus and maintaining contact with the limb was the most difficult part of the examination. Addition of a standoff pad for this structure increased contact and improved visualization of the UL at its insertion.

4. Discussion

There are a number of descriptions of injuries to the AL-SDFT throughout veterinary literature, with recent renewed focus on this topic.8,9 Injuries to the SDFT and AL-SDFT were the two most common injuries in a group of 121 horses with carpal sheath effusion.8 Early reports described a carpal canal syndrome, occurring most commonly in flat or steeplechase racehorses that was associated with fracture of the accessory carpal bone.1 This led to a syndrome of thickening of the tendons including the flexor retinaculum and secondary effusion of the carpal sheath and was treated with transection/removal of a portion of the flexor retinaculum. With improved technology and the increased use of ultrasound in veterinary medicine, knowledge of specific soft tissue injuries in the carpal canal are now identified. These injuries include injury to the AL-SDFT, characterized by thickening of the AL-SDFT, irregular fiber pattern, and tearing into the SDFT in combination with carpal sheath effusion.3 This injury is most commonly described in young horses with high levels of activity.3,4 Other abnormalities in this region include distal radial osteochondromas of the caudal distal radius as a cause of carpal sheath synovitis and associated desmopathy of the DDFT. These were reported predominantly in flat Thoroughbred racehorses (18/22), but a few horses (4/22) of other disciplines were also included.16 Tearing of the radial head of the DDF muscle with associated tenosynovitis of the carpal sheath has also been described, mostly in flat racehorses. This group had a slightly older population with a mean age of 4.5 years (range, 2–9 y).6 Most recently, ultrasound was valuable for diagnosis of intrathecal tears of AL-SDFT, with several tears causing intrathecal hemorrhage in the carpal sheath.9

As veterinary care improves, the geriatric or senior horse population has provided new focuses in veterinary medicine. Specifically, rupture of the SDFT in the carpal canal region in a group of aged horses was recently presented.7 An additional report describes similar findings in a group of nine horses ranging in age from 18–22 years.17 Interestingly, another study of horses with injury to the SDFT within the carpal sheath, specifically at the level of the carpus, reported these horses to be older, with a mean age of 18 years and predominantly Quarter Horses (9/12 cases). In contrast with the other 22 horses in the study, injuries distal to the accessory carpal bone occurred in younger horses (mean age, 6.3 y), that were predominantly Thoroughbreds used for flat racing.18

Injuries in this region are most common in Thoroughbred racehorses; however, additional indications for scanning this region include ruling out septic carpal sheath tenosynovitis from wound communication or associated tendon or ligament injuries from direct lacerations to this region.

The approach described allows for complete evaluation of the structures of the carpal canal and the technique can be readily learned by a novice ultrasonographer. A few things to keep in mind include when starting at home base, the approach is directly medial on the limb and just distal to the chestnut (Fig. 2). A common error is to start from a more caudal position on the limb. Also, using the fundamental ultrasound principle of keeping the structure of interest in the center of the image, improves evaluation of the structure along its length. Many structures are oriented in an oblique manner within the limb; if the transducer is moved directly distal when following the AL-SDFT, the structure will begin to “slide off” the screen. Small corrections to center the structure before moving distally again will improve exam quality and accuracy. Lastly, having knowledge of where tendons and ligaments insert is useful in case the ultrasonographer becomes disoriented or if normal anatomy is distorted due to pathologic changes. By identifying the structure at its insertion, a retrograde approach can be used to follow it proximally to the muscle belly in the distal antebrachial region.

One limitation of the study was that the cadaver limbs were imaged in nonweight-bearing positions, which created relaxation artifact in the AL-SDFT. Individual ultrasound skill and experience is also a limiting factor. With better ultrasound technique and hand-eye coordination, following smaller and obliquely oriented structures, such as the FCR or UL, was easier for more experienced ultrasonographers, but proved to be more difficult for those with novice skill level. Although this paper aids imaging of the normal carpal canal and caudal antebrachial structures, it does require practice and experience to identify pathologic changes, especially if subtle.

Approaching imaging of the carpal canal and caudal antebrachium as a complete, systematic examination, using a standardized approach will help increase familiarity with the anatomy over time. Identifying home base as a reference point helps guide the ultrasonographer to find the remaining structures, which may be unfamiliar. This technique expands on the previously described approach, but incorporates using an individual structure approach to evaluate the entire caudal antebrachium.

Acknowledgments

Declaration of Ethics

The Authors declare that they have adhered to the Principles of Veterinary Medical Ethics of the AVMA.
Conflict of Interest
The Authors declare no conflicts of interest.

References and Footnotes

*1.5 T magnet, GE Signa HDxT, Milwaukee, WI 53201.
*bOmnipaque, GE Healthcare, Inc., Shanghai, China.
*cLogiq9, GE Ultrasound, Milwaukee, WI 53201.
Abnormal Imaging Findings of the Femoral Third Trochanter in 20 Horses

Georgette E. Shields, DVM*; Mary Beth Whitcomb, DVM, MBA, ECVDI (LA Associate); Betsy Vaughan, DVM; and Erik Wisner, DVM, DACVR

Diagnosis of third trochanter injuries is challenging without multi-modality imaging. Due to clinical overlap, ultrasound of the pelvis and femur should be performed in horses suspicious for upper limb fracture. Lameness and return to function can be prolonged. Authors’ addresses: Department of Environmental and Radiological Health Sciences, Veterinary Teaching Hospital, Colorado State University, Fort Collins, CO 80523 (Shields); Department of Surgical and Radiological Sciences, Veterinary Medical Teaching Hospital, University of California, Davis, CA 95616 (Whitcomb, Vaughan, Wisner); e-mail: gshieldsdvm@gmail.com. *Corresponding and presenting author. © 2015 AAEP.

1. Introduction
Femoral third trochanter injuries are an uncommon but important source of lameness. Diagnosis is confounded by a lack of localizing signs and often requires nuclear scintigraphy or ultrasonography.

2. Materials and Methods
Retrospective analysis of medical records identified 20 horses with ultrasonographic or nuclear scintigraphic evidence of third trochanter abnormalities from 2004–2014.

3. Results
Ultrasound identified third trochanter fractures in 14/20 horses. Lameness was acute, insidious, or unknown. All but one was lame (Grade 2–4/5) at presentation. Ultrasound was the initial diagnostic modality in 5/14 fractured horses, whereas scintigraphic findings of intense, moderate, and mild increased radiopharmaceutical uptake (IRU) prompted ultrasound in 9/14 horses. Non-displaced fracture was suspected in one horse with intense IRU and negative ultrasound findings. In the remaining five horses, imaging findings included only mild IRU and lameness was localized to other regions. Six of 12 fractured horses with outcome data returned to function after a prolonged rehabilitation of 8–18 months.

4. Discussion
Scintigraphic findings directed focused ultrasound exams in the majority of fracture cases. Horses with third trochanter fracture had similar clinical characteristics to that reported for pelvic fractures. Ultrasonographic examination of both regions is therefore recommended, especially when scintigraphy is unavailable. Prognosis for return to function was less favorable than previously reported.

Acknowledgments

Declaration of Ethics
The Authors declare that they have adhered to the Principles of Veterinary Medical Ethics of the AVMA.

Conflict of Interest
The Authors declare no conflicts of interest.
Antimicrobial Activity and Cutaneous Sensation After the Addition of Mepivicaine Hydrochloride to Amikacin Sulfate Perfusate

Aimée C. Colbath, VMD*; C. Wayne McIlwraith, BVSc, PhD, DSc, FRCVS, DACVS, DACVSMR; Jenifer R. Gold, DVM, DACVIM, DACVECC; and Valerie J. Moorman, DVM, PhD, DACVS

A regional limb perfusate of 500 mg mepivicaine hydrochloride and 1 g amikacin sulfate diluted in 31 mL 0.9% NaCl does not affect amikacin synovial concentrations or antimicrobial activity and results in cutaneous analgesia. Authors’ addresses: Orthopaedic Research Center, College of Veterinary Medicine, Colorado State University, Fort Collins, CO, 80523 (Colbath, McIlwraith, Moorman); and Department of Clinical Sciences, College of Veterinary Medicine, Washington State University, Pullman, WA 99164 (Gold); e-mail: aimee.colbath@colostate.edu. *Corresponding and presenting author. © 2015 AAEP.

1. Introduction
The analgesic and antimicrobial effect of combining mepivicaine hydrochloride and amikacin sulfate in venous regional limb perfusion is unknown. The study sought to determine the effect of combining mepivicaine hydrochloride with amikacin sulfate on cutaneous analgesia, synovial amikacin concentration, and antimicrobial activity.

2. Materials and Methods
Fourteen forelimbs from nine horses received one treatment. Treatment A contained 1 g amikacin sulfate in 56 mL saline and treatment AM contained 500 mg of 2% mepivicaine hydrochloride and 1 g amikacin sulfate in 31 mL saline. Algometry readings were collected on the dorsal metacarpus at three different locations prior to sedation, following sedation, after tourniquet application, and 30 minutes after treatment administration prior to and following tourniquet removal. Thirty and 60 minutes following administration, synovial fluid was taken from the middle carpal joint for amikacin concentration; synovial fluid was inoculated on S. aureus and E. coli plates for zone of inhibition. Mann-Whitney U and ANOVA tests were used.

3. Results
Synovial fluid amikacin concentrations and zone of inhibition were not different between treatments. The amount of pressure to elicit a response was increased for treatment AM compared with treatment A, 30 minutes following treatment prior to and following tourniquet removal.

4. Discussion
The addition of mepivicaine to amikacin perfusate does not affect amikacin synovial concentrations or antimicrobial activity, and results in cutaneous analgesia.

Acknowledgments
Declaration of Ethics
The Authors declare that they have adhered to the Principles of Veterinary Medical Ethics of the AVMA.
Conflict of Interest
The Authors declare no conflicts of interest.

Research Abstract—for more information, contact the corresponding author

NOTES
Comparison of Two Diagnostic Tests Measuring Equine Serum Amyloid A Levels in Inflamed Septic and Inflamed but Nonseptic Synovial Structures

John David Stack, MVB, MSc, MRCVS; Matthieu Cousty, DMV, DECVS; Emma Steele, DMV; Ian Handel, BVSc, MSc, PhD, Stat, MRCVS; Antoine Lechartier, DMV; and Florent David, DVM, MSc, DACVS, DECVS, ECVDI Assoc., DACVSMR*

The synovial fluid (SF) serum amyloid A (SAA) point-of-care test evaluated here can aid differentiation between inflamed but nonseptic (IBNS) and inflamed but septic (IS) synovial structures. Authors' addresses: University College Dublin Veterinary Hospital, Belfield, Dublin 4, Ireland (Stack, Steele); Clinique Vétérinaire Equine de Livet, Cour Samson, 14140 St Michel de Livet, France (Cousty); Royal (Dick) School of Veterinary Studies and The Roslin Institute, Division of Veterinary Clinical Studies, The University of Edinburgh, Hospital for Small Animals, Easter Bush Veterinary Centre, Roslin, Midlothian, EH25 9RG United Kingdom (Handel); Clinique équine de l’Ecole Nationale Vétérinaire d’Alfort, Maisons Alfort, France (Lechartier); and Mid-Atlantic Equine Medical Center, PO Box 188, 40 Frontage Road, Ringoes, NJ 08551 (David); e-mail: flo_david@hotmail.com. *Corresponding and presenting author. © 2015 AAEP.

1. Introduction
Synovial sepsis, a life-threatening condition of horses, requires prompt diagnosis. SF cytology is commonly utilized to diagnose septic synovitis; however, not all cases are clearly discernible. Laboratory analysis takes 1–3 days. A rapid, reliable, point-of-care test diagnosing synovial sepsis would expedite early intervention.

2. Materials and Methods
Each structure was diagnosed as IBNS or IS based on SF cytology (nucleated cell count, percentage neutrophils, intracellular bacteria), culture, and synovial pressure-leak testing. SF SAA levels were measured by point-of-care semi-quantitative immunochromatographic and ELISA tests, by a blinded operator. Data were analyzed by means of receiver operating characteristic curves and optimal cutoffs appointed for each test.

3. Results
Seventy-two synovial structures (62 horses) were sampled (48 IBNS and 24 IS). An optimal SAA cutoff above which the test was considered positive for sepsis, was moderate for point-of-care test and 132 μg/mL for the ELISA test. Sensitivity (0.75) and specificity (0.92) were the same for both tests. Sensitivity (0.84) and specificity (0.92) improved for both tests when structures sampled within 6 hours of onset of clinical signs were excluded. Excellent

Research Abstract—for more information, contact the corresponding author

NOTES
correlation was observed between tests (Spearman’s rank correlation of 0.96; \( P < .001 \)).

4. Discussion
Sensitivity/specificity of SF SAA point-of-care test are very good when clinical signs of synovitis are present for more than 6 hours. This test, as an adjunct to traditional clinical methods, can assist equine practitioners to rapidly diagnose synovial sepsis.

Acknowledgments

Declaration of Ethics
The Authors declare that they have adhered to the Principles of Veterinary Medical Ethics of the AVMA.

Conflict of Interest
The Authors declare no conflicts of interest.
Biomarker Concentrations in Serum and Synovial Fluid Following Autologous Conditioned Serum Injection Into Osteoarthritic Coffin Joints

Dane M. Tatarniuk, DVM, MS*; Donna M. Groschen, CVT; Kelly Merritt, BS; Mike C. Maher, DVM, DACVS; Nicolas Ernst, DVM, MS, DACVS; Murray P. Brown, DVM, MS, DACVS; and Troy N. Trumble, DVM, MS, PhD, DACVS

Interleukin-1 receptor antagonist protein (IL-1ra) is elevated in autologous conditioned serum (ACS) vs unincubated, but not incubated control. However, IL-4, IL-6, and IL-8 suppression makes ACS globally distinct from incubated control. Following three ACS injections 7 days apart, IL-1ra in synovial fluid (SF) was not significantly different vs baseline and contained 1% of the concentration originally in ACS.

Authors’ addresses: Department of Veterinary Population Medicine, University of Minnesota, College of Veterinary Medicine, St. Paul, MN 55108 (Tatarniuk, Groschen, Maher, Ernst, Trumble); and Large Animal Clinical Sciences, University of Florida, College of Veterinary Medicine, Gainesville, FL 32608 (Merritt, Brown); e-mail: tatar014@umn.edu. *Corresponding and presenting author. © 2015 AAEP.

1. Introduction
The anti-inflammatory cytokine IL-1ra is considered central within ACS, but knowledge about ACS composition and effect on naturally occurring osteoarthritis is unknown.

2. Materials and Methods
Eleven horses with distal interphalangeal osteoarthritis (OA) were administered three injections of ACS 7 days apart. ACS was compared with unincubated and incubated control serum. Baseline SF samples were aspirated at Day 0, and post-therapy SF collected at Days 7, 14, and 21. All serum and SF samples were analyzed for cytokines (IL-1ra, IL-1β, tumor necrosis factor [TNF]α, IL-4, IL-6, IL-8, and IL-10), matrix metalloproteinases (MMP-1, MMP-3, MMP-9, and MMP-13), and tissue inhibitors of MMPs (TIMP; TIMP-1, TIMP-2, TIMP-3, and TIMP-4). A one-way ANOVA assessed statistical differences (P < .05).

3. Results
IL-1ra in ACS was significantly increased vs. unincubated but not incubated control. Suppression of IL-4, IL-8 (vs. both controls), and IL-6 (vs. 19-h control) as well as elevation of MMP-1 and MMP-9 (vs. 1-h control) was present in ACS. Following ACS administration, IL-1ra was unchanged in post-ACS SF samples vs. baseline.

4. Discussion
Clinical significance of IL-4, IL-6, and IL-8 suppression is unclear. Lack of change in IL-1ra within SF
may be from high receptor binding, or degradation and/or transportation outside of the joint.

Acknowledgments

Declaration of Ethics
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Conflict of Interest
This project was supported by the University of Minnesota Equine Center and The Minnesota Racing Commission with funds from the Minnesota Agricultural Experiment Station (2013); and the American College of Veterinary Surgeon Foundation – Surgeon In-Training Grant (2013).
Effect of Sensor Position on Kinematic Output of an Inertial Sensor System

Valerie J. Moorman, DVM, PhD, DACVS*; David D. Frisbie, DVM, PhD, DACVS, DACVSMR; Christopher E. Kawcak, DVM, PhD, DACVS, DACVSMR; and C. Wayne McIlwraith, BVSc, PhD, DSc, FRCVS, DACVS, DACVSMR

A 2-cm change in right front sensor location did not significantly affect inertial sensor system output. However, a similar change in pelvic sensor location significantly changed hindlimb but not forelimb lameness output. Authors’ address: Orthopaedic Research Center, Department of Clinical Sciences, College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Fort Collins, CO 80523; e-mail: valerie.moorman@colostate.edu. *Corresponding and presenting author. © 2015 AAEP.

1. Introduction
One widely utilized commercial inertial sensor system has demonstrated accuracy in lameness detection. However, the system’s accuracy has not been described when sensor locations are altered. The goal of this investigation was to determine the accuracy of the system when the position of right fore (RF) pastern and pelvic sensors were altered. We hypothesized that moving the RF sensor would not have a significant effect but that altering the location of the pelvic sensor would significantly influence pelvic variable kinematics.

2. Materials and Methods
Twelve horses were examined at the trot on a high-speed treadmill. The RF sensor was tested in three locations in random order: dorsal midline, 2 cm medially, and 2 cm laterally. During another session, the pelvic sensor was tested in five locations in random order: midline, 2 cm to the right and left of midline, 2 cm cranial, and 2 cm caudal. MaxDiff and MinDiff of the head and pelvis were ranked and analyzed using linear regression with significance set to $P < .05$.

3. Results
Altering RF sensor location had no significant effect on fore or hindlimb kinematics. Pelvic sensor location had a significant effect on MinDiff pelvis ($P < .0355$).

4. Discussion
Results of the study support that location of the pelvic sensor has a significant effect on output kinematics; thus, placement of this sensor must be anatomically accurate.

Acknowledgments
Horses used for this investigation were a part of a larger study funded by the NIH (NIH AR047702-07A1).

Declaration of Ethics
The Authors declare that they have adhered to the Principles of Veterinary Medical Ethics of the AVMA.

Conflict of Interest
The Authors declare no conflicts of interest.
Correlation Between an Inertial-Sensor System and Clinical Evaluation for Estimating Degree of Improvement Post-Diagnostic Analgesia

Josh R. Donnell, DVM†; and David D. Frisbie, DVM, PhD, DACVS, DACVSMR*

The evaluated inertial sensor system (ISS) demonstrated a strong correlation to clinical evaluation for estimating degree of improvement post-diagnostic analgesia. Authors’ address: Orthopaedic Research Center, Colorado State University, Fort Collins, CO 80523; e-mail: david.frisbie@colostate.edu. *Corresponding; †presenting author. © 2015 AAEP.

1. Introduction
Agreement between clinicians in identifying mild lameness is 50–60%. Agreement in change post-diagnostic anesthesia is poor between non-experts and only moderate between experts. This has lead to evaluation of objective measures that will provide strong correlation to expert opinion.

2. Materials and Methods
All horses in the study were examined for lameness and improvement post-diagnostic anesthesia by the ISS and an expert lameness clinician (blinded or unblinded to ISS). Percent agreement and 95% confidence intervals between ISS and clinician were estimated for limb of primary lameness. Correlation between ISS and clinician for estimated change post-diagnostic anesthesia was calculated.

3. Results
Thirty-six horses with 181 evaluations (5 ± 3 per horse) were enrolled. When blinded a 61% agreement and when unblinded an 81% agreement was observed between ISS and clinician for choosing the primary lame limb. Correlations (r) between the ISS and the blinded and unblinded clinician were 0.80 and 0.74, respectively for estimated change post-diagnostic anesthesia.

4. Discussion
Agreement of the blinded clinician with the ISS was similar to reports of agreement between clinicians in the presence of mild lameness. Clinician opinion combined with ISS demonstrated an 80% agreement, potentially suggesting an improvement in choosing a limb with primary lameness. When blinded the correlation between ISS and clinician was strong for estimated change in lameness post-diagnostic analgesia, suggesting this information could benefit clinicians of all experience levels.

Acknowledgments
Orthopaedic Research Foundation at Colorado State University provided the inertial sensor system.

Declaration of Ethics
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Conflict of Interest
The Authors declare no conflicts of interest.
Reversible Downregulation of Hypothalamic-Pituitary-Gonadal Axis in the Stallion With a Third-Generation Gonadotropin-Releasing Hormone Antagonist

Gabriel M. Davolli, DVM, MS*; Barry A. Ball, DVM, PhD, DACT; Alejandro Esteller-Vico, DVM, PhD; Anthony Claes, DVM, PhD, DACT; Igor F. Canisso, DVM, MSc, PhD, DACT, DECAR; Carleigh E. Fedorka, BS; Elizabeth M. Woodward, PhD; Mats H.T. Troedsson, DVM, PhD, DACT, DECAR; and Edward L. Squires, MS, PhD, DACT (hon)

Acyline, a gonadotropin-releasing hormone antagonist (GnRH), reversibly suppresses the hypothalamic-pituitary-gonadal (HPG) axis in the stallion. The recovery from the effects of acyline treatment supports its investigation as a possible treatment for androgen-dependent conditions, including the equine arteritis virus carrier state in stallions. Authors’ addresses: Department of Veterinary Clinical Sciences, School of Veterinary Medicine, Louisiana State University, Baton Rouge, LA 70803 (Davolli); Gluck Equine Research Center, Department of Veterinary Science, University of Kentucky, Lexington, KY 40546-0099 (Ball, Esteller-Vico, Fedorka, Troedsson, Squires); Department of Equine Sciences, Faculty of Veterinary Medicine, Utrecht University, Yalelaan 112, 3584 CM Utrecht, the Netherlands (Claes); Department of Veterinary Clinical Medicine, College of Veterinary Medicine, University of Illinois, Urbana, IL 61802 (Canisso); and New Bolton Center, School of Veterinary Medicine, University of Pennsylvania, 382 West Street Road, Kennett Square, PA 19348 (Woodward); e-mail: gdavolli@lsu.edu. *Corresponding and presenting author. © 2015 AAEP.

1. Introduction
The objectives of this study were (1) to evaluate the downregulation of the stallion HPG axis by a GnRH antagonist (acyline) based upon endocrine, seminal, and testicular effects and (2) to assess recovery after cessation of treatment.

2. Materials and Methods
Stallions were treated for 50 days (n = 4; 330 μg/kg acyline every 5 days). Controls (n = 4) received vehicle alone. Stallions were assessed for endocrine, seminal, and testicular parameters before treatment and for 72 days after the last treatment.

3. Results
Treatment induced a decline (P<0.05) in testosterone to castrate levels. Testosterone returned to control values within 9 days after the last treatment administration. Acyline-treated stallions failed to respond to follicle-stimulating hormones, luteinizing hormones, and an increase in testosterone after exogenous GnRH stimulation compared with pretreatment.
and control stimulation. Total sperm count, motility, and testicular volume were reduced in acyline-treated stallions ($P<0.05$). Testicular volume and most seminal parameters returned to normal values within 72 days after treatment discontinuation. Sperm output was normal by 7 months after the end of the experiment.

4. Discussion
Acyline reversibly suppressed the stallion HPG axis, and peripheral testosterone concentrations were reduced to gelding levels. These findings warrant the investigation of acyline in equine arteritis virus (EAV) carrier stallions as a possible means of eliminating the androgen-dependent carrier state of EAV.

Acknowledgments
This study was funded by Albert Clay, Shapiro and Koller endowments from the Gluck Center, University of Kentucky. The paper (abstract) was reviewed by the principal investigators responsible for the research directed endowments.

Declaration of Ethics
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Conflict of Interest
The Authors declare no conflicts of interest.

Note
There are no FDA-approved veterinary acyline products available so a compounded product was used.
In Vitro Efficacy of Nonantibiotic Agents Against Bacterial Biofilm From Equine Uterine Isolates

Kristen D. Loncar, DVM†; Ryan R. Ferris, DVM, MS, DACT*; Patrick McCue, DVM, PhD, DACT; Brad R. Borlee, PhD; Margo L. Hennet, BA; and Grace I. Borlee, PhD

To effectively degrade and kill bacteria in a biofilm state, accurately identifying the causative organism is important. No single agent tested in vitro was effective against all isolates of the four most common equine uterine pathogens. Authors’ addresses: Equine Reproduction Laboratory, Colorado State University, Fort Collins, CO 80526 (Loncar, Ferris, McCue, Hennet); and Department of Microbiology, Immunology, and Pathology, Colorado State University, Fort Collins, CO 80523 (B. Borlee, G. Borlee); e-mail: rferris@colostate.edu. *Corresponding author; †presenting author. © 2015 AAEP.

1. Introduction
Biofilm has been implicated in cases of chronic or recurrent endometritis. However, it is unclear if currently recommended therapies are effective against pathogens from the equine reproductive tract.

2. Materials and Methods
Equine uterine pathogens were utilized to form a biofilm in vitro and challenged for 6 hours with 9 nonantibiotic agents (Table 1) at concentrations suggested in equine and/or human medicine. Endpoints evaluated were disruption of the biofilm and killing of the bacteria. Positive controls were included in each replicate.

3. Results
Escherichia coli (N = 10) biofilm was significantly (P<0.05) degraded when exposed to Tris ethylenediaminetetraacetic acid (EDTA), 2-amino-2-hydroxymethyl-1,3-propanediol and disodium ethylenediaminetetraacetate dehydrate, acetylcysteine, dimethyl sulfoxide, and antimicrobial peptide mimic, and the bacterial load was significantly reduced when treated with acetylcysteine or hydrogen peroxide. Klebsiella pneumoniae (N = 10) biofilm was significantly reduced in mass (90%) after treatment with antimicrobial peptide mimic, and the number of bacteria was significantly reduced with hydrogen peroxide. Biofilm preformed with Pseudomonas aeruginosa (N =10) was significantly reduced in 50% of isolates when treated with either hydrogen peroxide or antimicrobial peptide mimic, and acetylcysteine significantly reduced the bacterial load. Biofilm preformed with Streptococcus zoopneumonicus (N =10) was significantly disrupted, and the bacterial load was reduced for all test agents except ozone.

NOTES
4. Discussion

Bacterial isolates recovered from the equine reproductive tract are capable of forming a biofilm in vitro. However, none of the routine non-antibiotic treatments currently used in clinical practice evaluated in these studies were capable of disrupting a preformed biofilm under the conditions tested in species of equine bacteria isolated from the uterus. Therefore, a critical need exists to identify the causative organism(s) of equine bacterial endometritis and determine which anti-biofilm treatments will be effective in vivo. None of the agents evaluated in this study were able to eradicate a biofilm in all species of bacteria tested.

Acknowledgments

This study was funded by the Grayson-Jockey Research Foundation and donations to the ERL Research Foundation.

Declaration of Ethics

The Authors declare that they have adhered to the Principles of Veterinary Medical Ethics of the AVMA.

Conflict of Interest

The Authors declare no conflicts of interest.

Footnotes

\(^a\)Tricide®, Sigma-Aldrich, St. Louis MO 63103.
\(^b\)Ceragyn®, Ceragyn LLC, Spanish Fork, Utah 84660.

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Table 1. Concentration of the Agents Exposed to a Preformed Biofilm for 6 Hours at the Highest Concentration Recommended in Equine Reproduction

<table>
<thead>
<tr>
<th>Agent</th>
<th>Concentration</th>
<th>MIC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antimicrobial peptide mimic</td>
<td>As directed for intrauterine infusion</td>
<td>1.5% K pneumoniae, 0.5% E. coli</td>
</tr>
<tr>
<td>DMSO (organosulfar compound)</td>
<td>30%</td>
<td>15%</td>
</tr>
<tr>
<td>H₂O₂ (reactive oxygen species)</td>
<td>1%</td>
<td>0.5%</td>
</tr>
<tr>
<td>N-acetylcysteine (interaction with</td>
<td></td>
<td></td>
</tr>
<tr>
<td>sulphydryl group)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>OmniPhase (hypochlorous acid)</td>
<td>As directed</td>
<td>N/A</td>
</tr>
<tr>
<td>Ozone (reactive oxygen species)</td>
<td>0.003%</td>
<td>N/A</td>
</tr>
<tr>
<td>Tris-EDTA (chelating agent)</td>
<td>50 mM Tris, 3.5 mM EDTA</td>
<td>50 mM Tris, 3.5 mM EDTA</td>
</tr>
<tr>
<td></td>
<td>20 mM 2-amino-2-hydroxymethyl-1,3-</td>
<td>20 mM 2-amino-2-hydroxymethyl-</td>
</tr>
<tr>
<td></td>
<td>propanediol, 8 mM EDTA</td>
<td>1,3-propanediol, 8 mM EDTA</td>
</tr>
<tr>
<td>Tricide (chelating agent)</td>
<td>As directed</td>
<td>N/A</td>
</tr>
<tr>
<td>Vetricyn (hypochlorous acid)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

If an agent was found to be effective the minimum inhibitory concentration (MIC) was determined. DMSO = dimethyl sulfoxide; N/A = not applicable.
How to Perform a Postmortem Examination of an Aborted Fetus in the Field

Luke Bass, DVM, MS*; Nigel Miller, MS; Hallie Noland, DVM; and Chad Frank, DVM, DAVCP

1. Introduction
The widespread use of transrectal ultrasound and fetal monitoring technology has allowed horse owners to attain more information about the mare and foal during gestation. Dependent on the report and population of horses, equine abortions still account for 8 to 19% of equine pregnancies.1–3 Equine abortions still remain a significant problem for equine breeders. Equine abortion is defined as a loss of pregnancy prior to 300 days’ gestation.2 Fetuses dying after 300 days are termed stillbirths given that foals born alive may be capable of survival.2 Common causes of abortion include both infectious and noninfectious conditions with noninfectious surpassing the prevalence of those caused by infectious agents.4 A group from the United Kingdom reported that noninfectious causes (umbilical cord disorders, twinning, and stillbirth) accounted for 58.5% when compared with infectious equine herpes virus, (EHV)-1 and -4 and placentitis which was only reported in 16.3% of cases. However, another report from France found that over a 24-year period, infectious causes were more common than noninfectious (67.3% vs 27.2%, respectively).5

When an equine abortion occurs, veterinary practitioners may be asked to evaluate the fetus and placenta in the field for a potential etiology. Performing a thorough and appropriate postmortem examination of an aborted fetus is a valuable skill for an equine practitioner. If no overt lesions are present, a practitioner needs to understand options for whole body submission compared with tissue submission. Furthermore, selection of appropriate samples to be collected will preserve tissues for evaluation once it has reached the laboratory. The goal of this paper is to review basic techniques of placental and fetal evaluation, determine what samples to collect for submission to a referral laboratory, and present common findings from review articles in the past on the subject.

2. Materials and Methods
Once a practitioner arrives to examine an aborted fetus, a decision must be made as to whether to examine the fetus on the farm, examine the fetus followed by sample collection and submission, or whole-body fetal and placenta submission to referring laboratory. Diagnostic laboratories will usually have a higher success rate if the examination and tissue sampling is performed by a diagnostic laboratory. However, there are advantages to performing the work yourself. Your direct involve-
the chorioallantois, amnion, and umbilical cord. Helpful (Table 1).

Samples to be taken and method of shipment is critical. In most cases per breeding season, a laminated checklist of appropriate tools allow for a necropsy to be performed easily.

<table>
<thead>
<tr>
<th>Tools for Performance of a Necropsy</th>
</tr>
</thead>
<tbody>
<tr>
<td>The basic requirements include:</td>
</tr>
<tr>
<td>Scalpel No. 10 or No. 20 or sharp knife with 6’ curved blade</td>
</tr>
<tr>
<td>10% formalin</td>
</tr>
<tr>
<td>Syringes with large-bore needles</td>
</tr>
<tr>
<td>Rat tooth forceps</td>
</tr>
<tr>
<td>Three aerobic culture swabs</td>
</tr>
<tr>
<td>Cutting board</td>
</tr>
<tr>
<td>Headlamp or other light source</td>
</tr>
<tr>
<td>Whirl-Pak bagsa</td>
</tr>
<tr>
<td>Sample submission containers</td>
</tr>
<tr>
<td>Permanent markerb (for labelling)</td>
</tr>
<tr>
<td>Non-sterile latex gloves, plastic boot covers</td>
</tr>
<tr>
<td>Garbage bags (for body cleanup)</td>
</tr>
<tr>
<td>Submission form (most are available online)</td>
</tr>
<tr>
<td>Rongeurs (to facilitate brain removal)</td>
</tr>
<tr>
<td>Sterile pack of instruments</td>
</tr>
</tbody>
</table>

The following samples should be taken and submitted for analysis:

**Culture:** Fetal lung, stomach contents, liver, placenta.

**Histology:** Adrenal glands, brain, heart, kidney, liver, lung, small intestine, spleen, placenta, and any other tissue that appears abnormal.

It is common for the placenta to be inverted with the allantoic surface exposed. If fresh, the chorionic side of the placenta should look burgundy to red in color with a velvety texture. This differentiates it from the allantoic surface, which will be pink, smooth, and has visible blood vessels. A normal amnion should be thin and white to translucent in color. Environmental contaminants should be removed by rinsing with cold water to allow for adequate visualization of the tissue. The placenta should be laid out in a ‘Y’ or ‘F’ shape (Fig. 1) and weighed if possible. The placenta should weigh approximately 11% of the foals' body weight.

Begin by inspecting the placenta completely to make sure no tears or sections are missing. Horn tips of the placenta can be torn and can cause complication in the mare if retained. Key features to note are the cervical star, gravid and nongravid horn, and body of the placenta. The gravid horn will be larger and thicker when compared with the nongravid horn. Identification of the cervical star should be completed next. It will be pale in color and contain no microcotyledons. Areas of inflammation and necrosis of the chorioallantois appear grossly as tan to brown, thickened regions may have necrotic exudate on the surface. Cytology can be useful to identify bacteria or fungal hyphae.

Ascending placentitis is common, in which case lesions will be present at the cervical star and may extend 10 to 20 cm cranially. Nocardioform bacteria cause lesions in a locally extensive pattern near the cranial ventral uterine body and base of the horns. Leptospirosis and Candida infections cause diffuse placentitis. Affective placentas may appear more red than normal and may have small irregular areas of pallor.

Samples of the placenta should be collected for aerobic culture and histology. Small squares (3–4 cm) of the gravid horn, nongravid horn, cervical star, body of the placenta, and amnion should be placed in a container with 10% formalin. The umbilical cord can vary in length and will have a mild-to-moderate twisting. Although the length can vary greatly, Whitwell states that 95% of Thoroughbred foals will have an umbilicus less than 84 cm. Torsion of the cord is pathologic and...
causes restriction of blood flow, which if acute can cause fetal death and abortion. Cross section of the umbilical cord should be taken and placed in 10% formalin.

After thorough inspection of the placenta and umbilical cord, examination of the fetus can begin. The fetus should be weighed and a crown-rump length (Table 2) should be taken with the fetus slightly bent in the natural position. Note any abnormalities before beginning the necropsy. Note the hair growth and body condition. The vertebral spinothalamic processes and femurs should not be prominent. The limbs should be evaluated for contracture or other congenital anomalies. Place the fetus in right lateral recumbency and examine the external surface of the fetus. Sharp dissection is made to reflect the right fore and hind limb of the fetus dorsally (Fig. 2). Next a single incision is made from the anus to the mandible along ventral midline. The skin should be reflected back before the abdominal wall is removed. Begin the incision at midline and make two cuts extending to the pubis and dorsal paralumbar fossa. Reflection of the rib cage can be completed by cutting along the costochondral junction. Reflect the abdominal wall and ribcage to fully expose the thorax and abdomen (Fig. 3). Once the thorax and abdomen are exposed, note any abnormalities. Is there any abdominal effusion? Are there any white-gray spots on the surface of the liver? Any effusion in the thoracic cavity?

Once a brief evaluation is complete, sample collection can begin. State Diagnostic Laboratory may offer an abortion panel that typically includes aerobic culture (fetal lung, stomach contents, liver, and placenta) and histology (adrenal glands, brain [entire brain], heart, liver, lung, small intestine, spleen, placenta, and any other tissue that seems abnormal). Care should be taken to collect tissue for culture without contaminating specimens. Clean gloves and instruments can minimize chance of confounding results. The fetal lung is sampled first using a sterile scalpel blade and forceps. The liver is sampled next using the same technique. Stomach contents are then collected using a syringe and large-bore needle (Fig. 4). Aerobic culture specimens are placed into a bag (Fig. 5) and labeled with mare name, owner name, date, and sample identification. Stomach contents are placed in a red top tube.

Once aerobic samples are collected, a full set of tissues should be collected for histopathology. For histopathology, fixation is important; a thin flat piece of tissue (< 1 cm in thickness) should be collected with care and placed in buffered formalin solution. The total amount of formalin should be

<table>
<thead>
<tr>
<th>Age, d</th>
<th>Fetus Length, cm</th>
<th>Fetal and Placental Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>120</td>
<td>15–20</td>
<td>External genitalia formed but scrotum is empty, placenta attached, ergots, and orbital areas prominent</td>
</tr>
<tr>
<td>150</td>
<td>25–37</td>
<td>May or may not have fine hair on orbital arch and tip of tail, prepuce not yet developed</td>
</tr>
<tr>
<td>180</td>
<td>35–60</td>
<td>Hair on lips, orbital arch, nose, eyelashes; no mane</td>
</tr>
<tr>
<td>210</td>
<td>55–70</td>
<td>Hair on lips, nose, eyebrow, eyelids, edge of ear, tip of tail, back, and mane</td>
</tr>
<tr>
<td>240</td>
<td>60–80</td>
<td>Hair on man and tail, back, and distal portion of extremities</td>
</tr>
<tr>
<td>270</td>
<td>80–90</td>
<td>Short, fine hair over entire body</td>
</tr>
<tr>
<td>300</td>
<td>70–130</td>
<td>Body completely covered with short hair, prepuce development, hair in mane</td>
</tr>
<tr>
<td>330</td>
<td>10–150</td>
<td>Complete hair coat with final color, testes descended</td>
</tr>
</tbody>
</table>

Adapted from Bergin WC et al.10
10 times the volume of the tissues being fixed. The necropsy is continued by removing the heart, lungs, trachea, larynx, tongue, and esophagus (“the pluck”) en bloc. The tongue, trachea, and esophagus are dissected free as gentle traction is used to lift and pull the tissue caudally. The trachea and esophagus are opened longitudinally, starting from the pharyngeal end. Each lung lobe is examined and sectioned. Does it sink in formalin? Is it excessively wet and heavy? The pericardial sac is opened and examined for effusion or adhesions. The heart is examined by making two incisions in the atria and ventricles. The right side of the heart is opened by incising the right atrium and ventricle, adjacent to the interventricular septum, to the apex of the right ventricle (Fig. 6). The right ventricular free wall is removed from the septum by extending the cut from the apex of the right ventricle through the pulmonary valves and into the pulmonary trunk.

The left side of the heart is examined by making an opening in the left atrium and then the left ventricle with a single incision through the left ventricular free wall to the apex. The blade or scissors are then placed under the atrioventricular valve into the aortic valve and a cut is made dorsally to expose both lining of the base of the aorta and left atria. Removal of the brain is usually not essential for infectious disease diagnosis but does allow for inspection if you suspect a congenital anomaly. The head can be removed and shipped chilled if you would prefer the pathologist to perform the removal. Alternatively, if you are removing the brain, start with a T-shaped incision through the skin on the dorsum of the cranium starting between the eyes. Extend caudally along the dorsal midline to the back of the head. Reflect incision cranial-laterally. The head is removed by making a deep cut ventrally through the muscles at the back of the neck. The brain is exposed by removing a dorsal section of the

Fig. 3. The ribcage and abdominal wall is reflected to expose the thorax and abdomen for sampling.

Fig. 4. Collection of stomach contents with 6-cc syringe and 18 × 1.5-in needle. Stomach contents are then placed in a red top tube.
calvarium using rongeurs or heavy scissors (Fig. 7). Transect and remove the dura mater prior to extracting the brain. The entire brain is submitted to the referral laboratory.

3. Results
Abortion can be a costly and devastating component to equine reproductive medicine. There are many causes of abortion and identifying them could aid in further prevention of subsequent abortions. Two main categories of abortion exist: infectious and noninfectious causes. In four retrospective studies, including a total of 7799 cases of abortion, infectious etiologies were determined to be the major cause of the abortions reported. Noninfectious causes include twinning, placental insufficiencies, umbilical cord problems, congenital abnormalities, fetal resorption, and body pregnancies. Unfortunately, not every cause is determined. Of the four retrospective studies 1345 of the cases (17%) could not be determined via diagnostic testing or characteristic lesions on the fetus or placenta.

Infectious Fetal Death
Bacterial placental infection is by far the most common cause of infectious abortions in horses. Of the 7799 total abortions, 1661 cases (21.2%) were attributed to bacterial etiologies. Majority of bacterial agents that cause abortion are more opportunistic bacteria and can be found normally in pregnant and nonpregnant mares. The main culprit in the four studies mentioned above was *Streptococcus zooepidemicus*. A majority of bacterial abortions are considered ascending placentitis (Fig. 8); however, some can be classified as nonascending placentitis or a hematogenous route. Placentitis can occur in two forms: acute diffuse placentitis or chronic focal placentitis. The acute form produces a much more diffuse pattern of neutrophil infiltration and occurs generally earlier in gestation than the chronic form. The chronic form produces a more focal pattern around the cervical star and tends to cause abortion in the later months of gestation. Typically bacterial infections are diagnosed via bacterial culture and can be determined from the fetus and the placenta. On occasion, however, multiple organisms can be found in the fetal and placental tissues, which indicates a mixed infection. Laugier et al. noted that mixed infections generally consisted of *Streptococcus zooepidemicus* and *Klebsiella pneumoniae* or *Streptococcus zooepidemicus* and *Escherichia coli*.

Viral causes of abortion are generally attributed to EHV-1, EHV-4, and equine viral arteritis (EVA).
with EHV-1 being the most common cause (4%) in the aforementioned reports with EHV-4 and EVA only responsible for few cases. EHV-1 is diagnosed via fetal necropsy with characteristic 1-mm white-yellow foci in the fetal liver as well as excessive pleural effusion in the fetal lungs. EHV-1 and EHV-4 abortions tend to occur between 7 and 10 months of gestation; however, some can continue onto term.13 These infections generally do not inhibit the mare’s ability to conceive again. EHV infections can cause abortions with or without clinical signs in the mare prior to the abortion.13 EVA is an infrequent cause of abortion but if mares are exposed to the virus, anywhere from 10 to 70% of them will abort. EVA infections generally cause abortions within a few days of the onset of clinical signs. EVA generally does not have any characteristic lesions on the fetus or the placenta and is diagnosed by viral isolation of placenta and fetal tissues as well as reverse transcription polymerase chain reaction assay.

Fungal etiologies are generally a minor component of abortions. Giles et al11 found only 1% of the reported abortions were attributed to fungal infections. Similarly, Laugier et al5 only found 1.8% of the abortions occurring due to a fungal infection with Aspergillus being the most common organism identified. As these infections tend to be very similar with regard to placental and fetal presentation as the bacterial placentitis, fungal culture is needed to identify organisms.

Noninfectious Fetal Death
Twinning can appear in a variety of ways: an apparently normal foal with a fetal remnant, premature foal (most likely deceased) with a smaller reabsorbing fetus, or birth of both twins (Fig. 9). The placenta will have abnormalities due to lack of chorionic villi in the areas where the two placentas come into contact with each other. Abortions caused by twinning generally have one fetus that is smaller than the other and showing more signs of retardation compared with the other twin. Also, the smaller twin usually shows signs of chronic placental insufficiency. All four retrospective reports agreed that twinning was the second most common cause (4.6%) of noninfectious abortions.4,5,11,12 However, the current use
Ultrasonography early in gestation (14–16 d) has decreased rate of twins.

Placental insufficiency is another major cause of noninfectious abortions. This component consists of placental edema, premature separation of the placenta (red bag), and developmental defects of the placenta. Abortions caused by placental insufficiency will show fetal retardation as well as physical problems in the placenta. Giles et al reported that 7.0% of abortions were attributed to placental insufficiency whereas Laugier et al discovered a much lower incidence (1.7%) of both of these causes of abortion.

Umbilical cord torsions were the major cause (60%) of noninfectious abortions according to a recent review in France while Hong et al in the United States showed that only 5% of noninfectious causes were due to umbilical cord pathology. The French study consisted of 49.6% Thoroughbreds while the American study was comprised of 75% Thoroughbreds. Whitwell has stressed that many fetal deaths of unknown cause in the last trimester of gestation coincide with cords longer than 80 cm, which suggests that these abortions could be linked to undiagnosed abnormalities of the umbilical cord. These include edema of the cord, sacculation of the cord, excessive torsion, and strangulation of the cord. All of which were noted to cause impairment of the blood supply to the fetus, thus resulting in abortion. Characteristic signs of torsion include excessive torsion of the umbilicus, stricture, dilations of urachus or umbilical vein, as well as hemorrhagic edema throughout the cord (Fig. 10).

Body pregnancies are generally not as common as other causes of abortion. Body pregnancies are defined as fetal development within the uterine body and can cause marked problems with the developing fetus. These pregnancies usually result in early gestation abortion, premature foal, or a dysmature foal. The placenta will have a very enlarged portion where it was in contact with the body of the uterus and the horns will be underdeveloped and the same size.

4. Discussion

There are many causes of equine abortion and identification could aid in further prevention of subsequent abortions. Performing a necropsy to diagnose the cause is very important as well as a relatively simple and straightforward procedure. Using this technique will enable the practitioner to evaluate a majority of the fetus, placenta, and umbilical cord during sample collection. Some diagnoses (ascending bacterial placentitis, excessive cord torsion, or EVH-1) may be evident during evaluation and tissue collection. This quick diagnosis will help the practitioner take appropriate preventative actions on the farm to prevent further cases. By performing this service, your practice cannot only profit from the procedure but have the potential of increasing revenue from additional services (vaccinations, ultrasound evaluations, medical therapeutics) for the remaining population of pregnant mares. Field necropsy of an aborted fetus can be time consuming during breeding season if the above technique is followed; therefore, submission of an intact fetus may be necessary in the busy season.

Proper tissue handling can decrease the amount of bacterial contamination and increase the number of diagnostic samples. Having a well-lit and clean environment to perform the procedure will improve your quality of work and increase the chance of getting an accurate diagnosis. Evaluation and tissue collection of the placenta and umbilicus is performed prior to fetal examination. The fetus is examined for congenital anomalies and tissue collection is performed with clean instruments and gloves. Most state diagnostic laboratories offer an abortion panel that will include testing for aerobic culture, virology, and histopathology. Aerobic culture samples are taken prior to those for histopathology. Bags and small containers with 10% formalin are used for culture and histopathology samples, respectively. Once the procedure is complete, proper cleanup and body disposal is imperative to limit contaminants to personnel and to the farm.

Acknowledgments

Declaration of Ethics

The Authors declare that they have adhered to the Principles of Veterinary Medical Ethics of the AVMA.

Conflict of Interest

The Authors declare no conflicts of interest.

References and Footnotes


*aWhirl-Pak bag, Nasco, Fort Atkinson, Fort Atkinson, WI 53538-0901.

*bSanford Brands, A Newell Rubbermaid Company, Oakville, ON L6J 3J3, Canada.
Diagnosis and Surgical Management of Uterine Tears in the Mare

Kevin M. Claunch, DVM, DACVS*; Rolf M. Embertson, DVM, DACVS; Brett Woodie, DVM, DACVS; Alan Ruggles, DVM, DACVS; and Scott Hopper, DVM, DACVS

Uterine lacerations should be suspected in periparturient mares with evidence of peritonitis. Early surgical intervention aids in definitive diagnosis of uterine tears, detecting concurrent pathology, and repairing the uterine defect. Authors’ addresses: Weems & Stephens Equine Hospital, 5960 Hospital Road, Aubrey, TX 76227 (Claunch); and Rood and Riddle Equine Hospital, PO Box 12070, Lexington, KY 40580-2070 (Embertson, Woodie, Ruggles, Hopper); e-mail: kevin@wseh.net. *Corresponding and presenting author. © 2015 AAEP.

1. Introduction
Uterine tears are a rare albeit serious complication of parturition in broodmares. Large-scale studies describing the presentation, treatment, and factors associated with prognosis of uterine tears are lacking.

2. Materials and Methods
Medical records of 92 mares diagnosed with uterine tears by vaginal exam, exploratory surgery, or necropsy were reviewed.

3. Results
The most common reasons for mares presenting for evaluation were colic, fever, and tachycardia postpartum. Most mares that presented ≤12 hours post-partum had clinical peritonitis characterized by peritoneal fluid with >10,000 WBC/dL. Short-term survival for mares that were treated surgically was 78% (69/88). Tears were more commonly located in the uterine horns as opposed to the body. Additional pathology besides a solitary uterine tear was discovered within the abdomen in 23% (20/86) of mares that were treated surgically. Of mares bred the same year as a uterine tear 64% (18/28) carried a foal to term and 77% (33/43) of mares bred the year following a uterine tear carried a foal to term.

4. Discussion
Early surgical intervention aids in definitive diagnosis of uterine tears, detecting concurrent pathology, and repairing the uterine defect. Mares with uterine tears treated surgically have a good prognosis for survival. Mares that survive uterine tears may successfully maintain pregnancies afterward and recurrence of uterine tears has not been observed.

Acknowledgments

Declaration of Ethics
The Authors declare that they have adhered to the Principles of Veterinary Medical Ethics of the AVMA.

Conflict of Interest
The Authors declare no conflicts of interest.

Research Abstract—for more information, contact the corresponding author

NOTES
Treatment of Uterine Cysts With Diode Laser Photoablation in a Thoroughbred Broodmare Population

Nicole Scherrer, DVM

In this study, a group of barren mares with uterine cysts were treated with diode laser photoablation. The foaling rate the year after removal of the cysts was similar to that reported for normal Thoroughbred mares in central Kentucky. The results suggest laser photoablation of uterine cysts improves fertility for mares adversely affected by uterine cysts and leads to no significant adverse effects. Author’s address: University of Pennsylvania, New Bolton Center, 382 West Street Road, Kennett Square, PA 19348; e-mail: scherrer@vet.upenn.edu. © 2015 AAEP.

1. Introduction
Uterine cysts have been suggested as a cause of infertility in mares. Uterine cyst laser photoablation was performed previously in 39 barren mares; 26 were reported to have conceived a foal after the procedure. The purpose of this study was to examine the results of photoablation of uterine cysts in mares adversely affected by uterine cysts, particularly foaling rates.

2. Materials and Methods
Medical records of 66 Thoroughbred broodmares that had uterine cyst photoablation were reviewed. Age, breed, parity, pertinent history, number of uterine cysts, and location of cysts were analyzed with postoperative foaling rates to identify factors that influenced ability to carry a foal to term.

3. Results and Discussion
A total of 45 uterine cyst laser ablation procedures was performed on 42 broodmares. In the first year after uterine cyst laser ablation, the live foal rate was 73.3% (33/45). The live foal rate per breeding season for all breedings combined was 66.7% (58/87). Younger mares (17 years or less) were more likely to carry a foal to term after laser cyst ablation than older mares (greater than 17 years): 96% versus 58%, respectively ($P = 0.0023$).

Acknowledgments

Declaration of Ethics
The Author declares that she has adhered to the Principles of Veterinary Medical Ethics of the AVMA.

Conflict of Interest
The Author declares no conflicts of interest.
Results of Treatment of Male Horses With Urethral Rents by Perineal Urethrotomy or Corpus Spongiotomy

Kati Glass, DVM*; Carolyn Arnold, DVM, DACVS; Dickson D. Varner, DVM, MS, DACT; Keith Chaffin, DVM, MS, DACVIM; and Jim Schumacher, DVM, MS, DACVS

Long-term resolution of hematuria caused by a urethral rent is possible after perineal urethrotomy (PU) or corpus spongiotomy (CS). Hemospermia may recur after PU or CS. A repeat procedure or primary closure may be required for resolution. Authors’ addresses: Department of Large Animal Clinical Sciences, College of Veterinary Medicine and Biomedical Sciences, Texas A&M University, College Station, TX 77843 (Glass, Arnold, Varner, Chaffin); and Department of Large Animal Clinical Sciences, University of Tennessee, Knoxville, TN 37996-4545 (Schumacher); e-mail: kglass@cvm.tamu.edu. *Corresponding and presenting author. © 2015 AAEP.

1. Introduction
Urethral rents in male horses typically cause hematuria or hemospermia.

2. Materials and Methods
Medical records from 1989 to 2013 of male horses with hematuria or hemospermia caused by a urethral rent and treated by PU or CS were reviewed. Signalment, clinical signs, urethroscopic findings, surgical treatment, and outcome were recorded. Long-term followup was obtained.

3. Results
Fourteen stallions and 19 geldings met the inclusion criteria. All geldings and one stallion were presented because of gross hematuria. Thirteen stallions were presented because of gross hemospermia. Twenty-one horses were treated by PU, and 13 horses were treated by CS. All horses with hematuria for which follow-up information was available had resolution of hematuria after PU or CS. Ten of 12 horses with hemospermia (83.3%) were successfully treated by PU or CS. Three of 12 horses with hemospermia (25%) required multiple surgeries to resolve hemospermia.

4. Discussion
Treatment of horses with hematuria caused by a urethral rent by PU or CS provides long-term resolution of clinical signs. Some stallions with hemospermia develop reoccurrence and require additional procedures to achieve resolution.

Acknowledgments
Declaration of Ethics
The Authors declare that they have adhered to the Principles of Veterinary Medical Ethics of the AVMA.
Conflict of Interest
The Authors declare no conflicts of interest.

Research Abstract—for more information, contact the corresponding author
Influence of Antral Follicle Count on Age-Related Changes in Follicular Parameters in Mares

Anthony Claes, DVM, PhD, DACT*; Barry A. Ball, DVM, PhD, DACT; Kirsten E. Scoggin, PhD; Edward L. Squires, MS, PhD, DACT (Hon); and Mats H.T. Troedsson, DVM, PhD, DACT, DECAR

Aging in mares is associated with changes in follicular parameters, which in turn are closely linked to differences in antral follicle count, suggesting a relationship with ovarian reserve. Therefore, determination of antral follicle counts in aged mares can provide valuable information about the reproductive aging process. Authors' addresses: Department of Equine Sciences, Faculty of Veterinary Medicine, Utrecht University, 3584 CM Utrecht, The Netherlands (Claes); and Gluck Equine Research Center, Department of Veterinary Science, University of Kentucky, Lexington, KY 40546-0099 (Ball, Scoggin, Squires, Troedsson); e-mail: a.claes@uu.nl. *Corresponding and presenting author. © 2015 AAEP.

1. Introduction
The number of antral follicles decreases with mare age but large individual differences in follicular populations exist between mares of the same age. Thus, the chronological age of a mare needs to be distinguished from its reproductive age.

2. Materials and Methods
Young (n = 10), middle aged (n = 15), and old mares (n = 15) of mixed breeds were examined using transrectal ultrasonography to monitor follicular growth and determine antral follicle count throughout two consecutive estrous cycles.

3. Results and Discussion
Older mares had a tendency to ovulate smaller follicles. Neither follicular growth rate nor the number of ovulations or the length of luteal phase was influenced by age. Interovulatory intervals and follicular phases were longer in aged mares while the day of deviation occurred later. As mare’s age increased, mares with low antral follicle counts had longer interinterovulatory intervals and follicular phases than mares with medium or high antral follicle count (AFC).

Acknowledgments
This experiment was supported by the Albert G. Clay Endowment of the University of Kentucky.

Declaration of Ethics
The Authors declare that they have adhered to the Principles of Veterinary Medical Ethics of the AVMA.

Conflict of Interest
The Authors declare no conflicts of interest.
Return to Cyclicity, eCG Levels, Ovarian Structures, and Post-Abortion Fertility in Embryo Transfer Recipient Mares

Javier J. Aguilar, DVM, PhD*; Jose Cuervo-Arango, DVM, PhD, DECAR; M.L. Vettorazzi, MV; and R. Martínez-Boví, LV

Mares that abort around Day 65 of gestation can: 1) return soon to normal cyclicity (37.5%), even with detectable levels of eCG; 2) have 1 to 3 anovulatory cycles before ovulation (56.3%); or 3) enter an anovulatory phase (6.2%). Authors’ addresses: Producción Equina, Facultad de Agronomía y Veterinaria, Universidad Nacional de Río Cuarto, Río Cuarto 5800, Córdoba, Argentina (Aguilar, Vettorazzi); and Departamento de Medicina y Cirugía Animal, Facultad de Veterinaria, Universidad CEU Cardenal Herrera, Alfara del Patriarca 46115, Spain (Cuervo-Arango, Martínez-Boví); e-mail: jaguilar@ayv.unrc.edu.ar. © 2015 AAEP.

1. Introduction
Induced abortion is a common practice among Polo horse breeders. The reproductive events after abortion are not clear.

2. Materials and Methods
Recipient mares from an equine embryo transfer center in Argentina were used. Mares were scanned to record ovarian structures and for fetal sexing. Mares carrying a male fetus (n = 32) were aborted by transcervical administration of cloprostenol (PGF). Mares carrying a female fetus (n = 25) were used as controls. Plasma samples were assayed for equine chorionic gonadotropin (eCG) and progesterone.

3. Results
Most treated mares expelled the fetus within 48 hours of treatment (27/32). The others required retreatment (n = 2) or had a dead fetus manually removed from the reproductive tract (n = 3). The mean interval from abortion to first ovulation was 28.5 days, range 5 to 65 days. Twelve mares ovulated within 15 days after abortion without forming a luteinized unruptured follicle (LUF). Two mares entered anestrus without ovulation or LUF formation. Of the mares with anovulatory cycles, 11/18 (61%) showed a well-defined estrus with low P4 and endometrial edema but with premature luteinization and hemorrhage of the dominant follicle without ovulation (LUF). The remainder (7/18; 39%), also had LUF cycles but without estrus signs. Aborted mares were reused and got pregnant again following embryo transfer (18/20; 90%).

4. Discussion
Levels of eCG at the time of abortion were extremely variable and did not correlate well with the number of supplementary corpora lutea (CL) (r = 0.25) or the interval from abortion to first ovulation (r = 0.38). After abortion, approximately 50% of mares had one, two or three LUF cycles, and their eCG levels were higher than those in mares with ovulatory estruses. The fertility of reused aborted mares was
high, even when some of them (25%) had detectable levels of eCG at the time of transfer.

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Declaration of Ethics

The Authors declare that they have adhered to the Principles of Veterinary Medical Ethics of the AVMA.

Conflict of Interest

The Authors declare no conflicts of interest.
Pregnancies can be obtained from equine blastocysts subjected to low-temperature storage prior to vitrification. Authors’ addresses: School of Animal Science, (Diaz, Gentry, Bondioli), and Department of Veterinary Clinical Sciences, School of Veterinary Medicine (Paccamonti), Louisiana State University, Baton Rouge, LA 70803; and Louisiana Center for Equine Reproduction, Opelousas, LA 70570 (Cramer); e-mail: fdiaz2@tigers.lsu.edu. *Corresponding and presenting author. © 2015 AAEP.

1. Introduction
Successful cryopreservation of equine blastocysts has been reported,1a yet no pregnancies have been reported following vitrification of large equine blastocysts (>300 μm) that have been previously subjected to passive cooling. The objective of this study was to evaluate the pregnancy rates obtained from vitrified blastocysts that were previously exposed to low-temperature storage.

2. Materials and Methods
Three cooling treatments were tested: no cooling, 12-hour, and 24-hour cooling. A total of 19 blastocysts (376 to 1584 μm) were used. The embryo distribution between treatments was: five Day 8 non-cooled, five Day 8 cooled for 12 hours, four Day 8 cooled for 24 hours, one Day 7 cooled for 12 hours, and four Day 7 cooled for 24 hours. Cooling treatments (7–10°C) were performed by the utilization of a passive cooling deviceb followed by a standard vitrification protocol with modifications.a

3. Results
Blastocysts had a mean diameter of 907.75 ± 326.24 μm for Day 8 and 490.68 ± 157.61 μm for Day 7. The pregnancy rates obtained from vitrified blastocysts at day 25 (heartbeat stage) were: Day 8 non-cooled, 100% (5/5); Day 8 cooled for 12 hours, 0% (0/5); 24 hours, 25% (1/4); Day 7 cooled for 12 hours, 100% (1/1); and Day 7 cooled for 24 hours, 75% (3/4).

4. Discussion
Pregnancies can be obtained from equine blastocysts subjected to low-temperature storage prior to vitrification. Results of this study show that medium-sized Day-7 blastocysts tolerate better low-temperature storage for up to 24 hours prior to vitrification resulting in higher pregnancy rates in comparison with larger Day-8 blastocysts (P < .05).

Acknowledgments

Declaration of Ethics
The Authors declare that they have adhered to the Principles of Veterinary Medical Ethics of the AVMA.

Conflict of Interest
Dr. Gentry served as a consultant for Genesearch, Inc. from June to July 2012. At the time of the study Dr. Gentry was not a consultant. Drs.
Diaz, Cramer, Paccamonti, and Bondioli have no conflicts of interest to declare. Genesearch, Inc. is in the process of developing and potentially commercializing the Embryo Cradle, a coaxial micromanipulation tool used for blastocyst collapse in this study. Genesearch, Inc. provided the micromanipulation tool and blastocyst collapse pipettes for this study.

Reference and Footnotes


*Equitainer, Hamilton Research, Inc., Ipswich, MA 01938.*
Morphometric Evaluation of Placental Vascular Network in Mares With Laminitis

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Mares with laminitis showed morphometric changes on placenta: lower vascular lumen diameter and higher vascular wall thickness. Authors’ addresses: Department of Veterinary Clinics (Pazinato, Nogueira, Santos, Feijó, Curcio); Department of Pathology (Fernandes), College of Veterinary, Federal University of Pelotas, Pelotas, RS 96160-000, Brazil; and Institute of Biological Sciences, Federal University of Rio Grande, Rio Grande, RS 96203-000, Brazil (Varela Jr); e-mail: curciobruna@hotmail.com. *Corresponding and presenting author. © 2015 AAEP.

1. Introduction
We analyzed the morphometry of the placental vascular network in mares with chronic laminitis.

2. Materials and Methods
Twenty-six pregnant Thoroughbred mares were selected (13 laminitis and 13 healthy). Arterial systolic pressure (ASP) and heart rate were measured. After deliveries, the placentas were weighed, and samples from the uterine body, gravid horn, and nongravid horn were collected to perform histopathologic evaluation. Digitalized images were taken from slides to perform vascular morphometry. The measures were made in vessels from the allantoic region: total vascular diameter (TVD), vascular lumen diameter (VLD), and vascular wall thickness (VWT). Data on gestational age and placental weight were recorded.

3. Results
Different results were found in laminitis and healthy mares (mean ± SE), respectively (P<0.05): ASP, 116 ± 6.73 mm Hg versus 98.3 ± 1.41 mm Hg; heart rate, 52 ± 4 bpm versus 44 ± 0.53 bpm; gestational age, 335 ± 2.94 versus 344 ± 2.47 days; placental weight, 5.8 ± 0.35 kg versus 6.9 ± 0.33 kg; VLD in uterine body, 45.77 ± 2.87 μm versus 60.41 ± 4.31 μm; VLD in gravid horn, 50.28 ± 2.78 μm versus 59.98 ± 2.77 μm; and VWT in gravid horn, 179.51 ± 7.54 μm versus 150.85 ± 8.34 μm.

4. Discussion
Pregnant mares with chronic laminitis showed hypertension syndrome, as found in pregnant women. These mares showed lower gestational age, lower placental weight, and morphometric vascular alterations (lower VLD and higher VWT), which could result from hypertension. However, more studies
are necessary to understand the impact of chronic laminitis in gestational mares.

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Declaration of Ethics
The Authors declare that they have adhered to the Principles of Veterinary Medical Ethics of the AVMA. This project was approved by the Ethical Committee on Animal Experimentation of the College of Veterinary - Federal University of Pelotas (number 510).

Conflict of Interest
The Authors declare no conflicts of interest.

Footnote
How to Use Umbilical Vessel Water Infusion to Treat Retained Fetal Membranes in Mares

Mark Meijer, DVM*; Margo L. Macpherson, DVM, MS, DACT; and Reinie Dijkman, DVM, DECVP

Using simple tools that are readily available to the equine practitioner (stallion catheter, hose adapter, and garden hose), retained fetal membranes can be easily and safely removed. Authors’ addresses: Dierenkliniek Zeddam, NL7038 EP Zeddam, The Netherlands (Meijer); Department of Large Animal Clinical Sciences, College of Veterinary Medicine, University of Florida, Gainesville, FL 32610 (Macpherson); and GD Animal Health, NL7400 AA Deventer, The Netherlands (Dijkman); e-mail: m.meijer@dapdz.nl. *Corresponding and presenting author. © 2015 AAEP.

1. Introduction

Expulsion of the fetal membranes after delivery of a foal is generally an uncomplicated event. Normal fetal membrane expulsion occurs 1 to 2 hours after delivery of the foal. Fetal membranes are considered retained in the mare if they are not expelled by 3 hours after delivery.1,2 The incidence of retained fetal membranes is not high, with reports of membranes being retained in about 2–10.6% of postpartum mares.1 Disturbances in foaling (dystocia, prolonged gestation, placentitis, Caesarean section, or hydropic conditions) have been known to result in retained fetal membranes although no direct relationship exists between foaling abnormalities and incidence of retained fetal membranes. Breed predilection for retained fetal membranes has been identified. Friesian mares are reported to have a high incidence (>50%) of retained fetal membranes.3 A similar rate of membrane retention (42% over 4 years) was recently reported in Standardbred mares from New Zealand.5

The pathophysiology of membrane retention in mares has not been elucidated; therefore, preventative and/or treatment strategies are largely empirical. It has been postulated that myometrial exhaustion1,5 after dystocia or prolonged delivery and/or inadequate oxytocin release contribute to retained fetal membranes in mares. Varying portions of the membranes may be retained with the non-gravid horn or the allantochorion being the most common portion of the membranes to be retained. Complications of retained fetal membranes can range from none to toxic metritis, septicemia, and laminitis.1,5 Given the life-threatening nature of these conditions, retained fetal membranes are considered an emergent condition requiring immediate treatment.

Treatment for retained fetal membranes centers on prompt removal of the membranes. The traditional methods used to recover retained fetal membranes include administration of oxytocin1,6 (repeated oxytocin injections [10–20 IU IM every 1–2 hours] or the administration of 1.0 to 1.5 IU oxytocin/minute [60–100 IU oxytocin in 1 liter saline IV over 1 hour], administration of calcium borogluconate,7 repeated uterine lavage, the
Burn’s technique [for intact membranes],\textsuperscript{8} and manual removal of membranes). Umbilical artery infusion of bacterial collagenase has also been used to treat retained fetal membranes in mares.\textsuperscript{9} The method selected for removal of retained fetal membranes is dependent on effectiveness, safety, cost, and convenience. Mares housed in hospital conditions or on well-managed farms can be administered drugs frequently such as multiple doses of oxytocin. Further, mares housed under intensive management conditions can be monitored closely for secondary conditions to retained fetal membranes such as toxemia, laminitis, and uterine prolapse. Mares housed in general field conditions without ready access to veterinary care may require more rapid, but safe, management of retained fetal membranes.

This paper describes a novel and practical approach to removing retained fetal membranes using a low-pressure infusion of water into the umbilical vasculature. This procedure causes stretching of the umbilical vessels, interstitial swelling of the membranes, and subsequent detachment of the microvilli, resulting in rapid and gentle separation of the fetal membranes from the endometrium.

2. Materials and Methods

Between 2007 and 2015, 147 broodmares with retained fetal membranes longer than 3 hours were treated by low-pressure infusion water into either the umbilical artery or vein. The mares were all located in the Netherlands and consisted of 47 Friesian mares (32%), six Draft horse mares (4%), 12 ponies (8%), 69 Warmblood mares (47%), and a mix of Arabians, Andalusian horses, Haflingers, or Iceland ponies (9%). All mares had normal parturition, dystocia, abortion, or stillbirth. Mares undergoing Cesarean section were not included in this report.

Initial treatment of mares consisted of a maximum of three oxytocin\textsuperscript{a} (10–20 IU, IM) injections every 1–2 hours. Mares that did not expel fetal membranes after oxytocin administration underwent infusion of the umbilical vasculature. Mares were restrained in stocks or in a box stall. The perineal area of the mare was cleaned prior to the procedure. Immediately before starting the procedure, mares were administered oxytocin\textsuperscript{a} (10–20 IU IM). A foal nasogastric tube or stallion catheter (with a maximum external diameter of 9 mm) was attached to a water hose using a hose connector with flow control valve (Figs. 1 and 2). An umbilical vessel (vein or artery, both are equally effective) was incised longitudinally using a standard scalpel blade (Fig. 3). The catheter, attached to the flow control fitting on the garden hose, was slowly advanced up the vessel under low water pressure until it could not be advanced further (Figs. 4 and 5). The veterinarian manually held the tube in situ and adjusted the water flow depending on the physical reactions of the mare (Fig. 6). In mares showing mild discomfort (shifting weight, mild efforts to kick), fluid flow was discontinued for a short period to allow the mare to relax and progress of placental release to be assessed. If the membranes remained firmly attached, low-pressure fluid infusion was resumed. After 3–5 minutes of intravascular fluid infusion, gentle traction was placed on the neck of the membranes at the mare’s vulva. Traction was continued until the membranes were released (Fig. 7), the mare demonstrated discomfort, or tearing of the membranes was suspected.

3. Results

In the majority of the cases (135/147; 91.8%), full separation and expulsion of the placenta occurred within 5–10 minutes of umbilical vasculature infusion. In four mares (2.7%), incomplete separation and tearing of the membranes occurred. In all four cases, the duration of the retained placenta was...
unknown but likely exceeded 12–24 hours after foaling. It was suspected that prolonged retention resulted in small, unrecognizable tears in the membranes causing subsequent tearing. In the cases of incomplete membrane expulsion, mares underwent repeated uterine lavage and oxytocin therapy over a 3–4-day period. For eight mares (5.4%), the time from onset of vascular infusion to expulsion of the membranes was more than 15 minutes but less than 30 minutes. Eight mares (5.4%) showed mild signs of discomfort, comparable with the discomfort experienced by postpartum mares after oxytocin administration or mild colic. By reducing the water flow infusion rate, the mares experienced less pain. Two mares were treated with additional uterine lavages for 3 days after placental removal by infusion because of intraluminal fluid retention in the uterus. Additional secondary side effects to the procedure (i.e., inverted uterine horn, uterine prolapse, uterine artery hemorrhage, unresolvable colic, metritis) were not noted after this procedure in any mare.

Follow-up pregnancy rates were available for only a small percentage of treated mares. It was recom-
mended that mares should not be bred on foal heat after retained fetal membranes (independent of treatment type). A total of 12 mares (all Friesian) were inseminated after the extraction of fetal membranes using this technique (three mares on prolonged foal heat (ovulation by day 15–16), five mares on the second estrus post-partum and four mares in cycles 2 or 3 post-partum). All mares became pregnant on the first cycle of breeding.

4. Discussion

Regulations dictated by the Royal Netherlands Association of Veterinarians are published for the treatment of retained fetal membranes in the Netherlands.\textsuperscript{10} Under these guidelines, manual removal of fetal membranes is mandated within 6 hours postpartum if traditional methods for membrane removal (i.e., oxytocin administration, uter-
ine lavage) fail. Because of the need to safely remove fetal membranes by 6 hours post-partum, removal using umbilical vasculature infusion was investigated. Traditional manual removal of fetal membranes in mares (manual separation of the chorioallantois from the endometrium combined with twisting of the membranes) is controversial because of the risks of hemorrhage, impaired uterine involution, tearing of the placenta, damage to the endometrium, and uterine prolapse. Similar complications were not noted in mares undergoing umbilical vasculature infusion. The degree with which the membranes were attached, as well as the duration of membrane retention, can affect the outcome of manual membrane removal using any procedure.

In the described procedure the infusion of water into the allantochorion via the umbilical vasculature appears to induce edema and swelling of the tissue (Figs. 7 and 8). It is postulated that the placental microvilli stretch under pressure causing separation of the fetal membranes from the endometrium. The weight of the placenta concurrently increases (but is controlled by support from the veterinarian) and which may also enhance the separation. Fluid may also “leak” into the space between the chorion and endometrium, thus facilitating further membrane separation.

In 2014, four placentas were examined histologically after performing this procedure. In addition, five normal placentas were infused with water or saline and samples for histology were collected be-
before starting the infusion of water, 2 minutes later, and again 5 minutes later. Macroscopic swelling of the placenta was seen and the maximum increase in weight was 100%. Histology showed interstitial edema in all layers of the placenta and distinct washing out of erythrocytes in the vessels in the placenta treated with plain water and saline (Fig. 8). In the water-treated group, additional hydropic degeneration of the epithelial cells, consisting of cell swelling and karyopyknosis could be appreciated when compared with the non-treated placenta, possibly due to the osmotic swelling of these cells (Figs. 8 and 9). Low water pressure seems to induce sufficient edema and cellular degeneration to facilitate detachment of the membranes. This osmotic swelling and cellular degeneration results in a more rapid separation of the chorioallantois from the endometrium (< 10 min), than is reported after hydrolyzing the placental structures by umbilical artery injection of bacterial collagenase (< 6 h)9 and is also more rapid compared with the Burn’s technique,8 which is somewhat similar in its approach. Furthermore, infusion of the umbilical vasculature of retained membranes is not fully reliant on an intact placenta. In those cases that resulted in delayed release of membranes or incomplete expulsion, the inability to induce the interstitial edema of the placental tissues may have occurred due to tissue autolysis (after delayed treatment) and leaking of the membranes. The water-induced degeneration of the epithelium, which probably enhanced the membrane separation, probably did not occur in these cases. It was clear from this group of mares that the umbilical vascular infusion procedure for membrane removal was more successful in mares having retained membranes less than 12 hours in duration.

There are several advantages to using this method for gentle but manual removal of retained fetal membranes in mares. A primary advantage is safety for both the mare and the veterinarian. Furthermore, the method is hygienic, noninvasive, rapid, and effective. It is very useful in small ponies or Miniature horse mares where management through uterine lavage or manual removal often leads to discomfort and resistance of the mare. Few mares undergoing this procedure showed significant signs of discomfort. In mares that became uncomfortable, reduction in water pressure quickly resolved signs of discomfort. A second advantage to this procedure is rapid removal of membranes in most cases. For veterinarians traveling long distances to treat mares with retained fetal membranes, prompt resolution of the condition can be essential to the wellbeing of the mare given that return trips can be challenging. In the reported group of mares, little subsequent treatment was necessary once the membranes were removed. Adjunct therapy including large-volume uterine lavage, antimicrobial and anti-inflammatory therapy, and oxytocin would be beneficial in some cases.

In summary, medical management of retained fetal membranes using oxytocin is the preferred first treatment method in most mares. However, the results of this report suggest that the treatment of retained placenta in 147 mares by infusion of water into the umbilical vessels is an effective and practical tool for the equine veterinarian. Little equipment is required other than a stallion catheter (or foal nasogastric tube), hose adapter, and garden hose. The procedure is easy to perform and results in expulsion of membranes in less than 10 minutes in most cases. Mares tolerate the procedure well, making it safe for both the mare and the veterinarian. The procedure was atraumatic and effective when performed in membranes retained for less than 12 hours in this group of mares.

Acknowledgments

Declaration of Ethics

The Authors declare that they have adhered to the Principles of Veterinary Medical Ethics of the AVMA.
Conflict of Interest
The Authors declare no conflicts of interest.

References and Footnote

*Oxytocin-jec REGNL 3030; Dopharma Research, BV, Raamsdonksveer, The Netherlands.*
How to Process Stallion Epididymal Sperm in Your Practice

Robert J. Stawicki, MS, DVM, DACT

Although many practitioners have shied away from processing stallion epididymal sperm as an in-hospital procedure, the possibility of achieving better fertility rates than previously thought possible with this sperm will likely entice a larger portion of your clientele to invest in this application. In addition, the minimal amount of equipment needed for performing this technique will make it a revenue-generating procedure for your practice. Author’s address: Department of Large Animal Medicine, University of Georgia, College of Veterinary Medicine, Athens, GA 30602; e-mail: rstawick@uga.edu. © 2015 AAEP.

1. Introduction

Pregnancies resulting from artificial insemination with cryopreserved epididymal stallion sperm were first reported in 1957, and the technique is now used in the clinical setting as a means of preserving the genetics of valuable stallions that have died unexpectedly, require castration of one or both testicles, or are otherwise affected by a condition that renders semen collection difficult or impossible. Since 1957, refinement of the technique for harvesting epididymal sperm has resulted in a significant increase in the number of sperm recovered from each epididymis, and consequently an increase in the number of insemination doses banked. This has made the process more cost effective for the stallion owner. In addition, it has been shown that epididymal sperm can be stored within the epididymis for up to 24 hours at 5°C before harvest and cryopreservation without a significant loss in post-thaw motility, which makes it more convenient for the practitioner.

Despite these advances in technique, however, difficulties associated with shipment and the high price tag that comes with the processing and cryopreservation in the referral setting has restricted its use for only the most valuable of proven breeding studs. What’s more, this procedure has recently fallen under scrutiny from the standpoint of fertility due to a wide range of published pregnancy rates in mares bred with cryopreserved stallion epididymal sperm.

The ability for veterinarians to process and cryopreserve epididymal sperm from stallions in house would likely make this service available for a larger portion of the practice population. In practices where ejaculated semen is already being collected and cryopreserved, there is very little additional equipment and subsequent monetary investment necessary for processing sperm from the epididymis. As such, successful cryopreservation of epididymal sperm is more directly related to the practitioner’s knowledge of the most current techniques and principles. One advantage that ambulatory equine veterinarians have over most referral practices is the easy access to testicles from routine castrations. The ability to practice this technique on discarded
testicles provides a gauge for individual results, and allows for a comfort level with the procedure before offering it to client-owned animals. Once a practitioner becomes comfortable with the technique, it is easy to see how bringing this procedure into the clinical setting reduces the cost not only from the standpoint of shipping, but also by bringing the pricing scale down from the specialist level.

The focus of this discussion is to outline the processes involved in obtainment and cryopreservation of epididymal sperm from the stallion. It will focus on methods for castration, storage of testicles prior to processing, techniques for processing of epididymides, sperm preparation, and sperm cryopreservation. The ultimate goal is to provide practitioners with the most current knowledge in this field as a first step toward being able to offer this service to their own clients in the clinical setting.

2. Materials and Methods

Candidates for Epididymal Sperm Recovery

In most cases, the obtainment of epididymal sperm for cryopreservation is considered for genetically valuable stallions that are suffering from a terminal medical condition. Regardless of the value of the stallion, it is important for veterinarians to consider the effect of prior medical issues on the potential quality of sperm that will be obtained from the epididymides. Stallions that have had prolonged periods of elevated body temperature, have been down for long periods of time, or are otherwise showing signs of long-term systemic compromise are less likely to have good-quality sperm that will result in a high number of progressively motile cells in the post-thaw sample. Additionally, there is a low likelihood of acquiring good-quality epididymal sperm from young stallions that are not sexually mature. Therefore, this procedure is best suited for sexually mature stallions suffering from acute illness or injury of less than 1–2 days’ duration.

Castration

Once the decision has been made to euthanize a terminally ill stallion and attempt to recover epididymal sperm, the next step will be obtainment of the testicles. This may be done by routine castration techniques; however, it is recommended that the stallion be castrated prior to administration of a lethal dose of thiobarbiturate. As such, a standard protocol of premedication with an alpha-2 agent for sedation, and induction of anesthesia with diazepam and ketamine is an acceptable protocol to facilitate castration. The injection of lidocaine into the testicle should be avoided to prevent laceration of the epididymal ducts or vas deferens within the spermatic cords. If additional relaxation is necessary to complete the castration, this should be accomplished by administration of additional anesthetic agents. Once anesthetized, an open castration should be performed to allow for optimal visualization of the spermatic cords. Care should be taken to place emasculators as high up on the cord as possible, as this will yield the largest length of vas deferens to be removed with the castrated testicle. Given that the vas deferens is likely to contain the most mature sperm leaving the epididymal tail, placement of a clamp across the cord just distal to the emasculator before it is engaged will prevent leakage of this sperm once the testicle is removed. After removal of both testicles, the stallion can be euthanized by humane techniques prior to recovery from anesthesia.

Preparation of Castrated Testicles

Once removed, the castrated testicles should be prepared immediately for processing. If castration was performed in the field, the epididymides should be prepared as soon as possible to prevent temperature fluctuations during transit. Once this technique is learned, both epididymides can be removed from the testes immediately after castration in the field, and placed in a single cooled semen transport device for further processing in the laboratory. The testicle and associated structures should be laid out on a clean, flat surface, and the ductus deferens must be identified within the spermatic cord where it was clamped and incised at its distal aspect. A circumferential ligature should be placed just proximal to the clamp to prevent leakage of sperm, after which the clamp can be removed. The ductus is then easily separated from the associated structures within the spermatic cord. The parietal vaginal tunic may be excised where it attaches at the ligament of the tail of the epididymis and discarded. Keep in mind that this tissue is directly attached to the epididymal tail; thus, great care must be taken not to cut into the epididymal ducts when removing the parietal vaginal tunic. Next, the epididymal tail and body must be freed from the testicle by incision of the proper ligament of the testis along its length between the epididymis and testicle. At this point, a circumferential ligature can be placed around the body of the epididymis just proximal to the epididymal tail to isolate the sperm between that ligature and the one at the distal end of the ductus. The epididymis is then transected through the body just proximal to the ligature, and the epididymal tail with attached ductus is placed in a clean plastic bag containing 1 to 2 mL of either sterile lactated Ringer’s solution or a skim milk and glucose-based semen extender to prevent desiccation. The same procedure is repeated for the second testicle.

Obtainment of Epididymal Sperm

Once in the laboratory, the first epididymis may be laid flat on a clean surface. The author prefers to use disposable bench paper to avoid potential contamination with spermicidal agents. The tissues may be kept moist by periodic irrigation with a room-temperature, skim milk and glucose-based se-
men extender. The visceral vaginal tunic covering over the surface of the epididymal tail must be removed to allow for expansion of the ducts during the flushing process. If this tunic is not removed, it will require increased pressure to force extender through the epididymal duct, which is likely to rupture the duct prior to complete perfusion and result in lower numbers of sperm recovered. The tunic may be grasped with Brown-Adson tissue forceps in an area of webbing between the epididymal tail and ductus deferens, and an initial nick is made using fine-pointed iris scissors. From this nick, the point of the iris scissors is inserted and the tunic is bluntly dissected away from the epididymis by spreading the scissors in the tissue plane parallel to the surface of the epididymal tail. This separation should be extended from this site to include the entire epididymal tail until the tunic is completely dissected away. The same process can be repeated with the second epididymis. The tunic over the ductus deferens may be left in place, as this does not seem to restrict expansion in this region during perfusion.

The distal end of the ductus deferens is then grasped between the thumb and forefinger, and the contents are squeezed proximally by 2 to 3 cm and held in place by manually pinching off the lumen. The ligature can then be cut off of the distal end of the ductus, and a semi-rigid, 14-cm polypropylene Tom Cat catheter can be inserted into the lumen at the incised end. Once inserted, the pinch can be released, and the end of the ductus can be pushed up over the length of the catheter until it is cannulated by 4 to 5 cm. A circumferential ligature should then be placed around the catheter and ductus approximately 1 to 2 cm from the catheter tip, and tied securely to prevent the ductus from slipping off the catheter during perfusion. A plastic syringe filled with 40 mL of room-temperature skim milk and glucose-based semen extender is attached to the distal end of the Tom Cat catheter. The proximal aspect of the epididymal duct must then be incised to allow for outflow of sperm. This is accomplished using iris scissors and cutting away the portion of epididymal body that was left attached to the tail. This cut should be made as close as possible to the junction of epididymal body and tail. The epididymal tail is then suspended within a 50-mL polypropylene conical-bottomed centrifuge tube and gradual pressure is applied to the syringe plunger to begin retrograde displacement of sperm by semen extender. It may take 4 to 5 minutes of gradual pressure applied to the syringe plunger before the caseous epididymal contents are seen coming from the proximal end of the epididymal tail, and another 4 to 5 minutes to perfuse all 40 mL of semen extender. Perfusion of the epididymal tail may be performed with the entire 40 mL of extender, or discontinued when the efflux from the proximal end of the tail changes from caseous back to the normal color and viscosity of the semen extender. Once all of the extender has been flushed through the duct, the surface of the epididymis should be rinsed into the tube with an additional 4 to 5 mL of semen extender. The same process can be repeated with the second epididymis.

If the epididymal duct ruptures during this process, this is likely the result of excessive forces placed on the syringe plunger in an attempt to expedite the flushing process. This will appear as a large bubble of semen extender in the superficial tissue plane of the epididymal tail or ductus. If this is observed, the only option at this point is to place the epididymis in a petri dish, cover it with skim milk and glucose-based semen extender, then make 20–30 cuts into the epididymis using a sharp scalpel blade. The minced epididymal tail and extender should be left for 30–60 minutes to allow the sperm to swim out of the ducts. After this time, the tissue is manually removed and rinsed back into the petri dish, and the remainder of this procedure followed. This will likely result in smaller sperm numbers recovered, as well as blood and tissue contamination of the final cryopreserved product.

Processing of Epididymal Sperm

Once the sperm has been flushed from the epididymal tail and resuspended in semen extender, do not be alarmed if the sperm motility seems poor upon initial microscopic evaluation. It is not uncommon for epididymal sperm to dramatically increase in motility after centrifugation, removal of the supernatant, and resuspension of the sperm pellet in cryopreservation media.

The tube(s) containing the epididymal sperm and extender is underlain with a total of 1 mL of room-temperature iodixanol-density-gradient medium. This is accomplished by pulling the media up into a 3-mL plastic syringe, attaching a 14-cm, polypropylene Tom Cat catheter and advancing the tip of the catheter to the bottom of the tube. The media is injected slowly to form a layer below the sperm and extender, which will protect the cells from compression during high-speed centrifugation. The tube should then be capped and centrifuged at 1000×g for 20 minutes at ambient temperature. Immediately following centrifugation, the supernatant should be aspirated off to within 2 to 3 mL of the sperm pellet. During aspiration of the supernatant, care should be taken not to inadvertently disrupt the pellet because this will result in loss of sperm into the supernatant, which will ultimately be discarded. Once the supernatant is removed, the polypropylene Tom Cat catheter is again inserted, now through the sperm pellet to the bottom of the conical tube, and the iodixanol is aspirated using a 3-mL syringe to remove as much media as possible without disrupting the sperm layer.

At this point, the pellet can be partially resuspended with the desired cryopreservation media. An evaluation of sperm morphology should then be performed to determine whether there are an acceptable number of normal cells to proceed with.
cryopreservation. Keep in mind that many sperm in the epididymis will still have cytoplasmic droplets, and in the author’s experience, pregnancies have been obtained using frozen-thawed epididymal sperm with high numbers of both proximal and distal cytoplasmic droplets. Once sperm morphology is deemed acceptable, the sample can be partially extended with cryopreservation media. Given that the total number of sperm recovered is not known at this point, only a small volume of media should be added to avoid overextending the sperm to a lower concentration than desired. The tube should be gently agitated until the fluid contents look uniform, and the sperm concentration should then be determined. The additional volume of cryopreservation media can then be calculated to reach the desired concentration, after which the epididymal sperm should be evaluated for motility. A dramatic improvement in sperm motility over that seen in the pre-centrifugation sample is often realized at this point, and it is not uncommon to find pre-freeze progressive sperm motilities of greater than 70%. If sperm progressive motility is less than 30% at this time, it is not likely that sperm motility in the post-thaw sample will meet industry standards set forth for frozen ejaculated semen. The technique for loading and freezing straws is identical to that for cryopreservation of ejaculated semen; therefore, package directions for the specific cryopreservation media should be followed.

3. Results
To date, the author has performed this procedure using the aforementioned technique on seven stallions suffering from terminal illness, and on three stallions for which the owners wished to preserve epididymal sperm after castration to supplement the bank of ejaculated sperm that had already been frozen. In addition, the author has processed epididymal sperm from 15 sets of stallion testicles as part of a study to evaluate the fertility of cryopreserved epididymal sperm.

For the stallions suffering from terminal illness, five of the seven cases resulted in the obtainment of cryopreserved epididymal sperm with a progressive motility greater than the minimum industry standard of 30%. The stallions in the remaining two cases were suffering from conditions that rendered them unable to stand for more than 4 days prior to euthanasia, and it was therefore not surprising that the epididymal sperm exhibited a progressive motility of less than 30% in the pre-freeze sample and was not worth moving forward to the cryopreservation process.

Of the 15 sets of stallion testicles processed as part of our study, 13 yielded cryopreserved epididymal sperm with acceptable post-thaw motility. The remaining two sets of testicles contained insufficient sperm numbers to provide the necessary number of breeding doses, and were therefore not processed further for freezing. Although these stallions fit the study requirement for age, the low sperm numbers were thought to be the result of delayed sexual maturity in these animals.

With regard to fertility of the epididymal sperm, breeding results from only one of the clinical cases has been reported thus far, and a pregnancy was achieved on the first breeding attempt with that sperm. Of the 13 sets of testicles processed as part of our study using standard techniques, seven of the 13 mares achieved pregnancy. Although most of the stallion epididymal sperm that has been processed in our clinical labs does not have fertility data to date, the results achieved as part of our project make it very clear that successful pregnancy can be achieved employing the technique previously outlined.

4. Discussion
Despite the ability to successfully cryopreserve stallion epididymal sperm, the assumption that it is less fertile than ejaculated sperm has been a major impediment to its widespread use in clinical practice. Looking back in the literature, only four studies have reported on pregnancy rates in mares bred with cryopreserved stallion epididymal sperm. The first two employed a variety of different sperm-processing methods, breeding dosages, and insemination techniques, and these studies reported pregnancy rates ranging from 7 to 30%.

These pregnancy rates are considerably lower than those typically reported for cryopreserved ejaculated sperm. The remaining two studies employed a recently developed stallion freezing extender that contains soy lecithin and dimethylformamide, among other proprietary ingredients, to process epididymal sperm. These studies employed the same sperm processing and insemination techniques, and reported a substantial increase in post-thaw per-cycle pregnancy rates ranging from 61 to 92%

These most recent pregnancy results equal or exceed those achieved with cryopreserved ejaculated sperm, which will likely make cryopreservation of epididymal sperm a much more clinically appealing technique.

Factors contributing to the higher pregnancy rates in these studies remain to be delineated, but could be attributed to a variety of factors including differences in study protocol, insemination dose, extender type, and inherent fertility of the individual stallion. The author is currently attempting to further validate the results of the two high-pregnancy-rate studies using cryopreserved stallion epididymal sperm. Our study employed the same techniques and commercially available freezing extender, and our findings seem to be in keeping with those studies. Given that the freezing extender used in this study has been shown to result in significant improvements in post-thaw epididymal sperm measures when compared with other selected extenders, it is tempting to speculate that this extender may be responsible for the improved pregnancy
rates, and therefore may offer advantages over other extenders for the cryopreservation of epididymal sperm. Although further research is necessary to validate this theory, as a practitioner offering this service, it is comforting to know that clients who invest the money to preserve their stallions’ genetics by cryopreservation of epididymal sperm may be able to expect higher pregnancy rates than previously thought possible.

Acknowledgments

Declaration of Ethics

The Author declares that he has adhered to the Principles of Veterinary Medical Ethics of the AVMA.

Conflict of Interest

The Author declares no conflicts of interest.

References and Footnotes


Equitainer, Hamilton Research, Inc., Ipswich, MD 01938.
Kendall sterile Tom Cat Cathether, Medtronic, Minneapolis, MN 55422.
50 ml polypropylene centrifuge tube, Corning, Inc., Corning NY 14830.
OptiPrep, Sigma-Aldrich, St. Louis, MO 63103.
Botu-crio, Botupharma Biotechnology, Botucatu, Brazil.
Comprehensive Review on Equine Placentitis

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Placentitis is estimated to affect 3 to 5% of pregnancies. In recent years substantial developments have been made on equine placentitis and a comprehensive review is timely. Etiopathogenesis, epidemiology, diagnosis and treatment, and research models for the four morphologic types of placentitis (i.e., ascending, nocardioform, diffuse, and multifocal) are discussed. Authors' addresses: Department of Veterinary Clinical Medicine, College of Veterinary Medicine, University of Illinois Urbana–Champaign, Urbana, IL 61802 (Canisso); Maxwell H. Gluck Equine Research Center (Ball, Squires, Troedsson) and Veterinary Diagnostic Laboratory (Erol), Department of Veterinary Science, University of Kentucky, Lexington, KY 40546; e-mail: canisso@illinois.edu. *Corresponding and presenting author. © 2015 AAEP.

1. Introduction

Placentitis, an important cause of late-term pregnancy wastage, is estimated to affect 3 to 5% of pregnancies in Thoroughbred mares.1,2 Late-term pregnancy wastage generates several million dollars of losses to the equine industry each year due to the lost expense of producing a pregnancy (e.g., stud fees, semen transport, feed, vaccinations, veterinary services, etc.). In addition, late-term pregnancy loss may cause emotional stress to the owner with high expectations for the foal from a particular mating. Therefore, means to manage and to prevent pregnancy loss are warranted.

Placental pathology/insufficiency was identified in a large retrospective study as being responsible for greater than 60% of pregnancy losses due to abortion, stillbirth, and neonatal death (up to 24 h post-delivery) in central Kentucky.3 Two additional retrospective studies conducted several years apart by the same referral diagnostic laboratory supported the finding that placentitis is the most common cause of late pregnancy loss in mares.4,5 Outside North America, placentitis has been identified as an important cause of pregnancy loss in mares in Brazil,6,7 France,8 and Australia.9 However, in the United Kingdom umbilical cord pathology (e.g., torsion and entrapments) was the leading cause of pregnancy loss in submissions received by one diagnostic laboratory.10 Differences between the United Kingdom and other parts of the world may be accounted for by the methodology applied to diagnose placentitis, type of placentas presenting lesions submitted for pathologic examination, and the presence of certain microorganisms (e.g., nocardioform organisms), as well as potential differences in true incidence.

In recent years, substantial advancements have been made regarding our understanding of equine...
placentitis through controlled and field studies carried out in different parts of the world. Reviews covering equine placentitis have been published, with one recent review emphasizing the immunologic aspects of the preterm delivery in mares. The following review aims to present a comprehensive review of classical and recent findings regarding equine placentitis.

### 2. Etiopathogenesis and Epidemiology

Several infectious agents have been recovered from placentitis cases; bacteria are responsible for the large majority of cases and fungi for the minority (Table 1). Bacterial agents commonly associated with placentitis include *Streptococcus equi* subspecies *zooepidemicus*, *Escherichia coli*, *Streptococcus equisimilis*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Leptospirosis*, *Crossiella equi*, and *Amycolatopsis* species *Amycolatopsis kentuckyensis*, *Amycolatopsis lexingtonensis*, and *Amycolatopsis pretoriensis* (Table 1). Fungi associated with equine placentitis primarily include *Aspergillus* spp. and *Candida albicans*. Mixed bacterial and fungal infections have been reported in ascending, however, less commonly. Anecdotally, it seems that some cases start with bacterial placentitis and then fungal secondary infections follow the primary bacterial infection.

The incidence of leptospiral infections causing abortions is highly variable across the years in central Kentucky. In North America, the serovar *Pomona* type *Kennewicki* is the most important type associated with Leptospiral abortions in mares; however, in other parts of the world, the serovars *Bratislava*, *Grippotyphosa*, *Copenhageni*, *Autumnalis*, *Hebdomadis*, *Arborea*, and *Icterohaemorrhagiae* are more commonly associated with abortion in mares. Environmental conditions such as precipitation and flooding are alleged to be important in the dissemination of the bacteria from wildlife to horses, which are considered incidental hosts. Wild animals including raccoons, white-tailed deer, striped skunks, opossums, and foxes are known to harbor the serovar *Pomona* and are thought to serve as a source of infection for pregnant mares.

Based upon morphologic lesions and suggested pathogenesis, there are four types of equine placentitis: ascending, focal mucoid (nocardiform), diffuse (hematogenous), and multifocal (Fig. 1). Ascending placentitis, the most frequent type of placentitis, is commonly associated with beta hemolytic streptococci and coliforms (Table 1). Nocardioform placentitis is commonly associated with Gram-positive branching bacilli, *Crossiella equi* and *Amycolatopsis* species (Fig. 2). Recently, two new species of *Streptomyces* (*Streptomyces atriruber* and *Streptomyces silaceus*) have been isolated in nocardioform placenta cases. A multifocal distribution pattern is rare but is sporadically seen in isolated cases in association with fungi or bacteria. Diffuse placentitis is diagnosed in isolated cases in association with bacteria or fungi, or during outbreaks in association with *Leptospirosis* (Fig. 1D).

Nocardioform placentitis has periodically been the predominant type of equine placentitis in central Kentucky with the 2010–2011 foal crop season in central Kentucky experiencing an exceptionally high number of nocardioform cases (Fig. 3). Conjecturally, *Crossiella equi* and *Amycolatopsis* spp. were responsible for 85% of the cases submitted during that period, with lesions compatible with nocardioform placentitis present without an identified isolate in the remainder of cases. Based on these cases it seems that *Crossiella equi* infections may be more likely to result in abortion, whereas infections with other type actinomycetes tend to result in live but premature foals. It is worth noting that, to date, nocardioform organisms have only been isolated from clinical placenta cases and not from the environment.

Bacteria causing nocardioform placentitis and other actinomycetes that affect horses differ in their propensity to infect not only the fetal membranes but also the offspring in utero. For instance, mares affected by *Cellulosimicrobium cellulans* in Kentucky present mucoid placentitis associated with severe fetal lesions. Similarly, the actinomycetes *Cellulosimicrobium sp* and *Cellulomonas sp*, along with other nonspecific bacterial species, have been associated with mucoid placentitis and fetal lesions in equine amnionitis and fetal loss, reported to occur with ingestion of processory needler caterpillars in Australia. Conversely, actinomycetes causing nocardioform placentitis do not infect the fetus in utero as reported in other types of mucoid placentitis; the bacterial infection in nocardioform placentitis is restricted to the chorioallantois.

Infectious agents associated with ascending placentitis seems to enter the uterus via the vagina/cervix and then colonize the caudal pole of the chorioallantois (Fig. 4). Once infection is established, clinical signs such as premature udder develop.

### Table 1. Ten Most Commonly Isolated Microorganisms in 236 Equine Placentitis Cases in Central Kentucky

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Streptococcus equi subspecies</em></td>
<td></td>
</tr>
<tr>
<td><em>zooepidemicus</em></td>
<td>39</td>
</tr>
<tr>
<td><em>Leptospira</em> ssp.</td>
<td>37</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>33</td>
</tr>
<tr>
<td>Fungi</td>
<td>16</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>11</td>
</tr>
<tr>
<td><em>Streptococcus equisimilis</em></td>
<td>11</td>
</tr>
<tr>
<td><em>Enterobacter agglomerans</em></td>
<td>5</td>
</tr>
<tr>
<td><em>Klebsiella pneumonia</em></td>
<td>2</td>
</tr>
<tr>
<td><em>α-hemolytic Streptococcus</em></td>
<td>5</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>2</td>
</tr>
</tbody>
</table>

Adapted from Hong et al.
Development (± streaming of pre-foaling mammary gland secretions), and vulvar discharge may become apparent. Normal mammary gland development begins approximately 4 weeks (2–6 wk) prior to foaling, with more pronounced development in the week immediately preceding parturition. Premature mammary gland development is not a pathognomonic sign of placentitis, but rather a nonspecific sign of pending late-term pregnancy loss, which can be associated with a variety of other conditions including twin pregnancy, umbilical cord torsion, and idiopathic/unspecific imminent abortion. Premature mammary gland development seems to occur concomitantly with increasing progestin concentrations (progestins are derived from the fetoplacental unit). Enlargement of the mammary gland may be observed in mares suffering with hydrops, pre-pubic tendon rupture (in which mammary glands may be filled with serosanguinous or bloody material and may be cranially displaced), and normal pre-foaling ventral edema. In mares with placentitis, premature mammary development might not be present until the placental lesions are well advanced (e.g., placental separation; Fig. 5). Vulvar discharge is highly variable as the discharge can be smeared by and accumulate under the tail and easily be missed, without close and frequent monitoring. If early treatment is not instituted the lesions will progress, and the infectious agent(s) may gain access to the fetus (Fig. 4). Anecdotally, it has long been suggested that infectious agents gain access through the fetus by infecting the allantoic and amniotic fluids. A large group of mares with experimentally induced placentitis had negative bacterial culture of the allantoic fluid despite extensive inflammatory changes in the fetus and chorioallantois and positive bacterial culture, suggesting that contamination of the fetal fluids are not a primary route of fetal infection.

Infection of the chorioallantois is associated with inflammation, which results in increased prostaglandin concentration in the fetal fluids and membranes with increased expression of the proinflammatory cytokines IL-6 and IL-8. Lesions in

Fig. 1. Representative photographs of the four morphologic types of equine placentitis. A, Ascending placentitis (focal extensive lesion in the chorionic surface of the chorioallantois); arrows indicate extensive funisitis and the asterisk indicates the chorionic surface affected by placentitis; #, amnionitis. B, Nocardioform (focal mucoid lesion in the chorionic surface) **, extensive chorionic surface covered by mucus. C, Hematogenous (diffuse erosive lesions present in the allantoic surface of the chorioallantois). D, Multifocal, in the allantoic surface view, multifoci areas of infection can be observed in this placenta. Courtesy of Dr. Neil Williams (B) and Dr. Rafaela De Negri (C).
Mares with ascending placentitis are not restricted to the chorioallantois and can be associated with both funisitis (i.e., inflammation of the umbilical cord) and amnionitis. Ultimately, prostaglandin release might lead to abortion or delivery of a premature foal (Fig. 4). Chronic placentitis leads to placental insufficiency, which may result in intrauterine fetal growth retardation and delivery of nonviable or weak foals. Foals born alive from mares with placentitis may be septic and may demand intensive veterinary critical care. In addition, despite treatment pre-partum and post-delivery there is no guarantee that the foal will be athletically sound. A recent retrospective study conducted in central Kentucky concluded that Thoroughbred horses delivered from mares suspected of having and treated for placentitis were not different from their cohort related to athletic outcome measured as number of starts, wins, places, shows, and amounts earned as 2-year-olds. This study illustrates that mares suffering with placentitis may produce a successful athlete. Intuitively, early diagnosis and appropriate treatment are likely to improve the odds of delivering a live and useful foal.

Placentitis accelerates the hypothalamic-pituitary-adrenal axis maturation, which is in contrast with premature labor associated with other causes. Mares with placentitis may deliver live and viable foals by 310 days of gestation, whereas dead or unviable foals are more commonly delivered from pregnancies of comparable gestational age of mares without placentitis that are interrupted early. The mechanisms associated with survival of preterm foals from mares with placentitis are unclear, but it has been suggested that it is related to a premature increase in fetal adrenocorticotrophic hormone (ACTH) and cortisol. Different from other species, final maturation of the equine fetal hypothalamic-pituitary-adrenal axis occurs in the few days immediately preceding normal parturition and continues during the first several weeks after delivery.

Fig. 2. Morphologic aspects of nocardioform microorganisms in culture and photomicrographs of Gram-stained microorganisms. A, Amycolatopsis ssp. Three species of Amycolatopsis have been identified in association with nocardioform placentitis: A. kentuckyensis, A. lexingtonensis, and A. pretoriensis. B, Gram staining of Amycolatopsis ssp, note the Gram + and branching aspect of the organisms. C, Crossiella equi culture morphology, note the donut shape of each colony. D, Gram staining of Crossiella equi, note the Gram + and branching aspect of the organisms.
Foals born prematurely have lower cortisol concentrations (< 3 µg/dL) within 2 hours post-delivery compared with normal full-term foals (12–14 µg/dL). In addition, premature foals showed remarkably higher ACTH concentrations in comparison with normal full-term foals, 650 pg/mL vs 300 pg/mL 30 minutes post-delivery.

In mares with placentitis, treatment strategies to prevent premature delivery should be instituted, prolonging fetal time in utero to allow greater fetal maturation and improve the possibility of neonatal survival. Conflicting opinions exist as to whether mares suffering with placentitis close to 340 days' gestation should have parturition in-
duced to minimize the fetal exposure to bacteria and toxins and inflammatory products; however, longer fetal residence in the uterus is more likely to support fetal maturation.

Nocardioform placentitis was first recognized approximately 20 years ago in central Kentucky, however, little is known regarding the pathogenesis of this disease. Lesions in this type of placentitis are located in the uterine body and base of the uterine horns with no association with the cervical star. As a consequence, vulvar discharge is not commonly observed in the mare with nocardioform placentitis unless the mare is about to abort. Based upon submissions to a referral laboratory in central Kentucky, Crossiella equi seems to be more pathogenic than Amycolatopsis species, as more mares infected with Crossiella equi tend to abort, whereas more mares infected with Amycolatopsis ssp tend to deliver live, premature foals. Because nocardioform infection is constrained to the chorioallantois (i.e., amnion, umbilical cord and fetus are not affected), chorioamnionitis and induced placental insufficiency (i.e., in-uterine growth restriction) are thought to be responsible for the fetal maturation. At present, the lack of an experimental model to assess changes induced by nocardioform placentitis preclude any further conclusion. Unfortunately, in a recent study involving a large group of mares we were unable to induce nocardioform placentitis in mares receiving the Crossiella equi intrauterine during the periovulatory period. In addition, intravenous, oral, and intrapharyngeal administration of Crossiella equi did not result in clinical, microbiological, or pathological evidence of nocardioform placentitis. These findings demonstrate that this type of placentitis is not induced simply by the presence of nocardioform microorganisms.

Little is known about the pathogenesis of diffuse or multifocal placentitis. It has been suggested that the microorganisms reach the uterus hematogenously; however, there has been speculation that multifocal placentitis is the result of a dormant chronic infection of the uterus that became active later in pregnancy. In multifocal placentitis, lesions can be confined to the chorion and may also be present on the allantoic surface. Leptospira, the most important cause of hematogenous (or diffuse) placentitis, is believed to gain access to the uterus through the systemic circulation. Leptospiral lesions include amnionitis, funisitis, and acute inflammatory changes in the chorioallantois. Interestingly, leptospiral abortions are associated with high immunoglobulin titers in maternal and fetal plasma, different than other types of placentitis. Leptospira is believed to cause abortion directly by infection of the fetoplacental unit, but it seems that systemic inflammation in association with hyperthermia and prostaglandin production may contribute to the pregnancy loss.

3. Experimental Model for Bacterial Placentitis in Mares

Experimental models for abortigenic diseases in domestic animals vary according to the animal species, infectious agent, and natural route of infection, as well as the ability of the organism to survive in the environment. Different routes of infection have been used to induce disease experimentally in various species of domestic animals; some models involve a vector that is important in the natural infection (e.g., Foot Hill Abortion in cattle), in other models, macerated tissues obtained from aborted materials are directly administered to susceptible animals. In the present review, only equine models will be briefly reviewed. To date there is only one effective model to experimentally induce bacterial placentitis in mares. The model was initially described by Dr. Michelle LeBlanc’s group at the University of Florida. The model involves the inoculation of a pathogenic strain of Streptococcus equi subspecies zooepidemicus (10^7 to 10^9 cfu) into the cervix of mares during the third trimester of pregnancy. Since the original description of the model, it has been used by other researchers with very predictable outcomes. One of the limitations of this research model is that a large inoculum is administered to mares intracervically, with a resulting acute ascending placentitis and rapid abortion. This clinical presentation seems much more rapid than seen in spontaneous placentitis, which tends to be insidious and chronic. Therefore, studies to address diagnostic markers and treatment strategies should be interpreted with caution regarding the differences in clinical presentation of spontaneous and experimental placentitis. Recently, our group modified the original model to induce placentitis in a method that seems to be less...
acute (Fig. 6).38 If present, a small amount of the cervical plug is removed with a gloved hand and then the bacterium inoculum is deposited midway intracervically with the usage of a semi-flexible artificial insemination pipet, the inoculum is contained in a 0.5-mL straw and deposited with the use of a stylet. We also reduced the inoculating dose to a 5 million colony-forming unit.38 The use of a small volume reduces spilling in the vagina and probable vaginitis. Mares in our study aborted on average approximately 10 days post-inoculation.38

4. Diagnosis

Diagnosis of placentitis is based on ultrasonography and clinical signs (i.e., premature lactation and/or vulvar discharge).9,11,12,39 Transrectal ultrasonography of the caudal placental pole is commonly used to diagnose ascending placentitis1 (Fig. 5). While performing this technique, the operator should make a slight off midline alignment of the transducer with the cervix and ventral aspect of the caudal pole to assure that a branch of uterine artery/cranial vaginal artery is imaged. Three measurements are usually taken of the combined thickness of uterus and placenta (CTUP) and the values can be compared with reported normal ranges.36,40 Increased CTUP associated with clinical signs and areas of placental separation are suggestive of ascending placentitis.12 Measures of CTUP outside the normal published ranges does not necessarily indicate placentitis,2 but rather normal variation, and at times artefacts associated with improper use of the technique. Interestingly, there seems to be small variations in normal CTUP between studies and breeds.1,36,40–42 In a Dutch study, parity was reported to have effects on values of CTUP, i.e., nulliparous mares presented higher values compared with multiparous mares.41 Despite the widespread use of transrectal ultrasonography to diagnose ascending placentitis, data on sensitivity and specificity are scant.

Doppler ultrasonography has become a popular tool in equine practice; however, its use to diagnose placentitis in mares remains questionable. A recent work addressed the hypothesis of whether arterial blood flow changes before other clinical and ultrasonographic changes associated with placentitis are noted; however, experimentally induced placentitis in pony mares did not change the artery blood flow.42 A recent work carried out with Warmblood mares in Germany suggested that the total artery blood flow was reduced preceding idiopathic abortion in four mares.43 These authors also suggested that three other mares suffering spontaneous placentitis had changes in blood flow. However, it is unclear whether comparisons were made across groups of healthy mares and mares suffering with placentitis. Results from this report are not unequivocal whether changes in blood flow precede other ultrasonographic changes (e.g., increased CTUP, placental separation) and clinical signs commonly seen in placentitis mares (e.g.,
premature mammary gland development and vulvar discharge).

Transabdominal ultrasonographic examination of the fetus and placenta has been used as a tool to assess fetal and placental health. However, given that a very limited area of the uterus is accurately visualized by transabdominal ultrasonographic examination, the lack of apparent lesions in the chorioallantois does not exclude the possibility of disease. Areas of placental separation with hyper-echoic exudate between the chorioallantois and the uterus are suggestive of nocardioform placentitis. Presumptive diagnosis of nocardioform placentitis can be challenging, especially if only a small area is affected.

Fetal heart rate monitoring and ultrasonographic character of the fetal fluids can also be evaluated by transrectal and transabdominal ultrasonography. Reduced or increased fetal heart rates (normal heart rate, ~80 beats/min during late pregnancy) are associated with poor pregnancy outcome. Increased echogenicity of the fetal fluids are associated with imminent abortion. It is worth noting that the echogenicity of the amniotic fluid increases with gestational age, probably related to the vernix being released by the fetus. However, fetal stress (e.g., colic surgery, fetal hypoxia by different reasons) may result in fetal diarrhea, and echogenicity of the amniotic fluid may increase remarkably as a consequence of solid particles floating in the amniotic fluid. The normal amnion appears as a thin membrane surrounding the fetus; amnionitis can be observed by ultrasonography as a thickened and irregular amniotic membrane.

Cytology and culture swabs obtained from the external cervical os in pregnant mares can be beneficial in cases of ascending placental infection, when the cervix is open and discharge is present (Fig. 7). Samples for culture and cytology can be collected with the use of a double-guarded cytobrush/swab via vaginoscopy or manually with a sterile sleeve. The presence of neutrophils, bacteria, and/or fungal hyphae will aid the presumptive diagnosis and give some clues regarding the type infection (i.e., Gram-positive/negative and shape, or presence of hyphae) while waiting for further diagnostic tests.

The definitive diagnosis of placental infection is attained by pathologic and microbiologic examination of the placenta. Ordinarily, the placenta should be laid out in an “F” shape, the amnion and umbilical cord should be spread out, examined, and sampled for pathologic examination. Affected areas, normal tissues, and areas of transition between the normal and affected tissues should be sampled from the chorioallantois, amnion, and umbilical cord.

As part of the diagnostic testing for abortion cases, swabs should be collected from affected placentas and then used for bacterial culture and polymerase chain reaction (PCR) testing for abortigenic infectious agents (e.g., Leptospira, equine herpesvirus type 1, equine arteritis virus). PCR testing for abortigenic infectious agents (e.g., Leptospira, equine herpesvirus type 1, equine arteritis virus) can be used for diagnostic testing. Fragments of placenta and fetal tissues can be collected, pooled, and used for culture and PCR testing. A combination of different diagnostic methods will improve the diagnostic accuracy. However, despite extensive testing, in approximately 20% of abortions submitted to a referral laboratory in central Kentucky, the cause of abortion could not be determined.

Lesions detected upon histopathologic examination of the placenta vary with the infectious agent involved and chronicity of the infection. Acute bacterial placental infection is characterized by neutrophilic infiltration of the intervillous space and/or necrosis of the chorionic villae. Chronic placental infection is associated with necrosis of the chorionic villi, presence of eosinophilic amorphous material in the chorion, and/or infiltration of mononuclear cells in the intervillous spaces. Other lesions reported in association with chronic placental infection include chorioangiogenesis, hyperplasia with or without squamous metaplasia of the chorionic epithelium, and adeno-
matous hyperplasia of the allantois. Leptospiral placentitis is characterized by edema, placental hemorrhage, brown mucoid material, green discoloration, cystic adenomatous hyperplasia of the allantois, vasculitis, amnionitis, and funisitis as well as by the presence of spirochetes (Fig. 8). Severe fetal lesions are also a remarkable feature of leptospiral abortion; the aborted fetus may be emaciated and icteric, present generalized edema and fluid accumulation and severe degenerative inflammatory lesions of lungs, liver, kidneys (Fig. 8).

In nocardioform placentitis, the macroscopic lesions are focal-extensive at the base of the uterine horn(s) and cranial uterine body on the chorionic surface of the chorioallantois, without involvement of the cervical star. A brown tenacious, mucoid material is found overlying white granular punctuate lesions. Histopathologic lesions are described as chronic-active; severely affected villi are often necrotic and coated with an extensive layer of eosinophilic amorphous material mixed with neutrophils and colonies of long filamentous branching bacteria. In less severely affected areas the same type of exudate is present in the crypts, and mononuclear cells are present in the villous stroma and chorionic stroma. Endometritis is a common finding in mares presenting with ascending placentitis. Adenomatous hyperplasia of the allantoic epithelium and hyperplasia with or without squamous metaplasia of the chorionic epithelium is frequently observed. Bacterial cultures of the affected areas are routinely used as part of the necropsy diagnostic testing in pregnancy loss cases (i.e., abortion cases, premature delivery, and suspicious placentitis cases). Methods and procedures for bacterial culture for nocardioform actinomycetes have been recently improved. Analyses by PCR for actinomycetes (i.e., Crossiella equi and Amycolatopsis ssp.) have been incorporated as a routine test for the diagnosis of nocardioform placentitis in central Kentucky. The perceived advantage of PCR in comparison with other diagnostic tools includes a rapid turnaround time; however, the specificity and sensitivity for PCR detection have not been reported. At present, as for other abortigenic diseases, the combination of the different diagnostic methods is highly recommended to maximize diagnostic accuracy.

Fungal placentitis is commonly associated with chronic extensive placentitis at the cervical star. Similar to chronic bacterial placentitis, some fungi induce multifocal granulomatous placentitis.
Adenomatous hyperplasia of the allantoic epithelium, with or without squamous metaplasia, can be commonly observed in chronic placentitis (e.g., fungal placentitis, *E. coli*, and nocardioform placentitis). Fungal placentitis can be found in association with bacterial placentitis. Anecdotally, treatment for bacterial placentitis may be associated with a secondary fungal infection; however, such associations have not been studied.

### Diagnostic Markers for Equine Placentitis

Several molecules have been measured in blood from pregnant mares to evaluate fetoplacental wellbeing. During the last three decades, steroids produced by the fetoplacental unit have received the most attention. Other molecules including relaxin and “equine feto-protein” have also been investigated in mares with pregnancy loss. Recently, serum amyloid A (SAA) has been investigated as a prognostic marker and diagnostic marker for mares with experimentally induced ascending placentitis. Therefore, different diagnostic markers for placental health in mares will be discussed herein.

#### Fetoplacental Steroids

During pregnancy several steroids (i.e., estrogens, progesterins, and androgens) are produced and metabolized by the fetoplacental unit. These steroids achieve extremely high concentrations in the maternal and fetoplacental circulation. At the present, we have very limited understanding of the function of the different steroids during pregnancy, particularly within the fetoplacental unit. Progestins are responsible for maintaining pregnancy; however, it is unclear what functions estrogens and androgens have during pregnancy in mares. Removal of fetal gonads did not affect pregnancy maintenance; however, concentrations of 13,14-dihydro-15-keto-PGF2α (PGFM), a metabolite of PGF2α, was remarkably reduced, and as a consequence, the second stage of labor was delayed. Progestins can be subclassified as pregnenes and 5α-pregnanes. Pregnenes include pregnenolone, progesterone, and 5α-pregnenes. Progesterone, and 5α-pregnenes-3β,20β-diol (P5ββ); 5α-pregnenes include 5α-dihydroprogesterone (5α-DHP), 3βP, 20α-P, βδiol, and 5α-pregnene-3β,20α-diol (βδiol); however, 5αDHP, 20αP, and βδiol predominate. During the first 100 days of pregnancy, progesterone is the main progestin, with 5α-DHP also being also present in high proportion starting from ovulation. It is produced by the primary corpus luteum, and secondary and accessory corpora lutea. Starting by 70–100 days of gestation the main source of progestins is the equine placenta. Removal of the maternal ovaries by 100 days of gestation did not affect pregnancy maintenance; however, removal of maternal ovaries by 70 days of pregnancy was associated with pregnancy loss in 50% of the mares. It is worth noting that in addition to progesterone, 5α-DHP is also present in the maternal circulation during early pregnancy and has been recently confirmed as a potent, bioactive progestin. Interestingly, from approximately 150 days of pregnancy on, progesterone is present in very low concentrations in the maternal plasma, and it seems that other progestins (e.g., 5α-DHP) are responsible for pregnancy maintenance. Throughout pregnancy, concentrations of progestins other than progesterone, particularly 5α-DHP increase with the advance of gestation. During the last 30 days of gestation, maternal concentrations of progestins increase until immediately prior to parturition. Measurement of progestins has been used to monitor placental health in spontaneous and experimental cases of placentitis. Chronic placentitis (> 7 d post-inoculation) in experimentally induced mares was associated with increased plasma progestin concentrations, however, in the same study, mares that developed acute placentitis (abortion < 4 days post-inoculation) experienced a rapid decline in plasma progestin concentrations. Spontaneous cases of placentitis have been reported to cause elevation in total progestin concentrations. This increase in concentration of progestins is thought to be linked to the accelerated adrenal gland maturation in the fetus of pregnant mares experiencing chronic placentitis. The adrenal gland is thought to produce pregnenolone, and this molecule is used as a precursor for progesterin synthesis in the equine placenta. It is assumed that placentitis increases the substrate (i.e., pregnenolone), which leads to an increased peripheral progesterin concentration in mares with chronic placentitis. It has also been suggested that placentitis affects the ability of the placenta to metabolize progesterone into other progestins; thus, progesterone will appear in higher concentration in plasma. Repeated measurements of plasma progestins in mares with placentitis have been proposed as a useful method to identify mares that may abort or deliver prematurely.

During pregnancy, the equine fetal gonads of both male and female fetuses undergo marked hypertrophy and secrete large quantities of androgens, particularly dehydroepiandrosterone (DHEA) and 7-dehydro-DHEA. DHEA serves as a precursor for classic phenolic estrogens (i.e., estrone, 17β-estradiol, 17α-estradiol, and their sulfoconjugates), and 7-dehydro-DHEA serves as a precursor for ring B unsaturated estrogens (i.e., equilin, equilenin, and their hydroxyderivatives, 17α-dihydroequilin, 17α-dihydroequilenin, 17β-dihydroequilin, and 17β-dihydroequilenin) by the equine placenta. In addition to DHEA, circulating testosterone is elevated in plasma of pregnant mares. To date, determination of fetoplacental androgens has not been critically assessed in mares experiencing pregnancy losses. Because androgens serve as precursors of estrogens, it seemed logical that placentitis affects androgen concentrations in the maternal circulation, but our recent study we showed that con-
Concentrations of DHEA-S and testosterone did not change in mares with experimentally induced placentitis. Likely, androgens are not useful diagnostic markers for placentitis.

The equine blastocyst begins to secrete estrogens approximately 10 days post-ovulation, there- after, estrogens are produced by the corpora lutea starting by 40 days of pregnancy. This ovarian secretion of estrogen is believed to be mediated by equine chorionic gonadotropin (eCG) and will last until the demise of the corpora lutea around 150 days of gestation. Concomitant with ovarian secretion of estrogens (i.e., by gestation d 40), the fetoplacental unit also starts to secrete estrogens. Concentrations of estrogens in maternal circulation will increase remarkably throughout the second trimester of pregnancy, and maximal concentrations are achieved by 210–240 days of gestation. From the peak of estrogen concentrations in maternal circulation, there is a progressive reduction in estrogen concentrations through the end of pregnancy with minimal estrogen concentrations at parturition, which is contrary to reports in domestic ruminants. The estrogen production parallels the enlargement of the fetal gonads. Given that estrogens are a final product of the fetoplacental unit, determination of peripheral estrogen concentrations in pregnant mares may provide utility in the diagnosis of equine placentitis.

Determination of estrone sulfate concentrations in maternal circulation has been suggested as a useful marker for fetal wellbeing during early pregnancy, however, studies involving estrone sulfate in advanced pregnancy are scant. There are no controlled studies assessing estrone sulfate in mares experiencing placentitis, despite its high popularity among practitioners which frequently use estrone sulfate as a test to assess fetal wellbeing and placentitis health. Field observations carried out by a commercial laboratory reported that aborting mares present significantly lower “total estrogen” (unreported crossreactivity) concentrations than do mares maintaining pregnancy. Controlled studies are warranted to confirm field observations concerning the utility of conjugated and unconjugated estrogen concentrations in pregnant mares as markers for placentitis.

Acute-Phase Proteins

Acute-phase proteins (APPs) are elevated when inflammation is present. This group of proteins is mainly produced by the liver in response to an inflammatory stimulus (i.e., cytokines). APPs are classified as minor, moderate, and major; minor and moderate (i.e., constantly present in the plasma and concentration increases 1–5-fold and 5–10-fold upon inflammation, respectively) and major (i.e., low or undetectable concentrations in the blood circulation and upon inflammatory stimulus increases >10 and often 100-1000 times). As a moderate APP, Hp is constantly present in plasma of healthy horses and concentrations are elevated upon inflammation. In horses, Hp has recently been shown to be a useful marker for a variety of acute-chronic clinical conditions (i.e., > 4 d duration). Recently, increases in peripheral blood concentrations of SAA have been reported in mares with experimentally induced bacterial endometritis and ascending placentitis. In healthy pregnant mares, SAA concentrations remained low throughout the last half of gestation while showing a significant increase beginning approximately 12 hours postpartum. Maternal SAA concentrations then return to baseline within approximately 60 hours postpartum. Interestingly, levels of SAA in peripheral blood have been reported to increase in mares affected by placentitis and decrease again in response to treatment.

Fibrinogen, the most widely used APP in the horse, is commonly used in association with white blood cell counts (WBC) as a means to assess inflammation in the horse, however, anecdotally it has been suggested that neither WBC nor Fb change in mares with placentitis. In a recent study, we demonstrated that concentrations of SAA and Hp increase rapidly subsequent to experimental induction of placentitis and remain elevated until abortion, whereas neither Fb nor WBC seemed to be useful markers for placentitis. In the same study, parturition did not trigger an increase in either SAA or Hp in normal foaling mares. In another study, we also showed that measuring SAA with a lateral flow device can be used to detect inflammation in mares with experimentally induced ascending placentitis.

Relaxin

Relaxin, a hydrophilic polypeptide hormone, is a member of the insulin-like peptide superfamily. Equine relaxin, which is the smallest relaxin among mammals, is composed of two subunits (i.e., A, α 20-residue; and B, β 28-residue) which are connected by disulfide bonds similar to insulin. It has been suggested that relaxin plays an important role during pregnancy, and the proposed functions include uterine growth, distensibility of uterus, and relaxation of the cervix and pelvic ligaments. To date the role of relaxin during equine pregnancy is not fully understood. Relaxin is produced by the equine placenta and can be detected in high concentrations in the peripheral blood from day 80 of gestation until term. It has been reported that relaxin is a useful biomarker to assess placental health, the authors reported that there was a positive relationship between circulating levels of relaxin and poor outcomes in high-risk pregnancies. These investigators suggested that because the placenta is the sole source of relaxin, blood concentrations could be used as a biomarker of placental function. It is worth noting that relaxin concentr-
tions vary greatly among breeds (e.g., Thoroughbred vs Standardbreds) and horse types (pony vs standard-size horse mares). In addition, the lack of a commercially available test at present precludes the use of relaxin clinically in mares.

**Alpha-Fetoprotein**

Alpha-fetoprotein (AFP) is a major protein present in the allantoic and amniotic fluids; AFP is a member of the albuminoid superfamily, the other protein members of this superfamily are vitamin D binding protein, alpha-albumin, and afamin. In mammalian fetuses, among other functions, AFP is associated with estrogen binding (allowing development of gonadotropin releasing hormone (GnRH) surge center in females), antioxidative activities (through binding of heavy metals), and immunoregulation. In horses, AFP is highly expressed during early pregnancy by the conceptus. Our recent study demonstrated that AFP is present in the fetal fluids of mares during the third trimester of pregnancy and that mares with experimentally induced ascending placenteritis have elevated plasma concentrations of AFP. However, it remains to be determined whether this protein is a useful marker for spontaneous cases of equine placenteritis.

**Biomarkers for Chorioamnionitis in Humans**

In recent years, microRNAs (miRNA), small noncoding RNAs (~22 nucleotides) have been the focus of human studies as potential biomarkers for diseases (e.g., cancer, inflammatory and cardiovascular pathologies). miRNAs are associated with a wide range of biological functions through regulation of gene expression either by degrading or transcriptionally repressing mRNA. miRNAs are relatively stable in the systemic circulation and represent a source of cell-free nucleic acid. In plasma, miRNAs are stable because they are transported within exosomes (noncellular vesicles), and recent findings have shown that miRNAs present in these vesicles control immune response mediated by T-cells. A number of studies have characterized pregnancy-associated miRNAs in women and some studies have suggested that changes in expression of miRNAs associated with abnormal or preterm labor, therefore indicating their potential diagnostic application. To date, miRNAs have received limited attention during equine pregnancy; a recent study demonstrated changes in miRNAs in the dam’s circulation during maternal recognition of pregnancy. Given that miRNA (i.e., miR223 and miR338) change in the placenta of pregnant women suffering with chorioamnionitis, it is reasonable to hypothesize that these or other miRNAs may change in mares with placenteritis.

Proteomics of the amniotic fluid and fetal circulation has been used in women to identify biomarkers for chorioamnionitis (intra-amniotic infection/inflammation). Ruetschi et al identified seventeen proteins that were overexpressed in amniotic fluid samples of women with chorioamnionitis. Among these proteins, three proteins were further identified as human neutrophil protein 1–3 as well as calgranulin A and B. Another study identified 39 proteins that are differentially expressed in amniotic fluid samples obtained from patients with intra-amniotic infections. To date, proteomics of the equine fetal fluids have not been described; thus, characterization of the protein composition of fetal fluids would be useful to identify potential new markers for equine placenteritis. Recently, a study involving proteomics of human neonates demonstrated that Hp and Hp-related protein immunoreactivity are useful markers for neonatal sepsis. Proteomics of the fetal fluids and the newborn foal may aid in identifying new diagnostic markers for placenteritis and neonatal sepsis.

**Macrophage migration inhibitory factor (MIF)**, an inflammatory marker, is elevated in amniotic fluid in chorioamnionitis. Interestingly, during early pregnancy, noninfectious early pregnancy losses in women were associated with reduced serum levels of MIF. Macrophage migration inhibitory factor is expressed in high levels by the embryo and endodermium during early pregnancy in horses. Unfortunately, our preliminary results measuring MIF in plasma of mares with placenteritis showed that MIF is highly variable across days and mares, and there were no associations between placenteritis and concentrations of MIF; therefore, measuring MIF does not seem useful as a diagnostic marker for placenteritis.

In human medicine, CRP has been the most widely studied APP, as a nonspecific biomarker for placental and fetal health. Changes in concentrations of CRP in the systemic circulation of women suffering with chorioamnionitis have been demonstrated by several authors. To date, CRP is a minor APP and has received limited attention likely due to the lack of remarkable changes in different diseases evaluated to date.

In mammals, infection of the placenta triggers the local innate immune response through activation of Toll-like receptors (TRL), a group of receptors responsible for recognition of pathogen patterns and tissue damage. Activation of TRL (e.g., TRL-2 and TRL-4) initiates a cascade of events of the innate immune response that results in migration of phagocytic cells to infected/damaged tissues and production of inflammatory mediators (e.g., cytokines, prostanoids, and many others mediators). Cytokines are small molecules (peptides, proteins, glycoproteins) that modulate the immune system/response, either by promoting or suppressing inflammation. Dozens of cytokines are known, and there are several cell-types that produce (e.g., macrophages, monocytes, fibroblasts, dendritic cells, B lymphocytes, NK cells, and epithelial cells) and secrete these molecules. Normal placental tissues of mammals including the mare and women constitutively express pro-inflammatory...
However, placental infection in women is associated with an increased production of several pro-inflammatory cytokines (e.g., IL-6, IL-8, and tumor necrosis factor [TNF]-α) not only in placental tissues, but also in the fetal and maternal blood. Pro-inflammatory cytokines have been shown to be useful markers for placental infection, and valuable prognostic indicators to determine whether a pregnancy will be carried to term in humans. However, there have been few studies evaluating pro- and anti-inflammatory cytokines in mares with experimentally induced ascending placentitis. It is unlikely that measuring cytokines in plasma will be a widely used tool in veterinary medicine due to the limited clinical availability of assays for equine cytokines.

### 4. Treatment

Bacterial infection of the equine pregnant uterus in the middle of gestation can result in acute abortions with no lesions in the fetal membranes and minimal to no outward clinical signs (e.g., vulvar discharge, premature mammary gland development). Therefore, treatment is often not an option to prevent this type of abortion. In contrast, bacterial infections of the uterus late in pregnancy tend to be associated with chronic placentitis. These mares will usually show the clinical signs as described above, thus if a diagnosis is made early enough, treatment can be applied and pregnancies can be potentially rescued.

Primarily, treatment for placentitis should be aimed to prolong maintenance of a viable fetus in utero to allow time for fetal development and maturation. To achieve this goal, therapy should:

1. control bacterial infection of placenta and fetus
2. maintain the myometrial quiescence
3. block the production of pro-inflammatory cytokines

The limited evidence-based treatment strategies come from mares with experimentally induced placentitis. Unfortunately, as noted above, experimentally induced placentitis does not necessarily mirror spontaneous infections; thus, data generated from experimentally induced placentitis should be interpreted with caution.

Treatment for placentitis is commonly based on a combination of antibiotics, anti-inflammatories, and progestins (Table 2). The rationale to use anti-inflammatory is based on the finding that mares with experimentally induced placentitis have increased uterine contractility as a consequence of the prostaglandin production and increased expression of pro-inflammatory cytokines. Similarly, progestin therapy for mares with placentitis is used to block myometrial contractions. Evidence for the use of anti-inflammatories and progestins is based on studies with cloprostenol-induced abortion during early pregnancy. In one study, mares receiving cloprostenol (250 mcg/d for 5 d) but also treated with altrenogest (44 mg/animal/d) maintained the pregnancy (8/8); however, mares given cloprostenol and also treated with progesterone (300 mg/animal day) resulted in five of eight pregnancies maintained, as expected all mares receiving daily cloprostenol (n = 5) but no other treatment aborted. An earlier study conducted by the same group did not observe any beneficial on exogenous flunixin meglumine in control luteolysis in mares treated with experimentally induced endotoxemia. From these studies and probable extrapolation from human literature, it has been assumed that mares with placentitis during late pregnancy will behave in a similar manner although this has not been critically evaluated.

Antibiotics are included as part of the therapy for the bacterial infection of the fetal membranes and to treat and/or prevent bacteria reaching the fetus.
To date, few antimicrobials have been shown to cross the placentation barrier and to achieve effective minimal inhibitory concentrations in the fetal fluids and fetus against common bacteria causing placentitis.11,12

Antimicrobials that have been shown to cross the placenta include sulfa-trimethoprim, penicillin, and gentamicin.146,147 Ceftiofur crystalline free acid, a drug that has been effective in vitro antimicrobial activity against many bacteria causing placentitis was recently tested.37 Unfortunately, administration of ceftiofur crystalline free acid to pony mares did not result in concentrations of desfurylecftiofur acetamide (i.e., the active metabolite) needed to effectively treat bacterial placentitis, and it did not improve survival of foals born from mares with experimentally induced ascending placentitis.37 These findings discourage the use of this antibiotic to treat placentitis in mares.

Controversy exists regarding the duration of antimicrobial therapy once a diagnosis of placentitis is made. Anecdotally, short antimicrobial treatment (i.e., 10–15 d) has been advocated to be an effective and thoughtful approach to treat placentitis as well as an approach to avoid bacterial resistance associated with prolonged antimicrobial therapy.11 However, evidence-based findings in mares with experimentally induced placentitis do not support the claim for effectiveness of short-term antimicrobial therapy. In fact, mares with experimentally induced ascending placentitis did not carry foals to term when treatment was discontinued after 2 weeks; however, an apparent increase in survival rates was observed when mares were kept on antimicrobials for a prolonged period of time.34,146 Another interesting finding about antimicrobial therapy from the same group demonstrated that mares kept on a prolonged treatment with antibiotics, present positive endometrial bacterial culture within 6 hours post-abortion or delivery of a viable foal.69 These findings suggest that antimicrobials administered to mares with experimentally induced placentitis may suppress bacterial growth but do not achieve complete bacterial elimination.

Empirically, estrogen supplementation has been advocated to treat mares with placentitis.72 As aforementioned, this author reported that mares with placentitis that subsequently aborted had remarkably lower “total estrogen” concentrations than mares of the same gestational age that maintained pregnancy. As estrogens are produced in high concentrations during the second and third trimesters of pregnancy, it is unlikely that estrogen supplementation will be able to restore normal estrogen concentrations. For example, it required continuous intravenous infusion of 126–231 mg of estrone sulfate per hour to achieve peripheral pregnancy levels of estrone sulfate.148 Therefore, to date, estrogen therapy has not been critically assessed by controlled studies or prospectively assigned field studies.

Other drugs that have been added to the treatment of equine placentitis include acetylsalicylic acid, pentoxifylline, and dexamethasone11,34 (Table 2). Acetylsalicylic acid has been given to mares with placentitis under the impression that this drug would improve the blood flow to the mare’s uterus; however, there is no evidence basis in the literature to support this practice. In human medicine, low doses of acetylsalicylic acid has been shown to reduce the risks of preeclampsia in women.149 Similarly, pentoxifylline has been included as part of the treatment for mares with placentitis with the idea that this drug possesses anti-inflammatory and rheologic properties,34 which might improve the oxygenation of the pregnant uterus. Pentoxifylline was found to be present in the fetal fluids of pregnant mares treated with the standard dose146 and has been added to the treatment of mares with experimentally induced placentitis.34 However, it is unclear whether this drug has any beneficial effect on the treatment of placentitis. In addition, uterine artery blood flow remained unchanged when pentoxifylline was given to early pregnant mares during a short treatment period.150 Dexamethasone has been suggested as useful for the treatment of placentitis11; however, it is uncertain whether administering this drug improves pregnancy outcome in mares with placentitis. At best, very limited to no evidence basis exists to support the practice to include these drugs (i.e., acetylsalicylic acid, pentoxifylline, and dexamethasone) in the treatment of placentitis.

Given that the pathogenesis of nocardioform placentitis is unknown, and there are no established experimental models to study this type of placentitis, treatment for nocardioform placentitis has been empirically applied and based on treatments used for other types of equine placentitis. To date there are no evidence-based reports supporting the treatment of nocardioform placentitis in mares. A recent retrospective study demonstrated in vitro antimicrobial sensitivity for Amycolatopsis ssp. and Crossiella equi14 (Table 3). However, it is unclear whether these antimicrobials are effective in vivo. It is also unclear whether addition of anti-inflammatory agents is effective or necessary for the treatment of nocardioform placentitis.

Tocolytic agents such as clenbuterol and isoxsuprime have also been used in practice as part of the treatment of mares with placentitis11; however, there is no evidence basis to support such practice. In fact, in a study carried out with normal prefoaling mares clenbuterol was not effective in delaying parturition, and treated mares foaled earlier than control mares.151 The lack of efficacy of clenbuterol to delay parturition should discourage the practice to give this drug to mares with placentitis.

Lately, a new practice of antimicrobial therapy has been introduced in which mares are administered antimicrobials for 10 days of the month throughout pregnancy even in the absence of a clin-
Table 3. In vitro Anti-Microbial Sensitivity for *Amycolatopsis* ssp (n = 38) and *Crossiella equi* (n = 22) Isolated From Placentas in Mares Aborting in Central Kentucky.

<table>
<thead>
<tr>
<th>Antimicrobials/Bacterial Species</th>
<th>No. of Isolates With MIC (µg/mL)</th>
<th>MIC&lt;sub&gt;50&lt;/sub&gt;/MIC&lt;sub&gt;90&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.06 ≤0.12 0.12 ≤0.25 0.25 0.5 ≤1 1 ≤2 2 ≤4 4 8 &gt;8 16 &gt;16 32 64 &gt;64 128</td>
<td></td>
</tr>
<tr>
<td>Amikacin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C. equi</td>
<td>2 13 13 4 1 2 3</td>
<td>64/8</td>
</tr>
<tr>
<td><em>Amycolatopsis</em> ssp.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amoxicillin-clavulanic acid</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C. equi</td>
<td>3 14 5</td>
<td>4/8</td>
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<tr>
<td><em>Amycolatopsis</em> ssp.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cefepime</td>
<td>7 7 18</td>
<td>8/16</td>
</tr>
<tr>
<td>C. equi</td>
<td>7 2 5</td>
<td>32/32</td>
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<tr>
<td><em>Amycolatopsis</em> ssp.</td>
<td></td>
<td></td>
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<tr>
<td>Cefoxitin</td>
<td>1 4 2</td>
<td>6/128</td>
</tr>
<tr>
<td>C. equi</td>
<td>7 18</td>
<td>8/128</td>
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<tr>
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<tr>
<td>Ceftriaxone</td>
<td>14 7</td>
<td>4/8</td>
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<tr>
<td>C. equi</td>
<td>15 20</td>
<td>8/8</td>
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<tr>
<td><em>Amycolatopsis</em> ssp.</td>
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<tr>
<td>Ciprofloxacin</td>
<td>1</td>
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<tr>
<td>C. equi</td>
<td>1 5</td>
<td>2/4</td>
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<td><em>Amycolatopsis</em> ssp.</td>
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</tr>
<tr>
<td>Clarithromycin</td>
<td>1</td>
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</tr>
<tr>
<td>C. equi</td>
<td>17</td>
<td>8/16</td>
</tr>
<tr>
<td><em>Amycolatopsis</em> ssp.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Doxycycline</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>C. equi</td>
<td>15 1</td>
<td>0.25/0.25</td>
</tr>
<tr>
<td><em>Amycolatopsis</em> ssp.</td>
<td></td>
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<tr>
<td>Imipenem</td>
<td>7</td>
<td></td>
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<tr>
<td>C. equi</td>
<td>12 6</td>
<td>0.25/8</td>
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<tr>
<td><em>Amycolatopsis</em> ssp.</td>
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<tr>
<td>Linezolid</td>
<td>4</td>
<td></td>
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<tr>
<td>C. equi</td>
<td>18 3</td>
<td>4/8</td>
</tr>
<tr>
<td><em>Amycolatopsis</em> ssp.</td>
<td></td>
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<tr>
<td>Minocycline</td>
<td>23</td>
<td></td>
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<tr>
<td>C. equi</td>
<td>19 2</td>
<td>4/16</td>
</tr>
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<tr>
<td>Tobramycin</td>
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<tr>
<td>C. equi</td>
<td>1 1</td>
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<tr>
<td><em>Amycolatopsis</em> ssp.</td>
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<tr>
<td>TMP-SMX</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>C. equi</td>
<td>8 7</td>
<td>0.5/2</td>
</tr>
<tr>
<td><em>Amycolatopsis</em> ssp.</td>
<td></td>
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<tr>
<td>Abbreviation: TMP-SMX, trimethophrim-sulfametoxazol.</td>
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<tr>
<td>The minimum inhibitory concentrations required to inhibit the growth of 50% and 90% of organisms (MIC&lt;sub&gt;50&lt;/sub&gt;/MIC&lt;sub&gt;90&lt;/sub&gt;). (Adapted from Erol et al. 14)</td>
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<td>Concentration ranges tested are shown in gray.</td>
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</table>
ical diagnosis of placentitis.\textsuperscript{11} The idea of such practice would be that antimicrobial treatment would treat subclinical/undiagnosed placental infection. This practice not only should be discouraged to the lack of proven efficacy, but also because it favors bacterial resistance. Thus, there is an enormous risk of creating super-resistant bacteria that may become a major threat not only to the horses but to public health.

It has been long suggested that mares with poor vulvar conformation are prone to developing placentitis. A field study conducted with Thoroughbred mares in Southern Brazil did not observe any association between vulvar conformation and occurrence of placentitis in the last 30 days pre-foaling.\textsuperscript{2} However, it is worth-noting that 87.7\% of the mares (i.e., 209/333) in that study already had Caslick’s stiches in place by the time the authors carried out the evaluations. It is our interpretation that an association between vulvar conformation and placentitis could not be assessed in that study. It remains best practice state of the art to place Caslick’s stiches in mares with poor conformation.

5. Conclusions

Bacterial placentitis is an important cause of pregnancy loss in mares. Bacterial infections are responsible for a large portion of abortion cases. There are four different morphologic types of placentitis: ascending, focal mucoid (nocardiform), diffuse (hematogenous), and multifocal, of which, ascending is the most frequent type of placentitis, whereby beta-hemolytic streptococci predominate. Pathogenesis of the different types of equine placentitis are poorly known. Acute infections of the gravid equine uterus during mid-gestation are commonly associated with fulminant bacterial infection of the fetus with minimal or no lesions present in the placenta. This type of infection is unnoticed and invariably results in abortion. In contrast, chronic infections of the placenta occurring from the mid to late term are associated with acute-chronic lesions present in the placenta.

Currently, the diagnosis of placentitis is based on ultrasonography and clinical signs. Often clinical signs and associated ultrasonographic changes are only present in well-advanced stages of placentitis. Recently, several diagnostic markers have been reported to be useful in experimentally treating placentitis (e.g., serum amyloid A, haptoglobin, estrogen, and alpha-fetoprotein); however, it remains to be determined whether these markers can be used to diagnose spontaneous cases.

Treatment of placentitis should be aimed at prolonging the presence of the foal in uterus by controlling placental and fetal infection, maintaining the uterus in a quiescent state, and by blocking the production of pro-inflammatory cytokines. Few antibiotics (i.e., penicillin, gentamycin, and sulfa-trimetoprim) have been shown to cross the placenta and achieve satisfactory inhibitory concentrations.

Acknowledgments

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Declaration of Ethics

The Authors declare that they have adhered to the Principles of Veterinary Medical Ethics of the AVMA.

Conflict of Interest

The Authors declare no conflicts of interest.

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Pneumonia Caused by *Klebsiella* spp. in 46 Horses

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*Klebsiella* spp. should be considered as a differential diagnosis for horses presenting with hemorrhagic pneumonia and for horses developing pneumonia after mechanical ventilation. Horses with radiographs revealing a sharp line of demarcation with severe ventral pulmonary consolidation did not survive and had evidence of pulmonary infarction. Authors' addresses: William R. Pritchard Veterinary Medical Teaching Hospital (Estell, Young, Swain), Department of Pathology, Microbiology, and Immunology (Byrne, Reilly), Department of Population Health and Reproduction (Kass), and Department of Medicine and Epidemiology, School of Veterinary Medicine (Aleman), University of California, Davis, CA 95616; e-mail: krista.estell@gmail.com. *Corresponding and presenting author. © 2015 AAEP.

1. Introduction

*Klebsiella* spp. are implicated as a common cause of bacterial pneumonia although few reports describe the clinical presentation and disease progression.

2. Materials and Methods

Medical records from 1993–2013 that were masked for peer review were reviewed. Exact logistic regression was performed to determine whether any variables were associated with survival to hospital discharge.

3. Results

Forty-six horses met the inclusion criteria. Overall survival in adults was 63%. For adults in which *K. pneumoniae* was the primary isolate, survival was 52%. Mechanical ventilation preceded the development of pneumonia in 11 horses. Complications occurred in 25/46 horses; thrombophlebitis and laminitis occurred most frequently. Multi-drug resistance was found in 47% of bacterial isolates. Variables that significantly impacted survival included hemorrhagic nasal discharge, laminitis, and thoracic radiographs with a sharp demarcation between marked caudal pulmonary alveolar infiltrate, and more normal-appearing caudodorsal lung. All horses with radiographs revealing a sharp line of demarcation with severe ventral pulmonary consolidation had a corresponding line of demarcation on postmortem with discrete, dark red regions consistent with pulmonary infarction.

4. Discussion

*Klebsiella* spp. should be considered as a differential diagnosis for horses with hemorrhagic pneumonia. Multidrug resistance is common in *Klebsiella* spp.

Acknowledgments

Declaration of Ethics
The Authors declare that they have adhered to the Principles of Veterinary Medical Ethics of the AVMA.

Conflict of Interest
The Authors declare no conflicts of interest.
Prevalence of Equine Coronavirus in Nasal Secretions From Horses With Fever and Upper Respiratory Tract Infection

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This study provides contemporary information on the frequency of equine coronavirus (ECoV) detected by quantitative polymerase chain reaction (qPCR) in nasal secretions from horses with acute onset of fever and/or respiratory signs. Given that the detection of ECoV by qPCR in nasal secretions is an infrequent event, the testing for ECoV should be restricted to feces from horses with fever, depression, anorexia, colic, and diarrhea and should not include horses with respiratory signs. Authors’ addresses: Department of Medicine and Epidemiology (Pusterla, Holzenkaempfer, Mapes) 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. © 2015 AAEP.

1. Introduction
Equine coronavirus has recently been associated with emerging outbreaks of pyrogenic and enteric disease in adult horses. Bovine coronavirus is closely related to ECoV and considered a pneumoenteric virus, causing not only enteric disease but also mild upper respiratory signs. Due to the inconsistent development of enteric signs in horses infected with ECoV, one can only wonder whether this virus has a tropism to non-enteric epithelial cells, such as the respiratory epithelium. The objective of the present study was to investigate the presence of ECoV in nasal secretions from horses with signs of fever and/or acute onset of upper respiratory tract infection.

2. Materials and Methods
Nasal secretions from 2,437 equids with acute onset of fever and/or respiratory signs were tested for ECoV and common respiratory pathogens by qPCR. Categorical analyses were performed using either the Fisher’s exact or the Kruskal-Wallis test to determine the association between observations (age, breed, sex, clinical signs, qPCR status for common respiratory pathogens) and ECoV.

3. Results and Discussion
ECoV was detected by qPCR in 17/2,437 (0.7%) of all index horses. Of the ECoV qPCR-positive horses, nine (52.9%) showed co-infection with either equine herpesvirus (EHV)-4, equine influenza virus (EIV), equine rhinitis B virus (ERBV), or S. equi ss. equi. Nasal discharge, fever, depression, anorexia, and coughing were the predominant clinical signs associated with ECoV qPCR-positive horses. No significant associations were found between ECoV qPCR-positive horses and the ECoV qPCR-negative index cases for age, breed, sex, use, specific clinical signs, and qPCR positivity for EHV-4, EHV-1, EIV, and S. equi ss. equi (P < .05). A higher-than-expected prevalence of ECoV qPCR-positive animals with

Research Abstract—for more information, contact the corresponding author

NOTES
concurrent detection of ERBV was found ($P = .01$) when compared with the ECoV qPCR-negative index cases that tested qPCR-positive for ERBV.

**Acknowledgments**

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**Declaration of Ethics**

The Authors declare that they have adhered to the Principles of the Veterinary Medical Ethics of the AVMA.

**Conflict of Interest**

The Authors declare no conflicts of interest.
Prevalence Factors Associated With Equine Herpesvirus-1 Infection in Equids With Upper Respiratory Tract Infection from 2008 to 2014

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This study provides new information regarding the frequency of equine herpesvirus-1 (EHV-1) detected by quantitative polymerase chain reaction (qPCR) in blood and nasal secretions from horses with acute onset of fever and/or respiratory signs. The results point to the fact that Thoroughbreds and racing horses have been overrepresented among the EHV-1 PCR-positive index cases. Authors’ addresses: Department of Medicine and Epidemiology, School of Veterinary Medicine, University of California, Davis, CA 95616 (Pusterla, Mapes, Akana); and Merck Animal Health, Summit, NJ 07901 (Barnett, MacKenzie, Gaughan, Craig, Chappell, Vaala); e-mail: npusterla@ucdavis.edu. *Corresponding and presenting author. © 2015 AAEP.

1. Introduction
Equine herpesvirus-1 (EHV-1) is a common respiratory virus of young horses associated with acute fever, depression, anorexia, mandibular lymphadenopathy, and profuse serous nasal discharge. In adult horses, the disease is generally mild or subclinical unless major complications arise such as abortion in pregnant mares or myeloencephalopathy. The objective of this study was to provide new information regarding the prevalence and epidemiology of EHV-1 shed by horses presented to veterinarians with clinical signs related to an upper respiratory tract infection from March 2008 to December 2014.

2. Materials and Methods
Nasal secretions from 4,210 equids with acute onset of fever and/or respiratory signs were tested by quantitative polymerase chain reaction (qPCR) for EHV-1. Categorical analyses were performed to determine the association between observations and EHV-1.

3. Results and Discussion
A total of 99/4,210 (2.4%) equids tested qPCR-positive for EHV-1, with most of the isolates belonging to the non-neuropathogenic genotype N752. EHV-1 qPCR-positive equids were overrepresented in Thoroughbreds and racing horses. Depression, nasal discharge, and coughing were significantly less frequently reported in the EHV-1 qPCR-positive equids compared with the EHV-1 qPCR-negative index cases.

Acknowledgments
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Research Abstract—for more information, contact the corresponding author
Declaration of Ethics
The Authors declare that they have adhered to the Principles of Veterinary Medical Ethics of the AVMA.

Conflict of Interest
The Authors declare no conflicts of interest.
How to Perform a Transtracheal Wash in the Field

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1. Introduction
The transtracheal wash (TTW) is a relatively non-invasive procedure that can be easily performed in the field. This is the ideal method for collecting respiratory secretions for culture in cases of suspected bacterial pneumonia. First described in horses in the early 1970s,1 the TTW procedure requires few supplies and can be performed in the sedated or unsedated horse depending on temperament.2–6 TTW avoids the need for an endoscope and reduces the chance of culturing upper respiratory flora by directly sampling the lower respiratory tract.7 Despite the ease of TTWs, many practitioners are hesitant to perform these procedures. It is the authors’ hope that practitioners will become reacquainted with this underutilized skill.

Respiratory secretions are brought up from the lungs via mucociliary clearance; thus, samples obtained from the tracheal puddle represent secretions from all areas of the lungs.5 A TTW is especially indicated in cases when localized lung pathology is suspected (e.g., an abscess or discrete pneumonia), because more directed respiratory sampling such as a bronchoalveolar lavage may obtain a false-negative culture result if a nonaffected portion of the lung is inadvertently sampled.8

It is highly recommended that TTWs be performed before commencing antibiotic therapy to increase the chance of identifying the causative bacterial pathogen. The TTW fluid may also be submitted for cytology, and reference ranges have been described.9–11 However, cells from the upper airway may be present in the tracheal puddle, and results may not be purely representative of the lower airway.7,8

2. Materials and Methods
Supplies (Fig. 1):
- Sedation
- Clippers
- Surgical scrub
- Alcohol
- 4 × 4 gauze
- 2% lidocaine neat
- 3- or 5-mL syringe
- 25-gauge needle
- Sterile gloves
- #15 scalpel blade
- Transtracheal wash aspiration kita

If no kit available (Fig. 2):
- Introduction catheter: 12-gauge intravenous catheterb
3. Preparation of the Patient—Sedation
Depending on the horse’s temperament and severity of illness, sedation may or may not be required or recommended to perform a TTW. If the horse is prone to moving around, light sedation or a twitch may help reduce the chance of contamination during the procedure. Because you will be in a relatively dangerous spot while performing the procedure, personal safety must always be a concern. Light sedation with xylazine and butorphanol is usually sufficient for the procedure because the procedure itself is not lengthy. The addition of butorphanol will also decrease the cough reflex. Coughing during the procedure will displace the tracheal puddle, making it more difficult to aspirate the fluid and potentially increasing the risk of contamination from the upper airways.

4. Preparation of the Site
Palpate the ventral midline of the horse’s neck. The sternocephalicus muscles will overlie the trachea caudally. As the muscles run cranially they move abaxially and lie lateral to the trachea, which allows for easy palpation of the tracheal rings. The TTW site is approximately halfway between the larynx and the point at which the rings can no longer be palpated because of the overlying sternocephalicus muscles. This will likely be approximately 1/3 to 1/2 the way down the neck. The area should be clipped and aseptically prepared. The authors prefer a wide clip area so that the trachea can be grasped and stabilized while maintaining sterility of the non-dominant hand. The horse’s mane should be braided or taped out of the way to ensure it will not contaminate your TTW site or hands during the procedure.

The lidocaine can be instilled into the TTW site at this point and followed by a second prep of the area. After donning sterile gloves, it could also be drawn up and infiltrated into the site using aseptic technique. When infiltrating the site with lidocaine, first create a bleb under the skin and then direct the needle slightly deeper to infiltrate the tissues adjacent to the trachea.

5. Transtracheal Wash Procedure
If possible, a small table or counter with the sterile field should be available within arm’s reach of the horse. All of the supplies should be prepared and organized before commencing the procedure. Sterile gloves should be donned at this point if they have not been donned already. Using aseptic technique, 20 to 30 mL of sterile saline is drawn up into each of two 60-mL syringes and placed onto the sterile field. Palpate the trachea and relocate the TTW site. Palpate the space between the two tracheal rings and make a small incision with the #15 scalpel. The incision is vertical—approximately 1 to 2 cm long or twice as long as the TTW introduction needle (or catheter)—along the skin over the middle of the trachea. If using an intravenous (introduction) catheter and polypropylene urinary (flushing) catheter instead of a TTW kit, this is a good time to ensure that the flushing catheter fits in the lumen of the introduction catheter (it would be very frustrating to find this out after the introduction catheter is positioned in the horse).
Hold the trachea firmly between two fingers while pulling the skin out on the side to maintain tension across the incision site. You can hold the trachea from above or from below the incision site. The introduction needle (or catheter) is then inserted through the skin incision between two tracheal rings. If there is a lot of resistance, it is because the catheter is hitting a tracheal ring. If this happens, pull it out, palpate the tracheal rings again, and reinsert the catheter a little bit lower or higher depending on palpation.

When inserting the introduction needle/catheter, do not insert the introduction needle/catheter perpendicular to the trachea. Keep the catheter parallel to the ground instead. This gives a downward angle to the catheter compared with the trachea (Fig. 3). In addition, hold the introduction needle/catheter with the bevel downward. This facilitates the insertion through the tracheal rings.

Once the introduction needle/catheter is in the tracheal lumen, angle the tip toward the lungs and advance to the hub. At this point the internal needle, or stylet, is removed, leaving the introduction catheter in place. Thread the flushing catheter through the introduction catheter toward the lungs. Ideally, the end of the flushing catheter will reach the thoracic inlet and be positioned in the tracheal puddle. Some kits have the flushing catheter within a sleeve that prevents inadvertent contamination of the tubing during insertion. If you are not using a TTW kit, it is very important to avoid contamination by touching the skin, hair, or any surface that is not sterile. The catheter is long and difficult to handle without contaminating it. It can be helpful to loop the catheter around your hand (Fig. 4).

When performing a TTW on a foal, many of the flushing catheters are too long and, if inserted fully, will pass the tracheal puddle. It is helpful to premeasure (aseptically of course) the catheter from the incision site to the thoracic inlet so that you have an idea of how far to insert the catheter.

Remove the flushing catheter stylet (if present) once the catheter is fully inserted. This stylet gives the catheter rigidity, which reduces the chance of it becoming kinked or turned cranially during threading. Attach the first 60-mL syringe (loaded with 20–30 mL of sterile saline) onto the flushing catheter. Inject the saline slowly and then start aspirating slowly. If the saline is injected too fast, it may...
be sprayed too caudally in the trachea and become difficult to aspirate.

If the catheter is placed correctly, there will be slight resistance during aspiration. The more viscous the tracheal secretions, the more resistance to aspiration you will feel. If there is only air coming back into the syringe while aspirating, it is because the tip of the tracheal catheter is not dipping into the secretion/saline pool. This usually happens if the catheter is inserted too far into the trachea (or less likely not far enough). In addition, sometimes the tip is bent upward instead of downward into the lumen of the trachea (resulting in a pharyngeal wash).

What to do in this case:

- Rotate the flushing catheter 180 degrees so that if the tip of the catheter was upward it is now facing ventrally. Aspirate and check if there is resistance.
- Disconnect the syringe from the flushing catheter, empty the air, reconnect it, and slowly withdraw the flushing catheter while aspirating. If you feel resistance when aspirating, you are in the right spot. Stop withdrawing the catheter and keep aspirating.
- If this doesn’t work, disconnect the syringe again to empty the air, reconnect it, and then aspirate while slowly advancing or withdrawing the flushing catheter further.
- Another option is to lower the horse’s head to move the tracheal puddle more cranially up the trachea.
- If none of these tips are successful, then more sterile saline from the second syringe can be instilled, and the aspiration procedure can be attempted a second time.

Once secretions are collected, empty the air from the syringe by keeping its tip up and prepare the sample for laboratory submission (Fig. 5).

When removing the TTW catheter, it is important to remove the flushing catheter first and then the introduction catheter. Because the contaminated flushing catheter is protected within the introduction catheter, this prevents it from contacting the skin and underlying tissue upon removal. An exception to this is if the introduction needle has a sharp end that may inadvertently cut the flushing catheter during removal. In this case, remove the introduction needle first.

After completing the TTW, a 4 × 4 gauze is held in place over the incision with a self-adhesive elastic bandage for at least 30 minutes to 1 hour after the procedure. This will help to seal the incision site and prevent air from tracking under the skin from the incised trachea. Some practitioners may apply a small amount of antibiotic or other wound ointment to the incision, although this is optional. The incision site should be monitored by the owners for any evidence of inflammation or infection over the next few days.

6. Sample Handling

It is important to understand what you want from the laboratory. Make sure you fill out the laboratory form appropriately and that you put the samples in the right milieu and at the right temperature for shipping.

- Do you want a Gram staining?
- Do you want an anaerobic culture?
- Do you want a sensitivity panel?

In optimal conditions, the answer is yes to each of these questions.

The advantage of a quick Gram staining is to give you an idea of the type of bacteria present and thus the type of antibiotics to use after the TTW procedure. However, the results will not be ready within 24 to 48 hours unless the staining is done at the clinic.

Although anaerobes are important in severe pneumonia and pleuropneumonia cases, they may not be as important in less severe cases. Most laboratories will not be equipped to test the sensitivity of anaerobic bacteria.

Last, the most important information you need is the sensitivity of the aerobic bacteria to antibiotics.
Options for a sample submission are as follows:

- Culturette swab (aerobic or anaerobic/aerobic)
- Bacterial culture collection and transport system
- Blood culture vials
- Red top tube

For cases in which you submit the sample for cytology, you should first make and then air dry some slides (similar to making blood smears). In addition, you should submit part of the sample in a lavender top tube.

7. Results

Summary of steps for the transtracheal wash:

- Sedate as required.
- Clip and aseptically prepare the site.
- Infiltrate the site with 2 to 4 mL of 2% lidocaine using the 25-guage needle and 3-mL syringe.
- Don sterile gloves.
- Draw up 20 to 30 mL of sterile saline into each of the two 60-mL syringes.
- Open the scalpel blade onto the sterile field.
- Move the sterile field with all equipment to within reach of the horse.
- Make a skin incision with the #15 scalpel blade.
- Locate the TTW site and stabilize the trachea with your nondominant hand.
- Insert the introduction needle or catheter into the trachea between two tracheal rings.
  - Hold introduction needle parallel to the ground with bevel down.
- Once inside the trachea, direct the introduction needle towards the lungs.
- Remove introduction needle/catheter stylet if present.
- Insert flushing catheter through the introduction catheter and direct it into the tracheal puddle.
  - Slowly inject sterile saline.
  - Slowly aspirate tracheal secretions.
  - Remove the flushing catheter.
- Remove the introduction needle/catheter.
- Place 4 x 4 gauze and self-adhesive elastic bandage over incision site for 30 minutes to 1 hour.

8. Discussion

Transtracheal Wash Cautions

Risks before the procedure if horses are in respiratory distress:

- If the animal is in severe respiratory distress or is hypoxic (usually because of a pneumonia), the sedation and saline injection into the trachea may be fatal.

- If the horse has a profuse amount of secretions in the lumen of the trachea, the diameter left for the air to flow through the trachea is tremendously reduced. Adding saline into such a tracheal lumen will decrease the circumferential area of the lumen further. This will increase resistance to airflow and worsen the respiratory distress.

Risks you have to keep in mind when performing the TTW procedure:

- Perforating the carotid artery or the jugular vein:
  - Can happen when the horse is not handled or sedated properly.
  - Can happen when the veterinarian does not hold the trachea and skin properly when passing the TTW introduction needle/catheter.

- Damaging a tracheal ring:
  - It is important to pass the catheter between the two tracheal rings to avoid possible damage or laceration to the cartilages.

- Damaging the dorsal tracheal wall:
  - When inserting the introduction needle/catheter, too much force may result in the sharp end perforating or lacerating the dorsal trachea, which can result in emphysema, chronic abscessation, or infection.
  - Care must be taken at this step to apply enough force to pop into the tracheal lumen between the cartilage rings, but control is required to stop the forward momentum once the lumen is entered.

- Cutting/breaking the flushing catheter and losing the catheter down the trachea:
  - This can occur if the introduction needle/catheter has a sharp end (this is less likely with new kits or when using an intravenous catheter). If your introduction needle/catheter has a very sharp end, withdraw the introduction needle/catheter first until the sharp end is completely out of the skin. Then withdraw the flushing catheter. This will reduce the chance of shearing the flushing catheter on the end of the introduction needle/catheter but does increase the chance of introducing infection into the tissue.
  - When injecting saline into the flushing catheter, the end of the syringe attachment may break off or become dislodged from the main catheter when bent or put under lateral forces (especially when using a dog urinary catheter). It is very important to hold the flushing catheter when injecting or withdrawing fluid using the syringe.
  - If a flushing catheter is lost in the trachea, most horses will cough up the catheter within 30 minutes. If the catheter is not
coughed up, endoscopy may be required to manually remove the catheter using biopsy forceps.

Risks After the Procedure

Infections:

- Infectious samples (usually associated with bacterial pneumonia) are aspirated from the lumen of the trachea during TTW. Problems may arise when bringing the catheter up through the skin, which can contaminate the subcutaneous and tracheal ring area with bacteria from the trachea.\(^1,2\) If the horse has been treated with antibiotics and did not respond to the therapy, the bacteria present in the lumen of the trachea may be resistant to the common antibiotics and may be difficult to treat.

- Risk of skin infection can also be reduced by use of a blunt, smooth-ended introduction needle/catheter so that the flushing catheter can be withdrawn through the introduction catheter without ever coming into contact with the skin or underlying tissue.

- This makes it important to make a skin incision to allow some drainage at the site where the catheter was passed through the skin and to have the owner monitor the site for an abscessation.

Emphysema

A rare complication of the procedure is secondary emphysema. This occurs when air escapes from the trachea through the incision site or from a dorsal tracheal perforation into the surrounding tissues. Air can then dissect through tissue planes, including entering into the mediastinum.\(^1,2\)

- Pressure applied to the incision site after completing the procedure will reduce the risk of emphysema.\(^2\)
- Limiting traumatic damage to the incision site through a single incision and needle puncture is also important.

Acknowledgments

Declaration of Ethics

The Authors declare that they have adhered to the Principles of Veterinary Medical Ethics of the AVMA.

Conflict of Interest

The Authors declare no conflicts of interest.

References and Footnotes


1*Transtracheal wash aspiration kit, Mila International, Erlanger, KY 41018.
2*Intravenous catheter, BD Medical, Sandy, UT 84070.
3*Polypropylene urinary catheter, Jorgensen Laboratories, Inc., Loveland, CO 80538.
4*Vetrap™, 3M, St. Paul, MN 55144-1000.
5*Port-a-Cul™, BD Biosciences, Franklin Lakes, NJ 07417.
Feeding a Bovine Colostrum Supplement Decreases the Duration of Upper Respiratory Disease in Thoroughbred Yearlings

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The data in this study show that a supplement that contains bovine colostrum is useful in yearlings for decreasing the duration of naturally occurring upper respiratory tract disease. Authors’ addresses: Equine Integrated Medicine, PLC, 4904 Ironworks Road, Georgetown, KY 40324 (Fenger, Langemeier); Gluck Equine Research Center, University of Kentucky, Lexington, KY 40546-0099 (Tobin); Mont Liggins Charitable Trust, Research Centre in Reproductive Medicine, University of Auckland, Auckland, New Zealand (Casey); Department of Statistics, University of Kentucky, Lexington, KY 40504 (Roualdes); Gunston Hall, Lexington, KY 40511 (Cowles); Department of Veterinary Microbiology, Western College of Veterinary Medicine, University of Saskatchewan, 52 Campus Drive, Saskatoon, SK S7N 5B4, Canada; and The Saskatoon Colostrum Co. Ltd., 30 Molaro Place, Saskatoon, SK S7K 6A2, Canada (Haines); e-mail: drfenger@hotmail.com *Corresponding and presenting author. © 2015 AAEP.

1. Introduction
Bovine colostrum (BC) is used in humans as a nutritional supplement for immune support and has been shown to reduce upper respiratory disease (URD). The aim of this prospective blinded randomized clinical trial was to evaluate the effects of a BC-based supplement on the proportion of the study period affected, duration of each event, and incidence of URD that occurred in yearlings.

2. Materials and Methods
A total of 109 yearlings on 2 farms were randomly assigned to treatment or placebo groups. Treatment yearlings were supplemented once daily for 17 to 25 weeks with 50 g of a commercial BC-based supplement applied as a “top-dress” to feed. Yearlings were observed weekly for signs of URD and weighed monthly.

3. Results
All 109 yearlings completed the study. The proportion of the study period that each yearling exhibited illness upon supplementation was considerably shorter (least squares mean = 23% of the study period) than yearlings on the placebo (least squares mean = 34% of the study period; P = 0.002). The average duration of illness was shorter for yearlings on the supplement (1.96 weeks) than yearlings on...
the placebo (4.39 weeks; \( P < 0.0001 \)). There were no differences in weight gains or incidence of URD in yearlings.

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**Declaration of Ethics**

The Authors declare that they have adhered to the Principles of Veterinary Medical Ethics of the AVMA.

**Conflict of Interest**

Dr. Fenger is a paid consultant for Animal Healthcare Products and Packaging, Inc., and Saskatoon Colostrum Company; Dr. Haines is an employee of Saskatoon Colostrum Company.
Wellbeing of New York City Carriage Horses

Sarah Mercer-Bowyer, BS*; David Kersey, PhD; and Joseph J. Bertone, MS, DVM, DACVIM

New York City carriage horses (NYCCHs) have no evidence of increased fecal nor salivary cortisol nor increased infrared thermography (IRT) medial canthus temperature throughout workdays. Authors' address: Western University of Health Sciences, College of Veterinary Medicine, 309 East Second Street, Pomona, CA 91766; e-mail: smercer@westernu.edu. *Corresponding and presenting author. © 2015 AAEP.

1. Introduction
Objective information concerning NYCCH lifestyle and physiologic status is lacking. We employed noninvasive approaches to assess stress in NYCCHs during the course of their daily routine to determine whether their lifestyle affects their wellbeing.

2. Materials and Methods
Samples were collected on three successive days from ten, eight, and nine horses at rest (time point [TP] 1), preparation for work (TP2), return (TP3) and post 1 hour (TP4). Fecal glucocorticoids, salivary cortisol, and medial canthus thermography (IRT) were collected. Feces was also collected from five pastured NYCCHs.

3. Results
No difference was found in fecal glucocorticoids between pastured (22.1 ± 9.8 ng/g) and NYCCH horses (19.5 ± 4.2 ng/g; P = .9855). Salivary cortisol differences were identified between TP3 (0.96 ± 0.06 ng/ml) and TP4 (0.77 ± 0.07 ng/ml; P = .02). All other time points were not different (P > .05). TP2 IRT (35.5 ± 0.64°C [95.9 ± 1.2°F]) was lower than TP3 (36.2 ± 0.64°C [97.1 ± 1.2°F]). No difference was found among other time points (P > .05).

4. Discussion
The NYCCHs investigated in this study did not exhibit physiologic responses indicative of a negative welfare state.

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Declaration of Ethics
The Authors declare that they have adhered to the principles of Veterinary Medical Ethics of the AVMA.

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Seroprevalence of Sarcocystis neurona and Neospora hughesi Among Healthy Horses in the United States

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This contemporary U.S. horse seroprevalence survey of S. neurona and N. hughesi, the two causative agents of equine protozoal myeloencephalitis (EPM), found upwards of 79% and 34% seroprevalence, respectively, in healthy horses. The distribution of these two protozoal parasites in healthy horses shows that S. neurona is ubiquitous across the country (with slightly lower prevalence in the West), and N. hughesi is evenly spread across all four regions (no significant difference between regions). These estimates are higher than previous studies suggest, and this high background seroprevalence should be taken into account when performing EPM diagnostic tests. Authors’ addresses: Department of Medicine and Epidemiology, School of Veterinary Medicine (James, Smith, Pusterla), and Department of Pathology, Microbiology and Immunology (Conrad, Packham, Guerrero, Ng), School of Veterinary Medicine, University of California, Davis, CA 95616; e-mail: kjames@ucdavis.edu. *Corresponding and presenting author. © 2015 AAEP.

1. Introduction
EPM is one of the most common infectious neurological diseases affecting horses in North America. The objective of this study was to describe the general seroprevalence of S. neurona and N. hughesi infection among healthy horses using the indirect fluorescent antibody test, as well as prevalence of risk factors.

2. Materials and Methods
Whole blood from 5250 horses was collected across 18 states in October 2013. Indirect fluorescent antibody test was used to determine antibody titers to the two parasites, and mixed effects logistic regression models were created to determine prevalence odds ratios.

3. Results
The percentage of S. neurona positive horses was 79%. N. hughesi had 34% positivity, the percentage of dual infections of S. neurona and N. hughesi was 31%, and the percentage of dual negative horses was 18%. Risk factors for seroprevalence included the geographic origin (South), Warmblood breed, and increasing age.

4. Discussion
Implications of these results are contemporary knowledge in the background infection rates, distributions, and risk factors associated with the two causative agents of EPM.

Acknowledgments
The study was supported by a grant from the Center for Equine Health at the University of California at Davis.

Declaration of Ethics
The Authors declare that they have adhered to the Principles of Veterinary Medical Ethics of the AVMA.

Conflict of Interest
The Authors declare no conflicts of interest.
Pathology of the Articular Processes in Horses With Cervical Stenotic Myelopathy

Jennifer Janes DVM, PhD, DACVP

Articular process skeletal pathology is not limited only to the site of compression in horses with cervical stenotic myelopathy (CSM) and involves both the articular cartilage and bone. Author’s address: Veterinary Diagnostic Laboratory, University of Kentucky, Lexington, KY 40511; e-mail: jennifer.janes@uky.edu. © 2015 AAEP.

1. Introduction
The exact etiology and pathogenesis of skeletal lesions in the cervical vertebrae of horses with CSM (Wobbler Syndrome) remains unclear. The study objective was to assess bone and cartilage lesions in articular processes observed on magnetic resonance imaging (MRI) to achieve a better understanding of their distribution and type.

2. Materials and Methods
Twenty Thoroughbred horses with CSM were compared with nine control Thoroughbred horses. Antemortem, subjects underwent a neurologic examination and standing cervical radiographs. Cervical column MRI studies were performed postmortem. Articular process lesions were identified on MRI and classified based on frequency, location, and severity. A subset of lesions was further characterized using microcomputed tomography and histopathology.

3. Results and Discussion
Lesions identified by MRI occurred with an increased frequency and severity in diseased horses and were not limited to the specific sites of spinal cord compression. The most common histopathologic lesions included osteochondrosis, osseous cyst-like structures, fibrous tissue replacement of trabecular bone, retained cartilage spicules, and osteosclerosis. This is the first report of osseous cyst-like structures in the articular processes of horses with CSM. The observed pathologic lesions and their generalized distribution provide additional support for a model of CSM pathogenesis in the cervical spine that involves primary developmental abnormalities of the vertebrae with secondary biomechanical changes.

Acknowledgments
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Declaration of Ethics
The Author declares that she has adhered to the Principles of Veterinary Medical Ethics of the AVMA.

Conflict of Interest
The Author declares no conflicts of interest.
Comparison Between Staphylectomy and “Tie-Forward” Procedures for the Treatment of Intermittent Dorsal Displacement of the Soft Palate in Standardbred Trotting Horses

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Analysis of racing speed and career results of horses with intermittent dorsal displacement of the soft palate (IDDSP) indicated no difference pre- or postoperatively between staphylectomy and tie-forward-treated horses. Authors’ addresses: Hallands Djursjukhus, 31168 Söinge, Sweden (Johansson); and Department of Large Animal Clinical Sciences, Western College of Veterinary Medicine, University of Saskatchewan, Saskatoon, SK S7N 5B4 Canada (Carmalt, Waldner); e-mail: james.carmalt@usask.ca. *Corresponding and presenting author. © 2015 AAEP.

1. Introduction
Comparative success rates for the treatment of IDDSP using a concurrent reference population using an established performance database have not been reported. The objectives were to compare the performance of horses with IDDSP treated with a staphylectomy with those treated with a “tie-forward” procedure and control horses. Secondly to determine whether there was a difference in postoperative performance between surgical techniques.

2. Materials and Methods
Standardbreds with endoscopically confirmed IDDSP (n = 56) and 48 with endoscopically normal upper airways were compared with 90 age and sex-matched control horses. Racing data were retrieved from online racing records (n = 4304 races). Generalized estimating equations controlling for horse were used to compare pre- and post-surgery racing performance (speed, m/s). Similarly, the effect of surgical procedure type on whether horses returned to racing, postsurgical speed, career race starts, and earnings were evaluated.

3. Results
Horses gained speed as a function of race number. There was no significant difference in race speed between IDDSP and endoscope-negative control horses. The percentage of horses racing post operatively, number of career races and career earnings, did not differ significantly between surgical methods or between IDDSP and control horses.

4. Discussion
There was no difference between the two surgical techniques used to treat IDDSP in this population of horses. Reported superiority of newer techniques may be biased by comparisons to historical reports using presumptive diagnoses.

Research Abstract—for more information, contact the corresponding author
Acknowledgments

Declaration of Ethics
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Conflict of Interest
The Authors declare no conflicts of interest.
Cerebral and Brainstem Electrophysiological Activity During Euthanasia With Pentobarbital Sodium in Horses

Monica Aleman, MVZ Cert., PhD, DACVIM*; Diane C. Williams, PhD; Alonso Guedes, DVM, MS, PhD, DACVA; and John E. Madigan, DVM, MS, DACVIM, DACAW

The goal of euthanasia is to end the life of equids meeting criteria for euthanasia in a painless and minimally distressful way. Time and sequence of respiratory, cardiovascular, and brain death was investigated. Intravenous overdose of barbiturates is an effective and fast method of euthanasia producing cortical electrical silence supportive of lack of conscious perception followed by loss of cardiac output, brainstem death, and lastly, loss of electrocardiogram activity. Authors' address: William R. Pritchard Veterinary Medical Teaching Hospital, University of California, Davis, CA 95616; e-mail: mraleman@ucdavis.edu. *Corresponding and presenting author. © 2015 AAEP.

1. Introduction
An overdose of pentobarbital sodium administered intravenously is the most commonly used method of euthanasia in veterinary medicine. Determining death after the infusion relies on the observation of physical variables. However, the time and sequence of events of respiratory, cardiovascular, and brain function loss is unknown.

2. Materials and Methods
This was a prospective observational study. Horses with untreatable neurologic, orthopedic, and cardiac illnesses were selected and instrumented for recording of electroencephalogram, electrooculogram, brainstem auditory evoked response, and electrocardiogram. Physical and neurological (brainstem reflexes) variables were monitored. The study was approved by an institutional animal care and use committee and owner consent was obtained.

3. Results
Loss of cortical electrical activity occurred during or within 52 seconds after the infusion of euthanasia solution. Cessation of brainstem function (loss of brainstem reflexes and brainstem auditory evoked response) happened within 4 minutes. Despite undetectable heart sounds, palpable arterial pulse, and mean arterial pressure, recordable electrocardiogram was the last variable to be lost after the infusion (mean, 9.3 min; median, 8.35 min; range, 5.5–16 min).

4. Discussion
Overdose of pentobarbital sodium solution administered intravenously is an effective and fast method of euthanasia. Brain death occurs within 73 to 261 seconds of the infusion. Although absence of electrocardiogram activity takes longer to occur, brain death has already occurred.

Acknowledgments
The study was funded by gifts from private donors to the Comparative Neurology Research Group.

Declaration of Ethics
The Authors declare that they have adhered to the Principles of Veterinary Medical Ethics of the AVMA.

Conflict of Interest
The Authors declare no conflicts of interest.

Daniela Luethy, DVM*; Perry Habecker, VMD, DACVP; Brian Murphy, DVM, PhD, DACVP; and Rose Nolen-Walston, DVM, DACVIM

Pheochromocytoma is a rare endocrine tumor that rarely causes the classic signs of paroxysmal catecholamine storm reported in humans and dogs. Usually an incidental finding at necropsy, pheochromocytoma may cause acute death from intraperitoneal exsanguination and should be considered in horses presenting with colic, tachycardia, and hemoperitoneum. Authors’ addresses: School of Veterinary Medicine, University of Pennsylvania, Clinical Studies, New Bolton Center, Kennett Square, PA 19348 (Luethy, Habecker, Nolen-Walston); University of California, Davis, School of Veterinary Medicine, Department of Pathology, Microbiology and Immunology, Davis, CA 95616 (Murphy); e-mail: dluethy@vet.upenn.edu. *Corresponding and presenting author. © 2015 AAEP.

1. Introduction
Pheochromocytoma is the most common adrenal medullary neoplasm of domestic animals, although rare in horses. Antemortem diagnosis in horses is difficult, with clinical signs often being vague and nonspecific. The purpose of this study is to describe the clinical, laboratory, and pathologic findings of pheochromocytoma in horses.

2. Materials and Methods
Records from two institutions were reviewed for horses diagnosed with pheochromocytoma on post-mortem examination from 2007 to 2014.

3. Results
Pheochromocytoma was identified in 37/4094 horses at post-mortem. Associated clinical signs were observed ante-mortem in seven cases, with the remainder being incidental findings. Colic was the most common presenting complaint (35%) and tachycardia was noted in 95% of cases (median, 86 bpm in clinical cases). Hyperlactatemia (median, 4.9 mmol/L) and hyperglycemia (median, 184 mg/dL) were the most common clinicopathologic abnormalities. Hemoperitoneum secondary to rupture was noted in 4/7 clinical cases. Concurrent endocrine tumors were found in 27/37 horses with 8/37 horses displaying lesions consistent with multiple endocrine neoplasia syndrome in humans.

4. Conclusions
Pheochromocytoma was diagnosed in 0.95% of cases presented for necropsy. The majority of these were incidental findings; however, pheochromocytoma was thought to contribute to clinical disease in seven of 37 cases (19%), and multiple endocrine neoplasms were commonly seen.

Acknowledgments
Declaration of Ethics
The Authors declare that they have adhered to the Principles of Veterinary Medical Ethics of the AVMA.
Conflict of Interest
The Authors declare no conflicts of interest.
Magnetic Resonance Imaging of the Pituitary Gland of Horses With Pituitary Pars Intermedia Dysfunction

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Magnetic resonance imaging (MRI) can effectively document pituitary gland (PG) and pars intermedia (PI) size as well as detail morphologic changes within the PI of equids with pituitary pars intermedia dysfunction (PPID). Authors’ addresses: Department of Large Animal Clinical Sciences (Schott, Pease), and Department of Pathobiology and Diagnostic Investigation (Patterson, Howey), Michigan State University, East Lansing, MI 48824; Department of Physiological Sciences, Center for Health Sciences, Oklahoma State University, Stillwater, OK 74074 (McFarlane); and Vetsuisse-Fakultät, Universität Bern, Bern, Switzerland (van der Kolk); e-mail: schott@cvm.msu.edu. *Corresponding and presenting author. © 2015 AAEP.

1. Introduction
PPID is the most common endocrine disease of aged horses. We hypothesized that enlargement of the PG in PPID-affected horses could be documented by MRI and that pathologic changes within the PI can be graded in a repeatable manner using MR images with results comparable to histological grading.

2. Materials and Methods
MRI was performed immediately prior to euthanasia in 21 horses: 13 with clinical signs of PPID and supportive endocrine test results and four aged and four young non-PPID-affected horses. The PG was removed for direct measurement of weight, height, and length. Total PG and PI areas were measured on midline sagittal MR images and histological sections and compared by correlation analysis. A MRI PI grading system (1–5) was used to grade MR images and mean MRI and histological grades were compared.

3. Results
PGs weighed 1.7 to 10.1 g and included PGs that ranged from 1–5 on both MRI and histological PI grades. Gross tissue, MRI, and histological measurements were highly correlated and mean MRI grade was highly correlated with mean histological grade (r = 0.83, P < .01). Both micro- and macroadenomas could be visualized by MRI. T2-weighted images provided the greatest contrast and anatomical detail.

4. Discussion
MRI is a useful tool to determine PG and PI size and morphologic changes within the PI of PPID-affected horses.

Acknowledgments
Declaration of Ethics
The Authors declare that they have adhered to the Principles of Veterinary Medical Ethics of the AVMA.
Conflict of Interest

Drs. Schott and McFarlane are consultants for Boehringer-Ingelheim, Vetmedica, Inc. This project was funded by the Equine Health and Performance Endowed Fund, College of Veterinary Medicine, Michigan State University, East Lansing, MI.
Review of Nonsteroidal Anti-Inflammatory Drug Selection in Horses

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Nonsteroidal anti-inflammatory drug (NSAID) selection in horses should be based on label indications, adverse effect profile, and efficacy, with consideration of the pathogenesis of the disease and mechanism of pain (i.e., inflammatory vs mechanical pain). Authors’ address: Faculty of Veterinary Medicine, University of Calgary, Calgary, AB T2N 4Z1, Canada; e-mail: hebanse@ucalgary.ca. *Corresponding; †presenting author. © 2015 AAEP.

1. Introduction
NSAIDs have long been used for treatment of pain, fever, and inflammation. The identification of isoforms of cyclooxygenase (COX) enzymes 25 years ago has allowed for development of COX selective drugs and assessment of the risks and benefits associated with using COX-selective compounds. Despite the advent of other pain medications, NSAIDs remain a central component of pain management in horses. NSAIDs are considered effective, relatively inexpensive, and convenient to administer. The purpose of this presentation is to review current evidence regarding safety and efficacy of NSAIDs in common equine disorders.

2. Mechanism of Action
NSAIDs work through inhibition of COX enzymes. Cyclooxygenase enzymes metabolize arachidonic acid into the common precursor prostaglandin (PG), prostaglandin H₂, which is then converted into a variety of eicosanoids depending on the enzymatic complement of the cell type. COX-1 is constitutively produced and is typically associated with the downstream production of PGs responsible for homeostatic functions, including gastrointestinal mucosal integrity, renal blood flow, and coagulation. COX-2 is induced by injury and inflammation, typically leading to the production of inflammatory and regulatory PGs. However, COX-2 is constitutively expressed in some tissues, including the kidney.¹

NSAIDs that inhibit both COX enzymes at therapeutic doses are termed nonselective, whereas those that inhibit primarily COX-2 enzymes are called COX-2 selective. Although there is no evidence for an overall class superiority when it comes to efficacy, there may be differences between patients or conditions. There is some evidence to suggest that COX-1 inhibitors may be more effective for mechanical pain, which is primarily centrally mediated,²³ whereas COX-2 selective drugs may be more useful for inflammatory pain, which is in part peripherally-mediated.⁴⁵

COX selectivity is also important with respect to adverse effect profile. NSAIDs are associated with a number of adverse effects, including renal medullary necrosis, oral, gastric, intestinal, and colonic ulceration, and coagulopathy primarily related to inhibition of constitutively expressed COX enzymes.⁶⁻⁸ In horses, there is also a report of ulcerative cystitis as-
associated with NSAID administration.

In most species, COX-2 selective compounds seem to be safer for the gastrointestinal (GI) tract, but there is no evidence that COX selectivity alters negative effects on renal function.

NSAIDs commonly used in horses include the systemic drugs, aspirin, phenylbutazone, flunixin meglumine, firocoxib, ketoprofen, meloxicam, and the topical agents, diclofenac, and flurbiprofen. In horses, flunixin meglumine, phenylbutazone, and ketoprofen are relatively nonselective, whereas firocoxib (265:1), robenacoxib (61:1), deracoxib (26:1), etodolac (4:1), and meloxicam (4:1) are relatively COX-2 selective at IC50. An overview of recommended indications and dose regimens for commonly used NSAIDs is provided in Table 1.

### 3. Adult Horses

Musculoskeletal Disorders

Musculoskeletal disorders may result in inflammatory pain, mechanical pain, or a combination of both. Anecdotally, phenylbutazone is probably the most commonly used NSAID for treatment of orthopedic pain in horses and is commonly cited as being more effective than other NSAIDs, although there is not strong evidence to support this.

Inflammatory Orthopedic Pain: Synovitis

Efficacy of NSAIDs has been studied in multiple experimental models of equine synovitis, including lipopolysaccharide (LPS) or irritant-induced synovitis (carregeenan or Freund’s adjuvant). In general, NSAIDs seem to relieve clinical signs and clinicopathologic abnormalities associated with synovitis. However, few studies investigating the relative efficacy of NSAIDs in these models have been performed. One study suggested that pretreatment with phenylbutazone was more effective than ketoprofen in attenuating clinical signs of carregeenan-induced synovitis at label doses.

In contrast, phenylbutazone and flunixin meglumine had similar improvements in clinical signs when administered following induction of synovitis using Freund’s adjuvant. Although both meloxicam and phenylbutazone have been demonstrated to improve lameness associated with LPS-induced synovitis, only meloxicam improved markers of inflammation-induced cartilage catabolism, suggesting that meloxicam may be a reasonable first choice for synovitis.

Mechanical Orthopedic Pain: Heart Bar Model and Navicular Disease

The heart bar model is a useful experimental model that is considered to produce mechanical pain. Only one study has directly compared NSAID efficacy in this model. There were no significant differences in efficacy of label doses of flunixin meglumine compared with phenylbutazone for 4 hours after induction of lameness in the heart bar model of lameness.

When evaluating naturally occurring disease representative of mechanical pain, such as navicular disease, there was a significant improvement in clinical signs of lameness in horses with navicular disease for up to 24 hours following treatment with either flunixin meglumine or phenylbutazone.

Taken together, these findings suggest that either flunixin or phenylbutazone are reasonable alternatives in treatment of mechanical pain.

Mechanical and Inflammatory Pain: Osteoarthritis

Pain secondary to osteoarthritis may result from both inflammatory and mechanical pain. Unfortunately, studies comparing NSAID treatments in naturally occurring osteoarthritis are limited. In a prospective clinical trial evaluating NSAID efficacy in treatment of chronic osteoarthritis, overall clin-
Mechanical and Inflammatory Pain: Laminitis

Depending upon the acuity and pathogenesis (endocrine vs inflammatory), laminitis may be characterized primarily by either inflammatory or mechanical pain. Inflammation-induced laminitis is associated with changes in platelet function and digital blood flow. Aspirin is a COX-1-selective NSAID at low doses that inhibits thromboxane synthesis from platelets, white blood cells, and blood vessels. Because aspirin irreversibly binds to the COX-1 enzyme, it prevents COX activity for the life span of the platelet. A recent study demonstrated that pretreatment with low-dose aspirin was effective in ameliorating both the LPS-induced decrease in digital blood flow and increase in platelet and leukocyte thromboxane A2 production. These findings suggest that aspirin may be a useful therapy to consider in cases of endotoxemia to prevent the onset of laminitis.

In horses with chronic laminitis, neither phenylbutazone (4.4 mg/kg, IV) nor ketoprofen (2.2 mg/kg, IV) changed lameness grade when compared with control over the subsequent 24 hours, although both ketoprofen and phenylbutazone improved hoof withdrawal response 6 hours after administration. This study only evaluated the effect of a single dose of an NSAID, and it may be that multiple doses are required before a treatment effect is observed. In chronic laminitis, centrally mediated or neuropathic pain has been theorized to play an important role in pain sensation. In other species, COX-1 has been shown to be an important component of mechanical pain or neuropathic pain, which may be ameliorated by COX-1 inhibition. Therefore, there may be benefit to using a nonselective NSAID, such as flunixin meglumine or phenylbutazone initially in cases of chronic laminitis. However, over the long term, transition to a COX-2 selective is recommended to limit gastrointestinal (GI) adverse effects.

Gastrointestinal Disorders

Colic is a common condition of horses that is often treated in part with NSAIDs. Although NSAIDs seem to be effective for managing GI-mediated pain, NSAIDs should be used judiciously due to their adverse GI effects. Both COX-1 and COX-2 are constitutively expressed in the jejunum and pelvic flexure. However, COX-2 seems to play an important role in GI healing. COX-2 gene expression is increased in ulcerated nonglandular gastric mucosa of horses and protein expression is increased in small intestinal mucosa subsequent to an ischemic event. Following small intestinal ischemia, COX-2 expression has been shown to increase rapidly (following 2 h of ischemia) while COX-1 expression seems to be slower to increase. This increase in expression was associated with an increase in transepithelial resistance, suggesting an increase in mucosal healing. Following an ischemic small intestinal event, the nonselective COX inhibitor, flunixin meglumine, impaired mucosal healing, as assessed by transepithelial resistance. In contrast, the COX-2-selective NSAIDs firocoxib and meloxicam did not seem to have a marked effect on rate of healing. A clinical trial comparing meloxicam and flunixin meglumine for management of pain following colic surgery suggested that there were no differences in overall pain scores between the two treatment groups, but more overt signs of pain in postoperative colic patients treated with meloxicam compared with flunixin meglumine. Importantly, meloxicam was administered at twice the label dose (0.6 mg/kg every 12 h). One study demonstrated that COX-2 selectivity ratio of meloxicam changed from 3.8 (at IC50) to 2.2 (at IC80) but the clinical significance of this change is unknown.

Although small intestinal mucosal response to ischemia and NSAIDs has been relatively well studied, limited data exists with respect to colonic responses. One study suggested that flunixin meglumine did not alter colonic mucosal healing responses. When evaluating the effects of NSAIDs on experimental endotoxemia, both flunixin meglumine and phenylbutazone have been shown to attenuate the effects of endotoxin when used as a pretreatment, although flunixin meglumine (1.1 mg/kg) seems to be superior to phenylbutazone (2.2 mg/kg). With respect to dose effect, low doses of flunixin (0.25 mg/kg) were shown to attenuate thromboxane production and overt signs of abdominal pain associated with endotoxin, without affecting other important surgical indicators. The lower dose may decrease the adverse effects associated with NSAID administration.

Collectively, these findings suggest that COX-2-selective drugs, such as meloxicam or firocoxib, may be preferred in small intestinal colic if there are no
signs of endotoxemia. Unfortunately, the efficacy of COX-2-selective NSAIDs in equine endotoxemia has not yet been adequately investigated. In the absence of such data, low-dose flunixin may be useful in the early treatment of endotoxemia, but should not be used in conjunction with another NSAID due to increased risk of toxicity when stacking NSAIDs. Additional study is required prior to determination of the preferred NSAID for large intestinal colic.

Ophthalmic Disorders
Limited data exist with regard to relative efficacy of NSAIDs for ocular pain and inflammation. Both systemic and topical anti-inflammatory drugs are used for management of pain and inflammation associated with corneal ulceration. When evaluating systemic NSAIDs, flunixin meglumine and firocoxib have been demonstrated to penetrate into the aqueous humor of healthy equine eyes. Anecdotal, flunixin meglumine seems to be more effective than firocoxib in ocular pain such as corneal ulceration. With regard to topical ophthalmic NSAID preparations, ketorolac, diclofenac, flurbiprofen, and suprofen are available. In dogs, there was no difference in the NSAIDs ability to stabilize the blood-aqueous barrier in an experimental model of uveitis using 1% ophthalmic solutions. There is not adequate evidence in horses to recommend one ophthalmic preparation over the other. However, in the authors’ experience, topical NSAID therapy alone is usually insufficient to control acute ocular pain and inflammation and systemic NSAIDs are required.

4. Foals
NSAIDs should be used judiciously in foals, particularly in sick or premature neonates who may have compromised renal function. A recent study of the COX-2-selective NSAID, meloxicam, suggests that this drug may be used in healthy foals < 6 weeks of age (0.6 mg/kg every 12 h) with limited adverse effects. Firocoxib at a dose of 0.1 mg/kg by mouth for 9 days did not produce any noticeable adverse effects in neonates (age, 36 h); however, plasma concentrations were much lower than those achieved in adult horses and whether those concentrations are therapeutic in foals remains to be determined. Regardless, research to date has been confined to a small number of healthy foals, and it remains a risk to use these drugs in hypovolemic, dehydrated, or premature neonates. It is the authors’ preference to avoid NSAIDs altogether in foals less than 30 days of age and use alternative pain management strategies.

5. Summary
Historically, flunixin meglumine has been the NSAID of choice for gastrointestinal pain, whereas phenylbutazone has been chosen for orthopedic pain. However, the advent of newer COX-2-selective compounds has broadened the opportunities for selection based on the relative importance of COX-1 or COX-2 in pathogenesis of and recovery from disease. Furthermore, evaluation of the current literature challenges the frequent selection of phenylbutazone for all orthopedic conditions. Selection of NSAIDs in horses should be made keeping in mind label indications, efficacy, and safety. When efficacy data are unavailable, the pathogenesis and mechanism of pain (inflammatory vs mechanical) should be considered.

Acknowledgments

Declaration of Ethics
The Authors declare that they have adhered to the Principles of Veterinary Medical Ethics of the AVMA.

Conflict of Interest
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References
44. Vol. 61 2015 / AAEP PROCEEDINGS
How to Analyze and Improve the Accuracy of Veterinary Inventory Demand Forecasting

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1. Introduction
The business-savvy veterinary practitioner recognizes that controlling costs associated with inventory is essential to the lean process management techniques necessary to improve gross profit margins, which ultimately lead toward increased practice profitability and value creation. Holding costs associated with holding too much inventory are a drain to cash resources due to tied-up capital and associated opportunity costs, together with the unavoidable factors of inventory shrinkage, expiration, and obsolescence. At the same time, shortage costs generated by the lack of adequate inventory required for the provision of services affect the profitability of the practice through the loss of revenue. This can be manifested through the loss of service provision, or even worse, the loss of a client’s customer lifetime value to the practice.

Inventory demand forecasting is the process of predicting demand for future service events and the corresponding inventory required for the facilitation and provision of the predicted demand for services. By nature, forecasting is affected by the practice’s external environment and is critically influenced by those factors that are out of the control of the practice such as economic, political, and industry conditions. In addition, forecasting can be greatly influenced by internal decisions associated with demand generation through the use of advertising for services during slow time periods, creating financial incentives for employees, or by temporary reduction in client pricing.

Whereas predicting the future inventory demand created by the normal running course of the business presents a difficult task, controllable and uncontrollable influence factors will affect the predictability of demand for future inventory even further. Business owners need the tools necessary to better predict the demand for future inventory that will ultimately decrease the associated costs of goods.

2. Forecasting Analysis
The first step in developing a system for demand forecasting is the analysis of previous forecasting performance through the measurement of past forecasting accuracy. Though actual methods of forecasting accuracy such as error, mean error, and mean absolute error tell us by how many units the forecast has been wrong, these methods are not measured relative to the total volume of demand. Knowing the quantity of units the forecast is off by provides little help when the information is analyzed outside the context of total demand volume; a
practitioner would be doing a great job being 10 units off in forecasting when the total volume is 1000, but would be doing poorly if the total volume was 25. In addition, unit error would fail to provide a proper point of reference for comparing different volume-sized inventory items.

Measurements of forecasting accuracy relative to a perfect forecast, on the other hand, describe the percent by which the forecast is wrong and are therefore best suited to the goals of the lean inventory management process. These methods are based on the measurement of the percent by which the forecasting technique is off in each period (percent error; PE) and on the measurement of the forecasting technique performance over multiple periods (mean absolute percent error; MAPE) (see Appendix). MAPE, specifically, will tell us how well we are doing over time in terms of error. As seen in the example presented in Table 1, the PE for each period is the percentage by which the forecast was over- or underestimated compared with actual sales or usage. The running MAPE is based on the sum of the absolute value of all the periods’ PEs divided by the number of periods. The example in Table 1 shows that after four periods, the forecasting technique has cumulatively been off by 21.03% (4.84 + 2.04 + 5.17 + 8.97), which translates to a MAPE of 5.26% (21.03/4). What this means is that, on average, the forecast is off by 5.26% each quarter through the total time period measured.

A percent-based error measurement allows for performance comparison among different volume-sized inventory items. By better understanding the effectiveness the forecasting technique has had over time, the practitioner can better adjust and improve the forecasting technique for future demand to achieve increased accuracy. An Excel spreadsheet calculator for PE and MAPE is provided for download at https://db.tt/xzw2oxvT.

3. Forecasting Techniques

There are qualitative forecasting techniques that rely on intuition, experience and judgment, and quantitative forecasting techniques that rely on analysis of historical sale patterns through time. Although the young practice will have to rely on qualitative techniques that, by definition, should incur a higher degree of forecasting error, the seasoned practice can incorporate quantitative techniques based on the practice’s own historical data. The ability to adapt the forecast based on observed seasonal changes and trend in sales patterns will allow for an improved forecasting accuracy. Notwithstanding the importance of data, qualitative techniques based on insights, experience, and knowledge of external factors should be factored into the forecast to further improve the forecasting accuracy. Qualitative techniques will not do a good job on their own, but they will substantially improve the effectiveness of the quantitative techniques which, by themselves, cannot foresee changes in the external environment.

Simple Exponential Smoothing

Exponential smoothing is a forecasting technique that places a percentage weight on the last period’s sales and the rest of the percentage weight on the last period’s forecast; a period being described as the unit of time being analyzed (e.g., if forecasting quarterly demand, “last period” refers to the previous quarter; if forecasting monthly demand, “last period” refers to the previous month). Exponential smoothing is described by the formula \( F_t = \alpha \times Y_{t-1} + [1 - \alpha] \times F_{t-1} \) (see Appendix) and indicates that this period’s forecast \( (F_t) \) is dependent on the last period’s actual sales \( (Y_{t-1}) \) and forecast \( (F_{t-1}) \). This enables the forecast to account for all of the older data’s forecasts that were developed from all of the older data’s sales. In simple exponential smoothing, a value called alpha (\( \alpha \)) sets the weight assigned to the last period’s sales \( (Y_{t-1}) \). The weight not applied to the last period’s sales is therefore applied to the last period’s forecast (through \( 1 - \alpha \)). The higher the alpha, the higher the weight of this period’s forecast \( (F_t) \) that is placed on the last period’s sales \( (Y_{t-1}) \) and the lower the weight placed on the last period’s forecast \( (F_{t-1}) \), which contains the historical data. The result of a high alpha is that a large weight is placed on the last period’s actual demand; therefore, the forecast is more reactive to the latest changes in demand. As seen in Table 2, when alpha is 1, the forecast for this period is identical to the sales in the last period (forecast for quarter 2 same as sales for quarter 1). The result of a high alpha is that the future forecast becomes more responsive to the last period’s sales and does not take into account the previous history. If the demand changes are trending, then a higher alpha will provide a better weighted average for forecasting because the forecast changes as it reacts...
to the changes in trending sales. Table 2 shows that, when sales are trending, a higher alpha provides a better forecast, as measured by the resulting MAPE than a lower alpha.

A low alpha, in contrast, places more weight on the historical data and the forecast is less responsive to recent changes in demand. As alpha reaches 0, the future forecast does not take into strong account the recent demand history; in fact, when alpha is 0, the forecast is identical to the last period's forecast. As shown in Table 2, a small alpha fails to properly respond in a timely fashion to trend changes and therefore results in a higher MAPE.

Table 3 shows that when sales are random, the reactivity of a high alpha will lead to a lower forecasting accuracy created by the bouncing reaction to changes in sales, whereas a lower alpha would dampen the effect of randomness by not being reactive; therefore resulting in a lower MAPE.

Exponential Smoothing for Seasonality
Most veterinary practitioners are faced with a repeating pattern of sales increases and decreases occurring within a 1-year period; a concept termed seasonality. In cases where there is seasonality in the demand pattern, a quantitative analysis of historical sales patterns must be performed so that a seasonality adjustment can be made to the exponential smoothing forecasting technique. After mathematically de-seasonalizing the data, the smoothing forecasting technique can be applied to the new data. Once a forecast is developed for a future period, the data can then be re-seasonalized.

Exponential Smoothing for Seasonality and Trend
The veterinary practice that is experiencing not only seasonality, but also a trending change, needs an exponential smoothing quantitative forecasting technique that incorporates adjustments for trend and seasonality through statistics to improve inventory demand forecasting accuracy.

The explanation of the mathematical calculation of exponential smoothing for trend and seasonality goes beyond the scope of this article but the calculation of such forecasts can be performed within the previously mentioned Excel spreadsheet that can be downloaded at https://db.tt/xzw2oxvT.

4. The Need for Improved Forecasting Accuracy
Excessive spending related to overestimation of demand or to loss of revenue through the shortage costs related to underestimating demand result in a negative cash flow that reduces operational efficiency. By definition, an increase in inventory investment is seen as an increase in working capital requirements that must be satisfied through tying up of existing cash reserves or through increased capital investments from outside sources of cash. A concurrent increase in cost of goods, or potential loss of revenues, leads to decreased margins that translate into decreased profits. The combination of reduced profits and increased capital investments leads to a reduction in returns and a reduction in shareholder value.

In contrast, an improved accuracy in demand forecasting leads to an increase in profits through a reduction in total annual inventory costs. Better
forecasting increases revenue by reducing shortage costs and decreases cost of goods sold by decreasing the holding costs associated with excess inventory. At the same time, the reduced inventory capital investment reduces working capital requirements, which reduces the practice’s cash flow toward inventory. More importantly, the increase in gross profit margin and decrease in working capital requirements lead to an increase in profitability and shareholder value.

5. Translating Improved Forecasting Accuracy Into Cost Savings

As previously described by the author, the total actual inventory cost is comprised of the purchase cost, holding cost, and order cost. As defined by the formulas presented in the Appendix, the forecasted demand is a key component of the calculations. The example that follows in Table 4 presents a real-life situation where minimization of PE and MAPE led to a better demand forecast and, in turn, a reduced cost of inventory. In addition, the example presents how going one step further within the process of lean inventory management through the calculation of the economic order quantity further enhanced the cost savings that was originally started by the improved demand forecast.

The example in Table 4 clearly indicates how improving the forecasting accuracy led to a savings of 8.3% on just this one inventory item. The generated savings go directly into increasing the bottom line by increasing gross profit margin and increasing profits net of taxes, a scenario that directly translates into increased shareholder value.

6. Measuring the Efforts

The efforts toward not having too much inventory on hand can easily be measured through the observed change in inventory turnover (see Appendix); a reduced amount of average inventory on hand (the formula’s denominator), thanks to better forecasting, resulting in an increase in inventory turns. Attention should be paid, however, to the concurrent efforts at reducing cost of goods sold (COGS) (through the use of economic order quantity analysis or through better pricing attainment, for example) as the reduction in the formula’s numerator (COGS) will artificially reduce the calculated inventory turns; to the point that an equal percentage reduction in COGS and in average inventory on hand will result in an unchanged calculation of inventory turns. For this reason, measurement of inventory turns should be made with knowledge of changes in COGS given that the costs of the inventory will affect not only the total cost of goods, but also the cost of the inventory on hand. A second method of measuring inventory turns that is only based on demand and order quantity takes away the effects created by unit cost on the previously described turnover calculation and gives a more precise evaluation of inventory usage. Re-arrangement of this formula provides the useful measure of days of supply, which will also allow the practitioner to monitor the results of improved forecasting.

Efforts toward avoiding shortage costs associated with too little inventory on hand cannot necessarily be measured through total revenues other than having personal knowledge of having lost a client’s business to a competitor. The effort to go lean through calculating the economic order quantity should be accompanied by the calculation of a reorder point (see Appendix), which will trigger an order and therefore avoid running out of inventory that allows the provision of service. Although measuring shortage costs cannot be done other than through gut feeling, implementation of a reorder point will prevent the shortage costs because the inventory will be reordered before running out.

7. Conclusion

To properly analyze forecasting accuracy, the veterinary practitioner must use simple statistics measurements that compare with a perfect forecast. PE and MAPE represent a percentage-based analysis of how far off the forecast was from the actual demand, and provide a continuous measurement to evaluate accuracy of estimation over time. Changes in the forecasting technique to reduce PE and MAPE will lead to
improved forecasting accuracy that leads to improved demand management.

Improved demand management will allow the veterinarian to better manage cash flow associated with invested capital, to take more precise advantage of distributor’s specials and promotions, and to improve operational efficiency through implementation of lean processes. Improvement of inventory demand forecasting will also have a significant positive effect on the ability to increase profits while decreasing working capital investments to the extent that a return on investment into inventory cost saving is usually worthwhile based on the resulting improvement to shareholder value. In all, the effect of improving the accuracy of the inventory demand forecasting process will translate into increased gross profit margins, which directly increase profits net of taxes, and thereby create an increase in the value of the practice.

Acknowledgments

Declaration of Ethics

The Author declares that he has adhered to the Principles of Veterinary Medical Ethics of the AVMA.

Conflict of Interest

The Author declares no conflicts of interest.

Reference


Appendix: Formulas

<table>
<thead>
<tr>
<th>Name</th>
<th>Formula</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percent error</td>
<td>$PE = \frac{\text{forecast} - \text{sales}}{\text{sales}}$</td>
</tr>
<tr>
<td>Mean absolute percent error</td>
<td>$\text{MAPE} = \frac{\sum</td>
</tr>
<tr>
<td>(Sum of the absolute value of all percent errors divided by the number of instances)</td>
<td></td>
</tr>
<tr>
<td>Simple exponential smoothing forecast</td>
<td>$F_t = [\alpha \times Y_{t-1}] + [(1 - \alpha) \times F_{t-1}]$</td>
</tr>
<tr>
<td>(Where $F_t$ is this period’s forecast, $Y_{t-1}$ is the last period’s sales, and $F_{t-1}$ is the last period’s forecast)</td>
<td></td>
</tr>
<tr>
<td>Total actual inventory cost</td>
<td>$\text{TAIC} = \text{PC} + \text{HC} + \text{OC}$</td>
</tr>
<tr>
<td>Purchase cost</td>
<td>$\text{PC} = (\text{demand}) \times (\text{cost} + \text{tax})$</td>
</tr>
<tr>
<td>Holding cost</td>
<td>$\text{HC} = (\text{order quantity} / 2) \times (\text{holding rate} \times (\text{cost} + \text{tax}))$</td>
</tr>
<tr>
<td>Order cost</td>
<td>$\text{OC} = (\text{demand} / \text{order quantity}) \times (\text{per order cost})$</td>
</tr>
<tr>
<td>Economic order quantity</td>
<td>$\text{EOQ} = \text{square root of } \left[ \frac{2 \times \text{demand} \times \text{per order cost}}{(\text{holding rate} \times (\text{cost} + \text{tax}))} \right]$</td>
</tr>
<tr>
<td>Inventory turnover</td>
<td>$\text{Inv. TO} = \frac{\text{cost of goods sold}}{\text{average inventory}}$</td>
</tr>
<tr>
<td>Or</td>
<td>$\text{Inv. TO} = \frac{\text{(annual demand) / (order quantity) / 2}}{2}$</td>
</tr>
<tr>
<td>Days of supply</td>
<td>$D \text{ of } S = \left[ \frac{\text{order quantity} / 2}{\text{(annual demand)}} \times (\text{No. of days open for business during year}) \right]$</td>
</tr>
<tr>
<td>Reorder point</td>
<td>$\text{ROP} = \left[ \frac{\text{(annual demand) / (No. of days open for business during year)}}{\text{lead time}} \right] \times \text{lead time}$</td>
</tr>
<tr>
<td></td>
<td>(where lead time is the time period between order placement and order receipt)</td>
</tr>
</tbody>
</table>
How to Mathematically Forecast Veterinary Inventory Demand

Jorge L. Colón, DVM, MBA

Author's address: Jorge L. Colón, DVM, PLLC, PO Box 11631, Lexington, KY 40576; e-mail: jorgecolondvm@me.com. © 2015 AAEP.

1. Introduction
A key component of lean processes in inventory management entails an accurate forecast of the demand for future inventory. Lean inventory management systems have been previously described and concentrate in the reduction of total annual inventory costs by reducing ordering costs and holding costs, both of which rely on demand quantity for their calculation. Whereas all practitioners have to utilize experience and knowledge of external factors to help forecast demand, qualitative techniques do not, by themselves, provide a significant level of accuracy. The established practitioner, however, can improve the forecasting accuracy by utilizing known data on previous demand. The use of a quantitative forecasting technique, together with qualitative techniques, will improve the accuracy of demand forecasting and help the practitioner improve the practice’s lean inventory management system.

2. Using the Data
Holt-Winters Seasonal Method (Triple Exponential Smoothing)
For the most part, the veterinary practitioner is faced with a demand that has both a trend and a seasonality component. Holt (1957) and Winters (1960) extended on Holt’s original linear trend method of demand forecasting to capture seasonality by creating a forecasting equation that includes three smoothing equations: level, trend, and seasonality; each being affected by the smoothing parameter α, β and γ, respectively. Utilizing historical data, the Holts-Winters seasonal forecasting method produces a demand forecast that takes into consideration the expected trend and seasonality of future demand. This paper utilizes a multiplicative Holt-Winters seasonal method due to the expectation that seasonal variations will change in proportion to the level of the data series.

A triple exponential smoothing forecasting calculator has been created and the Excel spreadsheet can be downloaded at https://db.tt/xzw2oxvT. Be sure to enable macros in your program.

Historical Data
For each inventory item to be analyzed, the practitioner should have on hand the historical sales data for the previous 4 years in order for the spreadsheet to define the trend and seasonality of demand. There are four columns and the quarterly data must be entered with the oldest year to the left and the newest year to the right. As newer yearly data becomes available, all previous years’ data gets shifted one column to the left and the previously oldest data gets removed from the chart. Some of the calculations cannot handle a zero value for any
sales data and therefore a number 1 should be entered for any quarter in which the sales data was zero. The spreadsheet will calculate the seasonal index for each quarter of data and will generate the corresponding level, trend and seasonal components based on the Holt-Winters Seasonal formula. Each smoothing equation will utilize a pre-populated arbitrary number for its respective smoothing parameter $\alpha$, $\beta$, and $\gamma$, which will be corrected during a process of optimization to follow. The resulting forecast for each of the 16 quarters of data will be compared with the actual sales data to determine the mean squared error of the difference between the forecast and the actual sale.

### Optimizing the Formulas

The mean squared error (MSE) is a statistics formula that measures the average of the squares of the errors in the data and, in this case, presents the difference between the forecast and the actual sales data. The smaller the MSE, the more accurate the calculated forecast for the actual historical demand. Microsoft Excel has a data analysis tool called Solver that can minimize the value of an objective cell by changing the values of variable cells, which are subject to a constraint. The objective cell for our model is the calculated value of the MSE provided by the analysis. The variable cells are the values of $\alpha$, $\beta$, and $\gamma$ that the smoothing equations use in the calculations. The value of these smoothing parameters are constrained to individual values between 0 and 1. Optimization of the smoothing parameters will provide the smallest possible MSE and therefore help improve the accuracy of the forecast.

The process of optimization has been enabled in a macro embedded within the spreadsheet (after entering the sales data click the Optimize button) but the process is as follows: in your Excel program, install the Solver analysis tool under File-Options-Add ins and make Solver Add-in an active application. Find Solver under Data menu. Set Objective as the cell containing the mean squared error value. Set TO to minimize (you want to reduce the value of the objective cell). Set the variable cells, separated by a comma, as the cells containing the values for $\alpha$, $\beta$, and $\gamma$. Add two constraints to each variable cell, one as “cell” $\geq 0$, and another as “cell” $\leq 1$ (sets each smoothing parameter to be constrained at $0 \leq value \leq 1$). Select the Solving Method as “GRG Nonlinear.” Click on Options and then on the GRG Nonlinear Tab and check the Use Multistart box, click OK. Once returned to the Solver Parameters window click Solve. Once the optimization is calculated, select Keep Solver Solution in the Solver Results window, and click OK.

### 3. Results

The values for $\alpha$, $\beta$, and $\gamma$ tell each smoothing equation how much weight to place on the different components of each equation for the process of exponential smoothing. Given that the forecast is based on the level, trend, and seasonality, optimization of the smoothing parameters leads to a more accurate forecast as Solver finds the values for $\alpha$, $\beta$, and $\gamma$ that lead to minimization of the calculated MSE. The end result being a projected forecast for the next four quarters that has been optimized for accuracy through exponential smoothing that took into account the level, trend and seasonality of the previously observed data.

Although MSE provides the statistics analysis to help us improve accuracy in the forecast, the statistics of mean absolute percent error (MAPE) can be used as a more easily understandable measure of how well we are doing over time in our forecasting. A statistics measurement that compares the forecast to a perfect forecast, MAPE expresses accuracy in terms of percentage so that we can see, on average, how far off the forecast has been. By entering into the spreadsheet the actual sales data for each quarter related to the forecasted period, the calculator will compute the MAPE over time to give an indication of the level of accuracy achieved. This will allow the practitioner to evaluate the forecast in light of the qualitative aspects of the forecasting that statistics cannot foresee and therefore cannot compute.

---

**Table 1. Savings Provided by Holt-Winters Seasonal Method Over Average-Based Demand Forecasting for a Single Inventory Item During the 2015 Breeding Season**

<table>
<thead>
<tr>
<th>Demand Based on Average Consumption Over Last 4 Years</th>
<th>Demand Based on Holt-Winters Seasonal Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Demand forecast</td>
<td>42 cases</td>
</tr>
<tr>
<td>Quantity per order</td>
<td>42 cases</td>
</tr>
<tr>
<td>Purchase cost</td>
<td>$2,713.94</td>
</tr>
<tr>
<td>Holding cost</td>
<td>$237.47</td>
</tr>
<tr>
<td>Order cost</td>
<td>$10.00</td>
</tr>
<tr>
<td>Total actual inventory cost</td>
<td>$2,961.41</td>
</tr>
<tr>
<td>PE from actual sales</td>
<td>23.53%</td>
</tr>
<tr>
<td>Savings over average-based forecast</td>
<td>$544.94 (22.55%)</td>
</tr>
</tbody>
</table>

EOQ, economic order quantity; PE, percent error.
4. Translating the Calculated Demand Forecast Into Cost Savings

Table 1 presents a real scenario encountered by the author during the 2015 breeding season. Historical data over the last four years showed that, on average, 42 cases of an inventory item were used over the first 6 months of the year. Normal routine would have been to place a single order during the American Association of Equine Practitioners convention for the expected 2015 breeding season average-based demand. Holt-Winters Seasonal Method analysis of the same 4-year historical data, however, suggested that the demand for the period would be 36 cases. In addition, economic order quantity analysis based on this calculated demand suggested that eight cases per order would minimize the actual inventory cost for this size of the demand. Actual sales during the 2015 breeding season were 34 cases. With a state sales tax of 6%, per order cost of $10, and using a holding rate of 35%, the calculated demand forecast translated into a 22.55% savings over what would have normally been incurred had the calculations not been performed.

5. Conclusion

Lean inventory management relies on cost reduction through the reduction of purchase cost and order cost. Both cost formulas rely on the forecasted demand. The third inventory cost component, holding cost, depends on the order quantity which, in turn, also relies on the forecasted demand. As such, improvement in the accuracy of inventory demand forecasting will serve as a major component of enacting and improving lean processes. The example presented clearly shows an opportunity to improve cash flows, to reduce invested capital, and to improve operational efficiency; all of which translate into improved margins, improved profits, and increased practice value.

The demand forecasting spreadsheet calculator should serve as a guidance tool to help accomplish the quantitative analysis of historical data. The goal is to help reduce the MSE of the calculations and therefore improve the accuracy of future forecasts. The data that a quantitative statistics analysis might provide, however, will be of minimal value if the qualitative components of demand forecasting are not taken into account. More accurate numbers will help improve forecasting accuracy only if the numbers are looked at through the eyes of experience that take into account all of the practice's external factors.

Acknowledgments

Declaration of Ethics

The Author declares that he has adhered to the Principles of Veterinary Medical Ethics of the AVMA.

Conflict of Interest

The Author declares no conflicts of interest.

References

2. Colon JL. How to mathematically decide if that distributor special is good for me, in Proceedings. Am Assoc Equine Pract 2014;60:380–381.

Appendix: Formulas

<table>
<thead>
<tr>
<th>Description</th>
<th>Formula</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seasonal index (where m denotes the period of the seasonality)</td>
<td>( s_{1-m} = \frac{\text{[(Sum of all data for specific quarter)/(Sum of all data for all years)]}}{4} )</td>
</tr>
<tr>
<td>Holt-Winters seasonal forecast (multiplicative; where ( Y_t ) = period's actual data)</td>
<td>( F_{1-m} = (l_t + bh_t) \times s_{1-m} \times \frac{Y_t}{Y_{1-m}} ), utilizes ( l_0 = \frac{\text{sum}(Y_1 \cdot Y_m)}{m} ) and ( b_0 = \frac{\text{sum}((Y_{m+1} \cdot Y_1) - (Y_m \cdot Y_{m+1}))}{(m+1) \cdot m} )</td>
</tr>
<tr>
<td>Level component</td>
<td>( l_t = [\alpha \times (Y_t/s_{1-m})] + [(1 - \alpha) \times (l_{t-1} + b_{t-1})] )</td>
</tr>
<tr>
<td>Trend component</td>
<td>( b_t = [\beta \times (l_t - l_{t-1})] + [(1 - \beta) \times b_{t-1}] )</td>
</tr>
<tr>
<td>Seasonal component</td>
<td>( s_t = [\gamma \times (Y_t/(l_{t-1} + b_{t-1}))] + [(1 - \gamma) \times s_{1-m}] )</td>
</tr>
</tbody>
</table>
Utilization of Medical Records in the Development of Clinic Strategy

Greg Andrews, DVM

Medical records have an obvious reason for being on the mind of all veterinarians: to record all history, signalment, pertinent notes, descriptions, observations, diagnoses, treatment protocols, and results. What do these objective and subjective parameters have to do with your strategy? It is your own “big data” right there on your computer. Let’s explore how we can strategically use this goldmine of information. Author’s address: Box 31, 4168 Elk Creek Drive, Ta Ta Creek, BC V0B 2H0, Canada; e-mail: greg.andrews@inovapartners.com © 2015 AAEP.

1. Introduction
What is strategy? The Oxford Dictionary defines it as “a plan of action designed to achieve a long-term or overall aim, under conditions of uncertainty.” The word originates from the Greek word for the art of a troop leader or the art of fighting. It is also front and center in Sun Tzu’s Art of War, a classic book often used in business and marketing discussions. Two of Sun Tzu’s most relevant quotes are as follows:

“Ponder and deliberate before you make a move.”
“If you know the enemy and know yourself, you need not fear the result of a hundred battles. If you know yourself but not the enemy, for every victory gained you will also suffer a defeat. If you know neither the enemy nor yourself, you will succumb in every battle.”

What I will try to demonstrate is how you can utilize what is in your medical records to allow you to develop a strategy to thrive and prosper. No, I don’t want you to go to war with other practitioners—just to present yourself well to the right market and with the right tactics. I will make the assumption that you practice ethical, high-quality veterinary medicine; you have to start with that or, in Sun Tzu’s thoughts, “the expert in battle moves the enemy.”

The mindset of “I just want to do good work and let it show for itself” does not work in the world of social media and the Internet. There is always someone with the magic elixir who is deemed to be better than you.

2. What Is the Link Between Medical Records and Strategy?
If you can’t measure it you can’t manage it. If you don’t know what you are making or not making money at all, how do you determine what to emphasize in marketing, continuing education courses, and hiring and firing? If you are spending most of your time and efforts on surgery, for example, but are losing money on it while still making a good margin on repro, then you can start to formulate a strategy going forward that focuses on where resources and
marketing will be targeted. The pertinent areas to consider for this strategy to work are as follows:

- Compensation and hiring: How do you compensate better for doing good work? In my opinion, a veterinarian who bills out more pure professional services and a higher percentage of lameness exams and blocks versus medications\(^b\) or joint injections is practicing better medicine that will endure the test of time and external scrutiny. Set up your records to allow these things to be separated and then monitor them. By using specific codes, any applicable software will show how much was earned. Put these data in an Excel spreadsheet and compare them among veterinarians. Over time patterns will evolve. These patterns will allow you to potentially reward your veterinarians for a higher standard of care and certainly a more profitable standard of care. Everyone wins—the horse, the client, the veterinarian, and the clinic. Setting up your codes properly, however, is imperative and will take some work.

- Quality control: Are standard operating procedures being carried out in your practice to the level that you expect? This can be followed and may affect compensation; it will allow for the issue to be corrected.

- Discounting: Discounting by veterinarians is both legendary and saddening. Simply tracking the percentage of discounts by veterinarians and factoring this into compensation has shown to decrease this habit.

- Marketing strategy: Medical records are not only just your notes on the diagnosis or treatment; they also need to involve many other things about the horse, client, and stabling. These data can be used for marketing of all types—whether through social media or otherwise. Where, when, and to whom you spend not only your marketing dollars but your time and focus, which are both valuable and limited, will go a long way in determining the success of your strategy.

- Retrospective studies: There is a wealth of information contained in your medical records regarding diagnosis, treatments, and outcomes. Setting up consistent nomenclature is critical for pulling specific scientific information from your data. There are many things you can do with this information other than publish it. You can make it work for you in managing your practice.

- Soundness management programs: Some veterinarians are developing comprehensive lame


n
ness wellness programs for their elite equine athletes. Actually what you are doing is creating a good medical record with everything that has been done and those yet to be documented. By setting up these type of programs you are creating a great medical record both with procedures that have been done and those scheduled. Your clients will pay for these good medical records. Another example is veterinarians caring for elite athletes who do many proactive examinations and provide their clients with a plan for managing each patient. This “performance or life story” can be used in many different areas of practice.

These are a few of the ways to utilize the records that we, by law, have to produce. There is a whole new science called informatics—the science of computer information systems. People dedicate their whole careers to this science, and one of the things they often lack are good data. When you have data, use it. You cannot afford not to.

Acknowledgments

Declaration of Ethics
The Author declares that he has adhered to the Principles of Veterinary Medical Ethics of the AVMA.

Conflict of Interest
The Author is chairman of Inova Partners, LLP - Veterinary Management Software and Management Solutions.

Reference and Footnotes

aOxforddictionaries.com
How to Determine a Price for a Procedure

Benjamin R. Buchanan, DVM, DACVIM, DACVECC

If one understands the cost of a procedure, one can make educated decisions about how to set a price. Calculating a rough cost of a procedure requires only an accurate income statement and a stopwatch. Author’s address: Brazos Valley Equine Hospital, 6999 Hwy 6, Navasota, TX 77868; e-mail: bbuchanan@bveh.com. © 2015 AAEP.

1. Introduction
All businesses must earn a profit to exist, expand, and grow. However, as a stereotype, veterinarians are compassionate individuals and not the most aggressive professionals at making large profits. Failure to truly understand the economics of business also affects profitability. No matter whether your practice is a high-volume/low-margin practice or a low-volume/high-margin practice, understanding your costs associated with a procedure are important to setting your price. Failure to understand a method to best set prices contributes to some practices with great customer service and client loyalty never reaching their full potential. In these proceedings some common methods of setting prices will be reviewed and an alternative pricing method based on looking at the true cost of producing a service will be proposed.

2. Price-Setting Methods

Call the Competition
This method involves discretely and anonymously calling competing practices and asking what they charge for a service. In many cases the caller then sets their fee just below in hopes of competing on price. Although this is an effective way to maintain competitive prices it has several drawbacks.

1. It may or may not be profitable. In an effort to offer more services, one may be losing money with each service.
2. It creates a downward pressure on prices in the area and can lead to price wars with the local practices.
3. The services being compared is frequently not something that is being price shopped by clients.

In some procedures or for a startup practice, this method can be beneficial to determine the local market. However, one should evaluate the cost of a procedure and determine whether they are willing to sacrifice profit for “shopped” services or potentially even lose money.

National Benchmarking (Services and Products)
Many consultants assist practices around the country. They often have a good feel for what is a profitable price for a service. They can also be very helpful in establishing a model for setting prices. However, not all equine practices are the same. Many are primarily ambulatory based and comparing prices to a large referral center in another state is not always helpful. Despite the differences, it is frequently enlightening to see what other practices charge and can help to adjust fees.
Cost-Based Pricing (Products)
This is a traditional-based method of developing pricing where the cost of a pharmaceutical is marked up 90 to 250%. In many cases, an administration or dispense fee is also added. Pharmaceutical products that have a lower turnover must be marked up higher. However, applying a flat markup to all pharmaceutical items means some expensive items can be priced above the market. The markup on the pharmaceutical is intended to pay for the drug, shrinkage, technician time for ordering and managing inventory, veterinarian time in choosing which products to stock, overhead, and to make a profit.

Charge by the Hour (Services)
Other service-based professionals charge a flat fee per hour. As a professional, veterinarians could do the same on a per-hour basis. Empirically, one could decide they are worth $X per hour and charge that amount in 1-minute to 60-minute segments. A charge per time makes financial sense as long as the charge is greater than the overhead. As pharmaceuticals continue to be lost to catalog and online sales, leveraging time and education as a service is a pricing strategy that will become more popular.

Charge by Overhead Cost Per Minute
Knowing the costs of running a business will allow an individual to set prices that cover the cost of business and generate a fair profit. This can be done on many levels; however, it does require an accurate measurement of how long a procedure takes. This can be measured with a stopwatch and should be done for procedures ranging from giving an injection to injecting a set of hocks, to inseminating a mare, to doing arthroscopic surgery. The time should include preparation, procedure, and cleanup. In addition, time for client communication can be included in some procedures. Essentially, this is a simplified version of activity-based costing, in which the cost of a procedure is determined by the resources actually used for that procedure.

3. Global Price Per Doctor Hour
Any overhead-based pricing requires a relatively accurate income statement (profit/loss statement). If there are personal costs (of owners, partners or others), the strategy should be adjusted to remove them from the calculation. The goal is to take the total cost of doing business and remove the drug costs and doctor compensation. This is the cost of overhead that can be used to create a price per hour to determine the cost of a procedure. For this discussion, a theoretical two-doctor practice will be used.

1. Start with expenses from the income statement ($1,040,000).
   a. Remove doctor compensation (−$260,000)
   b. Remove billable consumable supplies (−$260,000)
      i. Drugs, bandages, other inventory, supplies
      ii. How much detail is up to the individual.
      iii. Supply items removed should be billed to the client in addition to the procedure charge as either a separate line item or bundled into one price on the invoice
2. The remainder is the amount of money spent to operate the business in the last year minus doctor compensation and drugs ($520,000). This is the cost of overhead.

In this theoretical two-doctor practice each doctor averages 50 hours per week including time off for continuing education and vacation. Some weeks are greater than 50 hours and some weeks are less than 50 hours. However, the total time spent working on the practice by the two doctors is 5,200 hours.

If the cost of overhead ($520,000) is divided by the number of doctor hours (5,200) a cost of $100 per doctor hour for the overhead is calculated. Dividing that number by 60 equals a cost of $1.67 per minute. This is the minimum cost for a procedure to a client for a service. This number contains no supplies, doctor compensation, or profit. If a procedure is being charged at less than this number, the practice loses money every time it does that procedure.

To calculate a price per hour that includes compensation and profit, several things should be considered. How much profit? What is the expected commission or production bonus? Are any services traded out or discounted for marketing purposes? Are all charges collected? Are collections a problem and should accounts receivable be included?

Price per hour = cost of overhead/(1 − profit% − commission% − loss%).

Any additional markup should be included. If a target profit of 10%, a commission of 25% and a discount/marketing of 10% are used, one ends up with a price per hour of $181 ($100/(1 − 0.1 − 0.25 − .01). This is the minimum price that should be charged to cover the cost of overhead and to generate the commission and profit desired.

Many procedures include consumables. These were also removed from the cost of overhead and must be added back. However, that time associated with the inventory is included in the cost of overhead and the inventory does not need a large markup. In a perfect system, the inventory could be charged at cost. However, not everything has a turnover of less than 30 days, some inventory expires, breaks, is lost, or is stolen. In addition, not everything in the drugs and supplies category on the income statement is sold back to the client. For these reasons inventory is still marked up. When administered, the inventory items are paired with a 5-minute administration fee. The administration
fee will change as the cost of overhead and price per hour change. If a particular inventory suffers large loss/shrink one may must mark this up higher. Although, it is possible to separate out the inventory and the service on the invoice, the author’s experience has shown clients in our area prefer to see these bundled into a single price.

4. Profit-Center Specific

Hospitalization

The global approach to pricing does not work for some areas. One example is hospitalization. If one was to charge for 24-hour time at $181 per hour, it is likely a horse would never be hospitalized. To develop a more appropriate price for hospitalization one can again look at the income statement for items specific to the hospital and add that to a percentage of the cost of overhead. What this percentage should be can be examined in different ways: time spent on hospitalized patients, percent of income, or square footage. Again in the theoretical two-doctor practice:

1. Hospitalization specific (Total = $69,000)
   a. Feed, shavings (~$13,000)
   b. Barn crew (~$40,000)
   c. Barn supplies (~$10,000)
   d. Animal disposal (~$6,000)
2. Non-hospital specific (Total = $89,000)
   a. Rent (~$70,000)
   b. Insurance (~$10,000)
   c. Repairs/maintenance (~$9,000)
3. Hospital as percentage of revenue = 25%
4. Hospital as percentage of usable square footage = 33%
5. Number of hospital days billed in last 12 months
   a. This is the total number of hospital days charged. Average no. horses per day in hospital x No. of days = 2,700

If the total for hospital specific expenses and 33% of the shared expenses is used, a total expense of $98,370 is calculated ($69,000 + [89,000 x 0.33]). If this is divided by the total days of hospitalization, a cost per day of hospitalization of $36.43 is calculated ($98,370/2700). The practice is currently charging $38.50 for a daily board and examination. Reproduction boarding and rehabilitation/layup boarding being billed at $28 per day (less than cost).

It should be clear that the practice should re-evaluate how it is billing for hospitalization and the different levels of care. The author is currently charging a base price for hospitalization and then an additional bundled cost for higher levels of care, (i.e., cases requiring more treatments such as ICU cases, neonatal cases, eye cases, etc.) Considering that hospitalization is 25% of the practices revenue, other areas of the practice must be very efficient for the practice to generate the desired profit. This suggests that costs are being shifted to other areas to support hospitalization (overcharging in another area) or hospitalization is a lost leader. For patients requiring more than a daily examination and stall, the price for the hospitalization and level of care should reflect the increased cost.

Other Profit Centers

This same profit center approach could be applied to other areas of the practice. However, trying to separate out ambulatory from outpatient from surgery from medicine from laboratory can become very tedious and lead to different prices for the same procedure. One would have to take into account the number of staff dedicated to each service and assign some percent of the shared overhead. An alternative approach is to use a global price per hour and add extra cost and for services where additional staff are required. In many cases these are the specialty services for which there is not significant competition from other practices and a higher markup could be used.

5. Equipment

Procedures that use equipment may need to add an additional cost for using the equipment. If the cost of the equipment is expensed and included in the income statement, then the cost of the equipment is already considered as part of the global cost of overhead. However, if the equipment is financed and listed only on the balance sheet, an additional cost of the equipment should be considered similar to the use of inventory. Factors to consider include the cost of the equipment per year, the number of procedures, the life span of the equipment, replacement costs, maintenance costs, and any archiving/storage costs. Consider the number of times in a year that piece of equipment is used and a cost per use can be determined. Alternatively, the capital costs of equipment could be added into the cost of overhead calculation.

An example in the theoretical two-doctor practice would be the new digital radiograph machine.

Purchase price/replacement cost ($57,000)
Service fee ($5,000 per year)
Life span (3 year)
The annual cost of the equipment is $57,000/3 + $5,000 = $24,000.
Number total radiographs taken per year = 1,000

If the practice takes 1,000 radiographs a year with this unit the cost per radiograph is $24. Adding in a $1 per image archiving fee and the cost per radiograph is $25. The average time required to set up, collect images, archive images, and evaluate images is 10 minutes per radiograph session. At $181 per hour, that is $30.16 per image. This cost for the time plus the cost per radiograph indicates that an appropriate price per radiograph would be $53.00.
per image. The practice is currently charging $40 per radiograph.

6. Price Shopping
Certain procedures will always face downward pressure from lower-budget clients who price shop. Although it is not necessarily a bad thing to adjust prices down to accommodate for a higher volume, evaluating the true cost of a procedure can help to make informed decisions. Maybe one is willing to make less profit on vaccines. Maybe one is willing to take a loss on castrations. Whatever the decision, it should be made with a full understanding of what a procedure costs; this can help decide where to set prices, and how much one might be willing to discount for employees, other professionals, and youths.

In addition, as one elects to reduce profit on some procedures, they can elect to increase the profit on others. As referenced above, some procedures have less competition or a higher perceived value to the client and can sustain a higher markup. Examples would be gastroscopy, arthroscopy, regenerative medicine, abdominal ultrasound, or alternative therapies.

7. Profit Analysis
Understanding what the cost of overhead is and where a business’s profit comes from can allow for surprising decisions. In addition to adjusting prices, one may elect to focus the practice in a different direction or area. As practice management software continues to evolve, veterinarians will ultimately be able to set a price and cost for procedures allowing the software to calculate profit. Once that process is understood and transparent, it may lead to the next shift in compensation/commission strategies: paying doctors based on a percentage of the net profit from a procedure instead of percent of gross revenue production.

8. Changing Prices
Although not a focus of this exercise, it is worth noting that changing prices or culture can be difficult. The hardest sell is often internally. But with a clear strategy and the transparency that comes with using numbers from the income statement, it becomes easier for the staff to see how prices are created and why prices are set at the specific level. It is important to remember that many people do not understand the concept of profit, so providing staff education can be critical to a successful effort.

9. Conclusion
When considering pricing, a practice should consider its target audience. The price to the client will be different between a practice philosophy that is high volume but low margin compared with a practice that is high margin with lower volume. Regardless of the philosophy, no practice can survive if the price to the client is less than the cost of the service.

Moving to a cost-based pricing brings the focus of the practice onto services. Inventory becomes less important and less of a profit center. As inventory continues to get more competitive, it is important to find areas of profit in the practice. Although calculating out all the details of every procedure (gauze sponges, tech time, iodine, alcohol, facilities, doctor time, computer, equipment, etc.) can be daunting, the method proposed here is less complicated and will give practitioners a tool to use to get to the rough cost of a procedure. Knowledge is power and the tools to evaluate what makes a practice self sustaining, profitable, and healthy is already available to practitioners.

Acknowledgments
Dr. Charlie Buchanan developed the framework of this concept and continues to help refine it. Dave Shimanek and Dr. John Janicek provided critical feedback.

Declaration of Ethics
The Author declares that he has adhered to the Principles of Veterinary Medical Ethics of the AVMA.

Conflict of Interest
The Author declares no conflicts of interest.
Planning and Building Your New Equine Hospital—A Strategy for Success

Joe Martinolich, AIA*; and Rebecca Stinson, DVM

1. Introduction
The facilities where one practices has an effect on the quality and type of the veterinary services that can be offered. This applies to individual vets or large groups, established firms or startups, general practitioners or specialists. To enhance your practice’s ability to serve and attract clients, improve the quality of your services, and increase your business, there may come a time when the question arises about expanding or building a new facility. This presentation offers, through a case study, the considerations and steps needed to plan and build a new equine hospital.

2. Goals for the Session
By reviewing the activities that a small but growing equine veterinary practice (Carolina Equine Hospital located in Browns Summit, NC) followed as they built a new equine hospital facility, the important aspects of the planning and construction process can be learned and used by other practitioners as a strategy for their own building and facility expansion plans.

Determining Why You Need to Build
Dr. Stinson (the veterinarian) will lead the discussion on this topic:
- The practice’s internal discussions and decision making

Issues prompting decision to build
Strategy for managing the entire process, etc.

Selecting and Teaming With the Right Professionals
Dr. Stinson and Mr. Martinolich (the architect) will share the lead on this topic:
- Recognizing you can’t do this alone
- Researching architects
- Researching builders

Being Realistic in your Expectations, Scheduling, and Budget
Dr. Stinson and Mr. Martinolich will share lead on this topic:
- Expected costs and schedule
- Prioritizing wants vs needs
- Researching other facilities

Recognizing that the Planning Process Is an Investment Toward the Success of the Project
Mr. Martinolich will lead the discussion on this topic:
- Design process overview
- Communication during the process
- Effort and commitment during the process
**Staying on Course, Even When it Gets Bumpy**

Mr. Martinolich will initiate discussion on this topic:

- Unexpected delays
- Financing
- Local planning approvals
- Weather
- Subcontractor issues, etc.

3. **Takeaway**

Each of the session goals will be discussed using the case study to clarify and expand upon the means of implementing each issue. Drawings, illustrations, and photographs from the actual hospital design and construction project will be presented to add interest and relevance to the
discussion. Examples of some of these visual aids are shown in Figures 1–5.

The presenters will take listeners chronologically through the planning and building process, highlighting the key decisions and issues that were encountered. This firsthand accounting, presented by the veterinarian and architect who were directly involved throughout the process, will add credibility and personal insight, from both the owner’s and designer’s point of view, for each of the key points being discussed.

It is said that hindsight is 20–20, so the presentation will include a post-evaluation to reflect upon the process as well as the project, including what
worked well and what could have been done differently. Since its completion in the winter of 2013, the new hospital has proven to be profoundly beneficial to the practice and its clientele. Dr. Stinson will conclude with a summary of the financial and practical impact it has had since opening.

An overriding theme to the session and each key talking point is that of collaboration and strategic planning. From the group of owners’ initial decision to move forward with the project through the design and planning stages with the architect and into the actual construction work with the builder, regular communication, consensus building, mutual respect, and trust were key to the ultimate success of the project. Dr. Stinson and Mr. Martinolich, as co-presenters of this discussion, and involved in differing roles of the overall project team, will permit attendees at the conference to ask questions directly to either presenter for their objective and unique perspective.

Acknowledgments

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