Proceedings of the 67th Annual Convention of the American Association of Equine Practitioners

Program Chair: Emma K. Read, DVM, MVSc, DACVS

ACKNOWLEDGMENTS
Charlie Scoggin, DVM, MS, DACT, Educational Programs Committee Chair
Carey M. Ross, Scientific Publications Coordinator

Published by the American Association of Equine Practitioners

ISSN 0065-7182
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Policy Statement

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

Mission Statement

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

Future AAEP CE Dates

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

The Scientific Review & Editorial Committee (SREC) 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 2021 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 AAEP CE Needs Analysis surveys. 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 SREC.

Papers submitted by independent authors are each assigned 3 reviewers from the SREC. 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. This year 85 papers were submitted for the 50 available slots on the program.

Non-scientific sessions addressing business, 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 SREC for inclusion in the Proceedings.

The peer review process for the AAEP Proceedings is rigorous. It requires an enormous effort by over 50 members of the SREC to create the best possible program for the AAEP membership. Many volunteer hours were spent putting together the 2021 program, so please thank them for all their hard work creating this program for you.
From Your President

Dear AAEP Members & Guests:

Welcome to Nashville, The Music City, for the 67th Annual AAEP Convention and Trade Show. It is with great pleasure that I greet all of you to the return of our live convention. Although, there will be things that we have taken away from last year’s virtual convention, there’s no replacement for seeing all of you in person and getting to catch up on all the challenges of the last 2 years.

Dr. Emma Read, AAEP’s president elect and program chair for this year’s convention, has worked diligently to put together an outstanding program. Her program will be filled with a wide array of interesting and timely topics. Educational Programs Committee chair Dr. Charlie Scoggin and Vice Chair, Dr. Erin Contino have managed a large group of your colleagues to provide for the selection and review of papers presented in this program. I want to give a personal thanks to all the member volunteers that have contributed to this process. It is not an easy task.

This year’s meeting will once again feature a top-of-the-line trade show that the AAEP is known for. I am glad that we can welcome back all our exhibitors so that we can meet face to face and see what new and exciting things they have to offer. Please remember to thank them for their participation, especially our Educational Partners who are so important in helping us provide the state-of-the-art educational programs that we are accustomed to.

Nashville! What better place to get back together again! There will be no shortage of opportunities to enjoy the social events that are always a part of this convention and Nashville will be a great place to do them. From The Foundation for The Horse events such as the “Storytelling Nashville Style” and the ever-popular Alumni Receptions, all the way to the After Party and more, there will be something for everyone to enjoy. Please make the most of it!

The last year was full of challenges, some of which will pass. Yet other challenges remain and the leadership of the AAEP will continue to try to work through those challenges. It has been my pleasure and truly an honor to serve as the AAEP’s president during this year. With the help of the wonderful officers, the highly dedicated board of directors and you, the always willing volunteers of the AAEP, it has made things enjoyable despite the tasks at hand.

I also want to thank the staff of the AAEP led by Executive Director David Foley. Without this staff, it would be impossible for this organization to function, no less function as a well-oiled machine. They do their tasks in the background, often barely noticed. The staff of the AAEP is a truly dedicated group of people and I thank them wholeheartedly for making this organization run perfectly.

Sincerely,

Scott A. Hay, DVM
2021 AAEP President
Welcome to Music City! Nashville provides an ideal backdrop for the 67th AAEP Convention. There is something for everyone so bring your family and friends! From the hot chicken, to barbeque, to meat and three, to the Country Music Hall of Fame and Museum, and the Grand ‘Ole Opry. A great location with a terrific scientific and social program promises a fun-filled week!

As program chair for 2021 convention, I’ve had the privilege of working with a very dedicated Educational Programs Committee, led by Dr. Charlie Scoggin and Dr. Erin Contino. They have put together a program that will give everyone something to take home to improve their practice.

My deep appreciation goes to the AAEP staff as well as my colleagues on the Board for their support. The AAEP is lucky to have such wonderful people working hard behind the scenes!

Some highlights at the convention this year will be . . .

- Meagan Johnson will deliver the Keynote on “Generational Differences Examined.” Equine practice is struggling with retention and Meagan’s talk will help us to better know what we don’t know about ourselves and others!
- The Kester News Hour—A format including anchors, a forecaster, sportscaster, and field reporter promises to be a fast-paced bundle of the latest information.
- The Frank J. Milne State-of-the-Art Lecture will be delivered by renowned equine lameness expert and anatomist, Dr. Jean-Marie Denoix.
- In-Depth Sessions on donkey and mule medicine, athletic rehabilitation of equine athletes, and respiratory diseases.
- “How-to” sessions for maximizing use of ultrasound in the field, causes of poor performance, and dentistry for field uses plus a “Back to Basics” session on wound management.
- Sessions on infectious diseases, sports medicine, uterine health, medical therapeutics, rescue techniques and challenges, imaging, and responsible use of antimicrobials.
- A special session on Sunday dedicated to retaining practitioners in equine practice featuring findings from our retention task force’s work, a panel with some who have left our ranks, and practitioners who are trying novel methods of finding better balance.
- Business sessions on collaborative models, personal financial health, telehealth and telemedicine, impostor syndrome, new paradigms in equine practice, avoiding legal issues with pre-purchase and leveraging your team.
- Ethics will be an early riser session that includes practical case-based scenarios and helpful panel discussion.
- An AAEP/AAEVT Joint roundtable discussion will help us improve our partnership.
- Dry Labs on navicular bursa injections, endometrial culture and cytology, ultrasound guided spinal joint injections, and podiatry.
- A Trade Show of more than 300 exhibitors with a wine reception on Sunday, product demonstrations by exhibitors, and the AAEP General Store.
- Extended Student Program dry labs offering hands-on practical skills plus the Avenues Career Night networking opportunity for students to meet with practices offering internship and externship positions.
- Many interactive Table Topics sessions and Meet the Expert sessions.
- A Healthy Practice session on understanding generational differences, and every morning features Partner Sunrise Sessions that offer healthcare and practice topics along with a complimentary breakfast.
- Special events like Storytelling Nashville Style with proceeds going to The Foundation for the Horse and the After Party at the Wildhorse Saloon.

This program would not be possible without the Educational and Media Partners. They provide generous support to the AAEP and its many programs throughout the year. A huge thank you is extended to them and to the many sponsors for helping make the AAEP Convention possible.

Emma K. Read, DVM, MVSc, DACVS
2021 President-Elect and Program Chair
2021 AAEP Board of Directors

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2021 AAEP Awards

Distinguished Life Member – William A. Moyer, DVM
The AAEP Distinguished Life Member designation is awarded to a member in recognition of outstanding contribution to the association throughout their career.

Distinguished Educator Award (Academia) – Elizabeth M. Santschi, DVM, DACVS
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 Service Award – James P. Morehead, DVM
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.

Sage Kester Beyond the Call Award – Larry R. Bramlage, DVM, MS, DACVS
This award is named in honor of its first recipient, the late General Wayne O. “Sage” Kester, DVM, and represents the highest honor bestowed by the AAEP upon a current or former member. The award is presented to an individual who has made significant and long-lasting contributions to equine veterinary medicine and the community. This individual not only possesses the qualities of a leader with a strong commitment to the health and welfare of the horse, but also impacts and improves the lives of others through service above self.

AAEP Research Award – Joy Ellen Tomlinson, DVM, DACVIM
The AAEP Research Award recognizes an individual who has recently completed research that has or will make a significant impact on the diagnosis, treatment, or prevention of equine disease.
General Instructions for Authors
68th AAEP Convention
San Antonio, TX
November 18–22, 2022
ALL papers must be submitted online by March 15, 2022, 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 Scientific Review & Editorial Committee (SREC). 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 (contact cross@aaep.org for this criteria) 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 SREC 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 SREC 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, Abstracts, and Business papers can be found in their respective sections.

Format:
- 12 point, Times New Roman font
- Double-spaced
- 1” margins
Proceedings should be written in the third person. Avoid the use of the first person and pronouns such as I, we, my, mine, us, our, ours.

Example of First vs. Third Person:
Rather than stating in the first person: “We concluded from our results that A plus B did not equal C”, use the third person: “The results of the study indicated that A plus B did not equal C”.

Heads 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 concise summary 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. Normality of the data should be described, and statistical analysis should be appropriate for the distribution of the data (parametric or non-parametric). For weights and measures, metric units should be used. Dosages should be expressed entirely in metric units and with specific time intervals.

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

Results:
Actual results with numbers and data must be presented. When possible, quantify findings (mean, median, proportion) and present them with appropriate estimates of measurement error or uncertainty (such as standard deviation (SD), standard error (SE) or confidence interval) in addition to the results of hypothesis testing. If the data can be well represented with a graph or figure, these are encouraged if subsequent publication is not anticipated. If numbers and data are not presented due to concerns regarding publication in a refereed journal, indications of relative differences between groups such as odds ratios, % change, and significant differences must be included in the submission to be considered acceptable. In these instances, the authors should submit the data in the form of means, standard deviations, or other descriptions of comparisons among groups in an appendix, which will not be published and only used for review purposes.

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

The following items must be fully explained in the paper; the number of horses that have been worked on, how many will be affected, and evidence that the procedure works and is safe.

Recommended Nomenclature:
Anatomy and anatomic planes should be described using standard nomenclature following the guidelines developed by Nomina Anatomica Veterinaria [http://www.wava-amav.org/downloads/nav_2012.pdf]

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

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]

1. 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.

2. 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 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.

American Association of Equine Practitioners’ (AAEP) policy requires that authors must disclose and describe the nature of any actual or potential financial and/or personal relationships they have with companies that manufacture or sell products that figure prominently in the submission or with companies that manufacture or sell competing products. (This includes ownership, employment, consultancy arrangements, or service as an officer or board member.) As 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:

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

Examples:

Journal article:


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 (10–20 mg/kg IV) and butorphanol tartrate (0.01–0.02 mg/kg IV).

* Dormosedan® Orion Corporation, Espoo, Finland.

† 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.

Figures, tables, and text should all be included in the same document.

Tables:

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

Figures, tables, and text should all be included in the same document.

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).

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 an individual with expertise in this area. The individual will then make a recommendation to the SREC 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 SREC 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, 2022, 3:00 p.m. ET.; 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 is not known to each other. Papers will be reviewed, scored, and selected by the Scientific Review & Editorial Committee. Please follow the blinding guidelines below.

**Blinding Guidelines:**
- The title page and/or front matter of the blinded version of the 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 websites, etc
- Do not use author name or affiliation in the names of the submitted files

**Scoring Criteria:**
One goal of the SREC in choosing submissions for the AAEP Annual Convention is to combine the best available clinical research with clinical experience and expertise to meet the needs of our patients. The AAEP Scoring Criteria can be requested from cross@aaep.org.

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

**Honorarium:**
Presenting authors will receive one complimentary registration and a check for $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 who have agreed to volunteer their time to mentor an author who needs guidance. To see this list, email Carey Ross at cross@aaep.org.

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**Scientific Papers: Guidelines for Authors**

68th AAEP Convention
San Antonio, TX
November 18–22, 2022

ALL papers must be submitted online by March 15, 2022, 3:00 p.m. ET.

Authors who do not intend to publish in a refereed journal are welcome to submit a Scientific Paper.

Scientific Paper selection will be made by the Scientific Review & Editorial Committee (SREC). 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, with no upper word limit.

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

68th AAEP Convention
San Antonio, TX
November 18–22, 2022

ALL papers must be submitted online by March 15, 2022, 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 equine veterinarians the information they need to critically evaluate the pros and cons of the technique and implement it in their practice if they choose.
“How to” papers can be patterned after a modification of the style for a Scientific Paper supporting a scientific presentation. Refer to General Instructions for Authors as you prepare your submission. How to papers should be no fewer than 600 words, with no upper word limit.

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 if the currently used method can be improved, this should be explained.

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

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

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

Review Paper: Guidelines for Authors
68th AAEP Convention
San Antonio, TX
November 18–22, 2022

ALL papers must be submitted online by March 15, 2022, 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 “General Instructions for Authors” except where otherwise noted here. The paper should be titled “Review of Some Subject.” The content of review articles should be organized with headings and subheadings that provide a logical flow to the material presented. A “Take Home Message” is required for a Review Paper. The Introduction should define the subject matter and put it in context, explaining why the review is necessary. The purpose of the review paper should be clearly stated in the Introduction.

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

An appropriately complete reference list should be included. The format for references is the same as that described in “General Instructions for Authors.” Review papers should be no fewer than 600 words, with no upper word limit.

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

Abstracts: Guidelines for Authors
For those who intend to publish in a refereed journal
68th AAEP Convention
San Antonio, TX
November 18–22, 2022

ALL papers must be submitted online by March 15, 2022, 3:00 p.m. ET.

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 should be at least 250 words. These “abbreviated abstracts” should follow a structured format with the same subheadings (Take Home Message, Introduction, Materials and 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 1500-word long 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 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 author’s 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 abbreviated abstracts should be identified with the words “RESEARCH ABSTRACT” at the end of the title.
**Guidelines for Abstracts**
- Minimum of 250 words. Abstracts can be longer; however, this is dependent on the journal in which the author wishes to submit the full paper. Journals differ in what they consider to be “prior publication”, e.g., Equine Veterinary Journal (EVJ) currently will allow an author to submit an abstract up to 1000 words whereas some journals allow fewer words (250). It is the author’s responsibility to contact the respective journal to discuss their prior-publication criteria so that their accepted abbreviated abstract will not jeopardize their publication in the refereed journal.
- Subheadings should include Take Home Message, Introduction, Materials and Methods, Results, and Discussion.

**Guidelines for Full Papers**
- 1500 words
- 12-point font
- 1” margins
- When submitting online, please put both papers in one document; the abstract should be first, followed by the full-length scientific paper.

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

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**Business of Practice Papers:**
**Guidelines for Authors**
68th AAEP Convention
San Antonio, TX
November 18–22, 2022

**ALL papers must be submitted online by March 15, 2022, 3:00 p.m. ET.**

The general theme for the 2022 Business of Practice Sessions is “Increasing Profitability in Your 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. Papers are allotted a total speaking time of 20 minutes (15 minutes presentation time + 5 minutes questions). The following topic suggestions are intended to spark ideas that relate to the theme; however, also welcome paper submissions on any topic pertaining to the Business of Practice.

**Potential Topics:**
- Billing and collection strategies
- Inventory management/purchasing strategy
- Understanding practice finances from the associate’s perspective
- How to motivate staff (veterinarians, technicians, office staff)
- Employee retention (wellness component)
- How to foster a team environment
- Getting the most from your staff (ensure the right people are in the right roles)
- How to delegate services that do not require a license
- Increasing online presence - telehealth/virtual visits
- How to add ancillary services - things that are typically referred out
- Opportunities in technology: billing management software (paying through a patient portal, offering payment plans)
- Scheduling systems/patient appointment efficiency

**Guidelines:**
Failure to adhere to the following format will result in non-acceptance. It is the author’s responsibility to convince the Scientific Review & Editorial Committee (SREC) 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.

Heads 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 concise summary 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 multiple 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).

1. 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.
2. 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.) In addition, if the author provides veterinary business consulting services, or earns income through veterinary business educational offerings, this should be disclosed. 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 inappropriately influence, or be perceived to influence, their work.

The American Association of Equine Practitioners’ (AAEP) policy requires that authors must disclose and describe the nature of any actual or potential financial and/or personal relationships they have with companies that manufacture or sell products that figure prominently in the submission or with companies that manufacture or sell competing products. (This includes ownership, employment, consultancy arrangements, or service as an officer or board member.)

As 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:
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, 2022, 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 SREC. 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 un-blinded before final selections are made. This two-step review process was implemented to protect the association from selecting speakers whose presentations may have a strong commercial bend.

Blinding Guidelines:
- The title page and/or 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 websites, etc.
- Do not use author name or affiliation in the names of the submitted files.

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

Pre-Press Approval:
Authors will have final approval at the page proof stage. Changes/updates may be made within one week of receiving page proofs. Final grammatical changes will be the decision of the editors. Substantial changes or removal of any data will result in forfeiture of complimentary registration, honorarium, 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 who have agreed to volunteer their time to mentor an author who needs guidance.

“How to” Paper Submissions for Business of Practice Sessions:
“How to” papers are presented to describe and explain a technique or procedure that relates to the business of practice. The goal of these papers is to give 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.
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How to Evaluate the Foal Abdomen and Thorax Ultrasonographically in the Field

William F. Gilsenan, VMD, DACVIM (LAIM)

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1. Introduction
As technology improves, portable ultrasonography machines continue to become more affordable and more readily available. Ultrasonography is a safe and noninvasive diagnostic imaging modality. While ultrasonographic examination of the reproductive tract has become routine, this imaging modality is often underutilized in evaluation of the horse’s two largest body cavities. Unfamiliarity with internal anatomy and interpretation of collected images can seem daunting. However, with repetition, discernment between normal and abnormal findings becomes readily achievable using equipment that a practitioner often already owns. Because ultrasonography is limited by the depth that sound waves penetrate, the relatively smaller size of foals facilitates a more complete evaluation of the abdominal and thoracic viscera compared to their older and larger counterparts. Moreover, diseases affecting the respiratory, gastrointestinal, and urogenital tracts rank among the most common causes of morbidity and mortality for neonatal and juvenile foals. Skillful employment of the ultrasound probe will speed up the diagnostic process, lending to sooner and more targeted therapeutic intervention.

2. Materials and Methods
Prior to beginning an ultrasonographic examination, there are four recommendations that should be heeded to ensure a successful study. These recommendations include examination setting, physical and chemical restraint, selection of an appropriate ultrasonographic probe, and use of a conductive medium. First, the examination should ideally take place in a quiet and dimly lit environment to allow the practitioner to effectively view the ultrasonography machine’s display screen. Second, adequate restraint of the foal should be pursued whenever possible. In the author’s opinion, ultrasonography is best accomplished in the standing foal. Thoroughly imaging a down foal requires repositioning, decreasing the efficiency of the scan compared to performing the study in a tractable standing foal. Restraint can be accomplished by one or two assistants. It is less stressful for the foal and advantageous for the restrainer to place a hand cranial to the opposite shoulders and scapula rather than across the ventral aspect of the neck (Fig. 1). If the foal’s disposition requires chemical restraint, diazepam (0.1 mg/kg, IV) with or without butorphanol tartrate (0.02–0.04 mg/kg, IV) is recommended for sick foals less than 30 days of age and for all foals less than 14 days of age. Incorporation of butorphanol might induce recumbency. For older foals, xylazine (0.3–0.5 mg/kg, IV) will likely be more effective to sedate the patient for proper examination. Third, selection of an appropriate ultrasonographic probe is critical to completing an effective examination. In the author’s opinion, a small curvilinear, or microconvex, probe is
most appropriate to evaluate the neonatal or juvenile thorax and abdomen (Fig. 2). The microconvex probe’s higher frequency range allows increased imaging detail in relatively superficial planes. The microconvex probe can reliably penetrate up to 8 to 10 cm deep. The probe’s smaller footprint fits best in the intercostal spaces of young foals. While rectal probes and linear probes generally provide an even higher frequency range, their limited penetrative abilities can compromise assessment of deeper structures. Conversely, these probes provide excellent detail with a more suitable footprint for ultrasonographic evaluation of rib fractures in neonatal foals. A linear probe or microconvex probe is most suitable for evaluation of the umbilical remnants. The large curvilinear, or macroconvex, probe is limited by its lower frequency range and larger footprint. The depths to which this probe can image (up to 30 cm) are often not required during the first few months of a foal’s life. While the microconvex probe is ideal, any of these probes is appropriate, but the practitioner should be cognizant of the limitations of each. Most ultrasonographic probes have a ridge or LED light on one side of the transducer (Fig. 3). This indicator corresponds to the indicator on the display screen (Fig. 4). Depending on the plane that is being scanned, the indicator should be pointed either laterally, cranially, or dorsally. Lastly, because ultrasonographic waves do not travel readily through air, some sort of conductive medium is required during the examination to create a seal between the skin and the ultrasound transducer. In the author’s opinion, application of isopropyl alcohol to a foal’s coat, either directly or via a soaked cotton gauze pad, provides suitable images without requiring clipping. Some practitioners prefer to clip the coat, use ultrasonographic coupling gel, or both.
Ultrasonographic Examination of the Foal Thorax

In contrast to examination of the abdominal cavity, ultrasonographic evaluation of the thoracic cavity is comparatively simple due to the low acoustic impedance of air as compared to soft tissue and viscera. Specifically, with a normal aerated lung, only the visceral pleural surface is readily imaged. It should appear as a linear hyperechoic structure that glides easily along the parietal pleural surface during the respiratory cycle (Fig. 5). Disruptions of the visceral pleural surface are indicative of pulmonary disease. The author prefers to ultrasonographically evaluate the thorax in a caudal-to-cranial direction, starting dorsally in the most caudal intercostal space in which pulmonary tissue can be identified. The probe should be moved ventrally along each intercostal space until abdominal viscera are imaged. The probe should then be moved dorsally to the next intercostal space cranially; this should be repeated until the most cranial extent of the thoracic cavity is reached. It is generally recommended to decrease the depth of the ultrasonographic field during the thoracic examination as tissue deep to the pleural surface cannot be imaged in aerated tissue due to reverberation artifact. It is important to ensure that the pleural surfaces cranial to the heart (approximately third and fourth intercostal spaces) are imaged by placing the transducer in a frontal plane over the triceps muscles (Fig. 6). It becomes necessary to considerably increase the depth of the ultrasonographic field to assess the thoracic cavity in this region. The most common pulmonary pathologies identified by ultrasonography include comet tail artifacts, pulmonary consolidation, pulmonary abscessation, and pleural effusion. Comet tail artifacts indicate roughened regions of the visceral pleural surface and are indicative of current or previous pulmonary pathology (Fig. 7). While small numbers of comet tail artifacts can be identified in normal foals, coalescing comet tail artifacts are usually indicative of more severe pathology. Coalescing comet tail artifacts might be seen in foals with meconium aspiration pneumonia or interstitial pneumonia. Although ultrasonography cannot definitively diagnose either of these disease processes, these ultrasonographic findings should prompt further diagnostic efforts, such as clinical laboratory work, thoracic radiography, lower airway endoscopy, and tracheal wash, to identify the nature of pulmonary disease. Pulmonary consolidation allows the imaging of deeper pulmonary tissue due to the absence of air in the foal’s lower airways. It should be remembered that any air prevents ultrasonographic waves from penetrating more deeply, so partially aerated tissue may incompletely obscure deeper pulmonary consolidation. Consolidated tissue may therefore only be visible during certain phases of the respiratory cycle. Completely atelectatic pulmonary

Fig. 5. Visceral pleural surface in a normal foal.

Fig. 6. Placement of an ultrasonographic probe over the left triceps muscle to evaluate the cranial lung fields.
Parenchyma is more readily imaged due to the absence of air and is often described as "hepatized" due to its resemblance to the ultrasonographic appearance of the liver and increased conspicuity of the pulmonary vasculature (Fig. 8). Pulmonary consolidation is never considered normal. In most cases, pulmonary consolidation is indicative of pneumonia. If mild with a predominantly subpleural distribution, pulmonary consolidation might be related to decreased surfactant production in neonatal foals. Additionally, pneumothorax may cause lung collapse, leading to atelectasis. While some causes of pulmonary consolidation can be excluded based on history and signalment alone, the presence of this finding is most often indicative of pneumonia or, at the very least, compromised pulmonary tissue that is susceptible to infection. Pulmonary abscesses can be difficult to distinguish from pulmonary consolidation in many instances. Often the distinction is not critical as both findings are suggestive of a lower airway infection. Only superficial abscesses can be imaged unless overlying pulmonary parenchyma is not aerated. Pulmonary abscesses caused by Rhodococcus equi are often rounded, contain anechoic or hypoechoic debris, and are surrounded by a hyperechoic rim (Fig. 9). Identification of pulmonary abscesses in foals susceptible to clinical or subclinical Rhodococcus equi pneumonia (1–6 months of age) should prompt tracheal wash for confirmatory diagnosis. On breeding farms with endemic rhodococcosis, it has been demonstrated that the majority of foals with subclinical R. equi infection will experience resolution of disease without therapeutic intervention.\(^1\) A scoring system incorporating ultrasonographic findings has been developed to better differentiate affected foals that may require therapy. The reader is directed to the references for further information on this topic.\(^2\) Pleural effusion refers to the accumulation of fluid in the pleural space. Pleuropneumonia is considerably less common in juveniles compared to adults but is observed

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**Fig. 7.** Coalescing comet tail artifacts in a 60-day-old foal.

**Fig. 8.** Consolidated, or hepatized, lung parenchyma (Ø) in the left cranial lung fields of a 42-day-old foal.

**Fig. 9.** Superficial pulmonary abscess in a 45-day-old foal.

**Fig. 10.** Pleuropneumonia secondary to Rhodococcus equi infection in a 90-day-old foal. Echogenic pleural effusion (§), diaphragm (*), and liver (Ø) are imaged.
occasionally. Free fluid in the thorax is often anechoic. Echogenic pleural effusion should heighten concern of an exudate (Fig. 10). A swirling appearance of the effusion may be seen in cases of hemothorax that, in neonatal foals, would be observed most frequently in instances of rib fractures. Pleural effusion will induce atelectasis of ventral pulmonary parenchyma. Unless hemothorax is strongly suspected, thoracocentesis with fluid analysis, cytology, and culture would be the most logical next diagnostic step to assess the nature of observed pleural effusion.

Ultrasonographic Evaluation of the Foal Abdomen

The principles guiding ultrasonographic evaluation of the foal’s abdominal cavity do not deviate significantly from the examination of the adult abdomen. Examination of the abdomen is best described by breaking the cavity down into three regions: the left flank, the right flank, and the ventrum. Ultrasonographic examination of the left flank is best initiated caudodorsally in the left paralumbar fossa. The transducer should be oriented in a frontal plane and glided ventrally along the flank. This should be repeated in progressively more cranial planes while remaining ventral to the rib cage. In the normal foal, the spleen and left kidney should be readily imaged in the left paralumbar fossa. Along the ventral half of the left flank, the left dorsal and left ventral colon can be imaged. Normally, colonic contents include gas in addition to solid and liquid feed, so a reverberation artifact is expected deep to the colon wall. Purely liquid colonic digesta supports a diagnosis of enterocolitis. The colonic wall thickness can and should be measured in this location; normal wall thickness is less than that of an adult (2–3 mm). Following examination of the left paralumbar fossa and ventral colon, the intercostal spaces should be scanned in a caudal-to-cranial direction until no abdominal viscera can be identified, which usually occurs near the point of the elbow. Of primary importance in this region is identification of the stomach. The stomach can be recognized by its hypoechoic wall; normal wall thickness is 2 to 4 mm. The stomach is situated dorsal and slightly medial to the hilus of the spleen and typically does not extend further caudally than the 10th to 11th intercostal spaces. While the stomach might extend even further caudally immediately following nursing, a stomach distended with liquid contents in a colicky foal is an indication for immediate nasogastric intubation and decompression (Fig. 11). Lastly, a small segment of the liver can be imaged in the left craniolateral abdomen near the sixth to seventh intercostal spaces. In this location, the liver borders the spleen. This serves as a convenient site for comparing echogenicity of both viscera. In the normal foal, hepatic parenchyma should be hypoechoic compared to the spleen. To evaluate the right flank ultrasonographically, the same technique that is used on the left flank should be employed. In the caudodorsal right paralumbar fossa, the cecum can be imaged; as with the large colon, the cecum generally contains some gas, obscuring assessment of its contents. The right dorsal and right ventral colon can be identified in the ventral half of the right flank; assessment is similar to that of the left colon. The right kidney is imaged in the craniodorsal paralumbar fossa or at the 16th to 17th intercostal spaces. The right kidney serves as a helpful landmark for locating a cross-section of the duodenum, which is located just cranioventrally from the right kidney. The duodenum should be assessed for motility and wall thickness (2–3 mm). The duodenal lumen may dilate considerably with normal motility but should collapse frequently as peristaltic waves pass (Fig. 12). Turgid distension of the duodenum (greater than 2–3 cm internal diameter) is indicative of a functional or mechanical intestinal obstruction (Fig. 13). Marked thickening (>5 mm) of the duodenal wall might be identified in juvenile foals affected with impaired gastric emptying caused by...
gastroduodenal ulceration (Fig. 14). The presence of hyperechoic echoes in the bowel wall indicates intramural gas and is defined as pneumatosis intestinalis. This finding should heighten the practitioner’s suspicion of necrotizing enterocolitis. The liver is best studied along the ventral intercostal spaces of the right flank. The liver should be assessed for appropriate echogenicity and echotecture; the walls of the portal vasculature should be hyperechoic relative to the hepatic parenchyma, and the liver margins should be sharp, not extending beyond the costochondral junctions. Ultrasonographic examination of the ventrum, including the inguinal areas, is critical as identification of free peritoneal fluid and fluid-filled distended bowel is most frequently identified in this location. Evaluation of the foal’s ventrum is best accomplished by placing the ultrasound transducer in a transverse plane in the left craniolateral abdomen. The transducer should be moved in a cranial-to-caudal direction toward the pelvic inlet. This should be repeated in rightward planes until the entirety of the ventral abdomen has been examined. In the ventral abdomen, the practitioner should be able to identify the left and right ventral colons. Small intestinal loops are frequently identified in the ventral abdomen and inguinal regions; assessment of small intestinal loops in this area is identical to assessment of the duodenum (Fig. 15). Certain small intestinal abnormalities may heighten concern of a mechanical obstruction. These include the classic “target” lesion characteristic of intussusceptions or the presence of “two populations” of bowel in which some small intestinal loops are amotile and distended while other loops remain collapsed and devoid of ingesta. It has been demonstrated that intussusceptions may be identified incidentally in
normal Standardbred foals. However, identification of a “target” lesion in a colicky foal warrants abdominocentesis and probably exploratory laparotomy due to the potential for bowel incarceration and consequent devitalization. The bladder can be imaged in the inguinal regions at the pelvic inlet. The diameter of the bladder can vary considerably (up to 10 cm in a neonatal foal). Urine is typically anechoic but will have an echogenic appearance during the first 12 to 24 hours of life. Large volumes of free peritoneal fluid may be imaged in the ventral abdomen and inguinal regions; potential causes of increased peritoneal effusion are numerous and are best differentiated by fluid analysis, cytology, and culture via abdominocentesis. Lastly, and unique to the foal, evaluation of the internal umbilical remnants should be included in the abdominal ultrasonographic examination. Examination should begin with the transducer held in a transverse plane just caudal to the external umbilical remnants. Identification of the internal stump, including the urachus, umbilical arteries, and umbilical vein, is facilitated by angling the transducer slightly cranially (Fig. 16). The transducer can then be moved caudally to follow the urachus and umbilical arteries to their insertion at the bladder. To assess the umbilical vein, the transducer should be held in a transverse plane just cranial to the external umbilical remnants. The transducer should be moved cranially in a transverse plane along the ventral midline, following the single umbilical vein remnant. It may be necessary to move the transducer slightly to the left or right of the midline while coursing cranially to keep the umbilical vein remnant in the center of the screen. The remnant should be followed all the way to its insertion at the liver. The umbilical remnants should be assessed for size and structure. The internal umbilical remnants can develop into abscesses secondary to ascending infection or spread of hematogenous infection. Indications of an umbilical infection include enlargement of the internal structures or the presence of sedimented material or gas in the internal structures (Fig. 17). Importantly, a normal external umbilical remnant does not preclude the possibility for severe internal umbilical remnant infection. During the first week of life, the internal umbilical remnant diameter should be less than 24 mm, and the umbilical vein diameter should be less than 10 mm. The internal remnants should gradually regress and should be difficult to detect by 5 to 6 weeks of age.

3. Discussion

Ultrasonographic findings from the thorax and abdomen can provide the practitioner with a considerable amount of information when confronted with a sick foal in a stall-side setting. While ultrasonographic assessment is complex and requires skill and practice, approaching each examination in a methodical and consistent order should allow the practitioner to gain comfort in interpretation of images and differentiation between normal and abnormal. The reader is directed to the references for additional information on collection and interpretation of ultrasonographic images.

Acknowledgments

Declaration of Ethics

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

Conflict of Interest

The Author has no conflicts of interest.

References

Ultrasound of the Adult Abdomen and Thorax

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1. Introduction
Diagnostic ultrasound is widely used in equine practice for a diversity of applications. Sonographic evaluation of the equine thoracic and abdominal cavities provides diagnostic information, the value of which is heavily influenced by the preparation of the patient and the technique and experience of the examiner. These proceedings will outline key strategies for making the most of thoracic and abdominal ultrasonography in the horse with considerations of case selection, patient preparation, equipment requirements, and examination technique.

2. Materials and Methods
Case selection involves understanding both manifestations of disease and the limitations of sonography as a modality. By far, the most common application of abdominal sonography in equine practice is the horse with acute signs of abdominal pain. For these patients, specific information is desired to make a rapid determination as to how to achieve the best outcome for the patient (referral vs on-farm treatment, surgical vs medical management vs euthanasia). The most appropriate approach to these patients is a limited, rapid examination that efficiently and specifically addresses these concerns. The fast localized abdominal sonography for horses (FLASH) technique achieves these goals in a systematic examination in seven discrete windows. Evaluation of the anatomy in these windows gives the examiner important data about the likelihood of a colonic displacement, distention of the stomach and/or small intestine, and the presence or absence of peritoneal and pleural effusions and requires only around 10 minutes to perform. As effective a tool as FLASH is at providing information about acute colic, however, it is inappropriate to apply this technique to other applications for abdominal sonography, including fever, chronic colic, diarrhea, liver or kidney dysfunction, anemia, abdominal distention, weight loss, and hypoproteinemia. To obtain meaningful information for aiding in the investigation of these clinical presentations, a full abdominal ultrasound is indicated. A comprehensive abdominal ultrasound is time consuming; to fully scrutinize each intercostal space and the ventral abdomen, up to one hour may be required. To maximize the use of this technique, the sonographer must also possess a commanding knowledge of the anatomy and sufficient experience to accurately interpret subtle alterations of the anatomy. Indications for thoracic ultrasound include fever, tachypnea, cough, sternal edema, pleurodynia, and a history of trauma with suspicion of rib fractures, hemothorax, or pneumothorax. Pulmonary lesions are detected sonographically only if they extend to the pleural surface, and lesions that are confined to the airways, deep to air-filled parenchyma, such as equine asthma, are not sonographically accessible. Ultrasound evaluation of the thorax is most useful for detecting pleural effusion, rib
fractures, and infections and neoplastic processes that occur at the pleural surface. Familiarity with the anatomy of the pleural space and diaphragm is required to accurately interpret the images obtained. Regardless of the type of examination, proper, thorough patient preparation is essential for the acquisition of high-quality images. For ideal image quality, as much of the haircoat as possible should be removed by clipping with a #40 blade and the skin washed with soap and warm water. Application of gel to the skin provides a coupling substrate. Warming the gel prior to application promotes vasodilation in the skin and therefore improves transmission of sound waves. If clipping is not possible, the haircoat should at least be brushed thoroughly to remove dirt and debris, as dirt will block the transmission of sound waves. If not clipping, one should use isopropyl alcohol or warm water instead of gel to wet the hair and provide a coupling substrate (Fig. 1). When using alcohol, especially in cool environments, evaporation will cause piloerection and impede further imaging, even when alcohol is reapplied. Thus, applying alcohol to one small area at a time as that area is being evaluated is strongly recommended over dousing the entire side of the horse at the beginning of the examination. Visceral gas is a major impediment to visualization of the abdominal contents of the horse. Additionally, the small intestine is a common site for digestive dysfunction, resulting in weight loss, protein loss, and chronic intermittent colic, but is frequently poorly visualized due to colonic gas, its anatomic location, and the presence of ingesta within the small intestinal lumen. Fasting has been shown to have the effect of both reducing gas in the large colon and slowing the peristalsis of the small intestine, which favors it sinking to the ventral abdomen where it can be more readily examined. This effect has been demonstrated to improve measures of small intestinal visibility and definition, and although improvement of the visibility of other organs has not been critically assessed, fasting may also improve imaging access to other organs, such as the liver. Circumstances obviously prevent a pre-examination fast in acute colic, but all horses undergoing scheduled full abdominal sonography should be fasted (with free access to water) for at least 12 hours before the exam. If ultrasound and gastroscopy are being performed at the same visit, ultrasound would ideally be performed first to avoid the impediment of insufflated gas in the stomach and small intestine. The astounding advances in imaging technology have opened up a variety of options to the equine practitioner. To perform a thorough abdominal examination, a low-frequency transducer of either curvilinear or sector configuration is ideal. The advantage of these configurations is the pie-shaped image, which provides a wide image window through a relatively small transcutaneous footprint. Linear probes (e.g., “tendon” or “rectal” transducers) can be used for thoracic evaluation and can be used to obtain limited information about the abdominal cavity, but they have insufficient depth/power to penetrate the deeper tissues of the abdomen. Because the pleural surface is relatively close to the body surface, higher frequency transducers can be very useful for thoracic evaluation, but a curvilinear configuration remains the preferred choice due to the increased width of anatomy that can be evaluated at one time (Fig. 2). A linear transducer is often the ideal choice for evaluating the ribs when fractures are suspected, however. Many ultrasound systems have preset exam settings, which are a great starting place, but they cannot account for all of the physical (overall size, breed, and adiposity) and physiological (hydration and ambient temperature) differences between patients. This means that the examiner is required to “finesse” the settings to account for these differences and to account for the depths and densities of various organs. Settings that are frequently adjusted include depth, frequency, focus, gain, and time/gain compensation. Depth should generally be adjusted so that the organ of interest just fills the screen; this maximizes the information contained in the viewing...
Because of the various sizes and locations of abdominal organs within the body, depth is constantly adjusted in the course of abdominal sonography. The "frequency" refers to the number of sound waves that pass a fixed point per second. As frequency increases, the level of detail in the image increases, but the ability of the sound waves to penetrate tissue decreases. Therefore, frequency should be adjusted to the highest frequency that yields a diagnostic image at the required depth. Like depth, frequency is adjusted up and down to accommodate variable depths and tissue densities throughout the abdominal scan. Focus refers to the region of the screen that has the best resolution, which is analogous to the focus of a camera. For most systems, the zone in focus is indicated with a cursor on the right of the screen and may be single or multiple. Use of more than two focal zones slows processing speed, defeats the purpose of bringing the object of interest into focus, and is therefore not recommended. On certain systems, the zone in focus is always the area in the center between the most superficial structures and the limit of depth. When using these systems, adjusting the depth also has the function of changing the focus. Gain is the brightness of the image. It should not be altered until after the frequency has been optimized. Time/gain compensation changes the gain throughout the depth of the image, which is especially important to use when evaluating deeper structures to produce an image with even brightness throughout and not overly bright in the superficial structures and not bright enough in the deeper ones. This principle can also be used in reverse in cases of effusion when acoustic enhancement results in the deeper structures to be overgained. Thoracic sonography generally requires less manipulation of the images during the examination due to the fact that the pleural surface is generally the sole object of interest and occurs at a roughly consistent depth. In the case of pathology, however, adjustments will need to be made as in abdominal sonography. Regardless of what is being evaluated, labels are required to identify (at least) the anatomic location in the image and the orientation of the transducer with respect to the patient's body. The anatomic location seems logical and obvious when the anatomy is normal but is often neither in pathologic states. The label should indicate the side of the horse and the general region and provide more information if relevant. For thoracic imaging, it is useful to divide the trunk into thirds.
from ventral to dorsal and indicate that and the intercostal space number on the label. For the examination, as for any examination, the practitioner should follow the same pattern each time to maximize muscle memory and to avoid errors. This pattern should be logical and easy to the operator; no one system is perfect for everyone. The author begins every evaluation on the left side in the most dorsal imaging window; for the abdomen, this is the left paralumbar fossa, and for the thorax, this is the left 15th intercostal space just ventral to the epaxial musculature. The examination proceeds by sliding down each intercostal space from dorsal to ventral and then, for the abdominal evaluation, sliding from cranial to caudal (e.g., with the hair) along the ventral abdomen. When evaluating the thorax, the author interrogates each intercostal space starting dorsally at the epaxial muscles and terminating at the diaphragm. Care is needed in both thoracic and abdominal sonography to orient the transducer parallel in the intercostal space and perpendicular to the organ of interest. A fanning technique within intercostal spaces is useful to obtain images cranial and caudal to the intercostal spaces. When performing these examinations, images should be saved even when the anatomy appears to be normal. Representative images of the normal abdomen include at a minimum the nephrosplenic space, (including the entire left kidney), the tail of the spleen, the stomach, the liver/spleen interface, the right kidney and duodenum, the right dorsal colon adjacent to the liver, and the ventral abdomen. Representative images of the normal thorax include at a minimum a sampling of intercostal spaces at the level of the diaphragm; the author generally collects images at the 12th, 8th, and 6th intercostal spaces on each side of the thorax. When pathology is noted, more images are generally required to demonstrate the nature and extent of the abnormalities. Extremely abnormal findings may require additional annotation for clarity.

3. Results/Discussion
Abdominal and thoracic ultrasound provide useful diagnostic and prognostic information in both acute and chronic disease. Careful case selection, thorough patient preparation, knowledge of machine settings, accurate annotation, and well-practiced technique are all key elements of making the most of this imaging modality.

Acknowledgments

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

Conflict of Interest
The Author has no conflicts of interest.

References
Musculoskeletal Ultrasound of the Foal

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1. Introduction

Lameness in foals can be extremely frustrating when the source of lameness is not readily apparent. The clinical presentation of a traumatic insult can be similar to that of a septic process, so accurate and prompt diagnosis of the underlying problem is important to administer appropriate treatment to minimize morbidity and mortality. Proximal limbs are more challenging to assess, as soft tissue swelling and/or effusion can be difficult or impossible to palpate during a physical examination. Ultrasound examination of the lame foal can provide valuable information that assists with a definitive diagnosis of the cause of lameness using equipment that many practitioners have readily available to them.

2. Materials and Methods

If at all possible, the region of interest should be clipped to maximize image quality and diagnostic yield. The clipped area should be cleaned with isopropyl alcohol or soap and water and acoustic coupling gel applied. Sedation may be helpful if the foal is fractious. A linear tendon probe is generally appropriate for the distal limbs, stifles, elbow, and shoulder region. In younger or smaller foals, it may also be possible to image the pelvis with a linear probe. If a linear probe cannot provide the penetration required to image deeper structures, a lower frequency curvilinear probe may be necessary. The specific frequency, depth, gain, and other machine settings should be optimized for each patient and body region. A systematic approach is helpful for evaluating each body region. Specific anatomic structures to evaluate include the following: bony margins, physis, articular cartilage, synovial fluid, synovial membrane, tendons and ligaments, the subcutaneous tissues, and muscle layers. Particularly challenging areas in foals are any places where ossification has not been completed (e.g., physes, trochlear ridges of the distal femur, and proximal humeral tubercles). Comparing the limb of interest to the contralateral limb is extremely helpful for determining if a suspicious area is abnormal.

3. Results

Some general principles apply to ultrasonographic examination of the lame foal. A wide variety of abnormalities of a region can produce a similar clinical presentation, namely, lameness, soft tissue swelling, and sensitivity to palpation or flexion. The goal of the ultrasonographic examination is to identify specific abnormalities and therefore narrow down the list of differential diagnoses, while recognizing that additional tests (e.g., radiography, synoviocentesis and fluid analysis, and hematology) may be necessary as well. A region can be divided into the following four major categories: subcutaneous tissues/muscle, tendons and ligaments, synovial structures, and bones. One or more of these structures may be abnormal and provide clues as to the source of lameness. Generalized subcutaneous thickening is often a nonspecific finding. Lacy-appearing
Subcutaneous edema may indicate a cellulitis (Fig. 1), and a focal encapsulated pocket may represent an abscess or seroma (Fig. 2). Foreign bodies may be identified in any layer of tissue even if a puncture wound is not immediately apparent (Fig. 3), and ultrasonography is more helpful than radiography in cases of nonmetallic foreign bodies.1 Although tendon or ligament injury is relatively uncommon in foals, it does occur (Fig. 4). The injury may be traumatic in nature or secondary to sepsis. Synovial effusion and synovial membrane proliferation are hallmarks of synovitis due to a variety of causes (Fig. 5).2,3 In foals, a septic synovitis is often the culprit. Echogenicity of synovial fluid is a poor predictor of synovial sepsis in horses,2 and any suspicious effusion should be further...
investigated via synoviocentesis with fluid analysis to determine if sepsis is likely present. Although joints are often the first synovial structures that come to mind, bursas and tendon sheaths should also be assessed and sampled accordingly (Fig. 6). Ultrasound is an excellent tool with which to assess bony margins. Areas of irregularity consistent with osteomyelitis may be identified readily with ultrasound, sometimes prior to abnormalities becoming apparent radiographically (Fig. 7).4 Widening and irregularity of physes due to septic physitis may also be observed (Fig. 8). Bony margin discontinuities consistent with fracture can be appreciated, which are particularly useful in areas that are more challenging to radiograph, such as the pelvis (Fig. 9). Osteochondrosis lesions of the distal femur may also be identified using ultrasonography, even when radiographic images are normal or equivocal.5,6 The presentation of early osteochondrosis can be identical to that of a septic joint, with lameness and large amounts of synovial effusion. The finding of normal synovial fluid analysis with an ultrasonographically

4. Discussion

In the author’s practice, ultrasonography is routinely incorporated into the diagnostic approach for lame foals. It is readily available, noninvasive, and generally well tolerated by the patient (sometimes with the aid of sedation). The information gained often guides the subsequent diagnostic workup, which can efficiently focus tests that involve more invasive procedures on targeted sites of concern. For example, in a foal with diffuse fetlock region swelling, ultrasonographic findings can determine if a synovial structure is sampled, which synovial structure is sampled (e.g. fetlock joint, digital flexor tendon sheath, proximal or distal interphalangeal joint, or navicular bursa), or if an abscess or cellulitis is present in the absence of synovial effusion and synovial sampling is not indicated at the time but drain placement is indicated. As

Fig. 5. Longitudinal image of the tarsocrural joint from the dorsomedial aspect of the limb. There is effusion within the joint cavity as well as synovial membrane proliferation (arrows) and fibrin deposition (arrowhead). Proximal is to the right of the image.

Fig. 6. Navicular bursa effusion (arrows). DDFT, deep digital flexor tendon; NB, navicular bone; PII, second phalanx; arrowhead, navicular suspensory ligament. Proximal is to the right of the image.

Fig. 7. Transverse image of the scapular spine. There is focal irregularity of the bony margin (arrow). Cranial is to the right of the image.

Fig. 8. Longitudinal images of the caudolateral humeral head. A. There is widening and irregularity of the physis consistent with septic physitis (arrows). B. Contralateral limb demonstrating the normal appearance of the physis (arrowhead). HHI, epiphysis of the humeral head. Proximal is to the right of the image.
with any new technique, it does require some practice to become comfortable with ultrasonographic anatomy of new areas. This is particularly challenging in foals, who are constantly growing and changing. Comparisons between the limb of interest and the contralateral limb are extremely helpful when trying to determine if a finding is a true abnormality or is normal for a particular foal at its current stage of growth. For example, the thickness and appearance of cartilage is variable, even between foals of similar ages, and comparison to the contralateral (normal) limb can prevent a misdiagnosis or allow recognition of a clinically relevant abnormality.

Fig. 9. Image of the ilial wing and tuber coxae. A step defect in the bony margin of the ilial wing at the fracture site is present (arrow). A fracture should not be confused with the normal physis present at the tuber coxae (arrowhead). TC, tuber coxae. Lateral is to the right of the image.

Acknowledgments

Declaration of Ethics
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Conflict of Interest
The Author has no conflicts of interest.

References
Ultrasound of the Hind Suspensory Ligament

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1. Introduction

The suspensory ligament of the hind limb is an important source of lameness in the horse. Suspensory injury (or desmitis) most often occurs due to repetitive stress associated with work but may also occur secondarily to direct trauma. Often this trauma is associated with splint bone fractures.¹ A detailed clinical examination including diagnostic analgesia and diagnostic imaging is necessary to provide an accurate diagnosis. Determining the full extent of injury frequently requires multiple diagnostic imaging modalities. At a minimum, radiology should be used to assess bony involvement, whereas ultrasonography should be used to evaluate the soft tissue structures. It should be noted that these imaging tools provide complementary information about a suspensory ligament injury, as desmitis often occurs at the sites of insertion onto the metatarsal bones and/or the proximal sesamoid bones.¹ The suspensory ligament is composed of ligament fibers with fat and muscle distributed throughout. The fat and muscle are in similar locations in all horses but not always identical even between legs of the same horse.²,⁴ At the origin of the ligament on the third metatarsal bone the ligament begins in a triangular shape that quickly becomes heart shaped just distal to the origin. In this proximal region, the suspensory ligament is closely associated with the fourth metatarsal bone with the majority of the ligament lying lateral of midline. As the ligament continues distally into the body of the ligament, it becomes oval shaped until it bifurcates into a medial and lateral branch approximately two thirds of the way distal in the metatarsal region.² The branches begin proximally as circular structures and become more triangular shape in the mid aspect of the branch until they insert on the respective proximal sesamoid bone.

Ultrasound Machine

As with all ultrasound examinations, it is important to begin with the appropriate probe and preset selection on the ultrasound machine to optimize the image quality. A high frequency (7–13 MHz) linear transducer is used for the examination to assist in higher...
image resolution. Patient preparation with clipping and a detergent scrub of the skin are critical to optimize image quality. In addition, a standoff may be used to assist with contact (increasing the footprint) throughout the weight-bearing portion of the examination. The frequency, focal zones, depth, and gain should be adjusted to optimize the image throughout the examination.

2. Ultrasound Examination

A complete examination of the hind limb suspensory ligament includes an evaluation of the proximal suspensory ligament including its origin on the plantar aspect of the third metatarsal bone, the body of the suspensory ligament, the suspensory branches, and their insertion on the proximal sesamoid bones. A full examination not only includes all of these anatomic locations, but also a weight-bearing and a non-weight-bearing component. The examination should be performed in the transverse and longitudinal plane, especially when confirming the presence of a discrete lesion. The ligament should be compared between the right and left hind limb to assist in establishing normal patient variations, changes in size, or to confirm the presence of a lesion. Throughout its entirety, the suspensory ligament is evaluated for changes in size, shape, fiber pattern, and margins. Angled contrast ultrasonography has been proven an effective technique to more clearly delineate the ligament margins, as well as the surrounding tissues that may be associated with adhesion formation. Angle contrast ultrasonography is also useful in the proximal aspect as well as the body of the suspensory ligament to distinguish the ligament fibers from the fat and muscle dispersed throughout the ligament, as these tissues are less angle dependent than the ligament fibers.

Proximal

The proximal suspensory ligament is historically a more difficult region to ultrasound as well as confidently diagnose abnormalities. Getting a clear visualization of the proximal suspensory ligament can prove difficult due to its position deep to the flexor tendons, check ligament, and large tortuous vessels. All of the previously described structures between the suspensory ligament and the ultrasound transducer create significant edge artifacts within the ligament due to the differences in echogenicity, fiber orientation, and acoustic impedance. Because of these previously described difficulties, MRI examination remains the gold standard for imaging the proximal suspensory region when a horse’s lameness is localized to this region. It is important to also note that the tarsal extension is difficult to see ultrasonographically, and injuries in this location are normally diagnosed with an MRI. To begin the exam, the transducer is placed just distal to the chestnut or at the level of the tarsometatarsal joint. The fourth metatarsal bone is large and wraps around the plantar aspect of the proximal suspensory ligament; therefore the ultrasound transducer is placed on the plantar medial aspect of the leg to better visualize the suspensory ligament (Fig. 1). The origin of the ligament on the plantar aspect of the third metatarsal bone is an important part of the examination and should be assessed thoroughly both in the transverse and longitudinal plane. Fiber irregularities are most commonly seen at the osseous margin of the origin of the ligament. These changes can be seen as either hypoechoic areas consistent with fiber disruption or hyperechoic areas indicative of more chronic changes such as scarring and mineralization (Fig. 2). The osseous margin should also be carefully scrutinized for irregularities. A radiographic study of the proximal metatarsal region can further support these changes seen as sclerosis or resorption of the bone at the origin or potentially more severe changes such as an avulsion fragment or stress fracture. It is also important to evaluate for signs of soft tissue proliferation around the suspensory ligament as this may be suggestive of fibrosis and potential adhesion formation. One study comparing ultrasound to gross pathology found ultrasound to underestimate the presence of substantial adhesion formation between the suspensory ligament and surrounding structures which may influence the horse’s prognosis. As discussed previously, angle contrast ultrasonography or placing the ultrasound beam angle off-incidence may assist in separating out the margins of the suspensory ligament from surrounding soft tissues therefore potentially detecting adhesion formation. Visualizing the margins more clearly with angle contrast ultrasonography may also help confirm enlargement of the ligament. To visualize the suspensory ligament more thoroughly it should be evaluated with the limb off-weighted or non-weight-bearing. In the hind limb, this can be

Fig. 1. Transverse image of the suspensory ligament proximal body. While weight-bearing, the proximal suspensory is best visualized from the plantar medial approach. Lateral is to the left of the image. Second metatarsal bone (Mtt 2). Fourth metatarsal bone (Mtt 4). White arrows indicating the margin of the suspensory ligament.
done by resting the toe on the ground. This allows the flexor tendons to relax and move slightly to provide a more complete window to visualize the suspensory ligament (Fig. 3). In addition, the suspensory ligament becomes closer to the transducer providing improvement in the image resolution.2

**Body**

The body of the hind suspensory ligament is an ovoid tendinous structure with a central bundle of ligament fibers and a zigzag pattern of fat and muscle distributed across it.3 The body of the ligament can be visualized more easily than the origin of the ligament. Lesions in this area are seen most frequently as both fiber changes and enlargement in the cross-sectional area of the ligament. Because of the location in the limb and its association with the second and fourth metatarsal bones, the body of the suspensory ligament is commonly affected in association with splint bone fractures or axial osseous proliferation due to exostosis of the splint bones. Exostosis of the second or fourth metatarsal bones may have more axial bone proliferation than can be seen radiographically.2,9 The medial and lateral margins of the suspensory ligament should be scrutinized adjacent to the osseous changes for fiber changes as well as for surrounding soft tissue proliferation that can be adhesion formation. Ultrasound can be used to determine the degree of axial bone proliferation and if there is impingement of the bone into the suspensory ligament that may be a chronic source of lameness.1,9

**Suspensory Branches**

To optimize visualization of each suspensory branch they are evaluated from the respective medial and lateral aspect of the limb one branch at a time. Because of the superficial location of the branch and the contour of the leg in this region, a standoff is needed to get adequate contact and allow complete visualization of the margins. The branches should be evaluated in a systematic approach from the proximal aspect at the bifurcation to the distal aspect at the insertion on the corresponding sesamoid bone. The branches are evaluated throughout their length for changes, as discussed before, as well as more diffuse fiber abnormalities. The margins of the

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**Fig. 2.** Longitudinal image depicting the origin of the proximal suspensory ligament on the proximal plantar aspect of the third metatarsal bone. The red arrow is pointing to an area of hyper-echoic tissue indicating fibrosis and enthesopathy of the origin of the ligament. Proximal is to the left of the image.

**Fig. 3.** The images depict a non-weight-bearing image of an abnormal suspensory ligament in zone 2A (proximal to mid metatarsus). The abnormalities of the suspensory ligament in the images include moderate enlargement, seen as rounding of the ligament with displacement of the fat and muscle plantarly and diffuse central dorsal fiber heterogeneous fiber changes (noted as *). A, The suspensory ligament is viewed on angle. B, The suspensory ligament is viewed using angle contrast ultrasonography. Medial is to the right of the image. White arrows depict the border of the suspensory ligament.
ligaments should be scrutinized for abnormalities as well as the presence of periligamentous fibrosis (Fig. 4). Periligamentous fibrosis is seen more commonly in the hind limbs and is important to consider when determining prognosis as it is associated with a decreased prognosis. At the insertion of the branches onto their respective sesamoid bones, special attention should be given to changes in fiber pattern as well as the osseous margin. The longitudinal plane is helpful in this location when assessing the length of the insertion on the abaxial margin of the sesamoid bone. More chronic changes associated with enthesopathy include disruption of the bone suggestive of resorption and mineralization within the ligament due to abnormal healing (Fig. 5). When changes are seen at the insertion of the suspensory branch, especially when affecting the osseous margin, a radiographic study of the fetlock should be performed to see the extent of the osseous changes. With the addition of radiographic lesions such as extensive resorption, osseous cyst-like lesions, or avulsion fragments will be more easily identified. Other techniques have been described to evaluate the suspensory branches besides the traditional gray-scale weight-bearing approach. A non-weight-bearing approach has been described when evaluating suspensory branches to identify certain longitudinal splits as well as providing more accurate information when determining lesion severity in this region. It is important to note while evaluating the suspensory branches, ultrasonographic abnormalities do not always cause clinical lameness. In the author’s experience, besides using clinical judgment, Power Doppler can be beneficial when trying to determine if a lesion is active. Increased vascularity within the ligament can be correlated with clinical disease or lameness. There is little research currently on using Power Doppler in horses this way. However, there is one descriptive study that examined the suspensory branches of 13 horses with Power Doppler. In all branches that were abnormal on gray-scale ultrasound, Power Doppler signal was identified, whereas no Power Doppler signal was identified in branches that were normal on gray-scale imaging. The imaging findings were not correlated to soundness in this study, however, this study did provide justification to further investigate using Doppler in tendon/ligament injuries.

3. Conclusion
Ultrasound is a useful tool to examine the suspensory ligament in the equine hind limb. There are inherent difficulties while performing an ultrasound exam of the suspensory ligament and therefore more techniques than the standard plantar weight-bearing approach should be utilized.

Acknowledgments

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

Conflict of Interest
The Author has no conflicts of interest.

References
Ultrasound of the Pastern Region

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1. Introduction
Soft tissue injuries in the pastern region are a significant contributor to lameness and loss of use or performance.1–3 Ultrasound has been well established as a useful method for diagnosing soft tissue injuries in the horse. Ultrasound of the pastern requires slightly more technical probe handling skills and skills in recognizing variabilities than that of the metacarpal region, but with the current equipment capabilities and level of ultrasound familiarity amongst most practitioners, ultrasound of the pastern can and should be undertaken in the field with reasonable diagnostic expectation. Although magnetic resonance imaging (MRI) is considered the gold standard for musculoskeletal imaging of the distal limb, ultrasound as a primary method of soft imaging remains financially and often logistically more practical for many clients. The findings provided by a good-quality diagnostic ultrasound in the field may be able to provide a stand-alone diagnosis. If MRI is also an option, the ultrasound findings can be used as a platform to discuss the merits of additional MR imaging and help to better direct the area of interest of a potential MRI study. Post MRI, pastern ultrasound can be used to guide intraslesional therapy and is generally the standard method for monitoring lesion healing and guiding rehabilitation protocols at the frequency required for proper rehabilitation.

2. Area of Interest (P1/P2)
For practical reasons, examination of the pastern is divided into imaging of the structures of palmar P1 and very proximal P2 and imaging of the structures at mid-P2 and distally to the podotrochlear region. The soft tissue structures palmar to P1 are best imaged with a high-frequency linear transducer (7.5-10 MHz or 9-11 MHz), with variable use of a standoff pad. A small footprint rectal linear transducer can be used in some instances, but overall, the shape of the transducer is less amenable to imaging all structures in the longitudinal and transverse planes. To visualize structures distal to the very proximal portion of P2 (middle scutum), the probe has to be placed at the level of the heel bulbs, and a small footprint curvilinear or microconvex probe is required in order to have adequate contact. This presentation will focus on the basics of ultrasound of the palmar pastern region using a linear probe available to most practitioners with a brief example of what can be visualized at the level of P2. The fore- and hindlimbs are anatomically similar and treated as such for scanning technique.

3. Anatomy
The primary anatomic structures evaluated over palmar, palmaromedial, and palmarolateral P1 include the superficial digital flexor tendon (SDFT), the deep digital flexor tendon (DDFT), the straight sesamoidean ligament (SSL), the medial and lateral oblique sesamoidean ligaments (OSLs), and the digital flexor tendon sheath (DFTS). The proximal digital annular ligament, axial and abaxial collateral ligaments, and collateral ligaments of the proximal interphalangeal
joint may also be assessed but are outside the scope of this presentation. At the level of distal P2, the DDFT, navicular bursa, collateral sesamoidean ligament, and distal interphalangeal (DIP) joint can be assessed. Further anatomic detail is provided as referenced below. For labeling of images, the pastern is divided into three zones over P1 (P1A: proximal P1, P1B: mid-P1, and PIC: distal P1) and two zones over P2 (P2A: proximal P1 and P2B: distal P2/podotrochlear region). The combined OSL insertion provides the distal margin for P1B (Figs. 1 and 2).

4. Case Selection
Pastern ultrasound is indicated in horses who respond to palmar digital, abaxial, or low palmar/plantar

Fig. 1. Transverse anatomy specimens at the level of P1A (left image), P1B (middle image), and P1C (right image). A, Superficial digital flexor tendon. B, Deep digital flexor tendon. C, Straight sesamoidean ligament. D, Lateral and medial oblique sesamoidean ligaments.

Fig. 2. Transverse anatomy specimens at the level of P1C (left image), P1C (middle image), and scutum medium, P2 (right image). A, Superficial digital flexor tendon. B, Deep digital flexor tendon. C, Straight sesamoidean ligament.

Fig. 3. Clipped area for pastern demonstrating the recommended proximal extent. The distal interphalangeal joint is also clipped in this image.

Fig. 4. Photo demonstrating probe placement for transverse scanning from a palmar window for the DDFT and SSL. A, Shows the hand position to guide the probe most easily.

Fig. 5. Photo demonstrating probe placement for longitudinal scanning from a palmar window for the DDFT and SSL. A, Shows the hand position to guide the probe most easily.

Joint may also be assessed but are outside the scope of this presentation. At the level of distal P2, the DDFT, navicular bursa, collateral sesamoidean ligament, and distal interphalangeal (DIP) joint can be assessed. Further anatomic detail is provided as referenced below. For labeling of images, the pastern is divided into three zones over P1 (P1A: proximal P1, P1B: mid-P1, and PIC: distal P1) and two zones over P2 (P2A: proximal P1 and P2B: distal P2/podotrochlear region). The combined OSL insertion provides the distal margin for P1B (Figs. 1 and 2).

4. Case Selection
Pastern ultrasound is indicated in horses who respond to palmar digital, abaxial, or low palmar/plantar
diagnostic analgesia, horses with acute DFTS effusion or other localized swelling, or those with localized trauma or lacerations. Soft tissue lesions in the pattern may respond to palmar digital, abaxial (basi-sesamoïd), or even low palmar/plantar diagnostic analgesia depending on location, severity of the injury, or migration of the blocking agent; one must be careful to evaluate all structures that may potentially be affected by those blocks. Horses with a potential radiographic diagnosis but who are disproportionately lame or chronically lame in the face of appropriate therapy should also be evaluated for a soft tissue lesion. Lastly, soft tissue causation should be suspected in horses who have recurrent lameness in a shorter than expected therapeutic window following intra-articular or intrathecal medication/therapy.

**Table 1. Summary of Evaluation of Each of the Four Main Structures Over P1**

<table>
<thead>
<tr>
<th>Structure</th>
<th>Approach</th>
<th>Standoff</th>
<th>Confounders</th>
<th>Tips</th>
<th>Assess</th>
</tr>
</thead>
<tbody>
<tr>
<td>DDFT</td>
<td>Palmar</td>
<td>Helpful</td>
<td>Hypoechoic proximally due to angle of incidence; Central vessel causes artifact</td>
<td>Center over each lobe for longitudinal and fan axially and abaxially</td>
<td>Lobe symmetry Use off-incidence angle</td>
</tr>
<tr>
<td>SSL</td>
<td>Palmar</td>
<td>Less common</td>
<td>Lateral origin, P2 insertion; Insertion slightly inhomogeneous axially</td>
<td>Evaluate proximal portion; Evaluate insertion especially in longitudinal plane</td>
<td>Bilateral comparison helpful Compare medial and lateral</td>
</tr>
<tr>
<td>SDFT</td>
<td>Start palmar, follow medial and lateral branches separately</td>
<td>Helpful</td>
<td></td>
<td>Insertion slightly inhomogeneous</td>
<td>Compare medial and lateral</td>
</tr>
<tr>
<td>OSL</td>
<td>Under base of each sesamoid, move diagonally/axially</td>
<td>Variable</td>
<td>Origins can be inhomogeneous; Difficult to image distal third</td>
<td>Important to evaluate origin at sesamoid</td>
<td>Compare contralateral oblique</td>
</tr>
</tbody>
</table>

Abbreviations: DDFT, deep digital flexor tendon; SSL, straight sesamoïdean ligaments; SDFT, superficial digital flexor tendon; SSL, straight sesamoïdean ligaments; OSL, oblique sesamoïdean ligaments.

**Fig. 6.** Hand position to facilitate standoff usage.

**Fig. 7.** Transverse (2) and longitudinal (3) ultrasound images at the level of P1A, indicated on the sagittal anatomic specimen (1). On the transverse image, lateral is to the right; on the longitudinal image, proximal is to the right. The same orientation will be maintained for all ultrasound images to follow. Note narrow band of the SDFT and the off-incidence hypoechoic region seen on the DDFT. The OSLs are visible as separate medial and lateral bundles. A, Superficial digital flexor tendon. B, Deep digital flexor tendon. C, Straight sesamoïdean ligament. D, Lateral and medial oblique sesamoïdean ligaments.
5. Preparation

The pastern should be carefully clipped with a number 40 surgical blade, cleaned, and ample gel applied. The clipped area should extend from the distal metacarpus/tarsus to the heel bulbs along the palmar, palmaromedial, and palmarolateral aspects (Fig. 3). The pastern is a very difficult region to adequately image in an unclipped horse. Careful attention should be paid to the basi-sesamoid region and any whorls or unusual hair direction. Chronic dermatitis, thick skin, and scar tissue impede penetration of the ultrasound beam and can result in difficulty obtaining adequate images.

6. Technique

Each structure should be evaluated separately to allow for appropriate incidence angle, frequency, and focal zone location. The author finds starting with imaging of the DDFT and the SSL on the palmar aspect of the pastern, followed by the slightly medial and lateral palmar approaches for the SDFT branches and more lateral and medial approaches for the OSLs, allows one to progress in relative degree of imaging difficulty and manipulation (Figs. 4 and 5). As with any tendon or ligament, size, shape, echogenicity, and fiber, alignment should be evaluated critically. Cross-sectional area measurements and/or dorsal to palmar/plantar thickness can be performed on structures that are suspected of being abnormal. Contralateral
images can be compared for appearance and size if necessary. Sizes can be compared to published normal if available or the contralateral limb; cross-sectional areas can be compared across serial evaluations. A standoff pad can be helpful to evaluate the SDFT and other superficial structures and in petite horses or ponies (Fig. 6). Standoffs can be overly attenuating in horses who are more difficult to image, and the added
size can make the probe more difficult to manipulate in horses with short pastern conformation.

Deep Digital Flexor Tendon
The DDFT is one of the two most commonly injured structures in the pastern\textsuperscript{3,4} and is more commonly injured in the forelimb than the hindlimb. It is located directly palmar/plantar and normally demonstrates a homogenous echogenicity with symmetric lobular appearance and long linear fibers. Some twisting of the fibers is normally apparent on longitudinal views. Begin with the transducer in a transverse plane, focal zones at appropriate depth, and a standoff pad if needed. Angling upward just under the ergot, the probe should then be moved distally with adjustments for incidence angle until the level of heel bulbs (usually at the level of proximal P2). Proximally, the DDFT may appear hypoechoic due to off-incidence image, and care should be taken to manipulate incidence angle as best as possible and to understand the normal variation. While evaluating the DDFT at P1 and proximal P2, any lobe asymmetry or subtle differences in echogenicity or shape is
often of significance. If the classic echogenic appearance cannot be obtained at the level of P2, valuable information can be gained by rocking the probe distally and using the off-incidence view to evaluate shape and symmetry to obtain an index of suspicion of injury further distal. This type of off-incidence appearance is also helpful in determining the margins of the DFTS and the presence of any mineralization or fibrosis. The probe should then be turned 90° to the longitudinal view. Care should be taken to center the probe and evaluate each lobe separately in the longitudinal plane, particularly if a lesion is suspected in a particular lobe. Due to the narrow beam width relative to the width of the tendon when the probe is oriented in a sagittal plane, it can be difficult to confirm the lesion in both planes if one does not pay adequate attention to the lateral or medial positioning of the probe. If any abnormality or irregularity is suspected at the level of distal P1/proximal P2, further evaluation is recommended with curvilinear probe or MRI. If abnormalities are seen at the level of proximal P1, evaluation of the DDFT in the distal metacarpal/metatarsal region and non-weighted views are recommended to improve visualization in the palmar/plantar fetlock region. There is a vessel located centrally between the two lobes of the DDFT that can cast a significant side lobe artifact, usually at the level of P1B. This can be identified by the central reproducible location and the tortuous tubular shape (Table 1).

Straight Sesamoidean Ligament
This structure appears to be less commonly injured than the SDFT, DDFT, and OSLs, but injuries can result in severe lameness. It is located just dorsal to
the DDFT. Adjusting the focal zone a level deeper, begin in a transverse orientation angled proximally toward the base of the proximal sesamoid bones and slide the probe distally to the insertion on P2, adjusting incidence angle as necessary; then, repeat in the longitudinal plane. Cross-sectionally, the shape of the ligament changes from trapezoidal to square toward the P2 insertion. In distal P1/proximal P2, it may be isoechoic to the heel bulb/bulbar cushion in the transverse plane and thus may be easier to assess on longitudinal images. The size, shape, and appearance are somewhat variable between horses, so bilateral comparison is essential in most cases to confirm a suspected lesion. A hypoechoic reflection of the metacarpophalangeal joint is often seen on the lateral aspect of the origin in transverse and longitudinal images.
planes. A central hypoechoic region is almost always present at the longitudinal insertion onto the scutum medium of P2. Contralateral imaging is recommended in all cases prior to considering an insertional lesion (Figs. 7–12). Fig. 13 shows an example of a lesion of the medial lobe of the DDFT. Fig. 14 shows an example of a lesion of the SSL.

Superficial Digital Flexor Tendon

The SDFT is considered the third most commonly injured structure in the pastern, after the OSL and DDFT. Injuries occur most commonly in the forelimb. In the proximal pastern, it is a thin crescent shape that divides into medial and lateral branches, which insert separately onto distal P1 and the scutum medium of P2. Starting in transverse orientation with the focal zone positioned superficially, the SDFT is identified superficial to the DDFT at the level of P1. The lateral margin is then identified and the probe moved laterally to center the lateral branch in the image (Fig. 15). The probe can then be moved directly distally from that location to follow the lateral branch insertion. The branch changes from slightly teardrop in shape to a broad, somewhat inhomogeneous triangle, particularly axially (Fig. 16). With experience, the blending of fibers from the axial and abaxial palmar ligaments can be appreciated at the insertion. The probe is rotated to obtain a longitudinal image (Fig. 17). Care must be taken to ensure the probe is located over the SDFT branch itself in the longitudinal view and not a portion of another structure. The same technique is repeated for the medial lobe. Depending on conformation, it can be difficult to obtain the longitudinal image of the insertion onto P2. Fig. 18 shows an example of a large lesion of the lateral branch of the SDFT.

Oblique Sesamoidean Ligaments

The OSLs are increasingly recognized as a cause of lameness in sport horses, particularly dressage horses and jumpers. They are considered the most common abnormality identified in the pastern region, occurring more often in the forelimb of racehorses and in the hindlimbs of jumping and dressage horses. They can be technically more difficult to image well as they have a more heterogenous composition of ligament, adipose, and vascular tissue, as well as overlying vasculature that results in a more naturally inhomogeneous appearance. It is generally easier for the inexperienced operator to scan the lateral OSL and then move on to the medial OSL. Two approaches can be used to find the origin of the OSLs. In the first, the probe is placed in the transverse plane on a slightly proximal oblique angle at the base of the sesamoid bone. The ligament should appear as a slightly gumdrop-shaped structure originating from the base of the sesamoid (Fig. 19). The probe is then manipulated to an angle more parallel to the ground and the ligament followed distally on a slight diagonal toward midline, with P1 visible just deep to it. At about mid-P1, the fibers of the lateral and medial oblique blend to insert together directly palmarly; at that point, the probe is directly palmar or axial on the pastern (Figs. 20 and 21). The same technique is used for the longitudinal plane and repeated for the medial oblique. The second method for finding the origin of the oblique is to obtain an image of the corresponding SL branch in the transverse plane and follow it to its insertion on the proximal sesamoid bone. The probe then continues distally over the sesamoid and is angled proximally just after crossing the joint. The OSL origin should appear on the opposite side of the joint from the suspensory insertion. The ultrasonographer should be aware that the distal 1/3 of the OSL is quite difficult to identify in the transverse plane and it is also challenging to keep the probe fully in place over the OSL, making this region difficult to evaluate well on ultrasound. Additionally, the origin of the ligament can be somewhat inhomogeneous, and corresponding longitudinal views of this region take practice to obtain accurately. Adequate effort should be made to obtain good corresponding longitudinal images in order to help determine whether hypoechoic regions are due to incidence angle, anatomic variation, or pathology. When suspecting a lesion, care should be taken to obtain images in two planes, take measurements and evaluate the contralateral limb. When comparing measurements, it is important to note that the cross-sectional area of the lateral OSL can be up to 20% larger than the medial OSL normally. Figure 22 shows an example of an acute on chronic lesion OSL.

Podotrochlear Apparatus

It is possible to evaluate the DDFT, distal recess of the DDFT, NB, CSL, and palmar/plantar pouch of the DIP joint with a curvilinear or microconvex probe. If this type of probe is available to the practitioner, developing the skills to evaluate the podotrochlear region, in particular the DDFT, is recommended. Occasionally, a subtly abnormal DDFT has detectable lesions at the level of distal P2. A normal appearance of the DDFT over P1 does not rule out a lesion distally, especially if the clinical picture is suggestive of an injury to the DDFT. The region is scanned with the foreleg positioned behind the vertical as in a navicular skyline position for radiographs (Fig. 23). In the hindlimb, this region is generally more easily accessible than the forelimb. Longitudinal and transverse images can be obtained. Lesions most commonly present as lobular asymmetry or irregularities of the dorsal margin. Navicular bursa effusion may be present and symmetric or present and asymmetric (Figs. 24–26). Synovitis can be detected and, with experience, adhesions can be suspected.
Acknowledgments

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

Conflict of Interest
The Author has no conflicts of interest.

References
Reproductive and Urogenital Ultrasound of the Mare

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1. Introduction
With the increased availability and quality of ultrasound machines for the field practitioner, evaluation, diagnosis, and treatment of pathologic conditions of the mare reproductive and urinary tract has dramatically improved. Regarding the use of ultrasound in the reproductive management of the mare, the most significant positive impact was in the 1980s, when transrectal ultrasonography facilitated early and efficient management of mares with twins.1–3 This application has dramatically reduced the number of twins and twin-related reproductive loss in the equine breeding industry. This document will review some of the basic ultrasonographic characteristics of the mare reproductive and urinary tract as well as the caudal abdomen that are accessible by the transrectal and transabdominal approach. Although a detailed description of ultrasonography of the mare’s reproductive and caudal urinary tract is provided elsewhere,4–9 this document will help by highlighting techniques, landmarks, and measurements that will increase the repertoire of the practitioner in a field setting.

2. Methods for Evaluation of the Mare Reproductive Tract and Urogenital Tract
This discussion will be limited to structures that can be identified and evaluated using a standard mid-to-high frequency rectal linear array transducer (5-7.5 MHz) with a maximum depth of 15 cm. For a more detailed instruction on how to manipulate and optimize settings for ultrasound evaluation, the reader is referred to a more comprehensive review.6,10 For some machines, the ultrasound settings are preset, with a minimal ability to alter the settings. In most cases, the settings for reproductive transrectal ultrasound that are preset are usually appropriate for most cases to complete the examination described below. This document will review the highlights of mare reproductive ultrasound in the nonpregnant and pregnant mare, with a brief review on how to evaluate the urinary tract by using transrectal ultrasonography. Equine veterinarians are usually comfortable with performing a transcutaneous ultrasound evaluation on a horse for the purposes of a colic evaluation as well as transrectal palpation and ultrasound of the reproductive tract evaluation. In the case of a more in-depth transrectal evaluation or evaluation in a compromised or fractious animal, it is essential to prepare the patient for what may be a 20-minute procedure. Before the evaluation, the mare should be restrained safely in stocks or in a stall door by a handler competent and responsive to your requests and the horse’s behavior. Sedation may be required in the case of fractious or frightened horses or those unfamiliar with a transrectal evaluation. In the case of transrectal evaluation, chemical restraint is reliably achieved with 0.01 mg/kg detomidine HCl with 0.01 mg/kg butorphanol tartrate added in more fractious or anxious animals. Once the horse is compliant,

NOTES
and restrained, by using adequate lubrication, evacuate the rectum fully to the cranial extent one can reach with their fingertips. In horses that may have friable rectal mucosa (diarrhea, colic, and dehydration), 60 ml of sterile lubricant can be infused in the rectum before palpation to facilitate evaluation. In cases in which an infectious disease is suspected (e.g., Salmonella), the transducer can be placed in a palpation sleeve with lubricant to protect the transducer from becoming a fomite.

3. Ultrasound of the Nonpregnant Mare

Transrectal palpation and ultrasound of the reproductive tract include evaluation of the reproductive tract and also allow for evaluation of the caudal urogenital tract and peritoneal cavity. In cases of colic, suspected uterine or gastrointestinal (GI) viscera tear or hemorrhage, the transrectal exam can aid in this diagnosis and even identify the site of compromise. It is helpful during the transrectal evaluation to remember that any structure of interest can and should be viewed with the ultrasound in multiple orientations to better define it. For example, if there is a questionable structure identified in the cervix, the probe can be rotated from the cranial-caudal orientation to a transverse position and the exact location of the structure relative to the lumen can be identified. Evaluation of the following should be performed during each rectal exam for reproduction but also in cases of colic, chronic pain, and behavior issues that are often referred for evaluation. With ultrasound machines, there can be a delay in processing the image as one moves over an area of interest with the transducer. If moving too quickly with the machine set to a slower frame rate (less than 80 Hz), the operator will need to be cognizant to adjust or slow the movement of the transducer over areas of interest because if movement is too quick, small but significant abnormalities may not be identified. This is particularly important when evaluating for twins, and questionable vesicles should also be evaluated in multiple plans.

1. Pelvic inlet: Initial palpation should include evaluation for abnormal masses, palpation of the caudal aorta for dilations, confirmation of the normal location of GI tract, and sweeping of the pelvis and dorsal aspect of the pelvic inlet to try to identify any abnormal structures. It is also essential to ensure there is normal range of movement of the reproductive tract, bladder, and viscera. In cases of adhesions, they will often be identified by the inability to manipulate the structure in a normal fashion. Manipulation of viscera and sweeping the pelvis, under the ovaries and uterine horns and bladder, may be the only way to identify an issue. The ultrasound probe can be placed on any abnormal masses and doppler used to evaluate if blood flow is present (e.g., neoplasia). Abnormal masses (hematomas, neoplasia, and calcification) often disrupt the normal architecture and can be soft to firm and contain variable contents. Only neoplasia or the rare hematoma that encircles a vessel will have blood flow that can be diagnosed with doppler. Masses may be solid or fluid filled. The presence of free fluid is often determined by the echogenicity (usually hypoechoic, but may contain swirling or flocculent material). Fluid that is dense (purulent or mucoid) may appear to have architecture, but often when jostled or tapped, the fluid can be seen moving in a swirling fashion relative to organized tissue. Very hyperechoic flecks may represent air or mineralization. Adhesions often appear hyperechoic or isoechoic to the surrounding tissue and may be thick (1-3 cm) or thin 1- to 5-mm strands outlined by hypoechoic fluid.

2. Peritoneal fluid: One should always evaluate the ultrasonographic character and amount of the peritoneal fluid. Normally the largest measurable amount is small (<3 cm) anechoic pockets seen ventrolateral to the bladder and in some cases in small pockets around the ovaries and between viscera. Excessive free abdominal fluid (more than 10 cm at either side of the bladder, adjacent to the cervix and caudal uterine body) or change in echogenicity (normal is anechoic in appearance) should warrant further investigation (transabdominal ultrasound, bloodwork, or other diagnostics).10–15 One can often appreciate gas echoes as pinpoint hyperechoic dots that may coalesce in larger amounts dorsally, obscuring deep structures. This is useful in the case of ruptured GI viscera or uterus or recent surgery and should be searched for in the dorsal regions surrounding the ovaries and nephroplenic space. Increased echogenicity may indicate peritonitis, hemobadenon, or uroabdomen.14,15 Interestingly, in many systemically healthy animals that have had previous colic surgery, peritonitis, or cesarean section, one can see hyperechoic tags (usually <0.5 cm at thickest region) attached to serosal surfaces outlined by anechoic fluid. They can often be identified in pockets of fluid as being attached to the serosal surface of the digestive tract.

3. Ovaries: Ultrasound and palpation of the overall size, texture, and intraovarian structures (follicles and corpora lutea); paraovarian cysts; and presence of a palpable ovulation fossa should be noted.4,6 Moveability of the ovary and response of the mare during palpation may also be important in cases of abnormal behavior.16

4. Uterus: The uterus should be palpated for position relative to the pelvis (dependent and ventral positioning may inhibit normal
Endometrial edema: It is absent in diestrus or anestrus and increases under the influence of estrogen or inflammation. Presence of edema should be correlated to ovarian structures to make sure it is appropriate given the stage of the estrous cycle.4 Usually edema is graded from 0 to 4, with 0 being uniform echogenicity of the endometrial folds and 4 being significant or excessive edema with heterogenous “pin-wheel” appearance.6

Luminal fluid: The location, amount (usually measured in the dorsoventral plane in centimeters), and character (ranges from anechoic to hyperechoic) are evaluated. Normal uterine fluid is anechoic with only small amounts ≤1 cm in estrus.17,18 Fluid in diestrus is generally considered abnormal and should warrant investigation. Any echogenic fluid, unless recently foaled or mated should also be evaluated as it may represent purulent material, urometra, or other pathologic conditions. Again, intrauterine fluid should be compared to the ovarian structures to determine if it is appropriate.18–22 Slow, thorough evaluation of the entire uterus is critical to identify foreign bodies, retained endometrial cups, cysts, or areas with significant changes in echogenicity of the endometrium. Hyperechoic structures in the uterus can be associated with gas echoes (Fig. 1), scar tissue, mineralized material (endometrial cups can appear as multifocal pinpoint to 2 cm slightly hyperechoic to mineralized structures at the base of the uterine folds), foreign bodies, and urine sediment. In cases in which the uterine lumen requires further evaluation (suspected adhesions, cysts, and foreign bodies), the uterus can be infused with 1 to 3 L of sterile lactated Ringer’s solution with a cuffed bivona catheter, and transrectal ultrasound can be used to help outline the structures of question. Translumenal adhesions and foreign bodies will often appear as hyperechoic bands or structures outlined by the lavage fluid and in the uterine lumen. This technique may help differentiate if a structure identified on ultrasound is in the uterine lumen or the deeper uterine wall. This technique may also allow for evaluation of patency of the uterine horns.

1. Cervix: Transrectal ultrasonography can help identify anatomic defects that may not be appreciated by palpation.23,24 The inner linear longitudinal fibers of the cervix are easily seen surrounding the cervical lumen in the normal cervix during diestrus. To evaluate the cervix, the transducer is placed in the cranial-caudal orientation and directly above the cervix. The transverse image is obtained by rotation 90° within the pelvic canal. Disruption of the linear fibers, pockets of fluid, cysts, or regions of very different echotexture with blood flow present, may suggest pathology (muscular defects [Fig. 2], diverticula, neoplasia, cysts, or foreign bodies). The gold standard evaluation of cervical competency is digital evaluation during diestrus,4 but ultrasound evaluation may aid in identifying, characterizing, and defining anatomic relations of the structure of interest.24

2. Vagina: The vagina is important to evaluate during the transrectal ultrasound evaluation prior to any vulvar or vaginal manipulation that would introduce air. In the normal mare, the vagina is collapsed with no air present. It is seen just dorsal to the bladder in the 3- to 5-cm space that is thin and collapsed in the normal mare (Fig 2). The caudal aspect of the cervix should be evaluated, as pathology can be seen commonly in this area. This area should be evaluated for the presence of fluid (urovagina, vaginitis, cervicitis, or fluid from the uterus), air (abnormal in large amounts and suggests failure of the vulvar and vestibulovaginal fold barriers), or structures such as hematomas, foreign bodies, or abnormal masses. Accumulations of large amounts of fluid in the vagina may also suggest a persistent hymen or abnormal fluid evacuation. Evaluation of the vaginal wall with ultrasound is also helpful in the case of rectovaginal fistulas (air is often seen traversing the dorsal vaginal wall to the rectum). One can better characterize the extent and location of abscesses, neoplasia, and hematomas. In cases of vaginal tears.
due to breeding or foaling, the ultrasound again can be useful for identifying the extent and location of the tear, the presence of hematoma, and peritoneal fluid quality.

3. Vestibule/Vulva: Ultrasound can be useful in this region to help evaluate tears, abscesses, and hematomas. Air (hyperechoic) is often useful to help outline the path of a traumatic injury that can be better addressed once one knows the extent of the lesion.

In evaluation of the reproductive tract of the non-pregnant mare, the history, consistency of findings with stage of the estrous cycle, and behavior must all be considered to evaluate the animal. Additional diagnostics (e.g., uterine biopsy and hysteroscopy) or treatments are based on the consideration of all these data points.

4. Ultrasonography in the Pregnant Mare

The use of ultrasonography in the pregnant mare is important to determine the health of the pregnancy, identify twins for early management (13-16 days post-ovulation), and perform fetal sex determination. In early gestation, ultrasound imaging of the conceptus helps determine normal growth and normal development through familiarity of the “normal” ultrasound appearance of the conceptus and uterus. Using known developmental markers (28-day division of the conceptus with equal compartments of allantoic fluid and yolk sac), it is essential to rule out a nonviable pregnancy (lack of heartbeat or abnormal delayed growth) prior to endometrial cup formation at 35 days postovulation so that termination if necessary can be performed and the mare can have a chance to be bred back the same season. Fetal sexing can also be performed from 55 days postovulation onward in pregnancy, with the most efficient windows of sex determination at 55 to 75 days and 90 to 150 days. From 55 to 75 days (optimally 60 days), the location of the genital tubercle is used to determine sex, and later in gestation, the intra-abdominal gonad echotexture and external genitalia or mammary glands are used. Fetal sexing can be performed throughout gestation, but the recommended time windows are when the image is most reliably and rapidly obtained. For those trying to improve accuracy for sex determination, a stall-side phone app provides excellent images for immediate comparison. As the fetus and uterus enlarge with advanced gestation, imaging deeper structures becomes more efficient with a transducer of greater penetration (3-5 MHz) that can reach approximately 30-cm depth. Most linear transducers range from 5 MHz to 15 MHz and cannot penetrate these depths and a different transducer is needed. Despite this, fetal sexing can be performed with the linear rectal transducer by using a transrectal or transabdominal approach in some cases throughout gestation if the fetus is in the correct position. Below are some normal values and key time points that may be helpful when using transrectal ultrasonography to monitor a mare’s pregnancy and assess if there is pathology or abnormal development.

Embryonic Vesicle Size

The embryonic vesicle size (measurement of largest diameter of the vesicle) has been used to help predict if a pregnancy will not be maintained. Undersized vesicles indicated eventual loss in approximately 62% (21/34) of mares emphasizing that size is an important parameter in assessing early equine...
pregnancy. Figure 3 can be used as a reference for normal equine vesicle size by day of gestation and reflects the work done by Dr. Ginther. The position of the embryonic vesicle after 16 days of gestation should be at the base of a uterine horn. Vesicles that spent the majority of time in the uterine body during the mobility phase were associated with increased rates of loss. In addition to vesicle size, appropriate early developmental landmarks as the pregnancy develops and the fetal heartbeat become visible are reviewed in Figure 4.

In midgestation, the fetus and placenta can be evaluated by transrectal and transabdominal ultrasound using the linear transducer. For fetal evaluation in late gestation, the only limitation of this ultrasound technique is the depth of penetration. A lower frequency curvilinear transducer (3-5 MHz) can be used for imaging deeper structures, as is usually required for a detailed fetal evaluation. Again, the following discussion is limited to only the 15-cm depth linear transducer and what can be imaged and achieved with it. In the transrectal evaluation during mid- and late gestation, it is still critical to evaluate the vagina and cervical region to determine the presence of fluid, air, or any other type of contamination that may be abnormal. Evaluation of the cranial cervical region and any fluid accumulation between this and the choioallantois should be noted. The allantoic and amniotic fluid, the amnon, and often the fetus and fetal orientation can all be evaluated partially during the transrectal exam. Fetal fluids should be assessed for echogenicity and depth. The orientation of the fetus can be determined by locating the orbit or tail by transrectal evaluation or the orientation of the rib cage by transcutaneous ultrasound. Fetal heart rate, movement, and tone (are the limbs and neck flaccid or is there flexion and extension?) can be assessed, as well as character and thickness of the amniotic membrane.

Measurements of the combined thickness of the uterus and placenta (CTUP) taken just cranialateral to the cervix have been reported in normal mares and mares with placentitis and can serve, in conjunction with other clinical signs, as a way of identifying pregnancy compromise. The transrectal measurement is taken from the dorsal wall of the large vaginal artery to the allantoic surface just cranial and lateral to the cervix (Fig. 5). Below are the condensed estimates often used in practice for the upper limits of normal CTUP taken in late gestation. It is important to evaluate as much of the pregnancy as can be imaged, as the CTUP measurement alone does not describe regional pockets of separation, excessive edema in focal regions, or other pathology. For transcutaneous ultrasound evaluation of the pregnancy, one needs to minimally prepare the mare by removing any dirt or mud from the hair, as this will interfere with the image. Ideally one would shave the area but this is often not desired by clients. In the field, it is efficient to spray the abdomen with isopropyl alcohol (a one-gallon garden spray bottle works well), from the level of the udder to the xyphoid and approximately 1/3 of the lateral ventral body wall on the side of evaluation. The transducer is placed just lateral to the udder and moved cranially or in the direction of the hair in a systematic manner so that all regions where the uterus can be seen are imaged. Reapplying alcohol may help in regions with poor image quality. One of the most practical applications of the transabdominal ultrasound is determining if the mare is pregnant. In some situations, farms have moved to a rapid late transabdominal ultrasound pregnancy check that completely replaces the transrectal evaluation. To perform this, spray the area just cranialateral to the udder, and using the linear transducer, look for the presence of the fetus and placenta. This can allow for very rapid determination of pregnancy in the case of a field of mares when it is not known which animal aborted or for the purposes of expediency if the farm desires. Although some information is lost by foregoing the rectal palpation (distention of pregnancy, tone, and cervix), other information is gained (echogenicity of fetal fluids, fetal movement, and placental thickening). It may be obvious to some but remember that in the late pregnant mare the tissues that will be passed from outside to the fetus include skin >
body wall > abdominal fat > peritoneal fluid > uterine wall and chorioallantois (often appear as one structure) > allantoic fluid > amniotic membrane (approximately 2 mm thick) > amniotic fluid > and fetus (Fig. 6). With practice, the transabdominal assessment of the pregnancy using a linear transducer can be used and is helpful in the field setting. It is recommended that the following 3 parameters are evaluated on each transabdominal ultrasound, as together they give a more thorough assessment of the patient health and pregnancy status.

1. Peritoneal fluid: First evaluate the amount of peritoneal fluid (normal is approximately less than approximately 5 cm [author’s experience] with viscerae lying on the abdominal floor) in depth and its echogenicity (normal is anechoic or hypoechoic relative to a large vessel). In a case of a colic in the mare, it is prudent to ensure there is not excessive or abnormal peritoneal fluid (hyperechoic or flocculent fluid). Periparturient hemorrhage is always a concern in the late gestation mare, so ruling out hemoabdomen (swirling, moderately echogenic fluid with minimal to no particulate matter) is useful before progressing to a rectal evaluation. This does not guarantee there is not hemorrhage but likely alters the course of treatment.

2. Evaluation of the uterus and chorioallantois: This region in most healthy pregnancies appears as a relatively uniform echogenicity with a smooth wall on the uterine side and occasional thickenings that represent uterine folds. In some mares, there is a clear definition between the endometrium and chorionic surface. In others, it is not as clear and the significance of this is not known, but the author has seen both in compromised and normal pregnancies. The higher frequency and shallow depth of the linear transducer allow very effective evaluation of the endometrial-chorionic and allantoic membrane interface. Pathologic thickenings, cysts, allantoic vesicles, and large pockets of separation (placentitis, fetal demise, and placental necrosis) can be identified in this way. There is significant folding often associated with

Fig. 4. Determining embryonic age by diameter (mm) is depicted. From O. J. Ginther, Ultrasonic Imaging and Animal Reproduction: Horses. 92

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the nonpregnant horn in normal pregnancy and also at the region of the uterine bifurcation that can often be mistaken for “placental separation.” To differentiate between free fluid or parenchyma in the uterine-chorialic interface, increasing the gain and resolution helps visualize vessels and the echotexture of organized parenchyma consistent with uterine tissue compared to free fluid. Doppler can be used to assess questionable anechoic structures, and movement of the transducer into a different plane will help differentiate a tubular vessel from an irregular fluid pocket. One should optimize the ultrasound settings and understand how to change the doppler settings to identify blood flow in smaller vessels, which appear as anechoic circular structures in the placenta.\textsuperscript{16,36} If there is a question of the settings for doppler detection of blood flow, one can test the same settings on a region of vessels in abdominal muscle parenchyma. If it does not demonstrate blood flow in the abdominal musculature, the settings are inappropriate to assess blood flow in the uterus.

3. Thickness of the CTUP: This has been described for both transrectal (Table 1) and transabdominal evaluation.\textsuperscript{7,8,10,36–39} The transabdominal CTUP measurement is taken from the peritoneal surface of the uterus to the place where the allantoic membrane meets the allantoic fluid (Fig. 6). The maximal uteroplacental thickness was found to be $1.38 \pm 0.23$ cm in 33 mares evaluated with normal pregnancies in late gestation to term.\textsuperscript{8} Perhaps more important than the measurement is evaluation of excessive edema in any portion of the placenta (endometrium, chorion, allantoic membrane, or amnion), regions of placental separation, or greater variation in echogenicity and thickness. Edema often appears as heterogeneous echogenicity and areas of hypoechoic tissue (similar to the endometrium in estrus) with often the parenchyma stretched so that fibers, in extreme cases, make linear streaks (Figs. 5 and 7).

4. Evaluation of allantoic and amniotic fluid: In a group of 33 normal pregnancies evaluated in late gestation, the maximal vertical depth of amniotic fluid ($7.9 \pm 3.5$ cm) was less than allantoic ($13.4 \pm 4.4$ cm) and fewer echogenic particles were detected in amniotic fluid.\textsuperscript{38} The allantoic cavity is where hippomaneas are formed; these allantoic accumulations can

Fig. 5. The image demonstrates measurement of the increased and abnormal CTUP (1.5-2.5 cm) with transrectal ultrasound in a mare at 290 days of gestation that subsequently aborted. The left of the image is caudal and the right is cranial. In this image, there is significant edema of the placenta that allows for clear demarcation between the endometrium (which also has edema more clearly seen to the left and above the tab for CTUP), chorion, and allantoic membrane. The CTUP again is measured from the dorsal aspect of the large vessel imaged to the dorsal aspect of the allantoic membrane.
appear as flocculent material to a well-organized concentrically ringed hyperechoic structure 2 to 20 cm in length. Significantly increased echogenicity and very flocculent material in either allantoic or amniotic cavities may indicate compromise, meconium release from the fetus, or inflammation and infection. \(^{39-45}\) It is not uncommon on some evaluations for there to be flocculent debris in the allantoic cavity, surrounded by relatively anechoic fluid if the mare has just been moving (walked in from field for evaluation). 

5. Fetal evaluation: The ability to predict the outcome of the pregnancy based on biophysical markers diagnosed on ultrasound (fetal movement, character of fetal fluid, CTUP, fetal aortic diameter, and fetal heart rate) has not led to a universally accepted, highly reliable method to predict pregnancy outcome. A study in 2019 demonstrated that the biophysical profile that evaluated transabdominal CTUP, fetal heart rate, and fetal aortic diameter was reasonably sensitive (85.19%), specific (87.25%), and accurate (86.82%) in diagnosing compromised fetuses in 27/129 pregnancies. \(^{45}\) This topic has been explored in depth, with recent research suggesting fetal carotid ultrasonography may be helpful. \(^{46}\) While investigating the best way to identify pathology in utero, the following are a few useful pieces of information that can be obtained with just the linear array rectal transducer.

a. Orientation: Using the ribs and the narrowing of the ribs spaces or the location of the heart relative to the lungs and liver, the direction of the fetus can be ascertained. After 8 months of gestation, the fetus should be in anterior presentation and will likely not change orientation as the pregnancy progresses. \(^{4}\) Because of this, identification of a caudal fetal presentation at 310 days of gestation is helpful to appropriately prepare for foaling.

b. Fetal movement: By holding the probe still for several minutes, often gross fetal movement can be confirmed. The fetus goes through normal periods of rest, but if no fetal movement is appreciated, identification of a fetal heartbeat should be attempted.

c. Fetal heart rate: The ribcage is identified and followed cranially on the fetus (this usually corresponds to caudally on the mare). The maximum depth setting is useful in this scenario, as it allows one to see the widening and narrowing of the rib spaces (narrower cranially on the fetus). Once the heart is imaged, the practitioner can use either the M-Mode setting, doppler, or 2-D B-mode and count beats per

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Table 1. Normal CTUP Measurements of Healthy Pregnancies\(^ {8}\)

<table>
<thead>
<tr>
<th>Gestation Length (Days)</th>
<th>Normal CTUP (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>151-270</td>
<td>&lt;7</td>
</tr>
<tr>
<td>271-300</td>
<td>&lt;8</td>
</tr>
<tr>
<td>301-330</td>
<td>&lt;10</td>
</tr>
<tr>
<td>331-</td>
<td>&lt;12</td>
</tr>
</tbody>
</table>

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Fig. 6. Image of late gestation pregnancy with transabdominal approach using a linear array rectal transducer. Structures encountered from outside the body wall to the fetus include hair/skin > muscle > intra-abdominal fat > peritoneal serosal lining > peritoneal fluid > perimetrium (serosal lining of uterus) > myometrium > endometrium > chorioallantois (chorionic surface connects to endometrium, allantoic membrane is thin and faces the allantoic cavity) > allantoic cavity > amniotic membrane > amniotic cavity > fetus. In this image, the gain is too high, and so what is normally anechoic peritoneal fluid appears to have linear horizontal echogenic artifacts that traverse all the tissue layers deep to the peritoneal fluid pocket.
minute. Although there is significant variation in the fetal heart rate, in general, it is more concerning when the heart beat is very low (<60 bpm) as compared to an elevation in heart rate. Average late gestation fetuses with normal pregnancies demonstrated regular cardiac rhythm with a mean heart rate of 75 ± 7 beats/minute.\textsuperscript{38} Observing the fetus and re-evaluating heart rate is ideal, but in some cases, just identifying a heartbeat is the goal. In cases in which the heart cannot be identified, a large umbilical or fetal vessel can be identified and evaluated with doppler or magnification to detect ultrasonographic evidence of blood flow, and a heart rate can be determined.

d. Fetus: A thorough fetal exam in utero requires experience and time. For the purposes of most practitioners, a few key benchmarks are useful to take a preliminary assessment of the fetus.

i. Bladder: The bladder is normally collapsed or only slightly distended. A large round, anechoic fluid filled structure in the caudal fetal abdomen, not adjacent to the liver is most likely the bladder. This is abnormal if it appears distended, larger than the stomach, or obscured by the abdominal viscera. An enlarged bladder is often a sequelae to an umbilical cord torsion as the pressure elicited by twisting closes the urachal outlet into the allantoic fluid cavity.\textsuperscript{41–45}

ii. Umbilical cord: It is often recognized as a linear or coiled structure near the fetal abdomen or near the legs or the uterine bifurcation, containing 3 circular anechoic structures in cross-section (2 umbilical arteries, 1 umbilical vein).\textsuperscript{41–45} For the amniotic portion of the cord (nearest the fetus), the urachus can often be identified as an irregularly shaped structure not much larger than the diameter of the vessels. Extensive sacculations or dilations with no flow on doppler may signal pathology, and again, excessive sacculations and distention with an enlarged bladder may suggest umbilical torsion.\textsuperscript{41–45}

iii. Fetal stomach: The fetal stomach is located right behind the liver as a rounded, usually anechoic fluid filled structure. The presence of fluid in the stomach is a normal finding and suggests the foal is swallowing amniotic fluid.

5. Urinary Tract Evaluation in the Horse by Transrectal Ultrasonography

Indications for transrectal ultrasound evaluation of the urinary tract include hematuria, stranguria, or any other indication of pelvic pain or pathology of the urinary system. The urinary tract can be evaluated well with the linear array rectal transducer, and in some cases, the parenchyma of the kidney is better visualized with a transrectal approach (Fig. 7). The urethra, bladder, ureters, and both kidneys can usually all be imaged with patience and practice. The cranial aspect of both kidneys can be difficult to image completely, and due to positioning, the right cranial ureter and kidney are more difficult to image. A smooth muscle relaxant such as N-butylscopolammonium bromide\textsuperscript{d} can be used to facilitate rectal relaxation if the there is too much tension on the rectal tissue during evaluation.

Pelvic Urethra

In the mare, this is usually identified approximately 3 to 12 inches cranial to the anus and extends from the bladder cranially to the ventral aspect of the vestibulovaginal fold caudally. The urethra in the mare is usually collapsed and has a slightly echogenic line demarcating the lumen that runs from the caudal opening to the bladder (caudal to the cervix or just at

\textsuperscript{d} N-butylscopolammonium bromide is a smooth muscle relaxant used in veterinary medicine to facilitate rectal relaxation during ultrasound evaluation of the urinary tract.
the same level), to beneath the vestibulovaginal fold (often demarcated by air in the vestibule). The normal palpation of the equine bladder should not be distended so that it fills the pelvic inlet. Normally, the dorsal bladder wall does not extend above the brim of the pelvis, and if it is painful upon palpation or manipulation, this is abnormal. Often large bladder stones can be palpated in an emptied bladder. Ultrasound in the normal animal reveals a smooth bladder wall with uniform echogenicity and slightly wrinkled when collapsed. The ultrasonographic appearance of urine can be relatively anechoic to heterogenous swirling fluid with variable sediment in the ventral aspect. Bladder stones appear as smooth to irregular surfaced, hyperechoic structures that reflect the ultrasound such that tissue deep to the structure cannot be imaged. Movement of the bladder can help identify if the pathology is attached to the bladder wall or ureters and help to characterize and estimate size of the structure. Bladder rupture can have a variable appearance with collapsed bladder and increased free fluid in the abdomen. The continuity of the bladder wall is evaluated, and if there is no free fluid and the bladder is distended, rupture is unlikely.

Ureters

Ureteral trauma is rare and likely underdiagnosed, as it is not an area that is commonly evaluated. The easiest way to identify and evaluate the ureters is to identify the bladder and then place the transducer in transverse orientation, moved caudally to the region of the bladder neck and beginning of the pelvic urethra (Fig. 8). On the dorsal aspect of the bladder, the ureters will be small (<5 mm in diameter, wall thickness of <2 mm in the normal adult). The ureteral opening will appear as an irregular surface on the luminal surface of the bladder wall at approximately the 10 and 2 o’clock position. If one waits, as urine is emptied into the bladder, the ureters will distend and swirling fluid can be seen entering the bladder. The ureters can be followed cranially, sometimes all the way to the entrance to the renal pelvis. Once identified caudally, the transducer is manipulated to keep the ureter in cross section and is followed cranially. Intermittent filling with urine will help confirm their presence if one is unsure. Excessive dilation and hyperechoic contents that obscure the lumen warrant investigation of the kidney, and ureteral urine flow should be assessed (Fig. 8).

Kidneys

The kidney parenchyma can be evaluated well with the transrectal approach (Fig. 9). The limitation is that often the cranial aspect cannot be reached and imaged, and occasionally the right kidney is so far cranially that it is not easy to even image the caudal aspect. Renoliths, calcification within the kidney, and excessive dilation or trauma to the kidney have all been imaged with this transrectal approach.

The more one uses the ultrasound to evaluate their patients, the more familiar one becomes with what is normal. This is essential in later being able to differentiate normal from abnormal and help with early diagnosis of conditions that may have been missed without ultrasonic evaluation. The reader is encouraged to practice new ways of using their ultrasound equipment, as it will enhance the quality of care of the patients.
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Fig. 9. Transrectal ultrasound image of the left kidney using a linear array transducer. The normal cortex and medulla and renal pelvis can be seen. The transrectal approach can allow for a detailed exam of the left kidney and often the right, depending on the size of the horse. Ureters can be traced from the renal pelvis to the trigone of the bladder using the transrectal approach that can be useful in evaluation of the urinary tract for pathology.

Acknowledgments

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

Conflict of Interest
The Author has no conflicts of interest.

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*Dormosedan®, Zoetis, Parsippany, NJ 07054.
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Advanced Equine Repro Ultrasound, Veterinary Advances, Ltd., Ireland.
Buscopan®, Boeringer Ingelheim, Duluth, GA 30096.
How to Perform Plantar Non-Weight-Bearing Ultrasonographic Evaluation of the Equine Hind Proximal Suspensory Ligament

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1. Introduction

Suspensory ligament injury is an important source of lameness or poor performance in athletic horses.1–4 Injury to the suspensory ligament can affect the ligament and third metatarsal bone.1 Ultrasonography is commonly used to evaluate the suspensory ligament and may be a reasonably accurate predictor of suspensory ligament pathology.2 An accurate diagnosis of proximal suspensory desmopathy is of great importance as recommended options for management can be expensive and lengthy.3 Regions of fat and muscle create variations in the suspensory ligament’s echogenicity.1 This echo pattern makes it difficult to determine if these variations in echogenicity are the result of injury or normal suspensory ligament anatomy.1 In order to identify regions of suspensory ligament fibers versus areas of fat and muscle, off-angle or oblique-incidence imaging should be performed. When using off-angle imaging, the position of the probe is changed such that the ultrasound beam is no longer perpendicular to the longitudinal axis of the suspensory ligament fibers. This change in the position of the probe, and the resulting change in the echogenicity of suspensory ligament fibers and that of the regions of fat and muscle, can be used to identify the regions of different tissue types within the ligament.1 When the ultrasound beam is perpendicular to the longitudinal axis of linear fibers within a normal tendon or ligament, maximum echogenicity is created.1 When the ultrasound beam is not perpendicular to a normal tendon or ligament, decreased echogenicity can be created in that structure.1 The echogenicity of fat, and to a lesser degree, muscle, is not dependent on the ultrasound beam angle.1 However, the difference between the echogenicity of fat and muscle cannot be readily identified in the suspensory ligament.5 Therefore, comparing the appearance of the suspensory ligament with the beam both perpendicular (on angle) and not perpendicular (off angle) to the ligament allows identification of fibers versus areas of fat and muscle.5 Regions of mottling or decreased echogenicity identified in the suspensory ligament with ultrasound can then be further investigated using changes in beam angle to determine if the source of these regions is ligament fibers or fat.
This paper describes the ultrasonographic method of evaluating the equine proximal hind suspensory ligament using the plantar non-weight-bearing approach using on- and off-angle imaging as compared to the standard method.

2. Materials and Methods

Patient Preparation and Positioning

The hair should be clipped on the plantar aspect of the metatarsal region beginning just proximal to the chestnut. Beginning at the level of the chestnut, the skin should be clipped over the plantar and plantaromedial aspects of the metatarsus extending distally to the level of the suspensory ligament bifurcation. Following clipping, the skin should be cleansed and coupling gel liberally applied. When evaluating the hind suspensory ligament with ultrasound, the authors typically perform the plantaromedial approach first with the limb in a weight-bearing position, followed by the plantar non-weight-bearing approach. The plantaromedial weight-bearing approach is not described in this paper. For the plantar non-weight-bearing approach, the limb may be positioned in one of the following manners depending on the operator’s preference: (1) resting on the toe or (2) pulled forward with the foot resting on the operator’s knees; (3) the foot may be propped on a farrier stand, or (4) the foot may be held in a non-weight-bearing position by an assistant (Fig. 1). This creates laxity in the flexor tendons, resulting in a widening of the skin surface for increased probe contact, and decreases the distance between the ultrasound probe and the suspensory ligament, allowing adjustments in the ultrasound machine settings for a more detailed evaluation of the ligament.

Ultrasound Probe and Settings

High-frequency (7.5-16 MHz) linear array ultrasound probes are preferred for this examination. The frequency, focal zones, depth, and gain should be adjusted to maximize image quality at the level of the suspensory ligament. For an average-size Warmblood or Thoroughbred horse, a 4-cm depth setting and a frequency of 10 MHz are reasonable initial machine settings that can then be adjusted based on the individual patient. Although all structures at this level should be assessed during the ultrasound examination, the minimum depth should be selected that places the hyperechoic line representing the plantar third metatarsal bone margin clearly in the far field of the image.

Ultrasound Examination

First, the horse should be appropriately restrained and the limb positioned as described above, according to the operator’s preference. The ultrasound probe is placed in a transverse position on the plantar aspect of the limb, immediately distal to the tarsometatarsal joint at the level of the suspensory ligament attachment on the third metatarsal bone. The entirety of the proximal suspensory ligament is evaluated prior to evaluating the proximal branch of the suspensory ligament. This structure is also referred to as the accessory ligament or the proximal bundle in the literature. When the limb is in a non-weight-bearing position, manipulating the flexor tendons allows for ultrasound beam to be oriented as dorsally as possible while still visualizing the entire ligament. This is achieved by placing the probe surface parallel to the plantar third metatarsal bone margin. The initial image created should allow visualization of the axial surfaces of the second and fourth metatarsal bones with the plantar margin of the third metatarsal bone oriented in a horizontal position. Once the initial image is obtained, the ultrasound machine settings should be adjusted from baseline to ensure the correct depth, frequency, gain, and focal zones. The time gain compensation settings should be used to create uniform echogenicity in the image, which typically requires increasing the gain in the image far field. Normal suspensory ligament fibers will be echogenic when the ultrasound beam is perpendicular to the longitudinal axis of the fibers and will become hypoechoic when the probe position is changed such that the beam is no longer perpendicular to the fibers. Regions of fat and muscle will remain echogenic regardless of beam angle. Both on- and off-angle images are obtained during this examination, and corresponding longitudinal images can be obtained. Off-angle images of the ligament in its entirety should be obtained first, which allows...
identification of anatomic features of the ligament such as size, shape, and margins as well as the fat and muscle distribution, which correlates to gross and MRI findings (Fig. 2). Off-angle images are obtained by raising or lowering the probe cable at the site of skin contact. The probe position should be changed the minimum amount that decreases the echogenicity of the ligament fibers. This has been reported as approximately 10°.6 These images should be saved and compared to the on-angle images in order to identify and characterize pathologic change. In order to ensure that the on- and off-angle images are obtained at the same level, minor movements in probe position are necessary. Lowering the probe cable to create an off-angle image will require distal movement of the probe to match the on-angle image at the same level. Anatomic landmarks, such as the shape of the osseous margins, are used to ensure the off- and on-angle images are made at the same level. Setting the ultrasound machine to a split screen and obtaining off-angle images and on-angle images at the same level will assist with making these comparisons. A measurement system (zones or centimeters) should be consistently used. The entire suspensory ligament from the proximal branch continuing to the distal extent of the body should be evaluated for differences in echogenicity. When learning this technique, the proximal branch is most easily identified by placing the probe at the level of the suspensory ligament attachment on the third metatarsal bone and then following the fibers proximally to the fourth metatarsal bone. Comparison images of the opposite limb are imperative in all cases. The size and shape of the fourth metatarsal bone and its relationship to the third metatarsal bone can be used to ensure comparisons between the right and left hindlimbs are being made at the same level.1

3. Results
Since 2008, approximately 2000 horses have had ultrasound examination of the hind proximal suspensory ligament incorporating the plantar non-weight-bearing approach. The suspensory ligament anatomy has been well identified using this technique. In addition, suspensory ligament enlargement, fiber abnormalities, and alterations in the fat and muscle bundles have been diagnosed using this technique. Beginning the examination immediately distal to the tarsometatarsal joint, the suspensory ligament consists of a narrow, rectangular-to-square band of ligamentous fibers attached to the plantar surface of the third metatarsal bone. The fibers are more prominent lateral of midline (Fig. 3).4 The suspensory ligament has a relatively homogenous

Fig. 2. This is a comparison of the proximal suspensory ligament at the same level. A, gross dissection, B, MRI image, C, off-angle ultrasound image, and D, approximate location of the ultrasound probe at this level. Notice the similarity in fat and muscle bundles between the images.

Fig. 3. MRI (A) and off-angle ultrasound (B, C) images of the suspensory ligament at the proximal extent of the third metatarsal bone attachment and approximate location of the ultrasound probe at this level (D). Notice the rectangular shape of the suspensory ligament with a homogeneous echo pattern as no fat or muscle is present at this level. The perimeter of the ligament is outlined in image C so it can be compared to the remaining images.
echogenicity with no fat or muscle at the proximal extent of the third metatarsal bone attachment. In the distal half of the third metatarsal bone attachment, the suspensory ligament has small regions of fat and muscle, and the ligament becomes triangular in shape (Fig. 4). At this level, there is a focal bundle of ligament fibers originating from the fourth metatarsal bone that merge with the suspensory ligament. Distal to the third metatarsal bone attachment, there may be a partial sagittal cleft dorsally, giving the ligament a heart-shaped appearance (arrow). However, this sagittal cleft is not present in all horses, and in the absence of a sagittal cleft, the ligament is oval distal to the third metatarsal bone attachment (Fig. 6). As the suspensory ligament continues distally from the third metatarsal bone attachment, there is typically a prominent central area of fibers (central fiber bundle) as well as peripheral fibers with regions of fat and muscle that become more prominent. In off-angle images distal to the third metatarsal bone attachment, the suspensory ligament appears separated from the third metatarsal bone plantar margin by hyperechogenic connective tissue and vessels. This is how the distal extent of the third metatarsal bone attachment can be identified. Beginning at this level and continuing distally to the level of the suspensory ligament bifurcation, there are fat and muscle bundles located within the lateral and medial fiber bundles of the suspensory ligament. The lateral fiber bundle is slightly larger than the medial fiber bundle. These medial and lateral fiber bundles are triangular to oblong in shape, oriented in a dorsoplantar direction. However, random areas of fat and muscle can dissect through the peripheral ligament margin and can become zigzag in shape in the ligament body. Asymmetry of the fat and muscle pattern can exist between limbs, and these regions should not be mistaken for injury. The suspensory ligament has a larger amount of connective tissue separating it from the second metatarsal bone when compared to the distance between the fourth metatarsal bone axial margin and the lateral aspect of the ligament. A linear region of fascial tissue is sometimes identified plantar to the suspensory ligament, running between the plantar margins of the second metatarsal bone and the suspensory ligament.

**Fig. 4.** MRI images of the suspensory ligament, moving from proximal to distal. Ligament fibers are black and the fat and muscle bundles are intermediate to light gray on these images. A, The suspensory ligament immediately distal to the tarsometatarsal joint. The suspensory ligament is a narrow, rectangular-to-square band of ligamentous fibers attached to the plantar surface of the third metatarsal bone (arrow) with no fat or muscle. B, The suspensory ligament at the mid aspect of the third metatarsal bone attachment. Small regions of fat and muscle become visible medial and lateral to the central fiber bundle (arrow). Lateral fibers can be seen extending from the fourth metatarsal bone toward the central fiber bundle that will merge with the ligament (arrowhead). C, The suspensory ligament at the distal extent of the third metatarsal bone attachment. Prominent regions of fat and muscle are now present in the suspensory ligament, and the ligament has a triangular shape (arrow). D, Distal to the third metatarsal bone attachment, notice the partial sagittal cleft dorsally, giving the suspensory ligament a heart-shaped appearance (arrow). E, Continuing distally, the suspensory ligament becomes more oval (arrow) as the sagittal crest recedes.
and fourth metatarsal bones. The proximal suspensory ligament branch is oval in shape and homogeneous with no fat or muscle bundles, appearing similar in echogenicity to a tendon as it extends to the fourth tarsal bone. This branch lies medial to the plantar ligament (Fig. 7). This constitutes the appearance of the suspensory ligament using the plantar non-weight-bearing approach.

4. Discussion

This technique has been incorporated into all hind-limb proximal suspensory ligament ultrasound evaluations since 2008. It provides a method for determining the different tissue types within the suspensory ligament, which allows the identification of normal anatomic characteristics versus pathologic change. The echogenicity of the connective tissue surrounding the suspensory ligament is not beam-angle dependent, remaining echogenic regardless of beam angle. This is the reason that off-angle images are most effective at defining the ligament margins, differentiating them from the surrounding echogenic connective tissue. Additionally, the limb positioning and resulting displacement of the plantar soft tissues and vasculature reduces edge artifacts, which can obscure the suspensory ligament. This technique, which can be performed in the field, provides images that are similar in appearance to MRI images and gross pathology of the suspensory ligament, reflecting the true anatomic features of the ligament (Fig. 2). Utilizing the described technique places the ultrasound beam perpendicular to the bone-ligament interface, aiding in the identification of abnormalities at the suspensory ligament attachment on the third metatarsal bone. The dorsal aspect of the ligament and the central fiber bundle are commonly affected regions, and this technique improves visualization of these specific regions. Limitations to this technique include patient compliance with positioning. However, this is a consistent finding with any ultrasound evaluation and can be assisted with proper restraint and/or sedation. An additional limitation is that an in-depth knowledge of the suspensory ligament anatomic features is required in order to effectively utilize the technique. This technique may be considered by some to be challenging; however, with practice and knowledge of normal anatomic features, it can be mastered. Due to normal anatomic variations, there is the potential for asymmetry of the fiber and fat/muscle distribution when comparing opposite limbs. However, the opposite limb still provides a helpful guide for determining the anatomic features of the ligament. In addition, fiber disruption and/or moderate fiber abnormalities are both decreased in echogenicity to anechoic regardless of beam angle, distinguishing them from normal ligament fibers or other tissue types. A thorough clinical evaluation should be performed prior to ultrasonographic

![Fig. 6](image.png)

**Fig. 6.** MRI (A) with comparison on- (B) and off-angle (C) ultrasound images of the suspensory ligament distal to the third metatarsal bone attachment, with approximate location of the ultrasound probe at this level (D). The MRI image has been rotated to match the ultrasound images. The on-angle ultrasound image (B) has minor heterogeneity in the echo pattern. The off-angle ultrasound image can be used to identify the regions of fat and muscle (arrows). Notice the oval shape of the suspensory ligament at this level.

![Fig. 7](image.png)

**Fig. 7.** MRI (A) and ultrasound (B) images of the proximal branch of the suspensory ligament at the level of the fourth tarsal bone and approximate location of the ultrasound probe at this level (C). Notice the round shape of the proximal branch (outline), surrounding plantar ligament (star), superficial digital flexor tendon (arrowhead), and deep digital flexor tendon (arrow).
evaluation to ensure the findings can be correlated with the clinical presentation. This technique should be incorporated into the standard suspensory ligament ultrasound examination in conjunction with the plantaromedial approach. Subtle changes in shape as a result of ligament injury are often visible before fiber abnormalities are detected. Therefore, a comparison with the opposite limb is imperative to identify these subtle ligament shape changes. In conclusion, a thorough understanding of the normal anatomy in conjunction with a complete examination as described in this manuscript and comparison to the opposite limb will provide the most clinically relevant information in regard to the condition of the suspensory ligament.

Acknowledgments

Declaration of Ethics

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

Conflict of Interest

The Authors have no conflicts of interest.

References

Review of Non-Weight-Bearing Proximal Suspensory Ligament Ultrasound for Alterations in the Muscle/Fat Indicating Pathologic Change

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Ultrasonographic evaluation of the fat and muscle in the proximal suspensory ligament, with comparison of on- and off-angle non-weight-bearing images, can provide useful information about suspensory ligament injury. Alterations in the fat and muscle distribution on ultrasound images are an important and sometimes the only indication of pathologic change. Author's address: Equine Diagnostic Imaging, 14313 SW 79th Street, Archer, FL 32618; e-mail: equinedxim@yahoo.com. © 2021 AAEP.

1. Introduction

Proximal suspensory ligament disease is a common source of lameness in performance horses. Diagnosis of lameness localized to the proximal metacarpal or metatarsal region is typically achieved by physical examination, diagnostic analgesia, and imaging. Radiographs can be used to identify changes in bone density at the attachment of the suspensory ligament on the third metacarpal or metatarsal bone. Increased density resulting from sclerosis and/or decreased density resulting from lysis or resorption are radiographically identifiable abnormalities that can be associated with suspensory ligament injury. Ultrasound is typically used to evaluate the suspensory ligament prior to advanced imaging when lameness is localized to the proximal metacarpal and metatarsal regions. Ultrasound of the proximal suspensory ligament requires a thorough knowledge of anatomy and the normal anatomic variations of the suspensory ligament when comparing different horses as well as different limbs of the same horse. A complete ultrasound examination of the suspensory ligament has evolved over the last several years. New ultrasound methods have been developed that allow a more precise and complete evaluation of the complex suspensory ligament anatomy than can be accomplished using the standard on-angle weight-bearing technique. On- and off-angle non-weight-bearing ultrasound imaging is now considered an invaluable component of the complete proximal suspensory ligament ultrasound examination, and the advantages of this technique have been well established. This technique allows a complete evaluation of the suspensory ligament with identification of the different tissue types within the suspensory ligament. Identification of the complete suspensory ligament and the normal
There are a wide range of abnormalities that can affect the proximal suspensory ligament and the third metacarpal or metatarsal bone attachment resulting in lameness. Advanced imaging, such as magnetic resonance imaging (MRI) and computed tomography (CT), has been used for the diagnosis of pathologic change in the proximal suspensory ligament and is more effective than ultrasound for diagnosing certain components of injury in this region. MRI, CT, and ultrasound identify different types of pathologic change affecting the proximal suspensory ligament and its osseous attachment. MRI excels at soft tissue detail and has been a tremendous educational tool by clearly demonstrating the normal anatomic features and variations of the suspensory ligament as well as the different types of pathologic change. CT demonstrates bone detail and vascular patterns within injury, and the use of contrast increases the conspicuity of associated soft tissue injury. Advanced imaging is necessary to identify certain types of suspensory ligament injury, and this is typically dependent on the nature and severity of the injury. However, there are specific patterns of pathologic change that in the past were not diagnosed with ultrasound initially but were identified using MRI and then retrospectively identified with ultrasound. Eventually, with practice and experience, certain types of pathologic change can be more easily identified prospectively with ultrasound when the specific patterns of injury are better understood. One specific pattern of pathologic change in the suspensory ligament that is evident on MRI and can be identified well with ultrasound involves alterations and/or abnormalities in the fat and muscle distribution. In certain cases, these alterations and/or abnormalities in the fat and muscle distribution of the suspensory ligament can be the most evident abnormality on an ultrasound examination. Abnormalities affecting the fat and muscle of the suspensory ligament are typically accompanied by fiber abnormalities and often focal or diffuse suspensory ligament enlargement. A wide range of fiber abnormalities can be present in the suspensory ligament, with various degrees of clinical relevance. Some clinically relevant fiber abnormalities do not produce a detectable change in the echo pattern of the ligament fibers when examined with ultrasound. In these cases, abnormalities of the fat and muscle and ligament enlargement can be the most evident change identified with ultrasound. This paper will review the techniques for identifying abnormalities in the fat and muscle distribution of the proximal suspensory ligament as an indication of pathologic change using the on- and off-angle non-weight-bearing ultrasound examination.

2. Methods

The palmar or plantar non-weight-bearing approach using on- and off-angle ultrasound imaging with comparison to the opposite limb is recommended for specific evaluation of the fat and muscle distribution in the suspensory ligament. Although evaluation of the fat and muscle in the suspensory ligament can be done using on- and off-angle imaging with the limb in a weight-bearing position, placing the limb in a non-weight-bearing position for this examination will yield additional information that will aid in the diagnosis of injury. Assessment of the fat and muscle is not necessary for the proximal suspensory ligament branch in the hind limb, as it does not contain fat and muscle. However, on- and off-angle imaging will still provide additional information about the proximal branch of the suspensory ligament, which is best identified with the limb in a non-weight-bearing position. In both the front and hind limbs, the non-weight-bearing off-angle technique is first used to identify the entire peripheral margin of the suspensory ligament, which is achieved when the second, third and fourth metacarpal or metatarsal bones are identified (Fig. 1). This initial assessment of the suspensory ligament should be made with the third metacarpal or metatarsal bone visible as a horizontal echogenic line at the distal extent or far field of the image. The orientation of this line is extremely important as the horizontal positioning of the third metacarpal or metatarsal bone ensures that the ultrasound beam is interacting with the fibers at the same angle allowing comparison of the echogenicity within different portions of the ligament. Although the horizontal position of the third metacarpal or metatarsal bone is considered imperative for the initial images, there are reasons to change this osseous margin orientation when specifically evaluating different structures or features of the proximal metacarpal and metatarsal regions, such as the interosseous spaces or axial surfaces of the splint bones and their relationship with the suspensory ligament margins (Fig. 2). On the initial image, once the correct positioning of the suspensory ligament is achieved in the off-angle ultrasound image, the normal anatomic features of the ligament should be identified and evaluated. This includes the ligament margins, boundaries of the medial and lateral lobes in the forelimb or fiber bundles in the hind as well as the regions of ligament fibers versus fat and muscle. The purpose of this assessment is to determine if the pattern of ligament fibers and the fat and muscle distribution represent normal anatomic variation or pathologic change. This process consists of two steps. The first is to compare the anatomic characteristics of the ligament to the opposite limb. The second is to evaluate the ultrasonographic appearance of the ligament considering the patterns of normal anatomic variation that exist within the different limbs of the same horse (Fig. 3). If pathologic change is present in the ligament, comparison of the on- and off-angle images are used to determine the nature of the pathologic change. The opposite leg can be used as a guide for determining the size, shape, margins, and overall pattern of ligament fibers versus fat and muscle.
within the suspensory ligament on the off-angle images. Certainly, horses can be bilaterally affected. However, comparison to the opposite limb remains the most useful method for attempting to determine the normal characteristics of the suspensory ligament for a specific patient. For the purposes of this paper, the comparison leg will be referred to as if it does not have pathologic change in the suspensory ligament, which the author acknowledges may not be the case in all patients. When comparing to the opposite limb, the medial and lateral aspects of the suspensory ligament should be compared at the same level and region, with great care taken to ensure the levels match. Due to the marked changes that occur in the size, shape, margins, and tissue distribution of the ligament, minor differences in the level of the ligament when comparing limbs could produce marked asymmetry as a result of positioning, which should not be mistaken for pathologic change. Osseous landmarks provide the most reliable method of ensuring comparison images are made at the same level. Pathologic change within the suspensory ligament can alter all the normal anatomic features. Alterations in the appearance of the lobe size and shape, alterations in the distribution of the fat and muscle, as well as an abnormal echo pattern can be identified with suspensory ligament injury. For the purposes of this paper, the fat and muscle will be considered to have a similar echogenicity and similar response to changes in beam angle, such that they cannot be distinguished from each other but can clearly be distinguished from ligament fibers. They will be considered a single tissue type for the purposes of this technique. Off-angle images of the affected limb should be evaluated with specific assessment of the relationship between the

Fig. 1. Transverse off-angle non-weight-bearing ultrasound images of front (A) and hind limb (B) suspensory ligaments. On the corresponding images (A’, B’) the peripheral margin of the suspensory ligaments is delineated by the white line and the regions of fat and muscle are shaded with a dashed border. These images are representative of the initial images that should be produced and used to evaluate the fat and muscle within the suspensory ligament. The third metacarpal and metatarsal bones are horizontal echogenic lines in the distal extent or far field of the image. Due to the shape of the third metacarpal and metatarsal bones there is some curvature to the echogenic line.

Fig. 2. In this transverse, non-weight-bearing off-angle ultrasound image, the third metacarpal bone is angled as opposed to being a horizontal echogenic line as it is in Figure 1. This probe position and resulting beam angle are used to evaluate the medial interosseous space and axial margin of the second metacarpal bone. However, the ultrasound beam will interact with the suspensory ligament fibers at different angles which will impact the ligament echogenicity.
suspensory ligament fibers and the fat and muscle throughout the length of the ligament. This assessment should include the echogenicity of the fibers versus the fat and muscle, the clarity of the interface between the fibers and the regions of fat and muscle, as well as the position and the size of the fat and muscle. Several different abnormalities can occur in the relationship between the fibers and the fat and muscle when pathologic change occurs in the suspensory ligament. The clarity of the interface between the fibers and the regions of fat and muscle can decrease, the fat and muscle can be decreased in size or obliterated, and the regions of fat and muscle are often displaced as a result of enlargement of the fibers (Figs. 4, 5). The clarity of the interface between the fibers and the fat and muscle is the most difficult feature to evaluate and must be assessed in light of the overall quality of the images. There is variation in the quality of the images when comparing...
different horses affecting the amount of difference in the echogenicity of the ligament fibers versus the regions of fat and muscle, which directly impacts the clarity of the interface. Once abnormalities are identified within the suspensory ligament on the off-angle images, these specific regions are then evaluated on the on-angle images to determine if there is abnormal echogenicity within the regions corresponding to ligament fibers that would indicate fiber abnormalities are present.

3. Results

Using on- and off-angle non-weight-bearing ultrasound examination, generalized suspensory ligament enlargement or specific regions of enlargement can be detected. Furthermore, alterations in the echogenicity of the fibers as well as alterations in the margins, size, shape, and position of the fat and muscle bundles can also be identified. The severity of the abnormalities identified affecting the ligament fibers as well as the fat and muscle bundles can be used to assess the degree of pathologic change in the suspensory ligament. Enlargement or an abnormal shape can be identified by rounding of specific ligament margins or the entire ligament. Normal fore and hind limb suspensory ligaments have a specific characteristic size and shape. The fore limbs have a medial lobe that is longer in its medial to lateral dimension when compared to its dorsal to palmar dimension. In contrast, the lateral lobe in the fore limb is typically markedly shorter in its medial to lateral dimension and mildly longer in its dorsal to palmar dimension when compared to the medial lobe. The hind limb suspensory ligament goes through many characteristic shape changes beginning at the third metatarsal bone attachment and continuing into the suspensory ligament body. In the hind limbs, generalized suspensory ligament enlargement causes the ligament to appear rounded at a level where it should be triangular or oval (Fig. 4). In the fore limbs, the normal palmar margin of the suspensory appears flat and will become rounded with enlargement that can typically be attributed to a specific lobe, unless both lobes are affected. Suspensory ligament enlargement impacts the relationship between the ligament margins and the surrounding connective tissue and subsequently

![Fig. 4. Corresponding MRI and non-weight-bearing on- and off-angle images of right and left hind proximal suspensory ligaments in a case with right hind lameness localized to this region. The off-angle image of the right hind is duplicated so that the borders of the fat and muscle can be delineated (dashed outlines) and then compared to the unmarked image. The right hind suspensory ligament is enlarged and abnormally shaped based on the off-angle comparison images of the right and left hind. The left hind maintains a triangular plantar margin while the right hind has a rounded, wide plantar margin (white outlines). However, the most notable abnormality on the ultrasound images is the shape of the lateral fat and muscle bundle (arrows) when comparing the limbs. It is comma shaped in the right hind because it is medially displaced and compressed. There are extensive fiber abnormalities on the MRI image (arrowhead) in the lateral aspect of the suspensory ligament that do not produce a detectable change in the echo pattern of the suspensory ligament on the ultrasound images. These fiber abnormalities were only present on the proton density MRI images and not on the T2 fast spin echo or proton density fat suppressed images, which correlates with the lack of ultrasound visibility. The greatest indication of pathologic change on the ultrasound images is the alteration of the fat and muscle size and position, because the fiber abnormalities were not detectable with ultrasound. The white outlines denote the shape of the suspensory ligament margins have been moved plantar to allow visualization of the ligament margins.](image-url)
the splint bones. Enlargement affecting the medial or lateral aspects of the suspensory ligament will compress the surrounding connective tissue, decreasing the distance between the ligament margins and the axial surfaces of the splint bones. Dorsal ligament enlargement will compress or obliterate the connective tissue along the dorsal ligament margin. This will change the thickness of the dorsal connective tissue or the level at which the connective tissue becomes visible dorsal to the ligament when compared to the opposite limb (Fig. 3). The most effective way to identify enlargement of the suspensory ligament immediately distal to the third metatarsal bone attachment is to create images of both limbs at the same level using the fourth metatarsal bone shape and size as a level marker, and then compare the dorsal connective tissue thickness. The affected limb will have reduced dorsal connective tissue or no dorsal connective tissue at the level where the comparison leg has dorsal connective tissue. Once a region of enlargement is identified, close evaluation of the tissues in this region should then be performed because alterations in the fat and muscle bundles often accompany focal or diffuse suspensory ligament enlargement. The size, shape, and position of the fat and muscle bundles should be evaluated compared to the opposite limb. With enlargement of the dorsal suspensory ligament fibers, which seems the most frequently affected area, there will be palmar or plantar displacement of the fat and muscle bundles. Central fiber bundle enlargement occurs frequently in the hind suspensory ligament resulting in the peripheral displacement of the fat and muscle. Palmar or plantar fiber enlargement does occur and can result in dorsal fat and muscle displacement but is the least frequently encountered displacement of the fat and muscle (Fig. 6). One characteristic of pathologic change in the suspensory ligament that is evident on MRI and can be well identified with ultrasound is displacement, decreased size or obliteration of the fat and muscle bundles. In certain cases, alterations in the fat and muscle can be the most evident or only ultrasound finding in horses with lameness resulting from suspensory ligament injury. Decreased margin clarity between the fat and muscle bundles and ligament fibers can be detected but is more technically challenging and requires high quality ultrasound images. Abnormalities in the fat and muscle

Fig. 5. Corresponding MRI and non-weight-bearing on- and off-angle images of right and left hind proximal suspensory ligaments. The dorsal margin of each suspensory ligament is denoted by an asterisk on the off-angle images. The left hind suspensory ligament is enlarged and abnormally shaped based on the off-angle comparison images of the right and left hind. It has marked dorsal enlargement with focal moderate fiber abnormalities (arrows) in the dorsal extent of the enlargement that can be best identified in the on-angle ultrasound image. The fiber abnormalities in the dorsal aspect of the suspensory ligament produce a detectable change in the echo pattern of the suspensory ligament because of their severity. These fiber abnormalities can be identified on the proton density images with and without fat suppression. The severity of the fiber abnormalities causes them to be visible on ultrasound in contrast to the fiber abnormalities in Figure 4. In addition to the fiber abnormalities, alteration in the fat and muscle can be identified in the left hind. The fat and muscle bundles are plantarly displaced and the lateral bundle has a poorly defined interface with the ligament fibers. The marked dorsal enlargement of the left hind suspensory ligament is displacing the normal dorsal vasculature. However, this is not the case in the right hind, and the vessel (arrowhead) should not be mistaken for a lesion. It is outside the ligament margins on the off-angle image and its pathway can be followed or tracked in real time imaging.
Fig. 6. MRI and US images of the right hind (A, B, C) proximal suspensory ligament with comparison images of the left hind (D, E) in the proximal metatarsal region. The right hind suspensory ligament has focal plantar enlargement that can be well identified on the MRI and off-angle ultrasound images (white outlines on A and B). The shape of the plantar margin is quite different when compared to the left hind suspensory ligament at the same level (white outlines on D and E). Within the region of plantar enlargement affecting the right hind suspensory ligament, there are focal fiber abnormalities which can be identified on the MRI image (A) and in the on-angle ultrasound image (arrow on C). The region is less commonly affected than the dorsal aspect of the ligament. However, abnormalities can occur in this region. The white outlines denoting the shape of the suspensory ligament margins have been moved plantar to allow visualization of the ligament margins.
distribution and associated regions of fiber enlargement are noted, and these regions are then specifically evaluated on the on-angle images. Within the defined regions of ligament fibers identified on the off-angle images, there should be a normal, uniform echogenic pattern on the on-angle images if no detectable fiber abnormalities are present. Areas of ligament fibers that have decreased echogenicity regardless of beam angle, on both on-and off-angle images, should be interpreted as abnormal. The severity of the fiber abnormalities is proportional to the decrease in echogenicity as compared to a region of normal fibers. However, there can be clinically relevant fiber abnormalities in the suspensory ligament that do not produce a detectable change in echogenicity on ultrasound images (Fig. 4). These fibers can have an abnormal signal pattern on MRI images that is not detectable with ultrasound. In these cases, assessment of the fat and muscle becomes paramount as it can be the only indication of pathologic change in the suspensory ligament using ultrasound. In many cases it is accompanied by focal or diffuse ligament enlargement. The lack of decreased echogenicity in the fibers does not mitigate the clinical relevance of the abnormalities in the fat and muscle distribution.

4. Discussion

Evaluation of the fat and muscle in the suspensory ligament provides important information regarding the diagnosis of pathologic change. Using this technique over time will provide a knowledge base allowing a more accurate distinction between pathologic change and anatomic variation in the suspensory ligament. Whenever possible, comparison between ultrasound images and advanced imaging such as MRI should be performed, as this will provide a better understanding of the ultrasound images. MRI clearly demonstrates the normal characteristics and variation of all the salient anatomic features within the proximal metacarpal or metatarsal regions, which can then be correlated to ultrasound images. With experience, this information leads to a more accurate assessment of ultrasound images in the absence of an MRI. In difficult cases where alterations in the fat and muscle are not detected initially with ultrasound but are evident on MRI images, it is often possible to identify these changes retrospectively on ultrasound images which can be used to monitor abnormalities over time. The subjective assessment of differences in the appearance of the suspensory ligaments when comparing the fore or hind limbs, and whether to attribute these differences to anatomic variation or pathologic change, comes from experience in imaging different horses over time. There are rare cases with marked differences when comparing opposite limbs due to anatomic variation. However, the majority of horses fall within an expected range of variation, of which some subtleties cannot be detected with ultrasound but only with MRI. Therefore, the opposite limb provides a reasonable guide during the learning process. In cases where there is not a detectable change in the echo pattern of the suspensory ligament fibers, alterations of the fat and muscle bundles can be the only ultrasound visible indication of clinically relevant pathologic change. Incorporating assessment of the fat and muscle when using the on-and off-angle non-weight-bearing ultrasound technique adds valuable information for the initial diagnosis and monitoring over time. In addition, MRI is most important in these cases to accurately characterize the injury to the suspensory ligament. There are limitations to this technique. The suspensory ligament fibers curve dorsally at their osseous attachments, and this will create variation in the echogenicity of the ligament when comparing the dorsal and palmar or plantar aspects of the ligament. The amount of curvature varies in different horses and therefore requires experience to determine anatomic variation versus pathologic change. However, in most horses this variation should be relatively uniform and mild when comparing the medial and lateral aspects of the ligament. When there is decreased echogenicity in the dorsal aspect of the suspensory ligament, the other characteristics of the ligament should be closely evaluated for other evidence that supports normal anatomic variation or pathologic change. Unfortunately, there are cases with decreased echogenicity in the dorsal aspect of the ligament that is not representative of pathologic change. However, no other evidence of injury is present, such as enlargement or alterations in the fat and muscle, and this appearance is typically bilaterally present. Therefore, comparison images are quite helpful in these cases. Another limitation of this technique is that the tissue within the suspensory ligament margins that remains echogenic regardless of beam angle can be fat and muscle or scarring. There is not a completely reliable method to distinguish fat and muscle from scarring on ultrasound images purely using the echogenicity because these tissue types will have a similar echo pattern. However, scarring more typically occurs within in the regions of suspensory ligament fibers. Therefore, the location of persistently echogenic tissue within the suspensory ligament can be helpful in making this distinction. The complex anatomy of the hind suspensory ligament is a limitation. In addition, there is a vessel, the deep metatarsal vein, that extends along the dorsal ligament margin in the proximal metatarsal region that should not be mistaken for decreased echogenicity in the ligament. Color flow will not always create an appreciable signal in the vessel because it is quite difficult to place the ultrasound beam at the proper angle. The best way to detect the vessels is to follow from proximal to distal with the probe in an off-angle position. Tracking and demonstrating that the anechoic region extends outside the ligament margins are helpful ways to differentiate a vessel from
suspensory ligament injury. In conclusion, complete ultrasound of the suspensory ligament requires a knowledge of the anatomy and normal anatomic variation between limbs, as well as an understanding of how pathologic change alters the normal anatomic characteristics. Assessment of the fat and muscle can provide valuable insight into pathologic change, especially in cases that do not have obvious alterations in the echogenicity of the fibers.

Acknowledgments

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

Conflict of Interest
The Author has no conflicts of interest.

References
Controlling Wound Bacteria and Biofilm

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1. Introduction

Recognition of the role that biofilms play in the persistence of chronic wounds and the lack of response to treatment in horses is increasing. Preventing biofilm development includes the following three main strategies: effective wound cleansing and debridement to reduce bacterial counts in wounds, appropriate use of advanced dressings, and use of topical antimicrobial agents. Once biofilms are formed, eradicating them involves striking a balance between improving the wound environment without harming the native cells that are integral to the healing process, primarily through repeated lavage and debridement combined with topical antimicrobial therapy. The key points in this review are to understand why and how biofilms form, to recognize clinical indicators that biofilms have formed in equine wounds, and to be familiar with current diagnostic options and treatment strategies to eradicate biofilms. Clinical scenarios for cases in which biofilms developed and were treated will be discussed.

2. Understanding Biofilms

Bacterial biofilms are organized communities of bacteria that are typically attached to a surface (sessile) and enveloped in a 3-dimensional extracellular matrix (also known as extracellular polymeric substance [EPS]) that includes water, proteins, polysaccharides, glycolipids, bacterial DNA, and potentially other microbes that are benefiting from the protected environment.1–3 Bacteria that produce biofilms are capable of surviving and growing at a slower rate in local environments depleted of nutrients and oxygen, termed phenotypic heterogeneity.4 Biofilm formation is divided into the following three main stages: bacterial attachment, growth, and detachment.5 Planktonic (free-floating) bacteria adhere to a surface within minutes (stage 1), and the individual bacterium alter their phenotype and secrete extracellular matrix after 6 to 12 hours of attachment, growing and maturing based on cell-to-cell signaling called “quorum-sensing” (stage 2).6 Biofilms reach maturity within 2 to 4 days, and then begin to shed free-floating planktonic cells (stage 3), which disperse and attach to other areas of the wound bed. This distribution of cells activates the immune response of the host animal, stimulating the production of exudates that provides further nutrients and promotes survival of the biofilm.7–9 Once biofilms form, bacteria differentiate into a complex community with increased resistance to antibiotics, biocides, and environmental challenges such as desiccation and cells of the innate immune system.10,11 These obstacles in killing bacteria in biofilms may only be overcome if antimicrobials to which the bacteria are sensitive can be delivered at adequate concentrations for a sufficient time.9
3. Problems Caused by Biofilms

Polymicrobial biofilms have been reported in multiple types of equine wounds (acute and chronic, traumatic, and surgical). Factors that may predispose patients to biofilm formation in wounds include the immunocompetence of the patient (age, malnutrition, sepsis, corticosteroid administration, antibody deficiency, chronic stress, or diseases affecting the pituitary-adrenal axis such as pituitary pars intermedia dysfunction [PPID] or Cushing’s disease), inappropriate antibiotic sensitivity, reduced vascular perfusion to region, extensive wound contamination, or the presence of foreign bodies, surgical implants, or sequestra. Preventing biofilm development in acute wounds includes successful wound cleansing and debridement to reduce bacterial counts and the appropriate use of advanced dressings and topical antimicrobial agents. Development of infection involving biofilms has important implications for the management of chronic wounds in horses, as they present unique challenges in diagnosis and are more resistant to typical treatment methods. Wounds with biofilms may not exhibit signs typically associated with infection, although biofilms still prolong and impair healing as bacteria compete for metabolic resources and suppress the host inflammatory response in healing. The presence of biofilms has been shown to delay epithelialization and induce a chronic non-healing inflammatory phase in wounds. Furthermore, bacteria within biofilms are more tolerant to antimicrobial therapy administered topically (antiseptics) or systemically (antibiotics), as well as to the animal’s innate immune phagocytic response and environmental stresses as they are protected by their extracellular polymeric matrix. For example, Staphylococcus aureus has been demonstrated to be up to 100-fold more resistant to antimicrobial therapy when growing in biofilms versus in planktonic status. Mature biofilms are imperious to commonly used antiseptics, such as hydrogen peroxide, alcohols, bleach, acids, and generators of oxygen radicals, unless these products are used at concentrations toxic to the patient’s cells, which is not recommended. In addition, the ability of the animal’s immune response to effectively control microbes decreases as the biofilm matures. Consequently, infections involving biofilms frequently recur following discontinuation of antibiotics. Early recognition of the presence of biofilms in non-healing wounds and targeted treatment are key to the successful management of biofilms in equine practice.

4. Diagnosing Biofilms (Clinical Indications and Laboratory Testing)

Recent studies have demonstrated that biofilms associated with wounds are most commonly polymicrobial bacterial communities that include more bacterial species than are identified by routine culture and sensitivity methods. Traditional culturing techniques are frequently inadequate to comprehensively identify bacterial species associated with biofilms. Biofilms in wounds can only be definitively diagnosed using scanning electron or confocal microscopy techniques or molecular techniques to identify bacterial components within a biofilm, which are not readily available to clinicians. Biofilms have been identified in 60% of chronic wounds and 6% of acute wounds in one study, although more recent work suggests that biofilms are involved in most, if not all, chronic wounds. Multiple studies have documented evidence of biofilms in chronic wounds of horses specifically.

To review, typical methods to assess bacterial burden in wounds may be done qualitatively or quantitatively. Qualitative assessment involves determining types of bacteria in wounds, coupled with sensitivity testing to guide antibiotic choices in treatment. Quantitative bacteriology is rarely performed in veterinary medicine but should be considered when a wound is not progressing as anticipated or the skin graft fails. Bacterial counts greater than 10⁵ per gram tissue or mL exudate has historically been considered to indicate active infection, although the number of bacteria needed to produce infection may be dramatically reduced if bacterial virulence is high, foreign material (suture, necrotic debris, foreign body, and implant) are present, or host resistance is decreased. For example, as few as 100 bacteria per gram of tissue or mL exudate may be needed to incite infection with multidrug-resistant isolates, and polymicrobial infections with 2 or more different microorganisms act synergistically to result in greater virulence than infection caused by either species alone. Although a single species may make up a biofilm, recent studies have provided evidence that bacterial isolates from chronic equine wounds are frequently polymicrobial, with an average number of species identified of 3.02 ± 1.65 (range, 0–8), and with the bacterial genera involved being similar to those identified in human infections. One study identified Pseudomonas, Enterococcus, and Staphylococcus members as the most common organisms in chronic wound biofilms in humans. This finding further highlights the inadequacies of traditional culture methods, as molecular analyses of chronic wound samples has revealed far more diverse polymicrobial communities (up to 17 genera per wound), including anaerobic species that are not identified by traditional culture.

Currently, the best diagnostic method available to equine practitioners when biofilms are suspected is to submit a deep tissue biopsy, a swab of the deepest tissues available, or both. Following debridement of the wound, samples should be collected from multiple sites if possible, particularly from the deepest regions of the wound (e.g., pockets and fissures). When practitioners swab wounds for sample collection, the swab should be drawn across the surface of the wound with sufficient pressure to collect a sample of the biofilm itself. Drawing blood in the process of collecting the sample should be avoided, as blood itself contains...
antimicrobial elements that may affect culture results. Although tissue samples are more invasive to collect, they are more likely to yield reliable culture results. Ideally, all samples should be submitted prior to beginning or changing antimicrobial protocols. If deemed necessary to collect samples while horses are on antibiotics, it is recommended to notify the laboratory of the horse’s antibiotic regimen and when the last dose was received in relation to sample collection. If positive culture results are obtained, they should be interpreted with the assumption that the full microbial spectrum present within the biofilm may be underrepresented. In lieu of a positive culture result, diagnosis of biofilms in wounds may be made based on clinical suspicion (Table 1). Clinical indicators of biofilms within wounds include excessive moisture, poor quality granulation tissue, indicators of inflammation (heat, swelling, pain, and redness), history of persistent or recurrent infection despite antimicrobial therapy, negative culture results despite clinical suspicion of infection, or generally wounds remaining in a recalcitrant chronic inflammatory state despite addressing comorbidities (e.g., immunosuppression) that may affect wound healing. As culture results in diagnosing biofilms are unreliable, observation of clinical indicators that biofilms are present should prompt practitioners to use wound care techniques aimed at treating biofilms.

### Table 1. Indirect Clinical Indications of Wound Biofilm

<table>
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<tr>
<th>Clinical Observation</th>
<th>Biofilm Explanations for Clinical Observation</th>
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<tr>
<td>Excessive moisture associated with wound</td>
<td>Bacteria in biofilms secrete extracellular matrix, and biofilm presence promotes inflammation, resulting in increased exudate.</td>
</tr>
<tr>
<td>Autograft or allograft fails on wounds</td>
<td>Applying tissue grafts over biofilms provides a second growth surface and food source, leading to devitalization of graft tissue and increased exudate and inflammation.</td>
</tr>
<tr>
<td>Poor quality granulation tissue (e.g., hypergranular, friable)</td>
<td>Biofilm presence contributes to delayed epithelialization and is frequently associated with poor quality granulation tissue.</td>
</tr>
<tr>
<td>Indications of local infection (swelling, sensitivity, redness, heat)</td>
<td>Biofilms promote inflammation and may be a precursor to other clinical indications of infection.</td>
</tr>
<tr>
<td>History of persistent or recurrent infection despite antimicrobial therapy</td>
<td>Biofilm bacterial phenotypes adapt rapidly and may only demonstrate a 1 to 2 log reduction with antibiotic therapy at $50 \times$ to $1000 \times$ MIC. Biofilms contain persister cells that remain once antibiotic therapy is discontinued, seeding and contributing to subsequent biofilm reformation.</td>
</tr>
<tr>
<td>Negative culture results despite clinical suspicion of infection or signs of bacterial colonization</td>
<td>Biofilm bacteria metabolize more slowly and are phenotypically different than planktonic bacteria. Standard microbiological culture techniques are not capable of identifying all species present, making bacteria in biofilms difficult or impossible to identify by culture.</td>
</tr>
<tr>
<td>Wound remains in chronic inflammatory state and recalcitrant to therapy despite addressing comorbidities</td>
<td>Biofilms are resistant to host inflammatory responses and actually feed off exudate produced by inflammation, further promoting inflammation.</td>
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</table>

practical guidelines for clinical management for cases in which biofilms are suspected (Table 2). These strategies emphasize repeated debridement to physically disrupt the biofilm matrix and remove devitalized tissues that serve as nutrients to the microbes. Debridement disturbs the biofilm structure, allowing increased susceptibility to antimicrobial therapies for a period of time that may prevent bacterial reattachment, as immature biofilms are more vulnerable to antibiotics. Two principles of debridement in human wound care described by Wolcott et al. include debridging with the goal of altering the anatomy of the wound by undermining and removing all tissue surfaces that touch one another and removing devitalized and discolored tissue until normal bleeding tissue was encountered. Topical treatments are recommended to be applied within 4 hours of debridement or less, before biofilm reformation. An example of the successful treatment of biofilms (and how quickly they reform in the absence of consistent therapy) includes removal of plaque from dental enamel by using a combination of regular tooth brushing (i.e., debridement) in combination with topical antiseptic mouthwashes. Furthermore, antiseptic mouthwashes are of minimal benefit without preceding flossing and brushing. With this in mind, treatment of biofilms in wounds is recommended in three stages, as follows: physically debriding the biofilm, delaying or preventing reformation of biofilms, and repeating until full resolution of the biofilm is achieved. Debridement of biofilms may be performed sharply (or bluntly with a scalpel blade), using pulsed water-jet irrigation or low-frequency ultrasonic debridement, or mechanically with gauze.
swabbed across the surface of the wound. To minimize discomfort for the patient during this step, it is recommended that horses be sedated and the wound desensitized with local or regional anesthesia. In some instances, general anesthesia may be necessary for the first debridement, if dictated by the patient’s temperament, or if the wound is extensive or not easily accessed. Face protection or surgical masks may be recommended for the veterinarian to protect against multidrug resistant infectious organisms or if using pulsed water-jet irrigation, which may aerosolize organisms to a greater extent. The overall objective of debridement is to remove as much of the biofilm and associated extracellular matrix and devitalized tissue as possible to expose remaining bacteria to antimicrobial agents. Reducing or preventing reconstitution of biofilms following debridement may be achieved in several ways. Surfactants (polyhexamethylene biguanide [PHMB] or polyhexanide) can be used as adjunctive therapies at this time, as they reduce biofilm surface tension, facilitating degradation and removal. Antiseptic agents do not penetrate necrotic debris and are unlikely to reduce bacterial populations deep in the wound bed or without debridement. In general, they should be reserved for use on normal skin and not the wound bed. Examples include acetic acid, alcohols, aluminum salts, boric acid, chlorhexidine, formaldehyde, gentian violet, hexachlorophene, hydrogen peroxide, hypochlorite, iodine, povidone-iodine, merthiolate, permanganate, and silver nitrate. Unlike antiseptics, topical antimicrobial agents provide efficacy against bacteria within the wound bed and have minimal side effects on wound healing (depending on the vehicle). Ideally, antimicrobials are selected based on results of culture and sensitivity, and due to concerns with increasing incidence of resistance, ones used topically should ideally be different to those administered systemically. Topical dressings including silver sulfadiazine (1%) or silver-impregnated wound dressings are currently used to combat biofilms in humans and may be used if antibiotic sensitivities are not available (leaper). However, their efficacy is limited to the first 24 hours, so these dressings must be changed frequently to maintain their effect.7 If culture and sensitivity results are available, appropriate antibiotics may be applied topically with an enhanced effect. Additionally, topical application of plasma (natural or hyperimmune for the specific pathogen targeted) may be of benefit, as plasma inhibits the adhesion and growth of bacteria.36–38 The efficacy of antibiotics delivered regionally (e.g., regional limb perfusion) is also greater immediately after degradation of the biofilm, so timing of perfusion following surgical debridement may improve outcomes. In situations in which a positive antibiotic culture is not obtained or for interim coverage in severely infected wounds, broad-spectrum antibiotic therapy is generally indicated. Combinations of penicillin G (procaine penicillin or crystalline) or cephalosporins (ceftiofur, cefazolin, or cephalothin) and gentamicin are commonly used. Collecting a separate swab to perform in-house Gram staining may also help to guide interim antibiotic therapy. In general, severely contaminated wounds or those with biofilms are more effectively treated with bactericidal antibiotics (versus bacteriostatic), including penicillins, cephalosporins, aminoglycosides, metronidazole, quinolones, or rifampin.7 Despite treatment, biofilms associated with orthopedic implants frequently necessitate removal to eliminate the problem.39 In these situations, ideally, infection is controlled by systemic and local antimicrobial therapy until sufficient healing of fractures or arthrodesis has occurred. In situations in which both instability and infection are present, implants may be replaced or removed, sonicated, cleaned, autoclaved, and reimplanted using new screws and holes if financially feasible. Alternatively, implants may be removed and unconsolidated fractures managed with a transfixation pin cast or external fixator. The fracture site, bone, and surrounding tissues may be debrided during the sterilization process. The previous screw holes and surrounding region may be packed with absorbable antibiotic eluting materials. Use of surgical drains or leaving the distal aspect of the incision open may facilitate drainage in these cases.39 Debridement and efforts to impede reconstitution of wound biofilms should be repeated as frequently (daily or at least every other day) and for as long as needed until the infection is resolved. Mature biofilms reform as quickly as 24 hours (up to 72 hours) following debridement, allowing a window of opportunity in which bactericidal drugs may have a greater effect. It is recommended to monitor horses frequently and continue antibiotic therapy until signs of infection have resolved and wound healing is progressing as expected. If improvement is not seen within 3 to 4 days after the initiation of therapy, it is recommended to review all aspects of the case, including repeated physical examination, bloodwork, evaluation of 

Table 2. Key Statements on Biofilms in Wounds24

- Biofilms are present in most chronic wounds and are likely to be present on both the surface and deeper wound layers but may not be uniform across or within the wound.
- Biofilms are difficult to visualize macroscopically, and exudate, slough, or debris may be mistaken for biofilms.
- Wounds that contain biofilms may not be identified, leading to ineffective treatment and delayed healing.
- Important clinical indications that a wound likely contains a biofilm include a lack of response to treatment with antibiotics or antiseptics.
- Debridement is one of the most important treatment strategies against biofilms; however, biofilms reform rapidly, so debridement should be used in conjunction with topical antiseptics and surfactants.
suitability of antibiotics administered, and further exploration and debridement of the wound. Repeated bacterial culture and sensitivity may be indicated if signs of infection recur, response to therapy is less than anticipated, or during periods of prolonged antibiotic therapy, following discontinuation of antibiotics, if the infection included multidrug-resistant or polymicrobial agents. In communicating with clients about the cost of care, it is important to emphasize that the greater expense of aggressive debridement in the early stages of wound management typically reduces the duration of therapy and overall costs of treatment long-term. Limitations of current laboratory testing and definitive clinical signs indicative of biofilms make it impossible to determine categorically whether a wound is biofilm free. Therefore, until a stall-side test is available to identify biofilm presence, the clinical progression of wound healing with reduced slough and exudate are the most effective methods of determining response to therapy and resolution of biofilms in tissues.

6. Future Diagnostic Techniques and Treatment Strategies for Biofilms

Future diagnostic tests may indicate more definitively the presence of biofilms and where the biofilm is located within the wound. Stall-side or bedside tests to quantify wound bed protease activity may be one method to indirectly quantify the amount and viability of residual biofilm, as protease activity generally correlates to the amount of active biofilm. Novel techniques to reduce infection associated with biofilms are currently being investigated and developed further. They include quorum-sensing inhibitors (RNAIII-inhibiting peptide [RIP]), surfactants, hydrophobic polycationic coatings, sol gel coatings, covalent antimicrobial tethering, lactoferrin, xylitol, dispersin B, gallium, acetylsalicylic acid, bacteriophages (antimicrobial tethering, lactoferrin, xylitol, dispersin B, gallium, acetylsalicylic acid, bacteriophages (antibacterial viruses), ultraviolet light, low-voltage pulsed electric fields, and cellular therapeutic options including platelet rich plasma, and mesenchymal stromal cells. Further investigation of methods for improving outcomes in the management of biofilms in randomized controlled clinical trials is indicated.

7. Conclusions

Recognition that most chronic wounds likely involve bacterial biofilms is key to successful treatment. Clinical indications that biofilms may be present include exudate, poor quality granulation tissue, other indications of local infection, negative bacterial culture results despite clinical suspicion of infection, and wounds remaining in a chronic inflammatory state and calcitrant to therapy. The mainstays of therapy to reduce biofilm bacterial burden in wounds are repeated debridement and timely use of targeted topical antimicrobials and surfactants that have minimal cytotoxicity to native tissues. Improved diagnostic tools in the future to detect the presence of biofilms and monitor response to treatment in a more sensitive manner as well as adjunctive therapies to degrade biofilms may improve outcomes.

Acknowledgments

Stipend support for Dr. Lynn Pezzanite was provided by the CCTSI NIH/NCATS CTSA 5TL1TR002533-02, NIH 5T32 OD010437-19, and Carolyn Quan and Porter Bennett.

Declaration of Ethics

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

Conflict of Interest

The Authors have no conflicts of interest.

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Wound Debridement Techniques

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1. Introduction

Devitalized tissue, because it has no blood supply, has no chance of becoming viable. Accidental wounds of horses commonly contain devitalized tissue, biofilm colonized with multiple species of microorganisms, exudate, and foreign debris. Devitalized tissue delays healing by providing a focus for infection and by exacerbating the inflammatory response. Removing non-viable tissue or foreign material through debridement reduces bacterial populations on the surface of the wound, which enhances healing. However, for healing to proceed rapidly, as much viable tissue as possible must be preserved. The wound should be debrided when it is first examined, but debridement may need to be repeated throughout healing to address ongoing necrosis and infection. Although dirt and loose debris may be rinsed away by using copious sterile irrigation, wound debridement is often used in combination with irrigation to adequately prepare the wound for optimal healing. Various techniques of debridement available in clinical practice include autolytic, mechanical, enzymatic, surgical, biological, and negative pressure wound therapy. The method chosen depends on various factors, such as the type, size, location, and age of the wound; the extent of tissue damage and contamination; patient tolerance; economics; expertise of the caretaker; and available equipment. More than one type of debridement is often required to optimally prepare a wound for healing. The following subsections discuss the more common techniques of debridement available to the equine practitioner with an aim to provide an overview of the indications, cautions, and contraindications of each technique (Table 1).

Autolytic Debridement

Debridement occurs naturally within wounds by autolysis, a process whereby devitalized tissue is softened or liquefied by enzymes, which are released by macrophages, mast cells, endothelial cells, keratinocytes, and fibroblasts. Autolytic debridement is slow, selective (i.e., only devitalized tissue is liquefied), and virtually painless. This method uses the body’s own enzymes and moisture beneath a dressing, and nonviable tissue becomes liquefied. Dressing types commonly used are hydrocolloids, hydrogels, and transparent films (semiocclusive and occlusive).

Mechanical Debridement

Mechanical debridement by debridement dressings include adherent open mesh gauze, antimicrobial gauze dressing, hypertonic saline gauze dressing, calcium alginate dressings, and occlusive dressings. Adherent open mesh gauze may be applied to the wound, either dry to an exudative wound or wetted (generally with sterile saline solution) to a dry wound. A clean bandage covers this dressing. The dressing clings to the wound’s surface as it dries, so that when it is removed, tissue adhered to the gauze is also removed. Open mesh gauze mechanical debridement is nonselective because both healthy and unhealthy tissue are removed indiscriminately. Gauze dressings may disrupt angiogenesis, fibroplasia, and
epithelialization and, therefore, should be used exclusively for debridement and never beyond the inflammatory phase of healing. An in vitro study comparing the effectiveness of various dressings for debriding fibrin and blood clots in wounds of horses found that dressings hydrated with saline were better debriding devices than those hydrated with water because of their greater osmolarity. Gauze and hydrofiber dressings hydrated with saline were significantly (47%) more effective in breaking down protein (primarily fibrin) than were dressings impregnated with collagenase or papain/urea or hydrogel dressing. In that study, saline dressings reached a plateau in their rate of protein breakdown within 24 hours. Although this study did not consider the in vivo cellular effect on debridement, it did suggest that gauze, hydrofiber, and alginate dressings wetted with saline would be most effective in debriding wounds with a proteinaceous coagulum or those that have formed scabs. An Antimicrobial gauze dressing is an excellent dressing for treating heavily contaminated wounds because it is impregnated with polyhexamethylene biguanide, a powerful yet safe antiseptic that has a broad range effectiveness that has been shown to kill bacteria on the surface of the wound and decrease the amount of exudate formation. Hypertonic saline gauze dressing is ideal for treatment of necrotic, heavily infected exuding wounds. Calcium alginate dressings exert their bioactivity by stimulating macrophages within a chronic wound. In addition to providing a moist environment that is conducive to cell growth and migration, these dressings generate a proinflammatory signal that promotes fibroplasia via a cascade of mediators released from activated macrophages. Occlusive dressings are used for sealing particular types of wounds and their surrounding tissue off from air, fluids, and harmful contaminants. Occlusive dressings promote moist wound healing and “autolytic cellular debridement” and are best used on clean wounds primarily free of exudate.

Enzymatic Debridement

Enzymatic debridement uses an ointment or gel with enzymes that soften unhealthy tissue. The enzymes may come from an animal, plant, or bacteria. The papain-urea combination ointment may be more effective at degrading fibrin within the wound bed. Collagenase ointment derived from Clostridium histolyticum may be more effective at degrading collagen and elastin. Proteolytic enzymes dissolve collagenous tissue and cause superficial debris and devitalized tissue to slough. Collagenase or papain-urea-based agents may be used to speed the process of autolytic debridement in areas where surgical debridement is not possible or carries risk, such as in wounds that closely approximate nerves and/or blood vessels. Factors that optimize enzymatic debridement include a good delivery system that facilitates contact between the enzyme and the area requiring debridement, a sustained period of enzymatic activity, and an optimal wound environment. Frequently, proteolytic enzymes are applied along with other topical agents to address several objectives, including balancing moisture, managing exudate, controlling the measure of microbial contamination, and enhancing tissue regeneration. Enzymatic debridement may be inhibited when combined with other wound dressings. For example, iodine dressings and silver dressings have been shown to inhibit the activity of collagenase, resulting in ineffectual debride-ment. Because proteolytic enzymes cannot penetrate thick, hard, fibrinous eschar, they are more effective when the necrotic tissue is soft. A study in horses evaluated the debriding ability of enzymatic formulations. That study, using an in vitro model to simulate a dry, fibrinous chronic wound eschar, showed that traditional gauze dressings, hydrated with isotonic saline solution or various forms of hydrofiber, hydrocolloid, or alginate dressing (fluid-donating dressings), were more effective at removing fibrin and blood clots than were enzymatic formulations (collagenase, papain-urea, and streptokinase-streptodornase). Enzymatic wound debridement has limitations when treating accidental wounds of equids, which are often heavily contaminated and include a large amount of necrotic tissue; therefore, other forms of debridement may be preferred.

<table>
<thead>
<tr>
<th>Table 1. Debridement Techniques Available to the Equine Practitioner</th>
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<tr>
<td><strong>Autolytic Debridement</strong></td>
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<tr>
<td>Hydrocolloid dressing</td>
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<td>Hydrogel dressing</td>
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<tr>
<td>Transparent film dressing</td>
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Surgical Debridement

Surgical debridement includes the use of sharp, hydrosurgical and low-frequency ultrasound-assisted debridement. Sharp surgical debridement using a scalpel is the optimal method for rapidly cleansing wounds containing a large amount of devitalized tissue and microbial contamination of the tissues. This method of debridement is very selective because the surgeon has complete control over which tissue to remove and tissue to remain. The intent of debridement is to create a well-vascularized wound to support second intention healing or skin grafting. The ability to correctly identify tissue types and anatomic structures is required to avoid inflicting further injury during surgical debridement. Aseptic technique prevents iatrogenic contamination. Repeat surgical debridement may be necessary or, alternatively, may be followed by another form of debridement, such as dressing-enhanced autolytic debridement. Various methods of surgical debridement include excisional, en bloc, piecemeal, and staged debridement. Excisional debridement may be layered, which involves sequential removal of devitalized tissue, progressing from the wound’s surface to the wound’s depths, or en bloc, which entails excising the entire wound including its margin, so that all wounded and contaminated tissues are removed. En bloc debridement is often used for wounds with draining tracts. Piecemeal debridement is used for very large wounds, usually those of the trunk. Progressing from the beginning of the wound margin to the end, all devitalized tissue is removed a little at a time while preserving important anatomic structures. Staged debridement occurs over a number of days to avoid inadvertent removal of viable tissue. This approach is appropriate for most wounds on the distal aspect of the limb, where unwarranted removal of healthy tissue could have debilitating results. When surgical debridement is performed, the two governing criteria are color and attach-ment. White, tan, black, and green tissues, as well as those that are poorly attached, should be de-brided. Tissues that are pink to dark purple and that are well attached should be spared. Devitalized, nonarticular bone should be removed, whereas well-perfused bone with hardy soft-tissue attachments should be left. Fragments of bone with questionable viability or bone that has lost its per-iosteum should be aggressively debrided to reduce the risk of sequestration and to improve blood supply to the bone sur-face. Debridement of cortical bone is best accomplished with a bone rasp; however, a curette, bone chisel, or osteotome can be used. Hydrosurgical debridement involves the use of pressurized water or saline solution as a cutting/cleansing tool to rapidly and selectively debride a wound. A commercial hydrosurgical device generates a high-pressure (≤15 000 psi) jet of fluid (sterile saline solution) that emerges through a 0.127-mm orifice of the handpiece. Emergence of fluid from the outlet produces suction (Venturi effect) that draws devitalized tissue into a cutting chamber where it is shredded and evacuated. Hydrosurgery can often be performed with the horse standing, but sedation and/or regional anesthesia may be required for restraint. Hydrosurgical debridement is reported to be well tolerated because it is less painful than sharp debridement. If hydrosurgery is used to debride a fresh wound that is to be sutured, some form of local anesthesia is necessary; conversely, local anesthesia is not usually required if hydrosurgical debridement is used to debride a wound covered by granulation tissue that has been left unsutured to heal by second intention. A recent ex vivo study comparing the efficacy of different methods of debridement in reducing the load of Staphylococcus aureus from contaminated equine muscle showed that hydrosurgical debridement using the hydrosurgical device was more effective than irrigation with an isotonic saline solution, scraping with a scalpel blade, or a combination of irrigation and scraping. Low-frequency, ultrasound-assisted debridement uses low-frequency ultrasonic waves (20-60 Hz), emitted in contact or noncontact mode, produced by streaming saline solution through tubing into a sonotrode head. Microcavitation, resulting from oscillation of saline gas bubbles, cleaves necrotic tissue from healthy tissue, thereby debriding the wound and disrupting the biofilm; the necrotic tissue is suctioned or washed away. Healthy, more elastic tissue is preserved and stimulated to epithelialize. Because the recommended time of treatment is 20 to 30 sec/cm² of surface area, ultrasound-assisted debridement is likely less applicable to debride large wounds commonly found in horses.

Biological Debridement

Biological debridement involves the use of maggots, Lucilia sericata (green bottle fly), that are grown in a sterile environment and digest dead tissue and pathogens. Biological debridement is recommended for use in horses to enhance healing of subacute and chronic, nonhealing wounds when complete surgical debridement is difficult and to enhance healing of obviously infected wounds. The sterile maggots are applied to the wound bed with a dressing used to “confine” the maggots to the wound. The beneficial action of maggots on wound healing is attributed to producing potent proteolytic enzymes, upregulating expression of genes involved in wound healing, creating an antiseptic effect, and inhibiting formation of biofilm. During the process of dissolving fibrin and necrotic tissue, maggots also destroy and digest bacteria, and the excretions/secretions of L. sericata possess significant antifungal properties. Resistant Staphylococcus aureus in vitro. Sterile maggots can be applied to a wound by using a direct (free-range) or indirect (contained) contact method. With the direct contact method, maggots are applied
onto the wound with a dressing fixed to the surrounding healthy tissue. After the maggots are placed on the wound, a nylon mesh is fixed to the dressing to confine the maggots within the wound. In the indirect contact method, maggots are supplied within a closed polyester net filled with absorbent hydrophilic polyurethane foam and applied with the wound. Biological debridement using the direct contact method is superior in the horse. The direct method of applying maggots is also less expensive than the indirect method. In horses, biological debridement therapy is described as an alternative approach to the management of septic navicular bursitis, laminar necrosis associated with complicated laminitis, and subsolar infection-associated osteomyelitis of the distal phalanx. Biological debridement therapy has also contributed toward the successful healing of chronic nonhealing limb lacerations, orthopedic infections free of bony sequestrae, various soft-tissue abscesses, supraspinous bursitis, and dehiscence of the linea alba following exploratory celiotomy. In a retrospective study of 41 horses treated with biological debridement, a favorable response was noted less than 1 week after initiation of therapy. Lesions of the horses enrolled in this study were subacute or chronic and had been managed in diverse ways prior to receiving biological debridement. A preliminary surgical debridement usually precedes biological debridement, although this may not be necessary in the absence of foreign bodies and/or nonorganic matter in the wound. In some cases, after 3 to 4 days of biological debridement, a second treatment is required to reach the desired degree of debridement, disinfection, and fibroplasia (i.e., healthy granulation tissue filling the wound). Orthopedic shoeing, stent bandaging, and nonocclusive sleeve bandaging, typical to biological debridement, are adapted for each type of wound and anatomic location. For biological debridement to work effectively, free drainage of exudate is necessary and the maggots must have an adequate supply of oxygen.

Negative-Pressure Wound Therapy

Negative-pressure wound therapy (NPWT) is a method for controlling wound infection and enhancing wound healing. Indications for the use of NPWT in veterinary medicine include traumatic wounds (avulsions, degloving, and shear injuries), full-thickness burns, dehiscence of surgical incisions, myofascial compartment syndrome, and chronic nonhealing wounds. NPWT is also excellent for enhancing acceptance of free grafts, and it can be used effectively over sutured incisions to splint closures under tension, prevent formation of a seroma, particularly in closed incisional wounds with underlying dead space, and minimize edema. NPWT stimulates the rapid formation of granulation tissue, reduces the size of the wound, and effectively removes fluid from the wound. The early appearance of granulation tissue is remarkably consistent with the use of NPWT and correlates with observed increases in fibroblast activity and angiogenesis. Although NPWT should not replace the basic tenets of wound management, such as cleansing, debridement, irrigation, and treatment for infection, its use for debridement of an infected wound is likely to stimulate fibroplasia and increase resistance to infection early in the postwounding period. Other identified and validated mechanisms of NPWT include the removal of interstitial fluid; thereby reducing edema; altered blood flow to the wound; and upregulated gene expression of cytokines associated with wound healing. NPWT involves sealing the wound from the environment and delivering a controlled subatmospheric pressure, typically not exceeding ~125 mmHg, to the entire wound. Fluid produced by the wound is collected into a disposable reservoir canister attached to the pump. The open-cell nature of the foam and the cell size of 400 to 600 μm ensure an even distribution of negative pressure to the entire surface of the wound. NPWT may be particularly useful in the treatment of wounds of horses allowed to heal by second intention. NPWT prevents retraction of the wound margins, decreases the size of the wound, reduces wound edema, and stimulates early onset of fibroplasia. Granulation tissue forms earlier in wounded tissue treated by NPWT and is smoother, nonexuberant, and has well-organized collagen fibers, as demonstrated histologically. This makes wounds treated with NPWT less likely to develop “proud flesh” and more amenable to early reconstructive procedures, such as skin grafting. Furthermore, NPWT increases the likelihood of acceptance of a free skin graft by immobilizing grafts at the wound.

2. Conclusion

To promote healing, reduce risks of infection, and improve patient outcomes, an array of debridement methods can be included in the patient’s wound management plan of care. The use of more than one debridement method will provide consistency in wound bed preparation toward healing.

Acknowledgments

Declaration of Ethics

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

Conflicts of Interest

The Author has written wound care book chapters and has no conflict of interest with any manufacturers.

References and Footnotes


1. Introduction

Second intention wound healing is best performed with dressings that will keep the wound moist. Modern wound healing dressings are designed to provide the appropriate amount of moisture retention for the stage of wound healing. Moist wound healing occurs when the wound exudate is allowed to stay in contact with the wound bed. Studies on people have shown that full thickness skin wounds kept in a moist environment re-epithelialize in approximately 12–15 days, whereas the same wound exposed to the air will take 25–30 days to heal.1,2 The wounds are less inflamed, cause less itching, have less eschar formation, and are more likely to heal without scarring. Wound exudate in the absence of infection provides a substrate rich in enzymes, growth factors, and chemotactic factors. The enzymes are a byproduct of the breakdown of polymorphonuclear cells and macrophages. The enzymes allow the debridement of the devitalized tissue, improving the “foundation” for wound healing to proceed. This has been termed autolytic debridement. Autolytic debridement occurs between 72 and 96 hours depending upon the thickness of eschar and the size/location of the wound and is achieved under occlusive dressings, provided the wound bed remains in contact with the wound fluid. Growth factors provide a stimulus for the fibroblasts and epithelial cells. The chemotactic factors stimulate the migration of more neutrophils and macrophages to phagocytize bacteria and debris while releasing enzymes to further promote autolytic debridement. A moist environment allows better migration of neutrophils and macrophages than a dry wound environment. Occlusion provides a constant thermal regulated environment leading to healthier cells, and if the appropriate dressing is chosen then bacterial penetration is reduced or prevented. Disadvantages of moist healing include bacterial colonization, folliculitis, the possibility of trauma to peri-ulcer borders, and, at least in people, allergies to the dressing material. There can be a fine balance between drying out of the wound and maceration of peri-wound tissue. Fortunately, there are new dressings available to help the practitioner in determining what to use in each case to provide an optimum wound healing environment.1,2

Many dressings have been used in the treatment of lacerations and abrasions in horses. Nonadherent dressings and gauze dressings are probably the most common dressings used. Both of these dressings are porous, allowing fluid transfer from the wound to the overdressing and from the outside of the bandage to the wound surface. If woven gauze is used, the wound exudate is quickly absorbed through the gauze and into the overdressing. This is termed vertical wicking. If nonwoven gauze is used, the wound exudate tends to flow to the edge of the gauze quickly and then is absorbed into the overdressing. This is termed horizontal wicking. The amount of moisture retention is dependent upon many factors including the amount of exudate and the type of gauze, the secondary dressing, and the frequency of dressing...
change. It is difficult when using a gauze dressing to maintain a moist wound-healing environment even if a wet to dry bandage is used.\(^3\) Oclusive dressings isolate the wound from the external environment providing many benefits over a simple gauze dressing. The occlusiveness of a dressing is measured by the evaporation of fluids from the wound surface through the dressing and ranges from minimally occlusive to completely occlusive. The benefits of occlusion include rapid autolytic debridement with less necrotic tissue, a bacterial barrier, a waterproof barrier, a decrease in pain associated with wound/dressing, ease of use, fewer dressing changes, and decreased wound healing time. The fears about occlusion are mainly centered around infection. All wounds are colonized with bacteria while they may not be actually infected. Infection in general refers to invasion of bacteria to the \(10^5\) power in live healthy tissue. Signs of infection include edema, erythema, induration, and fever. Although there are concerns, studies have shown that occlusive dressings are not associated with increased rates of infection.\(^3\) The ideal dressing should keep the ulcer bed continually moist and the surrounding skin dry, or more simply stated, a dressing should manage the amount of exudate present. This determination will be dependent on clinical judgment.\(^3\)

2. Wound Dressings

There are probably as many different wound dressings as there are wound cleaning agents.\(^3\) This lecture will focus on the agents the presenter has the most experience with. Wound dressings should be chosen based upon the stage of wound healing the wound is in. 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.\(^1,2\)

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. In the author’s opinion, they are the most effective debridement dressings available.\(^8\) Hypertonic saline can be made by dissolving 200 g of salt in 1 L of boiling water. Lower concentrations of hypertonic saline do not appear to be as effective. This debridement dressing should be discontinued when the wound no longer appears infected.

**Antimicrobial Dressing**

One antimicrobial dressing\(^b\) 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.\(^3\) 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.\(^4\)

**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.\(^3,6\)

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.

**Granulation Tissue Development and Wound Contraction Dressings**

**Calcium Alginate**

In the past, exuberant granulation tissue has been the bane of the equine practitioner, and to think that one 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.

Epithelialization Dressings
Semi-occlusive foam dressings help to finish off the wound healing process. The foam dressings will increase the surface temperature of the wound by 1–2 degrees, 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 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. In summary, there are many options for 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. The author feels that the best healing results will occur with wounds that are covered and kept moist through the healing process regardless of the location of the wound on the horse. If uncertain what the dressing does, find out before using it. Many of these materials have quite detrimental effects on the wound.

Acknowledgments

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

Conflict of Interest
The Author has no conflicts of interest.

References and Footnotes
3. Hendrickson DA. *Wound Care Management for the Equine Practitioner*. Teton NewMedia, 2005

aCurasalt, Covidien/Medtronic, Minneapolis, MN 55432-5604.
bKerlix AMD, Covidien/Medtronic, Minneapolis, MN 55432-5604.
cAMD Foam, Covidien/Medtronic, Minneapolis, MN 55432-5604.
Skin Grafting Basics

Dean A. Hendrickson, DVM, MS, DACVS

1. Introduction
Skin grafting is generally reserved for wounds that have a very large skin defect that would not heal functionally or cosmetically with standard wound therapy intervention. Skin grafts not only cover granulation tissue and result in a more cosmetic end result, but they also have been shown to encourage wound contraction and epithelialization while decreasing exuberant granulation tissue. Skin grafting in itself is not a difficult procedure. Most of the effort involves appropriate preparation of the wound bed and skin graft prior to placement and effective immobilization of the grafted area after skin graft placement. The wounds should be treated with moist wound healing concepts to prepare the wound bed to receive the skin graft (see the presentation on wound care dressings). The most common circumstances that will lead to failure in skin grafting are infection, poor granulation tissue, and excess movement at the graft site. Consequently, the wound bed must be free of infection and have a healthy granulation tissue bed prior to grafting. If a previous graft has failed, quantitative bacterial analysis should be performed prior to placing a second graft. If the wound is over a site of excess movement, some type of wound immobilization, such as splinting or casting, should be performed. Regardless of the location of the wound, it is recommended to leave the original dressing in place for 7 days prior to changing. This will reduce the chance of dislodging the grafts. There are three main types of skin grafts used in the horse: autografts, allografts, and xenografts. Autografts come from the same animals, allografts are from the same species, and xenografts are from other species. Xenografts, such as porcine skin and fish skin, are not really integrated into the host animal; they are really just used as biological bandages.

2. Healing Stages of Skin Grafts
The healing stages of skin grafts are generally broken into four major categories: adherence, plasmic imbibition, revascularization, and final organization. Adherence occurs on day 1 before any other healing stages can occur. This is generally created by fibrin between the graft and the wound bed. Movement at this stage will often lead to graft failure. Plasmatic imbibition occurs from days 1 to 4 where nutrition for the graft comes from the fibrin clot around the graft. Cyanosis of the grafted tissue is expected during this phase. Revascularization occurs between days 3 and 7. There are at least three different methods for revascularization. Inosculation occurs when vessels in the graft anastomose with vessels in the recipient bed and is the most rapid method of revascularization. Capillary buds can penetrate from the recipient bed into the graft vessels, using them as a conduit to revascularize the graft. Capillary buds may penetrate the graft in areas other than old vessels to revascularize the graft. This is the slowest method.
of revascularization since the growth rate of capillary buds is approximately 1 mm/day. The greater the distance between the graft and the recipient bed, the more likely that capillary buds will have to form an entirely new vascular network and the greater likelihood of graft failure. The final stage of healing is organization where fibrous tissue replaces the fibrin of the original clot to hold the graft permanently in place. Fibroblasts are responsible for laying down the collagen during organization. The collagen fibers and neovascularization provide a firm attachment of the graft to the recipient bed within 9 days of grafting.

3. **Graft Thickness**

Full-thickness grafts are composed of both the dermis and epidermis. Partial-thickness grafts are composed of the epidermis and part of the dermis. Both full-thickness and partial-thickness grafts can be used in horses. Full-thickness grafts tend to give more cosmetic end results because they have all of the adnexal structures such as hair follicles and skin glands. However, they are more difficult to apply successfully because they are thicker and take longer to vascularize. Partial-thickness grafts tend to have a better success rate but have a less cosmetic end result. When choosing the donor site, it is best to select a site that will not leave a significant cosmetic blemish. Either the side of the neck under the mane or the ventral abdomen or pectoral region where there is redundant skin are the common sites. A secondary consideration is the hair color.

4. **Graft Types/Techniques**

Grafts can either be harvested manually or by using motorized equipment. Most practitioners will harvest using manual techniques. The benefit of even graft thickness when using motorized equipment is often outweighed by the cost of motorized equipment. No matter the method, the grafted tissue must have adequate capillary exposure by keeping the graft in close approximation to the granulation bed to allow in-growth of blood vessels. The recipient bed must have adequate capillary supply, and the practitioner must provide protection from mechanical disruption, hematoma formation, and infection. Skin grafting can be performed using many different techniques. The techniques will be listed in order of easiest to most complex.

**Pinch Grafting**

Pinch grafting requires the least amount of equipment of the listed techniques. The grafting can be performed with a number 12 scalpel blade, a thumb forceps, and a mayo scissors. The skin is tented with the thumb forceps and cut off with the scalpel blade. The grafts should be no larger than 8 to 10 mm in diameter. The subcutaneous tissue is removed with scissors. Pockets, approximately 1 cm apart, are made in the granulation tissue with the scalpel blade, and the grafts are placed into the shallow pockets. The graft site is dressed with a nonadherent dressing or petroleum impregnated dressings and a pressure bandage applied. This technique can be performed in the standing horse.

**Punch Grafting**

Punch grafting requires similar instrumentation as does the pinch grafting as well as 6-mm and 8-mm skin punches. The 8-mm punch is used at the donor site to cut through the epidermis and dermis. The subcutaneous tissue is trimmed from the graft and the graft placed on saline-soaked gauze. It is best to maintain normal orientation of the hair on the grafts for the most normal cosmetic result at the wound site. The 6-mm punch is used at the wound site to remove a granulation tissue plug. The granulation tissue plugs are removed, cleaned of blood, and filled with the skin plugs. The graft site is dressed with a nonadherent dressing or petrolatum impregnated dressings and a pressure bandage applied. This technique can be performed in the standing horse.

**Tunnel Grafting**

Tunnel grafting requires removing long narrow strips of skin from the donor site. A long forceps is tunneled under the granulation tissue and used to grasp the skin graft. The skin is pulled (taking care to maintain normal orientation) through the granulation tissue, and the ends are sutured to the surrounding skin. The wound is dressed as described above. Approximately 6 to 10 days later, the granulation tissue is surgically removed over the skin grafts. More skin is placed into the wound than with pinch or punch grafts, but less specialized equipment is necessary when compared to sheet grafting. This technique generally requires general anesthesia.

**Sheet Grafting**

Full-thickness sheet grafting will generally give the best cosmetic result. However, it requires the most specialized equipment and has the greatest chance for failure. The biggest reason for failure is movement of the graft over the wound bed inhibiting the ingrowth of capillaries. One way to reduce the problems with graft movement is to “mesh” the graft. Graft meshing is beneficial for two main reasons. It allows evacuation of exudate from between the graft and the wound bed, and it allows coverage of a larger area without having to remove as large of a graft from the donor site. Sheet grafting is best performed with a dermatome that will remove a similar-thickness sheet from the donor site. The dermatome can be set to remove a partial-thickness or a full-thickness graft. The harvested graft can then be placed through a mesher to “mesh” the graft for...
the reasons previously described. The graft is sutured into place around the periphery of the wound and then dressed as previously described. This technique requires general anesthesia. “MEEK” micrografting, which uses multiple small island grafts “glued” to an occlusive dressing, looks especially promising.

Pedicle Grafting

Pedicle grafting requires that there is enough skin close to the wound defect to be able to rotate skin into the area while still leaving at least some vascular supply intact. In most cases, this technique is used in the head region of horses, especially to cover fistulas over the maxillary sinus. The donor site may have to be left open to heal by second intention. The distal limbs do not have enough extra skin in most cases to rotate tissue. This technique requires general anesthesia.

5. Conclusion

Skin grafting can be a relatively simple technique for the equine practitioner to perform. The cosmetic end result is often superior to second intention healing. Care must be taken to minimize movement and bacterial numbers to provide the most successful results.

Acknowledgments

Declaration of Ethics

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Conflict of Interest

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References and Footnote


*Suggested Reading*

Finding Success and Satisfaction in Solo Practice

Caitlin Daly, DVM

Solo equine practice can be a rewarding career choice, allowing for a working life molded by personal values, mission, and vision. The weight of additional responsibilities and unique challenges is often well offset by the ability to shape one’s life and career, while still enjoying the benefits of a profitable small business. Author’s address: PO Box 1446, Waldoboro, ME 04572; e-mail: midcoastequine@gmail.com. © 2021 AAEP.

1. Introduction

In recent years, fewer graduating veterinarians are seeking a career in equine private practice, and about half of young graduates leave the equine sector within 5 years. A recent study has indicated that practices with more than four doctors have the highest number of departures, with close to 25% of respondents leaving. Remarkably, solo practices demonstrated the lowest rate of departure from the industry (10.45%). Reasons fewer solo practitioners are leaving the profession may include the numerous positive aspects of solo practice: the authority to take command of one’s professional and personal life, the ability to shape a specific brand identity that plays to one’s strengths, and the financial benefits of owning a profitable business. Solo practice presents its own unique set of challenges that include increased responsibilities, isolation, and the high likelihood of being the sole provider of emergency services. However, with creativity, flexibility, and collaboration, unique solutions can be developed. In the author’s experience, the benefits of solo practice have far outweighed any negatives and have led to an incredibly fulfilling career path, one uniquely its own.

2. Discussion

In solo equine practice, it can be easy to fall into the trap of trying to “be everything to everyone.” The financial and human capital of larger practices may allow them to successfully execute this paradigm and offer a wide array of specialized skills and services. A solo practitioner often does not have the personal or business resources of larger practices. Therefore, a solo practitioner must accept the limitations of being a one-doctor organization and realize that taking on a universal provider role (fulfilling all of a client’s needs for their horse) is unrealistic and could negatively impact their well-being. However, an advantage of solo practice is the ability to create a practice model reflective of one’s professional goals, unique skill set, and personal lifestyle.

Clients increasingly seek veterinarians with specialized training for certain aspects of their horse’s health. Today’s general equine practitioner may
provide a combination of basic skills and advanced education in one or two areas of interest such as lameness, dentistry, chiropractic, or acupuncture. It is advantageous when these advanced skill sets do not overlap amongst practitioners within the region. This provides an opportunity for referring cases to neighboring practitioners with different skill sets and equipment. For example, practitioner A may refer their dental extractions to practitioner B who has a large amount of advanced dentistry experience, while practitioner B refers their gastroscopy cases to practitioner A because of a lack of appropriate equipment. Solo practitioners with a specific special interest have the option to limit their practice—for example, providing only dentistry services or integrative therapy. By setting limits on the services they offer, these practitioners can decrease their frequency of emergency calls from clients, minimize their investment in equipment, and focus the development of their expertise in an area of practice that is highly profitable or of personal interest.

Practices that are limited in scope are increasing in frequency, often offering unique solutions to difficulties within the equine veterinary space. For example, emergency-only and relief practices are reducing the burden of emergency coverage for small practices and for those veterinarians with injuries, maternity leaves, or other needs to take time away from their practices. While practices such as these are few in number, they offer solutions to the problems that negatively influence the decision to leave equine practice. Other specialized practices, including those limited to dentistry and/or integrative medicine (chiropractic and acupuncture), often provide expertise without negatively impacting the general practitioner through competition for services. There are also unique solo practices that are transient and involve traveling to different venues and providing veterinary services to a niche horse population such as racehorses or show horses.

Once committed to a solo practice, there is complete freedom to determine how to present the practice within the industry. The solo practitioner can create a brand that is reflective of their uniqueness as an individual. Personal attributes such as kindness, compassion, empathy, humor, confidence, reliability, and effective communication possessed by the individual practitioner can establish the practice identity. Clients are in fact choosing the brand of the individual veterinarian when choosing the practice brand. When clients choose a veterinarian with whom they share values and priorities, their satisfaction with their experience may be improved. Likewise, the doctor’s work satisfaction may be increased by serving clients who appreciate their brand identity and have chosen it deliberately.

As they develop their practice, the owner must have a clear vision of what they want to create, for without a destination to aim for, progress is difficult to measure and action steps are hard to formulate. Branding helps to define the path to one’s pinnacle—often creating the image before it fully comes to fruition. Owners of solo practices have complete authority over the brand’s development and expression. Their dreams, values, and opinions determine the expression of the company logo, merchandise, website design, and social media presence. The process of branding can be incredibly exciting for a creative individual.

The strongest brands provide a consistent experience with every customer touchpoint. Daily interactions with clients, whether these are in person, over the phone, or by text or email, have the greatest impact as the solo practitioner is consistently reinforcing their brand with each interaction. A client’s trust builds with every positive interaction when their experience meets their expectation. This trust creates a strong, valuable brand that is in high demand. When your brand is “who you are,” it will be strong and consistent simply by just being yourself.

A solo practitioner is capable of earning compensation equal to or exceeding that of a high-producing associate while grossing only a fraction of the associate’s production. This is possible because the solo practitioner is paid not only for their effort as a veterinarian, as an associate is, but also receives the net profit from the business. Practice profits of 17% to over 20% are considered excellent and are not uncommon in ambulatory practice. Operating an ambulatory practice requires significantly lower upfront and overhead costs compared to its brick-and-mortar counterparts, with facility and equipment costs typically 5% to 6% less in ambulatory practices. Business management, which is crucial to success, may be a learn-as-you-go process for the solo practitioner, but it does not have to be. Fortunately, there are now veterinary peer groups aimed at educating the young practitioner. The financial benefits of business ownership are immense. It is one of the surest ways to financial freedom and success, including student loan repayment and retirement savings.

The life of an equine veterinarian can be hard, and it can be even harder for a solo practitioner that is responsible for all patient care, client communication, business management, and emergency duties. Relief from some of the challenges of equine practice can be gained from the formation of strong boundaries. Authority over oneself implies authority over one’s boundaries and does not require others’ approval. It is essential for the solo practitioner to clearly identify boundaries specific to their goals and lifestyle. While the skill of implementing boundaries is learned over time, it is much easier to have these boundaries in place when first starting. One of the positive aspects of solo practice is having the ability to set norms and boundaries that are unique to your needs.

Examples of questions the solo practitioner should ask themselves when creating boundaries include the following:

1. What or who has priority over my time or business?
2. What are my normal business hours?
3. What type of client communication, if any, am I comfortable with after hours?
4. Will I see emergencies for nonclients?
5. What can I do to assure that I am paid at the time of service?
6. What are my nonnegotiables for my practice of veterinary medicine?
7. What do I consider inappropriate or abusive behavior from clients? What will I do when I experience this?

By creating thoughtful boundaries at the outset of forming a practice, a veterinarian can communicate expectations to clients clearly from their first interactions, preventing misunderstandings and creating the foundation of a consistent client experience. By creating space for time away from the practice through strong boundaries, a solo practitioner can protect their sustainability in the equine space.

In addition to financial success, solo practice also provides the extraordinary opportunity to have sole authority over the work-life continuum as it changes throughout the life of the practitioner. The needs of an individual change as they age, if they choose to start a family, or if they get seriously sick or injured. In solo practice, there is no one from which to get permission should you need a day off for a doctor’s appointment, want to be at your kid’s basketball game in the afternoon, or want to skip town for a concert or vacation. Having this level of personal freedom requires a strong reciprocal relationship with a neighboring colleague or emergency cooperative so that the needs of your clients are taken care of during your time away.

The modern solo practitioner need not be the “lone wolf” but instead can develop collaborative relationships with their peers. Younger practitioners entering into this profession may find themselves at an advantage when developing relationships with their colleagues both near and far. “Millennial’s early (and constantly supervised) exposure to team sports has made them the best team players and collaborators in generations,” according to Jessica Brack of the Kenan-Flagler Business School. Collaboration fosters practitioners’ ability to learn from one another, ask for help when needed, and fulfill clients’ needs when their requirements are beyond a particular practitioner’s scope of practice. Examples of collaboration among neighboring colleagues include sharing emergency coverage (especially during times of injury or extended medical or maternity leave), sharing or renting equipment between practices, providing medication or supplies if a fellow practitioner runs out unexpectedly, or referring cases to another practitioner who has greater experience, skill, or necessary equipment.

Technology has completely changed how we connect with our peers. It has eliminated the physical barrier of distance, allowing the ability to source feedback from colleagues from nearly anywhere in the world. In the author’s experience, the degree of urgency required by the situation dictates the form of technology chosen. When immediate feedback is required, digital images sent through text and video chat allow for instantaneous communication between two colleagues. Both the American Association of Equine Practitioners listserv and various member Facebook groups (e.g., Equine Vet-2-Vet, Women in Equine Practice) allow veterinarians to connect and collaborate with colleagues without a previously established relationship. Both formats can create an environment that mimics hospital clinician rounds that were once inaccessible to solo practitioners. The personal opinions expressed within these formats are colored with years of experience. They provide practical advice, quick tips one never learns in school or a textbook, and most importantly, the sense that one is not alone in their struggles. Through these groups, one learns that we are all good at some things and not at others, we are all learning, and perfection is a facade. When a veterinarian practices by themselves on a daily basis, knowing they are not alone in these struggles is a lifeline. In fact, these days, all of us are more connected than ever.

3. Conclusion
Solo practice is not for everyone. But those who undertake its challenges can experience a life and career that is professionally, financially, and personally rewarding beyond measure. For those looking to define success and a life well lived for themselves, solo practice can be the right vehicle for them to execute their vision.

Acknowledgments

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

Conflict of Interest
The Author has no conflicts of interest.

References
Better Together: Utilizing an Emergency Cooperative to Prevent Burnout

Amanda McCleery, DVM

General equine practice requires round-the-clock emergency coverage for clients. This may understandably lead to burnout, especially in small one- or two-doctor practices, and is a top reason why practitioners are choosing to leave equine practice. The establishment of an emergency cooperative among local practitioners is one model that can be utilized in some areas to allow a better work-life balance while still providing an important service for clients. Author’s address: McCleery Equine Veterinary Service, PO Box 1280, Archer, FL 32618; e-mail: mccleerydvm@gmail.com. © 2021 AAEP.

1. Introduction

The equine profession in the United States is not retaining or replacing the veterinarians needed to service the population of horse owners. Fewer graduate veterinarians are choosing to enter equine practice, and retention of those who do is poor, with over half dropping their membership in the American Association of Equine Practitioners (AAEP) and presumably leaving equine practice within the first 5 years after their graduation (AAEP 2019 Survey Data). Many of those choosing to leave cite the lifestyle and number of hours of work required as the top reasons why. The current working conditions required of equine practitioners may simply be untenable for many professionals who are disproportionately female and burdened by more hours of household work and childcare. One specific cause of equine practitioners’ burnout appears to be a function of the demand of providing 24/7/365 emergency care, which requires a veterinarian to be available and ready to work at all hours. This is an essential part of veterinary care, and in many states like Florida, this is written into the state practice act. To address this burden, small animal medicine has turned to a model of after-hours clinics specializing in emergency care. However, this model has rarely been adopted by equine practitioners in the United States. Aside from leaving the field, equine veterinarians have dealt with this issue by joining larger practices, hiring relief veterinarians (i.e., veterinarians that provide services on an as-needed basis when full-time vets are away from their practice), or choosing to specialize in a service that does not require the provision of emergency care (e.g., dentistry, sports medicine, acupuncture, chiropractic, etc.). Another option to reduce the demands of emergency work is to share the burden by forming a local emergency cooperative. These cooperatives are groups of independent local practitioners that agree to share emergency coverage for each other’s clients, returning care to the client’s regular veterinarian during business hours. There are several advantages to this type of approach: (1) it has the potential to be easily and quickly put into action, (2) it...
allows practitioners to maintain their small one- or two-doctor independent practice while reaping some benefits of being in a larger practice, and (3) it provides increased collegiality in what can be an isolating profession. While there are clear advantages to the cooperative approach to emergency care, it has not been widely adopted. To help provide other practitioners with an example of a successful emergency cooperative, the author will provide the details of practice experiences—specifically, (1) discuss the agreements, makeup, and context of the author’s cooperative; (2) consider the pitfalls and limitations of such groups; (3) provide examples of how the author’s group has handled potential contentious issues; and (4) finish with a broad look at the personal and professional trade-offs that come with joining a cooperative.

2. The Author’s Emergency Cooperative

Emergency cooperatives have been part of the author’s local practice culture for decades, so formation of a cooperative was not a difficult subject to broach with other area practitioners. Other groups had formed and dissolved for a variety of reasons, including refusal of practitioners to attend nonequine cases, practitioners not reliably being available during their on-call rotations, and failure to follow up with a client’s primary veterinarian. If the author were to relocate to a new practice area, she would start with asking local colleagues if they would be interested in getting together once a month for case discussion. This would create a low-pressure environment to determine if the involved practitioners work well together and would make broaching the subject of forming a cooperative less of an obstacle. The author’s emergency cooperative, created in 2017, is made up of five ambulatory solo practitioners. All of the practices are predominantly equine focused, but several practitioners also work with cattle, small ruminant, camelid, and porcine clientele. The cooperative covers an area that is up to 80 miles in diameter, but most clients are concentrated within 40 miles of all practices. The veterinary members meet monthly as a group. This provides an opportunity for case discussion, addressing any scheduling issues, and discussing any concerns about the function of the cooperative. Although the author’s cooperative was not formed around legal agreements, the set of rules and guidelines below were agreed upon to clarify expectations and improve communication:

- The clients’ primary practitioner is to be updated on cases seen at the end of each on-call period
- Clients of a cooperative member will not be charged a nonclient fee when seen on emergency
- Cooperative members will provide services to equine, caprine, ovine, bovine, porcine, and camelid clients if they are current patients (generally defined as clients seen for a regular appointment in the last year)
- People who have recently moved to the coverage area and do not have a veterinarian should be provided service for emergencies, if possible
- All established clients, regardless of location, need to be provided service

Each practitioner covers emergencies for all the members of the cooperative one day of the week, on a rotating schedule. Similarly, the weekends and holidays are divided among the group members. The schedule is planned for 20 weeks in advance (each practitioner gets four weekends of emergency call in each planning period). When a cooperative member receives a request for emergency services from someone who is not one of their clients, the caller is asked to identify their regular veterinary provider to determine if they are associated with the cooperative. Although there is the potential that clients may lie, this has not proven to be an issue. When clients that are not associated with the cooperative request emergency services, it is at the discretion of the on-call veterinarian if they are willing and able to provide services. After the services are completed, all billing or payment is the responsibility of the veterinarian attending the call, who then provides an update on the case to the regular veterinarian via a phone call, text message, or e-mail of medical records. Access to the other practices’ records is not available. If a cooperative member wishes to provide emergency service to one of their own clients when they are not on call, this is allowed.

3. Limitations/Pitfalls

In the 2020 AAEP Emergency Coverage Survey, only 8% of the over 800 practitioners polled utilized the cooperative model for emergency coverage. Aside from larger practices not requiring such coverage, the top reasons cited for not joining a cooperative include the following:

- Concern over the level of care provided by other veterinarians
- Covering too wide of a geographic area
- Concern over loss of clients to other practices
- Possible loss of revenue
- Concern over other practices’ fee structures
- Fear of unreceptive clients
- No offer to join a group has been extended
- Mixed animal practice would be required

These are all legitimate concerns and challenges that were faced or considered in the author’s cooperative. However, all these issues can be addressed or at least mitigated. Below are the author’s experiences and approaches to each of these issues. One of the top concerns voiced in the 2020 AAEP survey was that the skill level of other veterinarians was not adequate to trust
with the care of one’s clients. Although the practice styles and strengths of each practitioner in different groups can vary, there has never been the concern that a colleague was not suited to provide adequate emergency care. Initially, this confidence was based only on the mutual respect one has for colleagues, but it has grown over time based on communication after emergencies and discussing cases and any concerns at monthly meetings. Bringing together multiple practices has expanded the geographical area that each member of the cooperative must cover when responsible for emergencies. While less than ideal, this is a trade-off that the author’s group has accepted. In return for occasional travel to further locations (> 1 hour), nights and weekends are more often available. For some cooperatives, the areas that require the furthest travel are generally underserved, and clients are happy a veterinarian is providing service, regardless of wait time. However, some clients in far-flung areas with more opportunities for equine veterinary care have been less accepting of longer wait times. While the clients’ desire for more immediate care is appreciated, it is not likely to modify the practice’s cooperative arrangement to service these desires, content that quality 24/7 care for all clients is provided. One concern that members of the cooperative and many veterinarians have is the potential to lose clients to other practices in the cooperative. It is a fact that clients may desire to switch practices, and when this happens in the cooperative setting, it can create feelings of distrust, undermine relationships and cause potential failure of the cooperative. To address this concern, clear communication with the clients and other veterinarians must be identified to increase trust and reduce the probability of clients switching practices. A general philosophy has been adopted that clients are not owned by a practice and may switch practices if they choose, but maintaining the trust of the other veterinarians in the group (and thus the ongoing success of the group) is valued above procuring a new client. If a client chooses to switch, a conversation between the two veterinarians generally takes place to prevent any misunderstanding. Face-to-face (currently outdoors while social distancing) monthly meetings are key to building good-faith relationships. If a practitioner is actively trying to steal another veterinarian’s clients by marketing to them, it would become increasingly uncomfortable to sit down and look that colleague in the eye every month. At the onset of the author’s cooperative, several veterinarians were worried about the potential loss of revenue from passing emergency work to their colleagues. These members were willing to join the group if they had the option to continue to see their clients’ emergencies at any time. This alleviated concerns about the potential for lost revenue yet allowed them to utilize the opportunity for time off when needed. To date, no member has had any issue with loss of revenue from joining the cooperative. In fact, many find the increased call volume seen when on call increases efficiency and makes up for any revenue lost when passing calls to the co-operative. This efficiency also makes it viable to pay an assistant to be available during emergency coverage periods. Without the cooperative, the 24/7 nature of being on call makes it too costly to maintain a technician’s availability in the event of an emergency, but with so few shifts, it is very doable. Like many veterinarians surveyed, some members have had concern over differing fee structures. Specifically, they were concerned that all members of the cooperative have similar emergency fees. This concern prompted the creation of a “no nonclient” emergency fee rule when seeing clients that were established with a veterinary member of the cooperative. It was also important to not discuss the pricing of any specific service as it is unethical and often illegal. Furthermore, in the author’s experience, unlike vaccines, castrations, and other similar services, clients are generally not price shopping emergency services. The members of the author’s cooperative have not experienced any feedback from clients on differing fee structures. With concerns about client receptivity, the cooperative addressed the issue head on. A group seminar was held for clients to promote the existence of the cooperative and give everyone a chance to meet the other veterinarians and see their working relationship in action. One member made a magnet with all of the practice logos and phone numbers that could be handed out to clients. This gives an excellent opening for discussing the existence of the cooperative with new clients. Because there is often a misunderstanding among clients about whether the vets all work in the same practice, the magnet makes it clear that each practice is still an independent entity. Additionally, when providing emergency services, it should be made very clear with clients that their veterinarian will be updated in the morning and their care passed back into the hands of their regular provider. Working on nonequine species was a concern for several of the equine exclusive practitioners when they joined the group, but the benefits of joining the group outweighed any discomfort of treating these species. To help familiarize these veterinarians with situations they may face, the cooperative held rounds on the most common emergencies seen in cattle and small ruminants. The veterinarians that work regularly on these species have also made themselves available for phone consults when needed. In addition to the initiative to develop an emergency cooperative, and the trust, respect, and clear communication required to keep it functioning, flexibility has been key to longevity. The rotation schedule has changed to allow several members to take maternity leave, deal with family emergencies, and travel. In the last year, the weekday emergency coverage has adapted to include daytime emergencies to help members deal with the unexpected increase in
childcare requirements that have come with the COVID-19 pandemic. The practitioner assigned to that evening’s call is ready to take on the other practitioners’ daytime emergencies with little notice. This has greatly reduced the stress that comes with unexpected family emergencies (i.e., a sick child, school closures, etc.). Emergency cooperatives have allowed the author to do something that is increasingly rare for solo practitioners—to own a practice without it owning the practitioner. After having a child, the author is quite sure equine practice would not have been possible without the support of the cooperative. If willing to make the trade-offs of occasional increased drive time, regularly entrusting patients’ care to a colleague, and perhaps working on occasional nonequine species, emergency cooperatives can be an excellent way to maintain the small general equine practice as a sustainable practice model. One will gain not only greater freedom with personal time but also stronger relationships with colleagues.

Acknowledgments

Declaration of Ethics

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

Conflict of Interest

The Author has no conflicts of interest.

References

Alternative Schedules and Practice Roles: Rethinking Industry Norms Can Promote More Valuable and Sustainable Businesses

Kelly A. Zeytoonian, DVM, MBA, CERP

Employee retention and practice growth are no longer easily achieved when following old business models. Business owners need to rework veterinary scheduling and employee roles to maximize profits and minimize burnout. Author’s address: PO Box 620071, Woodside, CA 94062; e-mail: info@starwoodveterinaryconsulting.com. © 2021 AAEP.

1. Introduction

The equine veterinary industry shows a steady decline in veterinarians entering the profession, with less than 2% of graduates entering private equine practice since 2014 (Fig. 1). Retaining these new graduates is difficult, with approximately 50% discontinuing their AAEP membership and presumably leaving equine practice within the first five years post-graduation. AAEP membership has been declining over the last several years (Fig 2 adapted from AAEP Annual Reports). The reduction in numbers is likely multifactorial: practice owners are retiring, and fewer new graduates are entering equine positions or they are leaving equine positions in pursuit of small animal jobs. To attract new professionals, business owners should take a new approach to an aged business model. An overview of the current business model’s perceived shortfalls and an introduction to alternative work schedules and practice roles will offer solutions providing practice owners a more sustainable future.

2. Discussion

Employee Pain Points

On-Call/Emergency Duty

A May 2019 survey administered by Dr. Amy Grice and published in AAEP 2020 Convention Proceedings summarized the key reasons why veterinarians choose to leave (or consider leaving) equine practice (Fig. 3).

In the 2016 AVMA AAEP Economic Impact Study, most equine veterinarians surveyed were responsible for more than 25% of emergency coverage at their place of employment (Fig. 4). A staggering 25% were the sole providers of emergency coverage. This fact is not surprising considering that approximately 38% of AAEP members designate themselves as sole proprietors. Comparable data in the small animal sector is not available since the AVMA excluded emergency coverage questions from non-equine respondents. However, companion animal emergency
centers are common in populated areas around the country.

Physical Demands/Dangers
In a study published in 2018, 80% of equine veterinarians working in the United Kingdom reported work-related injuries (in their career to date), with over 40% leading to loss of work.4

Debt to Income Disparity
The mean educational debt of students graduating from U.S. veterinary schools in 2019 was $183,302.5 Although this number is an average for all students, including those with no student loan debt, an income disparity quickly develops. It takes equine veterinarians approximately ten additional years of practice, compared to their small animal colleagues, to earn a mean salary of $120,000 (Fig. 5). Substantial debt coupled with lower lifetime earnings may exclude associate veterinarians from the ability to purchase a home, buy a practice, or feel they can meet the demands of their desired lifestyle.

Long Hours/Culture Work Ethic
The economic realities often lead equine veterinarians to work longer hours to earn additional income.6 This reality leads to a culture of new veterinarians associating their worth with how many hours they work or what sacrifices they are willing to make in their personal life. A practice culture that values hours of care over establishing healthy boundaries can become toxic and lead to burnout, medical mistakes, and physical injuries.

Owner Pain Points
The concerns of associates described above are also relevant to practice owners who must consider their succession plan, ability to hire associate veterinarians, and financing for retirement.

Fig. 1. Percentage of new graduates entering private equine practice.1

Fig. 2. Trends in AAEP membership among veterinarians and students.
Succession/Retirement Plan

Figure 5 also demonstrates the income disparity between retirement-aged equine veterinarians and their small animal colleagues. While the sale of a practice may be desired, newer graduates may not be positioned to purchase due to high educational debt burden (Fig. 6). As a result, older practice owners may face the need to continue working to meet their financial obligations.

Physical Pain or Fatigue/Inability to Ease Up with Increasing Age

Financial obligations and patient care do not decrease despite the onset of usual limitations of an aging body.

Finances of Hiring Additional Employees

Many practitioners are unsure they have enough business to support an additional employee and may not know how to assess their finances to make this decision.
decision. Inevitably, the owner should expect to transfer a portion of their revenue to a new associate, as they are introduced to clients and integrated into the practice. Owners should also expect associates to produce less revenue than themselves initially (Fig. 7).

**Limited Hiring Pool**

In early 2021, there were 396 postings on AAEP’s career center. An increase in job listings equates to added time for an actively searching practice owner to hire an associate. To summarize, the challenges faced by equine practitioners include a desire for the work to be less physically and temporally demanding while being more financially viable.

### 3. Solution

Consider the following options to address the general concerns that equine veterinary medicine faces.

**Charge Fees That Will Support Higher Salaries**

Without proper veterinary charges, practice owners will not have the funds to implement the suggested solutions. Associate veterinarians will have minimal incentive to work harder when they are not adequately compensated for their time. Practice owners should commit to reviewing their finances compared to industry benchmarks and their pricing strategies. A focus on increased profitability will make the following suggestions more obtainable.

**Alternative Work Schedules**

**Part-Time Associates**

Ten percent of respondents in the 2019 survey by Grice credited the inability to work part-time as a reason for leaving or considering leaving equine medicine. Hiring one or more part-time employees has several benefits:

- The practice can spread emergency coverage among multiple employees (Fig. 8).
- Part-time employees require fewer benefits.
- Higher utilization of resources when shared on alternating days (e.g., one work truck with all the supplies needed to be shared between multiple associates who are each scheduled 2–3 days a week instead of the typical 5–6).
- Greater flexibility and work-life balance.

**Shared Emergency Coverage or Emergency Associate**

Prior convention proceedings provide examples for establishing a shared emergency call schedule among neighboring practices. This allows emergency responders to be spread between many individuals with no additional financial burden and minimal time organizing and acclimating clients to new coverage protocols. If shared coverage is not possible in a particular area due to poor relationships among colleagues or lack of other equine practices, another solution is hiring a strictly emergency duty associate. Although this may seem logistically impossible, emergency-only after-hours practices have been prevalent in the small animal world for years. The author saw this model used in a local referral practice and noted that it was well-received by competing clinics because practice owners saw the emergency doctor as an emergency-only resource and not a daytime competitor. Practices looking for strictly

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![Fig. 5. Mean annual income reflecting the number of years since DVM graduation.](https://example.com/fig5)
emergency coverage could also fund a veterinarian to provide emergency coverage across multiple clinics.

**Seasonal Hires/Relief Veterinarians**

There are currently 61 veterinarians across the United States listed as relief veterinarians on AAEP’s Touch Relief Veterinarian Network. Whether a practice owner needs to go on vacation, an employee is out for illness or maternity leave, or it is during a busier season, relief veterinarians are a temporary solution for transient demands in doctor time.

**Using Licensed Technicians and Support Staff**

Hiring another veterinarian may not be feasible for a veterinary practice, but the addition of a support staff employee may be an option. Team members help address several of the primary concerns highlighted above: time efficiency, revenue opportunities, safety, desire to work part-time, etc. The 2016 AVMA AAEP Economic Survey found that hiring additional employees (vet or support staff) led to a negligible or positive financial impact in mean veterinary income (Fig. 9). Moving from one staff member to two to four staff members led to a $125 reduction in annual salary. When moving beyond two to four total employees, the mean veterinary salaries were positively impacted ($5,500–38,000 increase). For a solo practitioner, hiring an employee results in a minimal loss of income which is a reasonable trade for increased assistance, safety, and efficiency. Scaling in a large practice size creates higher salaries for veterinary staff. Although the

Fig. 6. Mean debt-to-income ratio of AVMA equine veterinarians and all AVMA veterinarians by graduation year.¹
Fig. 7. Comparison of gross revenue performance in equine specific vs. veterinary practice at large.¹

<table>
<thead>
<tr>
<th>Equine</th>
<th>Obs</th>
<th>Mean</th>
<th>Std. Dev.</th>
<th>1st Quartile</th>
<th>Median</th>
<th>3rd Quartile</th>
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<th>Max.</th>
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<tr>
<td>Owner</td>
<td>80</td>
<td>$418,863</td>
<td>$218,731</td>
<td>$262,000</td>
<td>$385,832</td>
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<th>Min.</th>
<th>Max.</th>
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<td>118</td>
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<td>$216,301</td>
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<td>$500,000</td>
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<tr>
<td>Associate</td>
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<td>$462,905</td>
<td>$171,098</td>
<td>$250,000</td>
<td>$450,000</td>
<td>$553,000</td>
<td>$100,000</td>
<td>$1,000,000</td>
</tr>
</tbody>
</table>

Fig. 8. Total veterinary time on-call relative to the number of veterinary coworkers.¹

Fig. 9. Veterinary income related to practice size.¹
The cited report did not assess the impact of practice size on revenue or profitability of the veterinary practice at large, one might presume that revenues will be higher as a reflection of increased salaries. There will also be intangible benefits (work-life balance, team culture, emotional support, perceived safety) that exist from increased assistance. There are currently 800 registered AAEVT members available to fill these roles and support the above efforts. For practices that already have support staff on their payroll, room for increased utilization of these employees exists. Figure 10 demonstrates everyday tasks performed by licensed and unlicensed technicians and the percentage of practices assigning these responsibilities to their support team.

Develop a Succession Plan

Approximately 40% of equine practitioners are over 50 years of age. These practitioners may not be prepared to retire despite the desire. Forming a relationship with a younger practitioner and developing a succession plan offers an opportunity to mentor while providing financial stability through a transition period. The succession plan may look different for every practice. Critical points should include the mentee/mentor relationship, employment period for a successful transition of clients, and proceeds from associate ownership percentage applying to the total purchase price. Dr. Ann Dwyer briefly reviewed the basics of practice ownership and acquisition in the AAEP Convention Proceedings from 2009.

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**Table: Common practice responsibilities and percentage performed by support staff.**

<table>
<thead>
<tr>
<th>Task Description</th>
<th>Licensed Technician (n=222)</th>
<th>Non-licensed Technicians/Veterinary Assistants (n=394)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Administer anesthesia</td>
<td>168</td>
<td>141</td>
</tr>
<tr>
<td>Setting up diagnostic imaging equipment (radiography, ultrasonography, endoscopy)</td>
<td>191</td>
<td>362</td>
</tr>
<tr>
<td>Obtaining radiographic studies</td>
<td>163</td>
<td>211</td>
</tr>
<tr>
<td>Perform other diagnostic imaging studies (e.g. nuclear scintigraphy, MRI)</td>
<td>54</td>
<td>45</td>
</tr>
<tr>
<td>Administer vaccinations</td>
<td>141</td>
<td>163</td>
</tr>
<tr>
<td>Place IV catheters</td>
<td>184</td>
<td>152</td>
</tr>
<tr>
<td>Administer treatments or set up fluids through catheter</td>
<td>189</td>
<td>258</td>
</tr>
<tr>
<td>Administer IV injections (no catheter)</td>
<td>172</td>
<td>171</td>
</tr>
<tr>
<td>Administer IM injections (other than vaccinations)</td>
<td>190</td>
<td>256</td>
</tr>
<tr>
<td>Perform routine treatments - e.g., apply foot wrap, apply leg wrap, apply topical medications</td>
<td>186</td>
<td>284</td>
</tr>
<tr>
<td>Collect venous blood samples</td>
<td>198</td>
<td>260</td>
</tr>
<tr>
<td>Perform laboratory tests in house - e.g., set up bacterial cultures, use CBC/chemistry analysis machines, perform quantitative fecal exams, cytology</td>
<td>196</td>
<td>280</td>
</tr>
<tr>
<td>Prepare aseptic preparation for procedures</td>
<td>201</td>
<td>333</td>
</tr>
<tr>
<td>Prepare injections for doctor use</td>
<td>191</td>
<td>305</td>
</tr>
<tr>
<td>Communicate with clients - e.g. Give care instructions, report laboratory results, make appointments</td>
<td>174</td>
<td>260</td>
</tr>
<tr>
<td>Write invoices for work performed by veterinarian</td>
<td>170</td>
<td>264</td>
</tr>
<tr>
<td>Assist in surgical procedures</td>
<td>162</td>
<td>245</td>
</tr>
</tbody>
</table>

1. Dr. Ann Dwyer reviewed the basics of practice ownership and acquisition in the AAEP Convention Proceedings from 2009.
Owners and new graduates/associates would benefit from reviewing these guidelines.

4. Example of Paradigm Change

My desire to build an equine practice that supported my lifestyle without becoming my life led to several shifts from the standard business model. While practicing as a solo veterinarian, I established a shared on-call schedule with a fellow practice owner. A registered veterinary technician (RVT) was hired to provide support during patient visits and administer solo appointments (bandage changes, laser therapy, injections, etc.). The benefit of having the RVT perform patient care included an afternoon off or an opportunity to see extra practice-building cases. Clients also accepted the idea of having other care providers besides the practice owner. The RVTs increased duties made a smooth transition for the first associate that was hired. The first associate, similar to 10% in the survey shared above, desired a part-time position. Her employment increased practice hours of availability, helped reduce owner on-call duties, and did not require the purchase of a new vehicle or additional equipment. Throughout the addition of new associates, a relationship was maintained with the solo practitioner who shared on-call. It became a natural progression to integrate both practices in a manner that offered excellent client care through the transition, increased caseload and additional mentorship for newer associates, and a path to retirement on the solo owner’s terms. There is still more to do, but the alternative scheduling, valued partnerships with solo practitioners, and shifting employee duties have been beneficial for both associate and practice health.

5. Conclusion

As a solo practitioner—ask yourself: Do I have a succession plan or am I making a financial retirement plan that will not rely on selling my practice? If not, is there a newer graduate you can take on as a mentee? In practices with associates, consider the expectations and scheduling parameters you place on your veterinarians. Examine your support staff roles and whether employees are “working to the top of their skill set.” Small changes can have a notable impact on employee satisfaction, retention, and the business bottom line.

Acknowledgments

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

Conflict of Interest
The Author offers and provides business consulting services to veterinarians and veterinary practices through Starwood Veterinary Consulting, Inc., of which she is a shareholder.

References
Investigation of the Bi-Weekly Administration of Diclazuril on the Antibody Kinetics to *Sarcocystis Neurona* in Healthy Horses

Nicola Pusterla, DVM, PhD, DACVIM, DAVDC-Equine*; Kaitlyn James, PhD, MPH; Fairfield Bain, DVM, MBA, DACVIM, DACVP, DACVECC; D. Craig Barnett, DVM; Duane Chappell, DVM; Earl Gaughan, DVM, DACVS; Bryant Craig, DVM; Chrissie Schneider, DMV, MS, DABVP-Equine; Wendy Vaala, DVM, DACVIM; and Mark G. Papich, DVM, DACVCP

The administration of diclazuril® pelleted top dress at half the label dose (0.5 mg/kg) twice weekly was able to maintain low titers to *Sarcocystis neurona* in healthy adult horses naturally exposed to the protozoal parasite. Further, trough diclazuril levels were in excess of the minimal concentration known to inhibit *S. neurona*. Authors’ addresses: Department of Medicine and Epidemiology, School of Veterinary Medicine, University of California, One Shields Avenue, Davis, CA 95616 (Pusterla); Department of Obstetrics, Gynecology and Reproductive Biology, Massachusetts General Hospital, Boston, MA 02114 (James); Merck Animal Health, 2 Giralda Farms, Madison, NJ 07940 (Bain, Barnett, Chappell, Gaughan, Craig, Schneider, Vaala); College of Veterinary Medicine, North Carolina State University, Raleigh, NC 27606 (Papich); e-mail: npusterla@ucdavis.edu. © 2021 AAEP.

1. Introduction

Prevention of equine protozoal myeloencephalitis represents an important challenge, and its focus relies on wildlife management, risk-factor manipulation, and use of antiprotozoal medication. A recent study showed that bi-weekly administration of diclazuril at half the current label dose produced steady-state plasma drug concentrations known to inhibit *S. neurona*. The aim of this study was to determine if bi-weekly administration of diclazuril at half the label dose would reduce seroprevalence and magnitude of titers to *S. neurona* in healthy horses naturally exposed to the apicomplexan protozoal parasite.

2. Materials and Methods

Twenty healthy adult horses were moved from a low-risk exposure to a farm with high exposure rate to *S. neurona* in their horse population. The
horses were randomly assigned to either a treatment or a control group. Treatment consisted in the administration of 0.5 mg/kg body weight of diclazuril pelleted top dress twice weekly (every 3–4 days) for 12 months. Prior to initiation of treatment and monthly thereafter, blood was collected for the detection of antibodies to \textit{S. neurona} using a quantitative immunoassay. Further, trough plasma diclazuril levels were determined every 60 days. Frequency distributions of the titers at each time point were compared with Fisher’s exact test between treated and control groups; repeated-measures analyses (analysis of variance and mixed-effects ordinal logistic regression) were also utilized to determine differences in raw titers between treatment and control groups over time.

3. Results and Discussion

All 20 horses remained healthy during the entire study period. At study commencement, the seroprevalence to \textit{S. neurona}, defined as the percentage of horses in each group with a titer $\geq 40$, was 70% and 80% in the control and treatment group, respectively. The initial seroprevalence decreased initially in the treatment group to 50% at 30 days post-treatment commencement, while the seroprevalence in the control group increased to 90%. This was followed by a slow increase in seroprevalence in the treatment group before reaching 100% in both groups by 90 days post-treatment commencement. The seroprevalence remained 100% in both groups from 90 to 360 study days. While titer distribution between the two groups was similar at study commencement, treated horses displayed significantly lower titers throughout the treatment period ($P < 0.05$). All treated study horses had detectable plasma trough diclazuril levels at the six time points, and the levels were above the concentration known to inhibit \textit{S. neurona} in vitro (1 ng/mL).

Acknowledgments

This study was supported by Merck Animal Health.

Declaration of Ethics

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

Conflicts of Interest

Drs. Fairfield Bain, Craig Barnett, Duane Chappell, Earl Gaughan, Bryant Craig, Chrissie Schneider and Wendy Vaala work for Merck Animal Health.

Footnote

* Protazil®, Merck Animal Health, Madison, NJ 07901.
Prevalence of and Risk Factors Associated with *Salmonella* Shedding Among Equids Presenting to a Veterinary Teaching Hospital for Colic (2013–2018)

Isabelle Kilcoyne, MVB, DACVS*; Gary Magdesian, DVM, DACVIM, DACVECC, DACVP, DCVA; Margherita Guerra, BS; Julie E. Dechant, DVM, MS, DACVS, DACVECC; Sharon J. Spier, DVM, PhD, DACVIM; and Philip H. Kass, DVM, PhD, DACVPM

The prevalence of *Salmonella* shedding in this colic population was low. Certain predictors such as development of a fever or reflux in hospitalized colic cases were associated with *Salmonella* shedding and may help the clinician to promptly identify horses likely to shed. Authors’ addresses: Department of Surgical and Radiological Sciences (Kilcoyne, Dechant), Department of Medicine and Epidemiology (Magdesian, Spier), School of Veterinary Medicine, Department of Medicine and Epidemiology (Guerra), Academic Affairs (Kass), University of California-Davis, Davis, CA 95616; e-mail: ikilcoyne@ucdavis.edu.

*Corresponding and presenting author. © 2021 AAEP.

1. Introduction

Colic has been previously associated with shedding of *Salmonella*. The purpose of this study was to determine the prevalence of *Salmonella* shedding in a colic population and identify factors associated with *Salmonella* shedding.

2. Materials and Methods

This was a retrospective case-control study. For each colic horse that was positive for *Salmonella* (n = 56), two colic cases (n = 112) that tested negative were enrolled as controls. Associations between variables and *Salmonella* shedding were identified using logistic regression. Univariate and multivariate models were developed pertaining to 1) presenting clinicopathological data and 2) clinical variables that developed during hospitalization.

3. Results

Of 1,917 horses presenting with colic, 1,585 had at least 1 sample submitted for *Salmonella* testing. Of these, 56 were positive for *Salmonella* yielding a prevalence of 3.5%. Horses shedding *Salmonella* were more likely to present with a history of fever (p = 0.01), increased lactate (p = 0.007) and/or...
neutropenia ($p = 0.02$). Hospitalized horses shedding *Salmonella* were more likely to be febrile ($p = 0.01$) and 10 times more likely to develop reflux ($p = 0.01$) compared to controls.

4. Discussion

The prevalence of *Salmonella* shedding in this colic population was low, presumably due to increased biosecurity measures. More *Salmonella* positive horses developed reflux versus diarrhea while in the hospital. These findings may warrant revision of relying on diarrhea as one of the primary signs of infection and revising the criteria for infectious disease control protocols.

**Acknowledgments**

*Declaration of Ethics*

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

*Conflict of Interests*

The Authors have no conflicts of interest.
Trends in Antimicrobial Susceptibility Patterns of Bacterial Isolates from Horses with Ulcerative Keratitis in Tennessee

Braidee C. Foote, DVM*; Diane Van Horn Hendrix, DVM, MS, DACVO; Dan Ward, DVM, PhD, DACVO; Sree Rajeev, BVSc, PhD, DACVM, DACVP; and Joe S. Smith, DVM, MPS, PhD, DACVIM, DACVCP

In horses with an infected corneal ulcer, use of two antibiotic classes targeting both gram-positive and negative bacteria led to a higher likelihood of in vitro susceptibility for the isolated organisms but no improvement in clinical outcomes was seen in this population. Authors’ addresses: Department of Small Animal Clinical Sciences (Foote, Hendrix, Ward), Department of Biomedical and Diagnostic Sciences (Rajeev); Department of Large Animal Clinical Sciences (Smith), College of Veterinary Medicine, University of Tennessee, Knoxville TN 37996; e-mail: bfoote@utk.edu. *Corresponding and presenting author. © 2021 AAEP.

1. Introduction
The objectives of this study were to document aerobic bacterial isolates from horses with ulcerative keratitis, characterize the antimicrobial susceptibility patterns, evaluate clinical outcomes, compare resistance patterns to previously reported data from the same hospital, and compare monotherapy versus combination therapy.

2. Materials and Methods
Medical records from horses with positive bacterial cultures from corneal ulcers treated at the University of Tennessee between March 2011 and December 2020 were compared to previously published data from January 1993 through May 2004. Data including bacteria isolated, history, cytology results, susceptibilities to selected antimicrobials, and case outcomes were collected.

3. Results
Thirty-one bacterial isolates were cultured from 27 samples (26 horses). The most common bacterial genera were Streptococcus (35%), Staphylococcus (29%), and Pseudomonas (13%). Use of topical corticosteroids prior to culture was significantly associated with Staphylococcus infections (p = 0.04). Compared to the earlier study period, there was a significant number of Streptococcus isolates reported with reduced sensitivity to gentamicin (p = 0.01) in the current data. Staphylococcus spp. and P. aeruginosa isolates had no significant changes in susceptibilities.

Research Abstract—for more information, contact the corresponding author

NOTES
over time. Antimicrobial susceptibility patterns suggest that combination therapy will cover ≥89% of all isolates, compared to ≤85% with monotherapy. Seventeen eyes (77%) healed by last follow-up [surgery (n = 2); medical (n = 15)]; whereas 5 were enucleated, and 4 were lost to follow up.

Acknowledgments

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

Conflict of Interest
The Authors have no conflicts of interest.
Gentamicin-Induced Auditory Loss in Healthy Adult Horses

Monica Aleman, MVZ Cert., PhD, DACVIM (LAIM, Neurology)*; Heather Knych, DVM, PhD, DACVCP; Alexander True, BS; Rebeca Scalco, DVM; Chelsea Crowe, BS; Lais Costa, DVM, PhD, DACVIM; and Munashe Chigerwe, DVM, MS, MPH, PhD, DACVIM

Gentamicin is an antimicrobial used for the treatment of Gram-negative infections. However, clinicians must be aware of hearing loss as a possible risk in some horses. Authors’ address: University of California-Davis, School of Veterinary Medicine, Department of Medicine and Epidemiology, Tupper Hall 2108, One Shields Avenue, Davis, CA 95616; e-mail: mraleman@ucdavis.edu. *Corresponding and presenting author. © 2021 AAEP.

1. Introduction
Gentamicin is a widely used antimicrobial in equine medicine for the treatment of Gram-negative infections. Gentamicin toxicity has included renal and ototoxic effects. Ototoxicity has been suspected in the horse, but such association has not been investigated at currently routine dosages. Therefore, the objective of this study was to investigate auditory and vestibular function in healthy adult horses administered gentamicin.

2. Materials and Methods
Ten healthy young adult horses were included in the study. Gentamicin sulfate was administered at 6.6 mg/kg in the jugular vein, alternating sides for 7 consecutive days. Vestibular and auditory function were evaluated through neurological examination and brainstem auditory evoked responses (BAER), respectively. Horses were sedated with IV detomidine hydrochloride to perform BAER studies. A BAER was done at the beginning of the study (day 1: baseline), at the end of the study (day 7), and 30 days after (day 37). Bone conduction was performed to rule out a conduction disorder.

3. Results
Seven horses developed auditory loss: complete bilateral \((N=1)\), complete unilateral \((N=2)\), and partial unilateral \((N=4)\). Absent bone conduction ruled out a conduction disorder and further supported sensorineural auditory loss in horses. Dysfunction was reversible in 4 of 7 horses. Vestibular dysfunction was not observed.

4. Discussion
Gentamicin might pose a risk for the development of auditory loss in horses, which might be irreversible.
Acknowledgments

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

Conflict of Interest
The Authors have no conflicts of interest.
Effect of a Combination of Butorphanol and Detomidine on Endoscopic Assessment of Laryngeal Function of Thoroughbred Yearlings

Hugo Almonte, DVM (Hons)*; James Schumacher, DVM, MS, DACVS, MRCVS; Christopher R. Johnson, DVM, MS, DACVS; Jeffrey T. Berk, VMD, MRCVS; and Rhodes P. Bell, DVM, MS, DACVS-LA

1. Introduction

This experimental, observer-blinded, crossover study examined the effects of intravenous administration of a combination of butorphanol and detomidine on left-to-right rima glottidis ratio (L:R RGR), cross-sectional area of the rima glottidis (CSARG), and grade of laryngeal function of Thoroughbred sales yearlings at rest.

2. Materials and Methods

Forty-six Thoroughbred yearlings underwent endoscopic examination of the larynx before (group 1) and after being administered butorphanol (0.01 mg/kg, IV) and detomidine (0.01 mg/kg, IV; group 2). L:R RGR and CSARG were measured from images captured from a video recording at the point of maximal abduction of the arytenoid cartilages. Recordings were reviewed by three clinicians to assess agreements of the grade of laryngeal function, using the 7-grade Havemeyer endoscopic laryngeal grading scale. Intraobserver and interobserver scores were analyzed to determine agreement.

3. Results

The L:R RGR and CSARG of group-1 yearlings did not differ significantly from that of group-2 yearlings. The mean intraobserver agreement of grade of laryngeal function of yearlings in group 1 was 93.1%, with a mean kappa statistic of 0.86. The mean intraobserver agreement of grade of laryngeal function of group-2 yearlings was 92.9%, with a mean kappa statistic of 0.88. The mean interobserver agreement of grade of laryngeal function of group-1 yearlings was 92.8%, with a mean kappa statistic of 0.84. The mean interobserver agreement of grade of laryngeal function of group-2 yearlings was 92.7%, with a mean kappa statistic of 0.87. The correlation between CSARG and grade of laryngeal function was significant for both groups ($P<.001$). All three observers assigned the same grade of laryngeal function to 35 of 45 (77.8%) of the yearlings in group 1 and...
33 of 45 (73.3%) of the yearlings in group 2. One video recording of a yearling was determined, by two of the observers, to be too brief to assign a grade of laryngeal function, which was excluded for statistical analysis. The median grade of laryngeal function in group 1 was II.1 and I in group-2 yearlings.

4. Conclusion and Clinical Significance
Administering butorphanol and detomidine to Thoroughbred yearlings, before examining the upper respiratory tract endoscopically, with the horses at rest, does not significantly affect the grade of laryngeal function. To provide a clinical scenario, a nose twitch was applied to all yearlings in group 1, which may have affected laryngeal movements.

Acknowledgments

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

Conflict of Interest
The Authors have no conflicts of interest.
Subclinical Colitis Following NSAID Administration in Healthy Horses

Rebecca C. Bishop, DVM*; Ann M. Kemper, DVM; Pamela A. Wilkins, DVM, MS, PhD, DACVIM-LA, DACVECC; and Annette M. McCoy, DVM, MS, PhD, DACVS-LA

Firocoxib administration in clinically healthy adult horses was associated with increases in colon wall thickness and edema, compared to flunixin meglumine administration. COX-2 selective nonsteroidal anti-inflammatory drugs (NSAIDs) carry a risk of developing subclinical colitis. Authors’ address: Department of Veterinary Clinical Medicine, University of Illinois, Urbana, IL 61802-4714; e-mail: rb17@illinois.edu. © 2021 AAEP.

1. Introduction

NSAID administration can cause gastrointestinal toxicity. Selective COX-2 inhibitors (“coxibs”) were developed to reduce risk of undesirable side effects when administering nonselective NSAIDs. It is unknown if coxibs are protective against the risk of subclinical colitis in horses.

2. Materials and Methods

Eight healthy adult horses were administered flunixin meglumine (1.1 mg/kg IV q12h for 5 days), then firocoxib (0.3 mg/kg PO once, then 0.1 mg/kg PO q24h for 4 days) with a 6-month washout period between. Omeprazole (1 mg/kg PO) was administered concurrently with each NSAID. Transabdominal ultrasonographic examination was performed at the beginning and end of each treatment week. Serum chemistry profiles and complete blood counts were performed.

3. Results

Clinical parameters and blood values were within normal limits. Colon wall thickness increased over time when horses received firocoxib ($p = 0.008$) but not flunixin ($p = 0.195$). Colon wall thickness was greater following firocoxib treatment compared to flunixin treatment ($p = 0.008$). Subjectively, colonic edema was present more frequently and was more severe following treatment with firocoxib, compared to flunixin.

4. Discussion

While no horses exhibited clinical signs of colitis, there was a significant increase in colon wall thickness following treatment with firocoxib that did not occur when the same horses were administered flunixin. A
larger sample size may be required to detect significant differences in other clinical parameters.

Acknowledgments
This study was funded by the U.S. Department of Agriculture under Section 1433 Animal Health and Disease, project ID ILLU-888-949.

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

Conflict of Interest
The Authors have no conflicts of interest.
Pharmacokinetics and Safety of an Oral Cannabidiol Product in Horses

Alicia F. Yocom, DVM†; Elsbeth S. O’Fallon, DVM, DACVIM; Daniel L. Gustafson, PhD; and Erin K. Contino, MS, DVM, DACVSMR*

Cannabidiol (CBD) administered orally has dose-dependent plasma bioavailability and is detectable in synovial fluid in horses. Authors’ address: Department of Clinical Sciences, College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Fort Collins, CO 80523; e-mail: erin.contino@colostate.edu. *Corresponding author; †presenting author. © 2021 AAEP.

1. Introduction

Cannabidiol (CBD) is widely marketed to reduce anxiety and pain, but limited safety, efficacy, or pharmacokinetic data is available in horses. The objectives of this study were to determine (1) plasma pharmacokinetics, (2) short-term safety, and (3) synovial fluid levels of CBD following oral administration.

2. Materials and Methods

Two groups of 6 horses were administered sunflower lecithin oil-based CBD at 1 mg/kg (group 1) or 3 mg/kg (group 2) for a 24-hour pharmacokinetic study. All horses then received 0.5 mg/kg or 1.5 mg/kg q12h PO for 6 weeks with steady state and elimination sampling performed up to 96 hours post final dose. Synovial fluid concentrations were evaluated at 12 and 24 hours and 5 weeks. Horses were monitored daily, and clinicopathologic parameters were evaluated.

3. Results

Mean ± SD Cmax and Tmax were 4.3 ± 2.1 ng/mL and 4.1 ± 4.1 hours and 19.9 ± 15.6 ng/mL and 5.0 ± 3.7 hours for groups 1 and 2, respectively. Following the final dose at 6 weeks, one group-2 horse still had detectable plasma levels at 96 hours. CBD was detectable in synovial fluid in 8 horses during steady state. Mild hypocalcemia was seen in all horses, and elevated liver enzymes were observed in 8 horses, but these changes decreased or normalized 10 days after the final CBD dose.

4. Discussion

CBD has dose-dependent, but variable, oral bioavailability at 1 mg/kg and 3 mg/kg daily dosing. CBD is detectable at steady state in synovial fluid at the higher dose. Further investigation is needed to establish clinically effective doses.

Research Abstract—for more information, contact the corresponding author

NOTES
Acknowledgments
Funding for this study was provided by a College Research Council Grant at Colorado State University.

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

Conflict of Interest
The Authors have no conflicts of interest.
Effects of a Supplement Containing Cannabidiol on Sedation and Ataxia Scores and Health Parameters

Frank M. Andrews, DVM, MS, DACVIM (LAIM); Michael St. Blanc, DVM*; Anna Chapman, DVM, MS, DACVIM; Michael L. Keowen, BS; Frank Garza, Jr., MS; and Lydia Gray, DVM

A supplement containing cannabidiol (CBD) (150 mg, treated once daily) administered for 56 days was palatable, safe, and did not alter mentation or cause ataxia in horses. In addition, blood concentrations were present in 78% of horses, and the supplement did not alter hematologic or plasma biochemical parameters. Authors’ addresses: Equine Health Studies Program, Department of Veterinary Clinical Sciences, School of Veterinary Medicine, Louisiana State University, Baton Rouge, LA 70803 (Andrews, St. Blanc, Chapman, Keowen, Garza); SmartPak, Inc., 40 Grissom Road #500, Plymouth, MA 02360 (Gray); e-mail: mstbla2@lsu.edu. *Corresponding and presenting author. © 2021 AAEP.

1. Introduction
Equine supplements containing cannabidiol (CBD) are commercially available, but data on the effects in horses are lacking. The purpose of this study was to determine if a CBD supplement would alter sedation or ataxia scores and blood parameters in horses.

2. Materials and Methods
Twenty geldings were randomly assigned to either the treatment (150 mg cannabidiol in a pelleted supplement) or control (pelleted supplement without CBD) group. Supplements were fed once daily for 56 days. A complete blood count and serum biochemistry were performed on days 0, 28, and 56. Sedation and ataxia scores were assigned weekly, and CBD concentrations were analyzed on days 0 and 56 in treated horses. A repeated-measures ANOVA with a mixed effects model was used to analyze the continuous variables with treatment, day, and their interactions as the fixed effects and each animal as the random effect. Sedation and ataxia scores were analyzed via Mann-Whitney test. Significance was set at P < 0.05.

3. Results
There were no treatment or treatment by day effects on blood parameters including bilirubin, alkaline phosphatase (ALP), and aspartate aminotransferase (AST). There were no significant differences in ataxia or sedation scores between groups (p > 0.05). Forty
percent of treated horses had detectable plasma CBD concentrations two hours after treatment on day 0, compared to 78% by day 56.

4. Discussion
Treatment was well-tolerated, and the results support further investigation of CBD use in horses.

Acknowledgments

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

Conflict of Interest
The Authors have no conflicts of interest.
A Look at Lameness Through the Eyes of Functional Anatomy (and Biomechanics)

J.-M. Denoix, DVM, PhD, HDR, Founder ISELP, LAIA-ECVDI, DACVSMR, DECVSMR, INRAE

The purpose of this paper is to show how the clinical manifestations of lame horses are correlated to functional anatomy and internal biomechanical data. A lot of information can be obtained from visual analysis of lame horses under standardized situations combining different gaits, ground surfaces and exercises. These data are not only useful for diagnostic purposes but are key to evaluating the significance of imaging findings and to establish an adequate rehabilitation program avoiding uncomfortable situations for the horse. In practice, analysis of video-recordings (at normal speed or slow motion) is recommended to confirm, complete, or extend analysis of clinical manifestations. More sophisticated methods have been used or are in development to give further objective data especially at the trot, but as the vast majority of functionally normal horses present gait asymmetries, there is a need for establishing the criteria to discriminate between physiological lateralization and lameness. Author’s address: CIRALE-NEV-EnvA, Goustranville, F-14430, France; e-mail: jean-marie.denoix@vet-alfort.fr. © 2021 AAEP.

1. Introduction

The diagnosis of lameness has been considered for a long time in many textbooks and papers, and symptoms as well as causes of musculoskeletal injuries have been extensively discussed and debated. Evaluation of the horse at the trot combined with diagnostic analgesia has been widely used, investigated, and recommended and is generally considered as the gold standard approach for establishing the origin of pain. Nowadays, the extraordinary development of imaging techniques and technologies has permitted the diagnosis and documentation of an unlimited number of causes of lameness. Constant improvement and increased availability of these techniques for field practice have enabled the practitioner to reach a more precise identification and documentation of lesions and abnormalities with the conviction or the hope for a better management of the affected horse. The drawback of having access to a variety of more sensitive techniques is the risk of overinterpreting imaging findings and overestimating their significance; it is also to bypass the essential step that consists of establishing the correlation between the clinical presentation and the lesion(s). The purpose of the presentation is to demonstrate how a horse alters its gait to decrease the pain induced by each type of condition. The basic concept presented in this paper consists of following each step of the dynamic
examination and to analyze gait and movement alterations of different types of lameness in the light of functional anatomy and biomechanics. A number of studies have been done to compensate the limitations of the subjective evaluation of lameness. Nevertheless, an interesting debate arose a few years ago about the use of quantitative gait analysis and the significance of the objective technological data obtained. Another objective of this paper is to demonstrate that there is still room for a visual (and physical) asymmetry needs to be identified (blocking of proximal regions is not possible). But functional (and physical) asymmetry is sometimes considered as separate conditions and inter-observer agreement of lameness is poor. Nevertheless, an interesting debate arose a few years ago about the use of quantitative gait analysis and the significance of the objective technological data obtained.

Lameness or Laterality?

In most cases, lameness is a clinical expression of pain, with the horse redistributing the load between limbs (kinetic aspect) and this results in asymmetrical gaits or movements (kinematic aspect). However, is asymmetry a lameness? Having examined sound and lame horses for more than 30 years, live and subsequently on video recordings, I still don’t know what a sound horse is. I have read numerous textbooks and an endless number of papers on different types and grades of lameness based on the severity of asymmetrical load distribution or manifestations, but I can hardly identify a physically and functionally symmetrical horse. Most of (if not all) the horses show some degree of asymmetry when examined at different gaits or at work. Asymmetry at the walk or at the trot is common and can be objectively identified using gait analysis systems. Is it possible to discriminate a low grade of lameness from asymmetry in horses? Contrary to the majority of lameness, asymmetry is not pain related. Asymmetry is not improved with rest, it does not worsen with the level of exercise or when the athletic demand increases; it can be compatible with adequate performance and it sometimes improves with exercise. Therefore, recognition of asymmetry versus lameness is easier when doing longitudinal rechecks of horses over prolonged periods (up to several years). Clinically, there is no change after performing adequate diagnostic analgesia (although this procedure is not entirely sensitive as complete blocking of proximal regions is not possible). But functional (and physical) asymmetry needs to be identified as it can predispose to secondary problems such as tendon disease on the limb carrying more weight. Differentiation between lameness and asymmetry or laterality is especially crucial when performing a single examination such as in a pre-purchase examination. It is sometimes difficult to conclude in this context and reexamination of the horse at its intended level of use is indicated to reinforce the decision. Origins of physical and functional asymmetry include the consequences of developmental conditions, sequelae of old trauma, and morphofunctional asymmetry of the nervous system command and control. Laterality in horses has been related to morphological, behavioral, occupational, neurological, or gender factors. Depending on the origin of the lameness, a differential diagnosis has sometimes been suggested to distinguish pain manifestations from mechanical lameness and neurological problems. However, in many horses two of these components are combined and are integrated in the diagnostic approach. Musculoskeletal lameness and neurological conditions are sometimes considered as separate conditions and inter-observer agreement of lameness is poor.

Basic Aspects and Terminology Used in This Paper

During a complete stride, each limb achieves a stance (weight-bearing) phase and a swing (non-weight-bearing, pendular) phase. The stance phase can be broken down into three parts: (a) the cranial part or load absorption phase, starting after landing; (b) an intermediate or mid-stance phase with full weight bearing on the limb; and (c) a caudal or propulsion phase ending with the break over (from heel off to toe off). The swing phase can be divided in a pulling phase (the limb being pulled forward) especially demanding at high speed, a mid-swing phase where maximal flexion angles take place, and a protraction phase ending at landing. From a clinical point of view, it is essential to analyze the chain of events happening during the cranial and caudal parts of the stride. The cranial phase (or part) of the stride is the part of angular displacement of the limb cranially to a vertical line dropped from the center of motion of the limb. This line is easily drawn on the hindlimb from the coxofemoral joint. On the forelimb, the scapula is linked to the thorax with muscles (synsarcosis) and slides over the thoracic wall. As a result, this line moves cranio-caudally and, as a practical approach, the spine of the scapula at the middle of this bone can be chosen to intersect with it. The cranial part of the stride begins with the protraction phase that ends at landing and continues with a load absorption phase up to mid-stance phase. During the caudal phase (or part) of the stride, the limb moves caudally to the vertical line, first during the caudal part of the stance phase when the limb is displaced caudally up to break over and then during the caudal part of the swing phase (pulling phase) when the limb is displaced cranially up to the mid-swing position. According to the timing of clinical manifestations, different types of lameness have been identified with some variations between authors.
Stance phase lameness conditions (or supporting limb or weight bearing lameness) are the most common ones and have been widely presented and discussed. The horse uses the inertia of its body mass to increase deceleration, bear more weight, and achieve greater propulsion from the sounder limb. On the lame limb, the horse reduces shock absorption (shock absorption lameness; decreased cranial phase of the stride), decreases load (mid-stance or weight bearing lameness), and/or reduces propulsion (propulsion lameness or push off lameness).

Swing phase lameness conditions (swinging limb lameness) are often underestimated. They show up during the pulling forward part (e.g., shoulder syndrome including scapulohumeral arthropathies and bicapital apparatus injuries), mid-swing (e.g., decrease flexion because of tendonitis of the extensor carpi radialis, fetlock partial ankylosis; or increase flexion angles: rupture of the extensor carpi radialis), and protraction (e.g., reduced protraction: shoulder syndrome, nerve and muscle paresis; increased swing of protraction: deficit of the digital flexor muscles).

Mixed lameness conditions are common. Even if the clearest manifestations happen during either the stance or the swing phases, many single causes of lameness may alter both (from prootrochlear syndrome to shoulder problems; Fig. 2).

The primary or baseline lameness is not always the most obvious and the primary cause of a lot of flexor tendon or suspensory apparatus injuries must be searched for an adequate management of the horse.

Secondary lameness is also called complementary or compensatory lameness. Pain in one limb induces redistribution of the load on the other limbs. As a typical example, a superficial digital flexor (SDF) tendon injury on a forelimb can be the consequence of pain on the opposite forelimb or the opposite hind-limb. But, considering load redistribution between...
forelimbs and hindlimbs, compensatory lameness should not be confused with secondary lameness due to asymmetry. This paper focuses on qualitative data. When mentioned, the severity of the lameness is expressed using the AAEP grading scale, although this scale is mainly based on stance phase alterations and other grading systems have been used. For more clarity, the gait characteristics of the lame or lamest limb (referred to as the ‘lame limb’) are considered. In multiple limb lameness, the combination of single limb characteristics may vary extensively depending on the number of sites of pain or mechanical defects. Flexion tests and diagnostic analgesia are not considered in this paper. Horse management during examination in hand, on the lunge, or when the horse is ridden or driven is important for both horse and human safety but is beyond the scope of the paper.

2. Hard (or Firm) Surface

Examination at the Walk

A common limitation of the clinical assessment of lameness is to restrict the examination at the trot and to rapidly move on to diagnostic analgesia. Examination at the walk can provide valuable information and is easily accessible as the movements are slow (pedagogical/didactic value). Besides, in very painful horses, this should be the only situation used to evaluate the lameness. As the walk is a slow gait with no suspension phase, there is less load impact and less maximum load at mid-stance phase, but there is an increased stance phase duration. Therefore, the clinical manifestations of a horse at the walk are more related to movement (kinematics) than to forces (kinetics).

Straight Line

Examination of the horse at the walk on a straight line on a hard and horizontal surface is probably the best situation to analyze the limb conformation, fetlock support, and foot landing. Looking at the horse from the front, with the horse coming toward the observer, is more reliable than the standing examination for assessment of limb conformation such as varus/valgus deformities of the carpus and fetlock. The cranial view is also useful for assessment of the foot landing and balance. Even moderate, angular deformities have direct consequences on the load distribution within the joints and their identification is an essential contribution to the management and even the prevention of associated or secondary injuries. Besides, as alternate muscle contraction highlights left to right asymmetry, identification of myopenia (muscle atrophy) is easier and more reliable at the walk than during the static examination, especially on the hindlimbs.

Forelimbs. Stance phase (weight-bearing) lameness conditions. To reduce the load absorption and maximum load on the affected limb, horses with very painful forelimb lameness demonstrate wide head oscillations (with higher velocity of the head dropping on the sounder limb) and decreased protraction during the swing phase (Fig. 3). Therefore, the affected limb shows a decreased fetlock extension during the stance phase and an increased fetlock extension on the sounder limb is observed. A hindlimb interference can be seen with increased protraction of the opposite hindlimb in an attempt to reduce the load on the affected forelimb. Horses with severe pain of the suspensory apparatus (e.g., proximal sesamoid bone fracture; Fig. 3) present a reduction of the cranial phase and an extended caudal phase of the stride. This is correlated to biomechanical data showing a higher tension of the suspensory apparatus and SDF tendon during the first part of the stance phase at the walk (Fig. 4). Several studies show that at the walk the peak of load on the deep digital flexor (DDF) apparatus (DDF tendon and its accessory ligament [AL-DDF tendon]) takes place at the end of the stance phase. This explains why horses with severe pain in the DDF apparatus or in the podotrochlear apparatus (navicular syndrome) show a reduction of the caudal phase of the stride and...
a wider cranial phase (Fig. 5). The same manifestations can be seen with injuries of the DDF tendon in contact with a radial osteochondroma or a fracture of the olecranon providing attachment to the ulnar head of the DDF muscle. A foot abscess at the dorsal aspect of the sole may also induce shortening of the caudal phase of the stride as pressure increases over the toe area at the end of the stance phase. A decrease in fetlock suspension on the lame limb may be present in cases of failure of the suspensory apparatus or SDF tendon rupture or elongation. But because of the limited load during the stance phase at the walk, most of the horses with suspensory apparatus desmopathies or proximal suspensory ligament desmopathies/enthesopathies are asymptomatic at this gait. In some horses, the reduction of fetlock extension may be the only dynamic manifestation of suspensory disease at the walk (Fig. 6). Extension of the distal interphalangeal joint at the end of the stance phase with sudden elevation of the toe is pathognomonic of DDF tendon elongation or rupture. Shoulder instability is often clearer at slow gaits compared to faster gaits. Suprascapular nerve paralysis (sweeney syndrome) typically induces a varus of the shoulder joint worse at the end of the stance phase. Indeed, the lateral stability is no longer achieved by the infraspinatus muscle body eccentric and isometric contractions. This manifestation is accompanied by a reduction of the forelimb protraction (cranial part of the swing phase) as a result of a combined paresis or paralysis of the supraspinatus muscle making it unable to extend the shoulder joint during the swing phase.

Swing phase lamenesses. Two typical examples of swing phase lameness can be observed in clinical cases. Some young horses with lesions of the shoulder joint (osteochondrosis, osteochondrodisplacement, fracture of the tuberculum supraplanoidea) show a marked decrease of protraction, wide head oscillations with a higher velocity during elevation of the head, synchronous of protraction of the affected limb. Pain during the swing phase is caused by the eccentric contraction of the supraspinatus muscle extending the shoulder joint as a Type 1 lever arm; the pressure over the articular surfaces of the humeral head and glenoid cavity of the scapula are therefore increased (Fig. 7). This lameness also has a stance phase component characterized by lowering of the head on the sounder limb, reduced fetlock

Fig. 4. Functional anatomy of the flexor tendons and suspensory apparatus of the forelimb during the stance phase. A, Cranial part of the stance phase: extension of the fetlock concentrates the stresses on the SDF tendon and the suspensory apparatus. B, Mid-stance phase: all tendons and accessory ligaments contribute to fetlock suspension. C, Caudal part of the stance phase: The distal interphalangeal joint (DIPJ) extension induces the highest tension of the deep digital flexor (DDF) apparatus (DDF tendon and its accessory ligament); the elevation of the fetlock reduces the tension on the suspensory apparatus, but active superficial digital flexor (SDF) muscle contraction still puts stress on the corresponding tendon. SDFT: superficial digital flexor tendon; DDFT: deep digital flexor tendon; TIOM: third interosseous muscle (suspensory ligament); AL: accessory ligaments of the flexor tendons.

Fig. 5. Five-year-old Standardbred trotter female presenting a fracture of the lateral palmar process of the distal phalanx on the right forelimb. There is a reduction of weight on the affected limb with a marked reduction of fetlock extension (image B). There is also a shortening of the caudal phase of the stride as distal interphalangeal joint extension puts stress on the deep digital flexor tendon partly inserted on the fracture.

Fig. 6. Seven-year-old Thoroughbred crossed gelding steeplechaser at the walk in hand. There is a defect of extension of the right fetlock compared to the left. This horse had a chronic proximal suspensory ligament enthesopathy on the right forelimb, was considered sound by the trainer, and went back to full training without recurrence of lameness.
extension, lengthening of the caudal part of the stride on the lamel limb, and hindlimb interference. Horses with injuries to the bicipital apparatus (biceps brachii, sulcus intertubercularis, bicipital bursa) also display a shortening of protraction and of the cranial phase of the stride. Horses with brachial plexus nerve root impingement caused by caudal cervical spine arthropathies may demonstrate moderate to severe reduction of the cranial phase of the stride due to paresis of the muscles achieving the protraction (Fig. 8). In most of these secondary neurological cases (with primary cervical arthropathies), there is less alteration of both head movements and fetlock suspension and reduction of the cranial phase of the stride is less visible at faster gaits. Developmental or acquired arthropathies of the caudal cervical articular process joints (C5-T1), responsible for brachial plexus nerve roots pathological and functional alterations are part of what can be called the equine cervicothoracobrachial (CTB) syndrome (Figs. 8–10). This syndrome overlaps what is described in human medicine for a long time as the cervicobrachial syndrome and the thoracic outlet syndrome, although horses lack a clavicle. Other lesions causing the same typical dyskinesia of protraction of the forelimb in horses include caudal cervical intervertebral disc lesions, arthropathy of the first costovertebral joint and congenital abnormalities of the first rib such as agenesia, hypoplasia, dystrophy, and synostosis with often incomplete and dystrophic fibrous union of the first two ribs. All these abnormalities or injuries are in close anatomical relation with the brachial plexus components, either with its nerve roots (ventral rami of C6 to T1 cervical spinal nerves at the level of the intervertebral foramen) or the brachial plexus itself, passing between the scalenus medius and the scalenus ventralis muscles and then crossing over and lying on the first rib. Resulting alteration of the nerve conduction is responsible for paresis of the muscles achieving the protraction. They include the supraspinatus muscle innervated by the suprascapular nerve (coming from the cervical nerves C6 and C7), the cleidobrachialis muscle innervated by the axillary nerve (coming from C7 and C8), and the biceps brachii and brachialis muscles innervated by the musculocutaneous nerve (coming from C6, C7, and C8) (Fig. 9). Involvement of the radial nerve roots (C7, C8, and T1) or pathway may induce forelimb

Fig. 7. Supraspinatus and biceps brachii muscles acting as a Type 1 lever arms for extending the scapulohumeral joint. Concentric contraction of these muscles pulls the distal part of the limb cranially and increases the pressure between the humeral head and the glenoid cavity of the scapula (broken arrows). 1, Supraspinatus muscle. 2, Biceps brachii muscle. Fss: force generated by the supraspinatus muscle contraction; d: distance between the tuberculi of the humerus and the center of rotation of the humeral head; Fbb: force generated by the biceps brachii muscle; d and d’: distance between the tuberculi and sulcus intertubercularis from the center of rotation of the humeral head; M: mass of the limb distal to the shoulder joint; l: distance between the center of rotation of the humeral head and the center of M; Fm: force generated by the acceleration of M as a result of the action of Fss and Fbb. All three forces are synergistic to increase pressure on the scapulohumeral articular surfaces.

Fig. 8. Eight-year-old Selle-Français femelle jumper presenting a marked reduction of the left forelimb protraction. Notice the elongated caudal phase of the stride of the same limb. The reduced fetlock extension and the high heels of the left forelimb are expression of the chronicity of this forelimb asymmetry. This horse had an arthropathy of the left C7-T1 articular process joint.
paresis (muscle weakness) further predisposing
the horse to faulty steps, stumbling or even
falling. These manifestations are often intermittent
but because of the danger for the rider a special

attention should be given to the quality of forelimb
protraction, which may be affected unilaterally or
bilaterally. Swing phase lamenesses are rarely seen
alone. With shoulder joint or bicipital apparatus
injuries, there is often a stance phase component of
the pain when load is put on the affected limb. For
horses presenting a CTB syndrome, there is often an
elongated caudal part of the stride compensating the
shortened cranial phase (Fig. 8).

Hindlimbs. Weight bearing (stance phase) lame-
nesses. Looking at the hind fetlock suspension dur-
ing the stance phase at the walk is essential as a
decrease in suspension can be compensated with acti-
vation of the flexor muscles and tendons at faster
gaits. The most common causes of this defect include
degenerative suspensory apparatus lesions and se-
quela of juvenile tendon laxity. A unilateral or bilat-
eral defect of fetlock suspension with a low pastern
axis is seen in degenerative suspensory disease (in
any location between its proximal insertion and its
branches). It must be noted that a horse that is lame
because of pain at the origin of the suspensory liga-
ment can present with a defect of fetlock suspension
despite a load reduction on the affected limb. These
clinical manifestations are the result of the functional
alteration created by the anatomopathological degen-
eration of the suspensory apparatus. A decrease in
fetlock suspension can also be seen in horses present-
ing with SDF tendonitis or laceration, or proximal
SDF tendon degenerative enthesisopathy at the level of
the supracondylar fossa of the femur. Contrary to
horses with prominent degenerative suspensory dis-
ease, horses with painful proximal suspensory enthe-
sopathies show a decreased fetlock extension. Many
other conditions can be responsible for the reduction
of fetlock extension during the stance phase at the
walk. The differential diagnosis includes foot
abscesses, fetlock problems (Fig. 11), severe suspen-
sory apparatus pain, severe distal tarsus pain,
femorotibial joint pain, stress fractures, and others. Most of hindlimb lameness conditions induce a reduction of the cranial phase of the stride (clearer at the trot), but reduction of the caudal phase with lateral rotation of the limb can be seen in some conditions such as coxofemoral joint injuries (fracture of the acetabulum or of the femoral neck). This manifestation is correlated to the tension on the capitis and accessory ligaments of the hip joint that increases with medial rotation of the femur and decreases with lateral rotation. It is often associated with deviation of the pelvis and hind quarters, with the horse walking sideways. Fractures of the tuber coxae (and some fractures of the tuber ischiadicum) may induce a reduction of the caudal phase of the stride. The ventral displacement of the affected tuber coxae induces a shortening of the tensor fascia latae and a limitation to hip extension. Looking at the horse laterally at the walk is the best situation to assess the hindlimb sagittal conformation and identify the “straight hock-low fetlock” syndrome. Although this condition can be the result of congenital or juvenile tendon laxity, it is often seen in horses presenting with progressive degenerative suspensory ligament disease. It is sometimes considered erroneously that horses with straight hocks are predisposed to suspensory disease. Biomechanically, the pathophysiology of this syndrome follows this sequence: a degenerative suspensory disease induces a fetlock suspension defect; hyperextension of the fetlock increases tension on the SDF tendon, which pulls the tuber calcanei cranially and induces secondarily hyperextension of the hock. Therefore, a straight hock conformation is not a predisposing factor to suspensory disease but is just the result of an existing elongation of the suspensory ligament (Fig. 12). On the other hand, a defect of hock extension can be seen in horses presenting with a luxation of the SDF tendon from its normal position over the tuber calcanei. Twisted hock syndrome is better identified at the walk when the horse is examined from the rear. This condition shows up during the caudal phase of the stride and has been seen in horses with femorotibial problems. A potential cause is an asymmetric radius of curvature of the medial and lateral femoral condyles, inducing a combination of lateral displacement and medial rotation of the tibia. This results in a varus of the hock at the end of the stance phase. The manifestation is often reduced at the trot or at faster gaits. A defect of hock extension combined with hyperextension of the stifles and dorsal subluxation of the fetlock has been seen in horses with sciatic nerve paresis. Paresis of the gastrocnemius muscle is responsible for hock extension defect and hyperextension of the stifles, both inducing tension of the SDF tendon responsible for fetlock flexion. A defect of stifle extension during the stance phase is typical of femoral nerve paralysis which induces a paresis or paralysis of the quadriceps femoris muscle. This muscle becomes unable to achieve its eccentric contraction during the first part of the stance phase and its concentric contraction to open the stifle during the propulsion. Increased extension of the stifle may be secondary to osteochondrosis of the lateral trochlear ridge, with the horse displacing the patella proximally to avoid pressure on the lesion or to reduce patellar instability over a dysplastic femoral trochlea.

Swing phase lamenesses. The typical example of a hindlimb swing phase lameness is the rupture of the peroneus tertius. The affected horse shows a pathognomonie lack of hock flexion despite a hyperflexion of the stifle; there is no or little incidence on the stance phase. This mechanical lameness is induced by rupture or elongation of the peroneus tertius, making it no longer able to achieve the passive solidarization between stifle flexion and hock flexion during the swing phase. It must be added that as the hock no longer flexes, the calcaneus lever arm does not act anymore on the SDF tendon to induce flexion of the fetlock joint which remains extended. Different conditions cause hyperflexion of the hindlimb joints in different parts of the swing phase. Hindlimb joint hyperflexion after breakover is seen in horses presenting foot abscesses over the toe area. When pressure is concentrated on the dorsal part of the foot at the end of the stance phase, pain is responsible for sudden active flexion and elevation of the foot arch at the beginning of the swing phase. In case of upward fixation of the patella, a sudden spastic high velocity hyperflexion of the hindlimb can be seen during the first part of the swing phase when the patella is removed from the tuberculum of the femoral trochlea after a sudden lateral rotation induced by contraction of the gluteofemoralis muscle. Sudden reduction of protraction at the end of the swing phase with caudal retraction of the distal limb just before foot landing is typical of fibrotic myopathy of the semitendinosus muscle (Fig. 13). Other muscles such as the semimembranosus and the gracilis muscles can be
affected with this condition and present similar manifestations. Although often considered as a mechanical lameness, this typical gait can be seen in horses without muscle fibrosis. Stringhalt is another swing phase lameness showing up at the walk. Hyperflexion of all hindlimb joints during the swing phase and worse during the cranial part of it, has been attributed to a lack of proprioceptive adjustment and muscle coordination. This disease highlights the passive coordination between all the hindlimb joint angles which flex in unison (Fig. 14).

Mixed Lameness at the Walk. Horses affected with proximal or distal peroneus tertius enthesopathies may present a swing phase lameness and a weight bearing lameness. A lameness induced by a foot abscess may present a stance phase and a swing phase component (see above). Neurological problems such as sciatic nerve paralysis or paresis can cause a lack of fetlock extension during the stance and swing phases.

Alternating Circles on a Firm Surface

Forelimbs. Horses reproduce on the circles the manifestations described on a straight line. Nevertheless, examination on alternating circles is essential as sometimes this is the only or the most demonstrative situation for detection of obvious to subtle changes of common conditions. As the limbs at the walk on a short circle combine a long stance phase duration with collateral motion and associated rotation, most of the horses affected with a podotrochlear syndrome (navicular disease) present with a reduction of the caudal phase of the stride (propulsion) when the lame limb is on the inside of the circle (Fig. 15). This typical gait alteration is induced by asymmetrical stresses on the podotrochlear apparatus (distal sesamoid bone, DDF tendon, and associated structures) as a result of collateral motion and rotation. These extra-sagittal movements are higher on the distal limb on the inside of the circle which is more oblique than the outside limb over the examination surface. Moreover, they are exacerbated at the end of the stance phase (Fig. 16). Horses with bilateral podotrochlear syndrome demonstrate this reduction of the caudal phase of the stride on both turns in an alternate manner; this results in a shortened gait. The swing phase protraction follows a longer pathway on the outside limb and reduction of protraction is sometimes clearer when the affected limb is outside of a short circle compared to the straight line (with the same causes: shoulder syndrome, CTB syndrome). On both the inside and outside limbs, special attention should be given to intermittent manifestations of carpus instability or of toe dragging as subtle manifestation of paresis.

Hindlimbs. Examining the horse on alternating circles at the walk provides another opportunity to check for joint motion, fetlock suspension, and changes in the cranial or caudal phases of the stride. As described for the forelimbs, collateral motion and...
rotation are responsible for asymmetrical distribution of stresses and exacerbation of pain on affected structures of the distal limb. However, generally speaking, as there is less load on the hindlimbs, the manifestations of distal hindlimb lameness at the walk are less demonstrative than on forelimbs. For example, in horses presenting with podotrochlear syndrome in a hindlimb, reduction of the caudal phase of the stride on the turns is less clear than on forelimbs. Nevertheless, pain can be exacerbated in case of injuries involving the distal and proximal interphalangeal joints as well as the fetlock joint especially when the proximal sesamoid bones are affected. The walk on alternating circles is one of the most informative situations to detect neurological problems on the hindlimbs and especially ataxia, paresis and dysmetria. A shortening of the hindlimb stride length accentuated on the turns may be seen in horses with lumbosacroiliac pain or dysfunction.91–92

**Axial regions.** A normal horse at the walk on alternating circles demonstrates relaxation and alternating lateroflexion of the axial regions. In the vertebral column, lateroflexion on one side is spontaneously associated to rotation on the opposite side.93 These combined movements induce shearing of the interspinal ligaments and spinous process margins. Besides, the sacroiliac joint undergoes greater range of flexion-extension, lateroflexion, and rotation at the walk than at the trot.94 Neck, thoracolumbar, or pelvic pain may induce a short and stiff gait, a lack of lateral bending, and sometimes counter-curvature (counter-bending).

Fig. 16. Functional anatomy of the distal interphalangeal joint of the left forelimb when the foot position is asymmetrical on a left turn. Elevation of the lateral quarter induces medial rotation of the distal phalanx (P3), tension of the medial collateral ligament (MCL) and relaxation of the lateral collateral ligament (LCL). As the lateral part of the condyle of the middle phalanx moves in a palmar direction, pressure is concentrated on the lateral part of the distal sesamoid bone (DSB) and the distal sesamoidean ligament (DSL) is stretched laterally. The lateral lobe of the deep digital flexor tendon (DDFT) receives pressure from the DSB and the medial lobe of this tendon is stretched.

Fig. 17. Ground reaction forces on a vertical (Fz-A) and horizontal cranio-caudal (Fx-B) axes of a 600-Kg body weight steeple chaser at the walk (1.4m/s), trot (4.3m/s) and canter (leading limb, at 6, 8, and 10m/s for Canter 1, 2 and 3). The vertical and horizontal forces increase with the speed (From Robin D, Chateau H, Falala S, et al. 2008, with permission).
trotting speed should be adapted and consistent on repeated occasions for diagnostic purposes and to check the horse after diagnostic analgesia or treatments.97,98

Forelimbs at the trot on a straight line. In most situations (except in perfectly bilateral lameness), to decrease the loading rate and load intensity on the lame limb the horse uses the inertia of the swing of the cervicocephalic pendulum (head and neck) and decreases the cranial phase of the stride. The result of this strategy is a reduction in fetlock extension. How is this strategy expressed clinically?

When examining a horse from the front (or the rear), alteration of the sinusoidal pattern of the head trajectory showing asymmetrical oscillations is the most obvious clinical manifestation of a forelimb lameness. These asymmetrical head and neck oscillations can also be seen from the side, but as head movements can disturb their regularity, this criterion can be difficult to assess, especially for low grade lameness. Altered sinusoidal pattern of the vertical displacement over time of the withers is often a more reliable manifestation of the horse’s strategy to put more weight on the sounder limb (Fig. 18).27 To decrease cumulation of vertical (z axis) and horizontal (x axis) loading rate after landing at the trot on a hard surface, the lame limb shortens the cranial phase of the stride. This is seen in many conditions including interphalangeal, fetlock, carpus, elbow, and shoulder conditions. In horses with pain in the suspensory apparatus, the increased loading rate combined with a peak of load in this apparatus during the cranial part of the stance phase67,71 induces a clear reduction of the cranial part of the stance phase at the trot compared to the walk. As mentioned above for shoulder and CTB syndromes, reduction of the cranial phase of the stride is often clearer at the walk. Even when a horse has demonstrated a reduction of the caudal phase of the stride at the walk (e.g., for podotrochlear syndrome), the cranial phase of the stride is more affected at the trot. It seems like the horse is more sensitive to the increased forces at the trot (kinetic effect) and less sensitive to the movement (kinematic effect). As mentioned above, at the walk, a slower movement and a longer stance phase duration without suspension phase increase the impact of distal interphalangeal joint extension on clinical manifestations. The decreased vertical load intensity and the reduction of the cranial phase of the stride induce a decreased fetlock extension on the lamer limb compared to the opposite limb (Fig. 18).58,59 This reduction of fetlock extension is more pronounced in horses with pain in either the fetlock or the suspensory apparatus. In horses affected by a functional deficit of the suspensory apparatus or flexor tendons (rupture or elongation), the difference in fetlock extension during the stance phase is not proportional to the uneven loading of the limbs as expressed by the asymmetrical sinusoidal trajectory of the withers (or tuber sacrale for the hindlimbs). Some more proximal joint or periarticular injuries of the forelimb induce changes in movements of flexion-extension. This is especially seen for shoulder injuries (e.g., osteochondrosis, bicipital apparatus injuries) and carpal, carpal canal or extensor carpi radialis injuries.

Hindlimbs at the trot on a firm straight line. When examining the horse from the rear, redistribution of the load from the lamer limb to the sounder limb is associated with an asymmetrical...
sinusoidal pattern of the tuber sacrale trajectory. Comparison of the left and right tuber coxae trajectories has also been used for diagnosing hindlimb lameness.99 Nevertheless, as demonstrated by most of the technical devices used for gait analysis, the tuber sacrale trajectory is a more reliable reflection of the horse’s strategy to put more weight on the sounder limb. This is expressed in an increase in downwards displacement of the tuber sacrale during the cranial part of the stance phase of the sounder limb which subsequently moves up the hindquarters (and tuber sacrale) using the inertia of the bodyweight to reduce the load during the stance phase of the lamer limb (Fig. 20). Discrimination between load absorption lameness and propulsion lameness can be done by considering the trajectory of the tuber sacrale.32,33 Alteration of the sinusoidal pattern of the head (cervicocephalic pendulum) movement can be seen with moderate to severe hindlimb lameness. Lowering of the neck is synchronous to the stance phase of the lamer hindlimb in an attempt to shift the load to the forelimb diagonally. For example, to reduce the load on a painful left hindlimb, the horse increases the weight on the right forelimb, the head exhibiting a lower position during the right diagonal stance phase. This has been confirmed using kinematic analysis of lame horses and of induced lameness on a treadmill.100,101

Examining the horse at the trot from the side facilitates identification of a reduction of the cranial phase of the stride. Just as on the forelimbs, the shortening of the engagement reduces the horizontal component of the ground reaction force and the duration (and therefore the impulse) of the load absorption part of the stance phase. As a consequence of the weight redistribution and shortening of the cranial phase of the stride, asymmetrical extension of the fetlocks can be seen from the side and the rear, with the horse exhibiting more extension of the fetlock in the sounder limb and less extension in the lamer limb (Fig. 20). However, in horses with alteration of fetlock suspension induced by elongation of the suspensory apparatus or the flexor tendons, fetlock extension can be exaggerated despite a reduction of load. In addition, asymmetrical flexion of the hocks during the stance phase can be observed with less flexion on the lamer limb due to the reduction of weight inducing less passive elongation of the SDF muscle and tendon as well as the superficial and deep aponeuroses of the gastrocnemius and lateral digital flexor muscles (Fig. 20 and 21). Dragging of the toe of the lame limb close to its mid-swing phase is a swing phase abnormality but not necessarily a swing phase lameness: it is the consequence of a lack of elevation of the croup by the lame limb during its propulsion phase, followed by a downwards displacement of the hindquarters during the stance phase of the sounder limb.
Axial regions. The trot is the most adequate gait for evaluation of the passive dorsoventral flexibility of the thoracolumbar spine. It is reduced in horses with back pain (F. Audigié, unpublished data) and during the stance phase of a lame diagonal. Its visual assessment helps to diagnose horses with pain or injuries of the thoracolumbar spine (kissing spines, articular process arthropathies, spondylosis) or of the lumbosacroiliac area.

A horse with back pain or caudal neck pain tries to reduce thoracolumbar flexion and extension movements by increasing the axial muscle tone. Clinically the horse demonstrates back (or neck) stiffness. It must be mentioned that horses with bilateral limb problems (especially forelimbs) reduce the elevation of the gait and therefore reduce visceral mass displacement and acceleration which consequently induces similarly a reduction of thoracolumbar flexibility. The differential diagnosis is then made by comparing the horse’s gait on soft ground which usually reduces distal limb manifestations and increases axial contribution to the gait (see below). Assessment of cervicothoracic flexibility is more difficult and less specific. It is more dependent on the forelimb locomotion.

Trot on the Lunge in a Circle on Firm Ground
Horses with symmetrical movement on the straight line have been shown to present vertical head and pelvic asymmetry during lunging and this should not be interpreted as lameness. In this study, less than half of the horses showing symmetrical gait on a straight line had inversed and equivalent asymmetrical movements of the head and pelvis when examined on the lunge in opposite directions. This demonstrates that examination on the straight line has limitations to detect pain or biological or acquired laterality of the horse.

Forelimbs at the trot on circles on firm surfaces. The majority of forelimb lamenesses are worse when the affected limb is on the inside of the circle. As the horse leans inside to balance the centrifugal force, and as the feet are closer than the proximal parts of the limbs, the inside forelimb and hindlimb are more oblique to the ground surface than the outside limbs (Fig. 23). Therefore, collateromotion and associated rotation are higher on the inside limbs. These extrasagittal movements explain why pain on distal joints and in the podotrochlear apparatus including the distal part of the DDF tendon, is exacerbated when the affected limb is inside the circle (Fig. 16). Clinical manifestations of some conditions involving the proximal limb are less affected by collateromotion and rotation but may also, to a lesser extent, be worse when the lame limb is inside the circle. Less commonly, the horse may be lamer when the painful forelimb is outside the circle because of the increased load on the outside limb (e.g., suspensory apparatus diseases) or increased pressure at the medial aspect of this limb (Fig. 24). This is observed...
with some fractures of the medial palmar process of the distal phalanx, subchondral bone pain in the medial part of the metacarpal condyle, medial splints (second or medial intermetacarpal syndesmopathy), medial third carpal or radiocarpal bones injuries, with the horse being sound or mildly lame at the walk for these last conditions. 59,116 Propulsion lameness induced by a DDF tendonitis can occasionally be worse on the opposite circle (Fig. 25). As the suspensory apparatus is more affected by the loading rate and peak of load than by collateromotion and rotation, lameness induced by suspensory disease is usually clearer on a straight line and on the opposite circle. Horses presenting dyskinetic protraction induced by CTB syndrome or shoulder syndrome (scapulohumeral arthropathies or bicipital apparatus injuries) show a slower swing phase ending with a reduced cranial phase of the stride when the affected limb is placed outside the circle. Lameness severity can be similar on both circles in case of desmopathies of the lateral collateral ligaments of the interphalangeal and metacarpophalangeal joints, subchondral bone trauma of the medial part of the metacarpal condyle, as well as some elbow and shoulder joint conditions. For (subchondral) bone conditions, pain is generated by collateromotion and rotation when the limb is inside the circle and by concentration of the pressure at the medial aspect of the affected limb when it is outside the circle. In lateral collateral desmopathies and enthesopathies, pain is generated by increased tension on the ligament when the affected limb is outside the circle and by extrasagittal movements of the painful joint when this limb is inside the circle.

Hindlimbs at the trot on circles on a firm surface. A sound horse trotting on a circle puts more load on the outside hindlimb to counteract the centrifugal force.117 It is essential to

Fig. 23. Orientation of the forelimbs on a circle on a hard surface. The horse’s body leans toward the center of the circle; the inside limb (A) is adducted and is more obliquely placed over the ground surface inducing more collateromotion (here lateromotion) on its distal joints than the outside limb (B) whose distal joints are subjected to mediomotion.

Fig. 24. Trajectories determined from inertial measurement units at the trot on a hard surface of the head, withers, and tuber sacrale of a 12-year-old jumper female presenting a subchondral bone trauma of the medial part of the metacarpal condyle on the left forelimb. On a straight line, the mare showed a very mild reduction of the load on the left forelimb (LF) and puts more load on the right hindlimb as a potential expression of diagonal compensation. On the left circle the trajectory of the withers is symmetrical but the head drops on the right forelimb (RF); after elevation of the croup by the inside LH, the tuber sacrale drops more on the right hindlimb. On the right circle, although there should be more load on the outside LF, the head and withers markedly drop during the RF stance phase (red line). The subchondral bone pain at the medial side of the fetlock is responsible for this stance phase lameness, exaggerated on the opposite circle. As the head drops during the RF diagonal stance phase, there is less load on the outside LH. The different curves represent the trajectories of successive strides.
compare the asymmetrical movements of the pelvis and fetlock extension of both hindlimbs on the left vs right circle to avoid overdiagnosis of lameness. This can be demonstrated establishing the asymmetrical vertical movement pattern of the tuber sacrale using inertial sensors (inertial measurement units) showing more downwards displacement of the tuber sacrale during the stance phase of the outside hindlimb.\textsuperscript{114,118–120}

Compared to the normal range of hindlimb asymmetry on circles, horses presenting interphalangeal or metatarsophalangeal arthropathies are lamer, with a reduction of the cranial phase of the stride when the affected hindlimb is inside the circle as limb obliquity increases collateral motion and associated rotation causing pain.\textsuperscript{87} As the load is higher on the outside limb,\textsuperscript{117} horses presenting with pain in the suspensory apparatus may be lamer when the affected limb is outside in a circle on a hard surface. In horses with severe pain, the downwards displacement of the tuber sacrale can be seen on the sounder inside hindlimb. Nevertheless, horses with desmopathy or enthesopathy of the medial branch of the suspensory ligament may stay lamer when the affected limb is inside the circle as lateromotion increases the stress of this branch. Other conditions involving the medial aspect of the hindlimb such as distal tarsus osteoarthritis or spongy (subchondral) bone cyst in the medial femoral condyle may be lamer when the affected limb is outside the circle, as pressure forces are increased medially.

Axial regions on circles. On the circle, the vertebral axis of the horse combines movements of flexion-extension movements with lateroflexion and rotation and the range of motion is greater compared to the straight line.\textsuperscript{93,100,118} Therefore, visual assessment of the amount of motion is facilitated especially when the horse decelerates from trot to walk. At the trot on the lunge in a circle, the sinusoidal pattern of the thoracolumbar oscillation is different for the two diagonal stance phases. As there is more load absorption on the outside hindlimb, there is more extension during the stance phase of this limb than during the stance phase of the inside hindlimb. These asymmetrical flexion-extension movements combined with lateroflexion and rotation can exacerbate thoracolumbar pain and create discomfort resulting in back stiffness and reduction of the stride length and suspension.

3. Soft Surface on the Lunge

When examining a horse in a circle on soft ground care should be taken by the operator holding the lunge as horses can buck from happiness on soft surfaces and may be dangerous, especially if they had a reduction of physical activity before the examination.

Examination at the Trot on the Lunge on Soft Ground

Comparing the gait abnormalities on soft and hard surface contributes significantly to the differential diagnosis of the cause of lameness and to the critical
assessment of the significance of imaging findings at the end of the examination. A soft surface is chosen to change the biomechanical stresses on the limbs; deep surfaces should be avoided for the routine diagnostic approach as the muscular demand for propulsion is increased and active efforts mask diagnostic information. Trotting speed and the radius of the circle may influence the grade of asymmetrical movements. Generally speaking, on soft ground surfaces the loading rate and loading intensity on the limbs decrease (Fig. 26) but propulsion forces and therefore the load on the tendons increase (Fig. 27). Moreover, on soft deformable surface, collateral motion and associated rotation are reduced as the foot penetrates more and the position of the center of pressure moves toward the center of the foot (Fig. 28). Therefore, the clinical manifestations of foot problems and distal or intermediate limb arthropathies improve more than those of proximal limb arthropathies or tendinopathies. Lameness induced by recent tendinopathies tend to be worse on a soft ground.

Discussion on the properties of different types of surface is beyond the scope of this paper.

**Forelimbs**

When the affected limb is on the inside of the circle, horses presenting with chronic foot problems or distal arthropathies are improved on soft ground compared to the clinical manifestations on a hard surface. Improvement is less clear for intermediate arthropathies and mild or absent for proximal lameness. The lameness is rarely worse on soft ground, but this can be seen for recent and severe distal DDF tendon injuries. When the affected limb is on the outside of the circle, horses presenting recent injuries of the suspensory apparatus or flexor tendons (SDF and DDF tendons) are not improved or can be worse on soft ground. Several biomechanical factors are responsible for that including the higher load on the outside limb, the increased need for propulsion forces on soft ground, and the interphalangeal flexion reducing the DDF tendon contribution to the fetlock support.

As seen in a circle on a hard ground, horses presenting dyskinetic protraction induced by CTB syndrome or shoulder syndrome show a slower protraction during the swing phase ending with a reduced cranial phase of the stride when the affected limb is placed outside the circle.

**Hindlimbs**

Many lame horses with distal or middle hindlimb injuries improve on soft ground compared to similar situations on hard ground as a result of the reduction in concussion, load absorption and extrasagittal movements, especially when the lamer limb is on the inside of the circle. Horses presenting pain in the suspensory apparatus typically present an exacerbation of the lameness when the affected limb is on the outside of the circle. As for forelimbs, this is explained by the higher load on the outside hindlimb, the increased propulsion forces on soft ground and the lower DDF tendon contribution to the fetlock support. The same observation can be made with horses presenting lesions of the gastrocnemius or digital flexor (SDF, DDF) tendons. When the affected limb is on the outside of the circle, horses presenting with recent episodes of pain in the distal tarsus (bone spavin) or in the medial femorotibial joint are not or mildly improved as the reduction of loading rate on soft ground is canceled by the concentration of load at the medial aspect of the joint.

**Axial Regions**

In horses with pain or sensitivity in the distal joints, the suspension phase is reduced and passive vertebral axis flexibility decreases on the circle on a hard ground (see above). As these horses are more comfortable on a soft surface and display more active gaits, examination of the horse trotting on the lunge on soft ground facilitates assessment of the passive...
thoracolumbar, lumbosacral as well as cervicothoracic flexibility. \textsuperscript{104,111} Criteria include the amount of motion, relaxation, and lateral bending on the circle (Fig. 30).\textsuperscript{112} They are altered in horses with back, neck, or lumbosacroiliac pain or stiffness (Fig. 31).

Examination at Canter on the Lunge on Soft Ground
In a study performed on 12 galloping horses on a straight line, no significant difference was observed in head and pelvis acceleration and rotation, limb timing, and stride duration measurement, before and after induction of lameness in the forelimb and hindlimb.\textsuperscript{37} Comparing left and right circles, asymmetry can be detected in forelimb and hindlimb movements as well as in axial positioning and mobilization. Canter provides another opportunity to assess the horse's coordination as ataxia and hypermetria are sometimes revealed or clearer at this gait.

Axial Regions
At the canter, the thoracolumbar and lumbosacral regions are actively moved by extensor epaxial (erector

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Fig. 27. In vivo evaluation of the force in the superficial digital flexor tendon at the walk and trot on asphalt (dark curves) and on a mixed fiber sand track (light grey curves). The peak of tension takes place during the first part of the stance phase at the walk; it is higher and around the mid-stance phase at the trot. Maximal tendon force was higher on sand than on asphalt at both gaits. The duration of tendon loading was longer on sand and the area under the curve (impulse) was larger at both walk and trot. Blue frame: stance phase (From Crevier-Denoix et al. 2013, with permission).

![Graph of tension force vs time for walk and trot on asphalt and sand](image)

Fig. 28. Mean trajectory of the center of pressure (right) and vertical and longitudinal forces (left) recorded from an instrumented horseshoe placed on the right fore foot (RF) when the horse was circling at trot (3m/s) to the left (LC: left circle) or right (RC: right circle) on 4m radius circle on asphalt or sand and fiber mixed surfaces. On both circles, the trajectory of the center of pressure of the foot is closer to the sagittal plane on the soft surface. The vertical forces are higher on the soft surface and higher when the RF is outside the circle (LC). The longitudinal breaking down forces are higher on the soft surface. (From Chateau et al. 2013, with permission).
spinae and multifidus) muscles and flexor hypoxial (iliopsoas and abdominal wall) muscles.57,100,127,128 Active lumbosacral flexion and extension movements are wider and therefore easier to assess than thoracolumbar mobilization which remains proportionally limited57,100,127,129,130. The canter on the lunge (except for racing trotters!) is an adequate situation to evaluate head and neck carriage and motion as well as neck and back coordination and lateral bending.57,131 Pain or pain adaptation may affect the relaxation of the gait, the stride length, the balance of the suspension phase, the positioning and mobilization of the axial regions and the quality of lateral bending on turns. Horses with neck or back pain tend to pull on the lunge or be counter-incurvated (Fig. 31). In horses with back pain, a lack of thoracolumbar lateroflexion (lateral bending, incurvation) predisposes the horse to display a disunited or rotary canter as the inside hindlimb is not placed adequately to achieve protraction.104

Forelimbs and Hindlimbs
Biomechanical studies at the canter demonstrated that the vertical load was higher on the diagonal (trailing forelimb-leading hindlimb). There was more deceleration on the leading limbs and more propulsion on the trailing limbs.132–134 The canter highlights some defects in limb movement and especially protraction. Dyskinesia of the forelimbs induced by neurological deficits related to cervicothoracic arthropathies (part of the CTB syndrome) is usually more pronounced on the outside forelimb (Fig. 32). When the most affected limb is placed inside the circle, the horse may canter on the wrong sounder leading limb (leading limb outside) in a reiterated manner.
As mentioned above, a wrong placement of the hindlimbs (disunited canter) can be seen in horses with inadequate thoracolumbar lateroflexion. A protraction deficit induced by any injuries of the inside hindlimb can also result in a disunited canter. Looking at the horse performing downward transitions from canter to trot and from trot to walk is particularly informative on forelimb and hindlimb coordination and on how the horse is distributing the deceleration forces within the different limbs.

Small Jumps on the Lunge on Soft Ground

Examining jumpers doing small jumps on the lunge may provide additional information on forelimb and hindlimb lameness manifestations. Other objectives of this simple exercise include checking the owner or trainer’s concerns as well as anamnesis and getting information on the horse’s athletic capacities and style. Axial problems involving either the cervical or the thoracolumbar spine or the lumbosacroiliac junction need to be considered when the horse’s technique, power, and style are altered.

Abnormalities observed during jump approach and take-off include poor forelimb placement, propulsion and impulsion, lack of forequarter elevation, and poor neck mobilization or impulsion. These manifestations can be seen in horses with forelimb problems and horses with cervical or thoracolumbar injuries. A defect in hindlimb propulsion can be seen in jumpers with hindlimb, lumbosacroiliac or thoracolumbar injuries. A decrease in cervical and thoracolumbar mobility is the most obvious manifestation of back pain during the jump suspension or airborne phase. Unilateral or
bilateral deficit of forelimb or hindlimb flexion can be related to intrinsic limb injuries or to injuries of the corresponding vertebral segment and nerves. Eccentric contraction of the muscles of the proximal forelimb controlling the flexion of the shoulder and elbow joints, and eccentric contraction of the serrati and pectoral muscles as well as dorsal cervical muscles have an essential contribution to load absorption at landing after the jump (Fig. 34). 57

Neck stiffness at landing can be a manifestation of neck or thoracic pain (e.g., thoracic spondylitis). Extension and subsequent flexion of the lumbosacral area can be altered by lumbosacral pain (Fig. 35). A leading preference at landing can be the manifestation of several conditions as explained by biomechanical data (Fig. 36).137,138 As the impulse (combining load and duration represented by the area under the curve) is larger on the leading limb, a horse with suspensory apparatus or fetlock pain prefers placing the painful limb as a trailing limb (contact limb) at reception (Fig. 37). Horses with reduction of protraction as a manifestation of CTB syndrome tend to prefer landing on the lead opposite to the lesion, the trailing limb being then on the more clinically affected side.

4. Examination of the Horse at Work

Ridden Sport or Pleasure Horses

Manifestations described above can be reduced or exacerbated, not only because of the saddle and rider interference, but also because of a different behavior of the horse at work.139–142 Only key data are shortly presented in this paper.

Walk

Putting the saddle on and tightening the girth may reduce unilateral or bilateral protraction of the forelimbs. Several causes of this manifestation have been documented or suspected such as the CTB syndrome, withers, and thoracic injuries as well as costo-vertebral arthropathies as pressure over the sternum can reproduce the symptoms.

Trot on Alternating Diagonals Between Opposite Turns

Compared to the trot in hand and on the lunge, lameness at the trot on the ridden horse may be worsened because of the rider’s weight and technique or improved by appropriate rider’s management.143,144 Intensification has been seen with suspensory apparatus conditions in the forelimbs and hindlimbs. As the horse puts more load on the outside hindlimb on a circle (Fig. 29),117 trotting on diagonals alternating left and right turns is recommended to evaluate the
inversion of the hindlimbs asymmetry on opposite turns or to get more data on lameness manifestations. The influence of the rider at the rising trot should be considered.\textsuperscript{139,145,146,147} During the sitting phase of the rider, the range of motion decreases between T12 and L2 and increases between T6–T12 and L2–L5.\textsuperscript{148} Besides, the roll of the pelvis is reduced and the load increases on the sitting diagonal (Fig. 38).\textsuperscript{144,148} When a forelimb limb presents a decreased fetlock suspension during the stance phase, sitting on the opposite diagonal may decreased fetlock extension asymmetry. Therefore, when a lame forelimb presents a decreased fetlock extension during the stance phase, sitting on the corresponding diagonal can attenuate kinematics manifestations of lameness; sitting on the sounder forelimb diagonal tend to increase forelimb extension asymmetry. On a right turn, compared to symmetrical sitting trot, at rising trot a left hindlimb lameness tends to be clearer as the rider adds weight on the right hindlimb. On the left turn, the rider sits on the right diagonal (right forelimb + left hindlimb) and a left hindlimb lameness tends to be reduced.\textsuperscript{146}

Canter

Additional clinical and athletic information can be obtained at the canter including the horse’s intrinsic capacities and limitations and the rider’s influence.\textsuperscript{143} Equestrian aspects of assessment of the canter are beyond the scope of this paper.\textsuperscript{149} As for the canter in lunge, the quality of the gait, the mobilization and coordination of the limbs and joints, the positioning and mobilization of the axial regions are evaluated. Special attention should be paid when the horse is changing leads. When no obvious lameness is observed at the trot, difficulties or defenses during this exercise (to be assessed according to the level of education of the horse and rider) are usually related to proximal limb or axial problems such as the CTB syndrome for the forelimbs, thoracolumbar injuries or the lumbosacrall arc syndrome for the hindlimbs.

Jumping and Dressage Exercises

Examination of the horse at work is routinely done to investigate athletic problems or to check anamnèsis when lameness is described as happening in specific situations or exercises.\textsuperscript{140,143} This is sometimes required to evaluate diagnostic analgesic techniques in horses showing insufficient manifestations during the routine clinical examination in an attempt to get as objective an assessment of the block as possible. This can also be useful to evaluate the horse’s tolerance to different athletic exercises in order to adjust a rehabilitation program. Several studies have investigated the mechanics and kinematics of the horse while jumping.\textsuperscript{57,100,136,143,150} Influence of the height of the fence has been investigated using kinematic and kinetic analysis of the limbs of jumping horses.\textsuperscript{138,150–152}

In dressage horses, pain induced by collateromotion and rotation of the distal joints can alter the regularity of adduction and abduction movements during lateral work (e.g., half pass, shoulder in). Horses presenting a CTB syndrome tend to present a head nod on the sounder side (limb) to facilitate adduction during the swing phase of the more affected side. During a pirouette, as the horse concentrates the weight on the engaged inside hindlimb working as a pivot, the increased ground contact of the foot exacerbates the stresses induced by the medial rotation of this limb. Just as examples, medial rotation of the hind fetlock joint increases pressure on the lateral proximal sesamoid bone and tension on the deep layer of the medial collateral ligament. Medial rotation of the tarsus increases transverse shearing of the distal tarsal joints, tension of the calcanean fasciculus of the medial collateral ligament and stresses on the grooves of the tibial cochlea. Medial rotation of the femorotibial joint increase pressure on the cranial horn of the medial meniscus and tension of the cranial cruciate ligament. The tension of the capitis ligament and pressure on the articular surfaces of the coxofemoral joint are also increased with medial rotation of the corresponding hindlimb.

Thoroughbreds Ridden (at Canter) on the Track

Examination of horses being ridden at the trot on the track is useful when only mild to moderate asymmetry or lameness can be seen at the walk and the trot in hand. Movement analysis using slow motion video at different gaits is particularly useful to detect or confirm visual observations (Fig. 39). Looking at the horse cantering on both left and right leads is essential to identify the horse’s preferred lead, which may be in relation to existing clinical or subclinical conditions or may predispose to secondary injuries induced by asymmetrical load on diagonals (Fig. 40).\textsuperscript{66,132,153,143}
As the canter is an asymmetrical gait, evaluation of the head, withers, and tuber sacrale trajectories does not provide clear clinical data on the redistribution of load between limbs in a lame horse. On the forelimbs, there is a higher fetlock extension on the trailing forelimb than on the leading one during the stance phase. Therefore, to assess clinically potential reduction of load on one limb, it is essential to evaluate fetlock extension of the forelimbs or the hindlimbs at the left and right leading canter at the same speed (using slow motion video-recordings). When ridden at a canter, evaluation of the thoracolumbar mobility is limited but manifestations of back pain or discomfort may be exacerbated (Fig. 41). Visual assessment of the lumbosacral flexion and extension movements is easier and the amount of motion increases with the speed.

Harness Racing Trotters on the Track
Examination of racing Standardbred trotters in hand is often frustrating and observations are not always correlated to the complaint and anamnesis. Examination of the horse being driven on the track is an essential part of the evaluation of lameness and discipline related problems. Only the most common remarks are mentioned in this paper.

1. It is quite common to observe a lameness on one side when the horse is examined in hand and a lameness is on the opposite side on the track. The primary lameness seen at high speed induces an overload of the opposite limb at work. At slow speed when the stresses responsible for the manifestations of the primary lameness are lower, the secondary lameness shows up. Therefore, it is crucial to identify the primary cause of the athletic manifestations at training or high speed to direct the diagnostic approach and adequately manage the horse.

2. A frequent gait alteration at speed called *traquenard* or *hiking lameness* can be seen with pain involving the forelimb or the hindlimb on the same side (Fig. 42). The forelimbs are at the trot and the hindlimbs seem
to canter. The use of slow-motion video is essential to analyze the fast sequence of limb placement. In a horse presenting with a left forelimb lameness as in Fig. 42, the right hindlimb increases protraction and anticipates ground contact to reduce load on the painful left forelimb. As the right hindlimb lands before the left forelimb, the distance between the limbs of the left diagonal increases. As propulsion made by the right hind is more effective, landing of the left hindlimb is delayed inducing shortening of the right diagonal distance.\(^5\) The same features are observed in a horse presenting with a left hind lameness. Complementary analysis of this gait alteration can be performed using a video-camera placed over the helmet of the driver (Fig. 43).

3. A common manifestation on turns is to put more load on the inside hindlimb at medium and high speeds. Therefore, a hindlimb lameness is often worse when the affected
A deficit in forelimb protraction is responsible for an asymmetrical forelimb gait called aubin.\textsuperscript{153} The hindlimbs are at the trot and the forelimbs seem to canter (Fig. 44). The protraction deficit (swing phase lameness) is worse when the affected limb is on the outside of the turn. Different conditions can induce this gait abnormality ranging from distal limb injuries up to CTB syndrome.

5. Hindlimb asymmetry induces a corresponding forelimb asymmetry. Therefore, a complete clinical and imaging evaluation of the corresponding lateral (forelimb and hindlimb of the same side) is required.

6. The lumbosacral joint is highly specialized in flexion and extension. Rotation and, above all, lateroflexion are limited. Unlike in Thoroughbreds showing wide lumbosacral flexion-extension movements at fast speeds, in trotters protraction of one hindlimb is synchronous to propulsion of the opposite limb and rotation becomes the main movement of the lumbosacral junction. This explains why intertransverse lumbosacral arthropathy is a common condition in racing trotters.\textsuperscript{92,113}

5. Conclusions

Lame horses are live illustrations of functional anatomy and biomechanics. The trot is often considered as the reference gait for lameness evaluation and to assess diagnostic analgesia. Much information can be gained when the horse is examined at the walk, some of which (such as forelimb protraction or fetlock suspension) becoming less clear or even disappearing at faster gaits. Looking at the horse in different situations in hand and on the lunge, combining different surfaces and different exercises of training and even competing allows for a complete clinical and athletic assessment of the horse and understanding of its gait alterations. Identification of the best as well as the worst situations for the horse is not only essential from a diagnostic point of view but also provides adequate pieces of information to establish the best rehabilitation program for each individual patient. At present kinematic and kinetic technologies are being developed to provide objective data to evaluate lameness in horses; these devices are well adapted for assessment of a symmetrical gait such as the trot. In practice, evaluation of the different gaits and exercise conditions can be done using video-recordings in the field. Their analysis enables a global and detailed evaluation of the horse’s locomotion as every joint can be assessed independently. Moreover, based on these recordings, a more objective follow up of the horse’s locomotion can be done at several months or even yearly intervals allowing a more accurate assessment of the evolution of the disease process, of the efficacy of a treatment and/or of a rehabilitation program. No doubt that instrumental analysis of locomotion in horses will bring new data on the manifestations of pain, mechanical, and neurological disorders in the future. We must keep in mind that few horses display symmetrical gaits, so the new challenge is to determine what is an acceptable degree of asymmetry. As locomotion is the expression of an endless number of biological factors, the answer varies with individual horses and the diagnosis and prognosis of locomotor variations and troubles is not dependent only on figures, graphs, or statistical analysis but must consider the interindividual capacity of tolerance to pain as well as to the physical and functional asymmetry. The mixture of objective data combined with individual expression make this field of equine locomotion and its alterations such an exciting and fascinating discipline.

Acknowledgments

The Author wishes to acknowledge his colleagues of the INRAE-BPLC unit for providing scientific support to the clinical field and his colleagues working at CIRALE for their assistance in performing imaging procedures and contribution to the examination of our equine patients. A special thanks to the referring practitioners sending cases contributing to build the collective experience of our profession. The author of this paper has no actual or potential conflict of interest including any financial, personal, or other relationships with other people or organizations that could inappropriately influence, or be perceived to influence, his work.
Clinical activities and research of CIRALE-ENVA are supported by the Conseil Régional de Normandie, the Calvados Department, the FEDER and the Eperon Funds.

Declaration of Ethics

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

Conflict of Interest

The Author has no conflicts of interest.

References


Collaborative Practice Models

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1. Introduction

There is an incredible flurry of corporation aggregators currently buying equine veterinary practices. At first these groups were focused on acquiring companion animal practices, but as more and more of the prime companion animal practices have been purchased they are turning their attention to other species. The acquisition of veterinary practices in North America is relatively new, but it has been going on for many years in Europe. Presently, 50% of companion animal veterinary practices in the UK and 60% of practices in Sweden are corporate owned. More recently consolidators are quickly buying practices of all species in the UK, Netherlands, and Germany. In North America, 25% of companion animal practices are expected to be corporate owned by 2023. Regardless of which region of the world is being targeted for corporate acquisitions, purchase amounts seem to be exceptionally high, and many veterinary practice owners wonder if they should jump in and be part of the bonanza of seemingly high valuations placed on practices. At the same time, selling a practice to an outside group can be the basis of many questions. Will things remain the same? Will the vets and staff be taken care of? Will our legacy be preserved? Most practice owners would prefer to sell to an associate, but the high load of student debt in the United States can often make a younger vet cautious about increasing their debt load to buy a practice. In addition, the purchase prices offered by consolidators are often far higher than what an associate could afford to pay. Many veterinary practice owners who previously thought they would never sell to a corporate aggregator are forcing practice owners to re-evaluate their opposition due to the absence of internal purchasers and the high price offered by these groups. Like any high stake transaction, it is helpful for practice owners to understand who they are selling to and if the promise of a high payout is actually what it seems to be. The purpose of this presentation will be to discuss the business model of these aggregators, why veterinary businesses are attractive to these corporate groups, determine if their offers to purchase are as lucrative as they appear to be and finally the pros and cons of selling to a corporate aggregator.

2. Business Models

There are three types of entities that are buying veterinary practices. The first are family owned private entities like Mars Inc, the owners of VCA, Blue Pearl, Banfield in North America and Anicura in Europe, and JAB Holdings, the investment vehicle for a German family that owns NVA, a large group of companion animal hospitals in North America, Australia, and New Zealand. The latter group has recently purchased many equine practices in North America over the past 2 years. Typically, they prefer to buy 51–100% of a practice so that they can have a controlling interest in the acquisition. These types of entities typically have a long vision for their portfolio companies and since they are privately owned do not need to respond to short term market forces facing public companies or private equity firms. Often, the...
acquisition of veterinary hospitals is complementary to other assets. For example, Mars also owns Royal Canin prescription pet food as well as Sound/Antech. The second type of aggregators are public companies that are listed on stock exchanges. The largest of these groups is CVS Group PLC based in the United Kingdom. They own over 480 practices and over the past 2 years have moved into the equine veterinary market. Like Mars Inc, they have a diverse animal health care portfolio that includes diagnostic laboratories, crematoriums, and e-commerce. Like other public companies, it has to answer to shareholders and their expectations for financial returns that ideally outperform the market. The final group of aggregators are private equity firms. Their basic business model is to collect a fund of money from investors in order to buy a business, or group of businesses, and then sell them within 4-6 years for a much higher value than the original purchase value.3,4 Investors in a private equity fund can range from high net worth individuals to institutional investors like pension funds, hedge funds and insurance companies. The private equity company will identify a business or industry that is poorly managed or has undervalued assets. They develop a business case that tells these investors that they can acquire these assets and with proper management or consolidation of a portion of industry can sell them within 4-6 years for a far larger sum. In other words, they do an excellent job of buying at a low price and selling at a much higher value. Private equity acquisitions are often financed through debt. They do this by using a portion of the invested fund to make some purchases and then rely on debt to acquire the rest of the targeted businesses. The debt is held by the acquired companies and not the private equity company. In the case of financial difficulty or bankruptcies, the acquired businesses are liable for debt repayments. This was more pronounced years ago and was responsible for the bad reputation associated with private equity companies. Many business analysts think the downfall of Toys R’ Us was related to the enormous debt burden placed on it by a private equity firm. It left the company cash starved and unable to compete against the likes of Amazon since any excess profits were used to pay back their debt.5 The other relevant aspect about private equity firms is how they are compensated. Typically, they charge the fund 2% of revenue annually and receive 20% of the profit from the sale. In the case of vet practices that collectively gross $40,000,000 annually and are sold for $80,000,000, they would receive $800,000 annually as a management fee and 20% or $8,000,000 of the excess profit of $40,000,000 when they sell. In practice this means the private equity managers are incentivized to maximize revenue, through acquiring more businesses or raising prices and maximizing profitability through a combination of increased prices charged to the end user and minimizing expenses. A critical aspect to consider of the private equity business model is that to accomplish their end goal of selling their businesses for a higher value, they are actively working to sell their portfolio of veterinary practices to another company. The first 1-3 years of their plan will often be to acquire as many practices as possible. They will then spend years 4 and 5 nurturing these companies to increase revenue and profitability and then by year 6 sell the practices at a maximum value. The timeline may be a bit shorter or longer, but ideally the funds they are working with have a finite life span in order to get the expected returns. Private corporate groups will have a much longer horizon for their holdings and may keep them for decades, or until a more promising investment opportunity occurs. At such a time they will restructure their portfolios by selling some assets to finance the acquisitions for their new focus. Unlike private companies like Mars Inc and Jap Holdings Inc, acquirers of veterinary practices that are listed on stock exchanges or are private equity firms are in the business of maximizing shareholder value. Compensation of these companies are often dependent on meeting or exceeding market expectations, or what was promised investors.

3. The Allure of Equine Veterinary Practices to Consolidators

Now that we know about the business model of the main types of companies buying veterinary practices, the obvious question is why are they so attracted to the veterinary industry? The simple answer is that veterinary practices are relatively recession proof and offer financial returns that are far higher than conventional investments like bonds, or equities in the stock market. In other words, veterinary practices are low risk vehicles that carry high financial returns. This is the goal for any investor. Fundamentally, these groups are buying future cash flows of a business. This is primarily determined by the EBITDA of a company, or earnings before interest, taxes, depreciation, and amortization. The latter two items are accounting terms that refer to how a business will spread the cost over time of large purchases, like digital radiology systems or leasehold improvements. According to a financial overview of 24 equine veterinary practices,6 the average EBITDA was 12.7%. This means if a practice has revenue of $1,000,000, they would have an EBITDA of $127,000. One way that veterinary practices are valued for sale is to determine the average normalized EBITDA over the previous three years. A normalized EBITDA accounts for situations where certain business expenses or revenues that are not directly related to the operation of the business for sale are added or deducted from EBITDA. To ensure that their investment in the future cash flows of company is secured, the buyer will assess how risky the investment could be. For example, a practice with three plus veterinarians with a broad range
of clients spread over a number of veterinarians is a safer investment than a one-veterinarian practice. Once the single veterinarian leaves the practice, there is a very good chance the clients would leave too. There is a greater chance that if one of the veterinarians left the multi-practitioner practice that the loss of clients would be minimal, thus the latter situation is a safer investment. Valuations of a practice will often include a multiplier that is directly related to the risk involved in purchasing future cash flows. It reflects the ability to recoup the investment on a yearly basis. The one veterinarian practice would have a multiplier of 1 or less indicating that the risk is high. The multi-veterinarian practice could have a multiple of 4 or 5 because there is less risk, and the buyer feels confident that their investment could be recouped in 4-5 years. Currently, corporate consolidators are using a multiple of between 6-10 EBITDA when purchasing lower risk large group practices. If they are planning on selling in 4-6 years, why are they paying a rate when they won't see their return in 6-10 years? The answer is simple, if they can aggregate a large number of practices, they reduce the risk even more and can sell to a larger aggregator for greater than double the multiple they initially paid for the original practices. If they bought numerous practices with a combined EBITDA of $20,000,000 and used a multiple of 8 they would buy the group of practices for $160,000,000 with the hope of selling the aggregate for at least 16 times multiple, or $320,000,000. Ideally, they would also improve the overall EBITDA during their ownership by 1-3%. Going back to the original EBITDA of $20,000,000 a 3% improvement by the time they sell again will give them an EBITDA of $20,600,000, which when multiplied by 16 gives a selling price of $329,000,000, a $9,000,000 improvement. In addition, a consolidator will be paid 20% of the final selling price above the initial purchase price. If there was no improvement in profitability, they would pocket 20% of $160,000,000 or $32,000,000 and if they improved profitability by 3% they would gain an extra $1,800,000 from the additional $9,000,000 selling price. There are conditions that are often applied by the buyer. They will want the selling veterinarians to stay on for 2-3 years to help with the transition to newer veterinarians. They will hold back 25-30% of the sale price and pay that once this period is over. Associated with this may be performance metrics that once reached in 2-3 years allows the seller to get a bonus on top of the initial selling price. Some buyers will insist that this 25-30% of the selling price be invested into the group of practices. The payback to the selling owners will be when the aggregate of practices is sold to the next buyer. This could be a substantial reward to the practice sellers if the new purchasers are willing to pay a much higher multiple of EBITDA. All of these are incorporated to mitigate risk for the buyer. They don't want a practice owner to sell the practice and then start up a new business in the area and they want them invested in the success of their own practice, but also that of a combined group of practices. Finally, many corporate consolidators will welcome an associate that owns a small percentage of the practice to maintain their ownership at the time of the sale, but there is often wording in the sale agreement that when the group of practices are sold that the minority shareholders have to sell as well. We can see how the veterinary profession could be so attractive to corporate consolidators because of the low risk and high returns. The questions then arises if they are offering a fair price to selling veterinary practice owners.

4. Are the Prices Paid to Purchase Equine Veterinary Practices Sufficient?

The reality is that the high numbers that are being offered to practice sellers seem lucrative but don’t necessarily lead to a pot of gold. There is a very good financial argument that practice owners with more than a 5 year horizon before they want to sell would receive less overall compensation compared to what they would recoup if they sold in 5 years or more. Consider the scenario where a practice is sold today. Let's say, for example, the practice is valued at $2.5 million. They will ask the sellers to stay on for 2-3 years to help with the transition to the new owners. For the three years, the past owners will get a veterinary salary, but afterwards they are living on the proceeds from the sale. They may get a 5-7% return on their investment if they are lucky, but they will also begin to draw down on their nest egg for living expenses. Not a bad life for someone entering their golden years. A just reward for all of the years being a vet is to have 20-30 years with their spouse and family doing whatever they want to do. Now imagine they are 55 or younger, and they have many years of practice ahead of them. Is selling now the best option? The reality is that there are very few financial scenarios that make sense to sell now. A seller will make more money by holding on for several years and reap the real value of their practice. The math is simple. Collect a vet salary. Collect the annual profit from the business, work on the business a bit more to find 1 or 2 percentage points of profitability, and then sell to an associate or a corporate group when they really want to retire. Even if someone sells in 10 years at a lower multiple than they would now, they will reap the benefits by waiting. Here is what math tells us: if we compare selling now for $2 million and selling in 10 years for $1.5 million, the seller would accumulate $3 million more in 10 years by holding on to their business than selling now. The larger return comes from the accumulation of annual
profit and salary over the 10 years plus the ultimate sale. Even practices that have been offered 15 times EBITDA, can earn more money by selling in 10 years at a much lower multiple of EBITDA. Here is the uncomfortable question we have to ask ourselves when a corporate aggregator comes knocking on our doors. If they are in the business of making money, what about our practices do they like? The simple answer is that they are buying low and selling high. It’s obvious that if someone is about to retire and can sell to one of these groups, they would be wise to take what is typically offered. But if their career has a longer horizon, it is much more profitable to hold on and reap the rewards down the road. One should ask, would you like $2,000,000 now or $5,000,000 in 10 years? Warren Buffet states “Investing is laying out money now to get more money back in the future.” If you own your own business, you just need to invest time to get more money in the future. Your business is working for you to maximize your investment. Just like rolling a snowball, it just keeps getting bigger and bigger.

5. Pros and Cons of Selling to a Consolidator

Money is not the only reason why someone would sell a practice. Many would value some of the management services a corporate group can offer or want the security of knowing their retirement is assured. Let’s review some of the pros and cons of selling to a corporate aggregator.

Pros
• Ability to sell in the absence of internal prospects
• Administrative support with accounts receivables and accounts payables
• Recruiting of new veterinarians and support staff
• Human resource support
• Marketing support
• Equipment purchasing support
• Peace of mind that the sellers can retire
• Potential bonus when shares held in overall group are sold

Cons
• Reduced overall compensation if owners want to practice for more than 5 years
• Resistance of veterinarians to work for corporate owners
• Lack of control over management
  ○ Prices
  ○ Equipment purchases
  ○ Employee compensation
• Risk that performance targets are not met resulting in a reduced purchase price
  • Potential new practice owners in 4-6 years
  • Potential conflict of interest between patient care and financial return to investors
  • Potential loss if final sale price is lower than expected

6. Conclusion

Selling a veterinary practice is a major life event. Owning a practice reflects many years of risk and sacrifice from building a practice, the long-term care given to clients and their horses, a sense of community, and responsibility to all employees. The practice stands as a legacy of an owner’s dreams and ambitions. Selling to an outside corporate group is fraught with risks for both the seller and buyers. It is imperative that the seller recognize the business model of the purchaser to ensure that they are compatible with the legacy that is important with the sellers. The money received for the sale may or may not be sufficient depending on the seller’s situation. It could be a lifeline for those at the end of the career or insufficient for those who have a longer horizon. The main thing for all sellers to be aware of is that the goals of the corporate groups are not necessarily the same as a veterinary practice. While it is important for an aggregator to treat the practices they purchase well, at the end of the day, they are ultimately responsible to their investors. As veterinarians, our goal is to care for our patients. Yes, we need to be profitable, but it isn’t the only thing. An investor wants to be known for caring for their purchased practices, but their reputation and livelihood depends on fulfilling the needs of their investors. The trade off with selling to an aggregator is that the selling practice owners can receive fair financial compensation, but in return there is a loss of control. Ultimately, it is up to the seller to be aware of the business model of the purchaser so that they can recognize the potential benefits and disadvantages of working with the new owners and determine if the price offered is worth it, especially if the sellers want to continue practice for more than five years.

Acknowledgments

Declaration of Ethics

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

Conflict of Interest

The Author has no conflicts of interest.

References


Understanding Endocrinology and Pharmacology in Donkeys and Mules

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There are endocrine, metabolic, and pharmacological differences between donkeys, mules, and horses. Endocrine and metabolic disorders are frequent in donkeys, and when diagnosed, the disease process and complications (hyperlipemia, insulin dysregulation, obesity, endocrinopathic laminitis) are often advanced. In donkeys suspected of insulin dysregulation with equivocal insulin concentrations, dynamic testing may be indicated, taking into consideration donkey-specific values. Basal adrenocorticotropic hormone and the thyrotropin-releasing hormone (TRH) stimulation test are the preferred methods to diagnose pituitary pars intermedia dysfunction (PPID) in donkeys. Pergolide is an effective treatment for PPID in donkeys. Donkeys have faster metabolic rates and higher cellular water content than horses, which may contribute to their ability to metabolize many drugs differently than horses. Extrapolation of doses and dosing intervals from horses to donkeys and mules could be ineffective or lead to side effects. For most drugs used in equine practice, donkeys and mules need higher doses and/or more frequent dosing intervals than horses. Other drugs (e.g., fluoroquinolones, pergolide) have longer half-lives, requiring lower doses or longer dosing intervals. Authors’ addresses: Department of Veterinary Clinical Sciences, College of Veterinary Medicine, The Ohio State University, Columbus, OH 43210 (Toribio); Department of Animal Medicine and Surgery, University of Cordoba, Campus Rabanales, 14104 Cordoba, Spain (Perez-Ecija, Mendoza); e-mail: toribio.1@osu.edu. *Corresponding and presenting author. © 2021 AAEP.

1. Introduction

The donkey (Equus africanus asinus) plays important economic, social, and cultural roles throughout the world, particularly in developing countries. The donkey and mule were central to the development of countries such as the United States as well as many others in Europe and Asia. With industrialization, the use of these animals for economic development decreased to the point that they became secondary and, over time, even irrelevant. Uses such as recreational activities, ecotourism, hippotherapy/onotherapy, as well as companion animals have been increasing in recent decades, mainly in developed countries. The donkey continues to be a valuable asset in developing countries, where the livelihood of many families and local economies depend on these animals. Donkeys are also used as a source of meat, milk, and byproducts for allergic conditions and for the cosmetic industry. Unfortunately, demand for donkey hide from China to produce “Ejiao” and other...
products has led to a decline in the worldwide donkey population.\textsuperscript{2,3} To date, more donkeys are being admitted to veterinary hospitals or are receiving specialized care as a result of economic growth, cultural shifts, awareness of animal welfare, and programs to rescue breeds close to extinction. Therefore, there is a need to increase donkey- and mule-specific knowledge in veterinarians and professionals involved with their use and care. Veterinarians practicing in this species should be familiar with anatomical, physiological, endocrine, and pharmacological differences considering that data extrapolation from horses can lead to misdiagnosis, inadequate treatments, complications, unnecessary expenses, and, in some instances, even death.\textsuperscript{1,4–6}

2. Endocrinology

Hyperlipemia

Hyperlipemia refers to the clinical condition associated with increased triglyceride concentrations (hyperlipidemia).\textsuperscript{1,7} Hyperlipemia is more frequent in donkeys than other equids, and it seems its higher incidence is a consequence of their efficiency to store lipids and rapid ability to mobilize fat stores.\textsuperscript{1} It is more frequent in older donkeys, jennies, and smaller donkey breeds.\textsuperscript{1,7,8} Hyperlipemia is not restricted to donkeys with obesity and insulin dysregulation (ID) because it occurs in donkeys of any age and in good or poor body condition, as well as in donkey foals. As with other equids, predisposing factors include conditions associated with a negative energy balance (e.g., anorexia, fasting), increased energy demands (e.g., late pregnancy, lactation), and concurrent diseases (e.g., stress, endotoxemia, parasitism, liver disease, gastrointestinal disease, laminitis).\textsuperscript{1,7,8} Obesity, stress, and pregnancy are major risk factors for hyperlipemia in donkeys. Mortality rates in affected donkeys can be up to 80\%, which are similar to ponies and miniature horses.\textsuperscript{1,7,8}

Physiologic or pathologic processes that increase energy demands activate hormone-sensitive lipase (HSL) in adipocytes to induce lipolysis and the release of free fatty acids (FFA) into circulation.\textsuperscript{1} In the liver, FFA are re-esterified into triglycerides, which are released as very-low-density lipoprotein (VLDL) into systemic circulation. If lipolysis persists and VLDL production exceeds peripheral triglyceride uptake, hyperlipidemia (hypertriglyceridemia) will ensue, resulting in hepatic fatty infiltration, liver dysfunction, and increased risk of liver rupture. In severe hyperlipemia, other organs (kidneys, pancreas, heart, intestines, skeletal muscle) can be infiltrated by triglycerides, compromising their function (Fig. 1).\textsuperscript{1,7,9}

Catecholamines, glucocorticoids, adrenocorticotropic hormone (ACTH), growth hormone (GH), glucagon, and pro-inflammatory cytokines (IL-6, TNF-\(\alpha\)) increase lipolysis, while insulin inhibits lipolysis and
promotes lipogenesis. Increased concentrations of lipolytic factors (cytokines, hormones), decreased lipogenic hormones (insulin), and insulin insensitivity combined with reduced VLDL removal will ultimately lead to hypertriglyceridemia and clinical hyperlipemia (Fig. 1). Insulin is the main lipogenic hormone, and impaired signaling indirectly facilitates hyperlipemia, particularly in animals with metabolic syndrome and obesity. Hyperlipemia is usually secondary to other conditions, and initial clinical signs are the result of the primary condition. In fact, hyperlipemia often goes unnoticed. Lethargy and anorexia are common findings of hyperlipemia. Fatty infiltration of various organs leading to dysfunction can exacerbate disease progression, additional clinical signs (e.g., diarrhea, dysrhythmias), and laboratory abnormalities (e.g., increased liver enzymes, azotemia, acidaemia, hyperbilirubinemia). Hyperlipemia is easily diagnosed by measuring serum triglyceride concentrations. Equids can be classified as hypertriglyceridemic (200–500 mg/dL [2.26–5.65 mmol/L]; no evidence of tissue fatty infiltration) or lipemic/hyperlipemic (> 500 mg/dL [5.65 mmol/L]; there could be fatty infiltration in various organs). Severe hyperlipemia is evident when triglyceride concentrations are > 1000 mg/dL [11.3 mmol/L]. Gross lipemia can be noted with triglyceride concentrations > 500 mg/dL in sitting ethylenediaminetetraacetic acid (EDTA) and serum tubes. However, these cutoff values are somehow arbitrary, are mainly used in ponies, and may not apply to donkeys. In donkeys, lipemia may become grossly evident at higher triglyceride concentrations than ponies and horses, likely due to a different lipid profile. Lipemic serum may interfere with the measurement of various chemistry analytes, falsely changing their values (e.g., pseudohyponatremia). The therapeutic principles for hyperlipemia are aimed at halting fat mobilization, controlling the primary disease, reducing hepatic triglyceride synthesis, avoiding stressful conditions, and restoring a positive energy balance. In pregnant animals, it is essential to provide a balanced caloric intake, although excessive calories can predispose to other disorders (e.g., insulin dysregulation). In extreemisituationes, pregnancy termination should be considered. In lactating animals, early weaning is highly recommended. Hyperlipemic donkeys should be encouraged to eat by offering a variety of palatable foodstuff that may stimulate hunger (e.g., honey, apples, carrots). Placing a feeding tube should be considered if gastrointestinal function is normal. Intravenous dextrose may be necessary to reduce lipolysis. Partial parenteral nutrition without lipids may be required in some animals. If hyperglycemia persists or triglyceride concentrations continue to increase over 24 h, regular insulin (0.05–0.1 IU/kg/h, IV) or slow-release insulin (0.10–0.15 IU/kg, q 12–24 h, SQ) should be considered. In donkeys where insulin proves effective, a continuous rate infusion (0.05 IU/kg/h starting rate) together with intravenous dextrose can be implemented. Close glucose monitoring is essential to avoid hypoglycemia. The benefits of heparin or low molecular weight heparin to increase lipoprotein lipase remain to be evaluated. These products should be used with caution in animals with liver dysfunction due to their increased risk of bleeding.

Donkey Metabolic Syndrome

Donkey metabolic syndrome (DMS) was recently recognized and appears to be highly prevalent in donkeys, particularly in developed countries or where food is readily available. DMS shares key features with equine metabolic syndrome (EMS), including obesity, ID, and endocrinopathic laminitis. Clinical signs reported in horses such as reproductive disturbances, diabetes mellitus, and pancreatic insufficiency are poorly characterized in donkeys. Similar to horses and ponies, not every obese donkey has ID, and lean animals can be affected. The incidence is higher in jennies and middle-aged to old donkeys (> 8 years of age); however, this condition can be seen in much younger donkeys. Multiple factors likely contribute to the pathogenesis of ID and endocrinopathic laminitis in donkeys. Their energy efficiency associated with reduced physical activity results in obesity, which is a major complicating factor for these disorders. In addition to excessive body weight, adipose tissue produces hormones and cytokines that interfere with insulin signaling and promote a systemic pro-inflammatory state. Inflammatory mediators and prolonged hyperinsulinemia disrupt lamellar cell function, which together with excessive body weight contribute to the development of endocrinopathic laminitis. Insulin concentrations are often elevated in obese donkeys. Insulin insensitivity interferes with glucose uptake, promotes fat mobilization (lipolysis), and alters endothelial integrity. Cytokines also reduce insulin signaling in the liver, worsening hepatic fatty infiltration. Adipocyte factors (adipokines) such as leptin and adiponectin may contribute to the pathogenesis of ID and endocrinopathic laminitis. The diagnostic principles of EMS also apply to DMS. Donkey-specific body condition score systems have been developed, ranging from 1 (very thin) to 5 (obese) or from 1 (emaciated) to 9 (obese). A neck score system was also developed (0 = thin neck without pal-pable crest; 4 = thick neck, rounded and gross cresty). Fasting glucose and insulin concentrations as well as dynamic tests are the main methods used to diagnose ID in donkeys. Factors that could interfere with the diagnosis of ID include stress, fasting time, carbohydrate-rich diets, physical activity, pain, transport, endocrinopathies (pituitary pars intermedia dysfunction [PPID]), concomitant diseases, and a2-adrenoreceptor agonists. Fasting insulin concentration is the main test used to diagnose ID in horses, with a cutoff value of > 50 μIU/mL suggestive of ID. Insulin concentrations differ between assays, and extrapolation can be misleading. Dynamic tests should be considered when baseline insulin results are inconclusive (20–50 μIU/mL). Dynamic protocols have been evaluated in donkeys, demonstrating differences with horses for the intravenous glucose.
tolerance test (IVGTT) and the combined glucose-insulin test. For the IVGTT, glucose (300 mg/kg, 50% dextrose solution) is administered IV as a bolus, and glucose and insulin concentrations are measured at 0 (baseline), 5, 15, 30, 45, 60, 75, 90, 105, 120, 150, 180, 210, 240, and 300 minutes. In insulin-sensitive donkeys, glucose should be back to the normal range by 180 minutes. This test is time-consuming. For the combined glucose-insulin test, glucose (150 mg/kg, IV) is administered as a bolus and immediately followed by regular human insulin (0.1 IU/kg, IV). Glucose and insulin concentrations are determined at the same time points as the IVGTT. While horses are considered ID if glucose concentrations are above baseline at 45 minutes, donkeys would be considered ID if glucose concentrations are at or above baseline at 60 minutes. The test can be simplified by collecting blood samples at baseline and at 60 minutes. A serum insulin concentration of > 100 μIU/mL at 60 minutes also supports ID. The intravenous insulin tolerance test and frequently sampled intravenous glucose tolerance test (FSIGTT) have also been investigated in healthy and obese donkeys. FSIGTT results are comparable between donkeys and horses. The FSIGTT is not a practical test, and its main use is in research. Oral carbohydrate tolerance tests, whether using dextrose or corn syrup, remain to be evaluated in donkeys, but dynamics likely differ between species. Leptin and adiponectin cutoff values for donkeys with evidence of ID and endocrinopathic laminitis have not been reported. Therapeutic approaches for DMS have not been established, and clinicians rely on protocols for EMS. They are focused on promoting weight loss, improving insulin regulation, and controlling signs of endocrinopathic laminitis. To promote weight loss, caloric restrictions and increased physical activity are central to success. Due to their energy efficiency and ability to subsist on poor quality feedstuffs, dietary management should rely on low-quality hay and elimination of access to grain, concentrates, and carbohydrate-rich pastures. Commercial diets to manage ID and obesity in horses may not be appropriate for donkeys. Access to paddocks to promote physical activity is encouraged. Grazing should be avoided in early morning or after rainfall. Grazing muzzles may be used with caution, and some clinicians avoid their use in donkeys because of concerns of inducing stress, which could trigger additional problems. The presence of other animals (donkeys, horses, goats) is recommended to reduce stress and promote physical activity. Weight loss in donkeys must be a very slow process due to risk of other conditions such as hyperlipemia. Information on the pharmacology of drugs to manage ID or obesity in donkeys is lacking, and equine doses are generally used. Levothyroxine sodium is used in donkeys at equine doses (0.1 mg/kg, q24h, PO). Metformin has not been evaluated in donkeys, but it is occasionally administered (15–30 mg/kg, q12h, PO). Thiazolidinediones (pioglitazone) and sulfonylureas (glyburide) have not been assessed in donkeys. Considering the clinical benefits of these and other drugs to manage energy dysregulation in other species, including horses, evaluating their pharmacologic properties in donkeys would be valuable.

Pituitary Pars Intermedia Dysfunction

PPID is common in equine species, partly due to their longevity. The condition is often underdiagnosed, and epidemiological studies are lacking. Breed and gender do not appear to be risk factors. The pathogenesis of PPID in donkeys and mules is likely similar to horses and ponies, but mechanistic data are lacking. Clinical signs are similar between donkeys, horses, and ponies, with hyperhidrosis being common. It is important to note that some donkey breeds have long haircoats. Lethargy could be overlooked due to their calm behavior. Laminitis is a consistent finding in donkeys with PPID. Hyperhidrosis, ID, abnormal fat distribution (supraorbital, neck, shoulder, tail base), muscle wasting, reproductive problems, predisposition to infections, endoparasitism, and orthopedic problems also occur in donkeys. Polyuria is infrequent. Information on PPID in mules is minimal. Baseline ACTH concentrations and the TRH stimulation test are the main diagnostic tests for PPID diagnosis in donkeys. ACTH concentrations in healthy donkeys are higher than in healthy horses and mules. Similar to horses, donkeys exhibit seasonal variations in ACTH concentrations that should be taken into consideration when measuring baseline ACTH concentrations or performing the TRH stimulation test. Donkeys and mules have seasonal variations in ACTH concentrations (donkeys: August–October = 19.5–143 pg/ml; November–July = 5.0–55.4 pg/ml; mules: August–October = 9.8–68.7 pg/ml; November–July = 5–37.1 pg/ml). Donkeys have higher plasma ACTH values in the fall compared to horses. Basal cortisol concentrations are similar in healthy donkeys and horses. Similar to horses, hypercortisolism is a rare finding in donkeys with PPID. Blood samples to measure ACTH should be collected in EDTA tubes, processed rapidly, and shipped on ice. The ACTH chemiluminescent immunoassay works well with donkey samples. It is important to take into consideration stressful conditions and drugs (e.g., α2-adrenoreceptor agonists, glucocorticoids) that could alter ACTH concentrations. The dexamethasone-suppression test and the combined dexamethasone-TRH test are not reliable methods in donkeys. For the TRH stimulation test, a donkey is considered PPID positive if plasma ACTH concentrations are higher than 110 pg/mL 10 minutes after injecting 1 mg of TRH intravenously. It is important to note that this study had a small number of animals, and a large population of donkeys would be necessary to further validate these values. Pergolide, a dopamine (D2) receptor agonist, is the drug of choice to treat PPID in donkeys. The pharmacokinetics of pergolide differs between donkeys and horses. After repeated dosing,
pergolide achieves higher plasma concentrations in donkeys, indicating that lower doses may be sufficient to ameliorate the signs of PPID in donkeys. However, pharmacodynamic information is lacking, and donkeys are typically treated with 0.25 to 0.5 mg/250 kg, q24h, PO often with clinical improvement. It is recommended to monitor animals early in treatment for potential side effects. In animals with anorexia, the dose should be reduced or discontinued. Bromocriptine, another dopamine D2 receptor agonist, and cyproheptadine, a 5-HT receptor antagonist, have not been evaluated in this species. Repeated measurements of basal ACTH concentrations may be necessary to assess response to treatment and adjust dosing.

Thyroid Gland

Information on thyroid diseases in donkeys is lacking. Donkeys have higher plasma-free and total triiodothyronine (fT3, tT3), free and total thyroxine (fT4, tT4), and reverse T3 (rT3) concentrations than values reported for horses. No gender differences for thyroid hormones have been documented in donkeys. Similar to horses, young donkeys have higher fT4, tT4, and rT3 concentrations. Drugs such as phenylbutazone and dexamethasone reduce thyroid hormone (TH) concentrations in horses; however, their effects on asinine thyroid function have not been investigated. Their use could potentially lead to a misdiagnosis of hypothyroidism.

Parathyroid Gland

Serum total calcium, total magnesium, and phosphorus concentrations in donkeys are within the reference range of values reported for horses. One study found higher ionized calcium, complexed magnesium, and calcitriol concentrations but lower parathyroid hormone (PTH) concentrations and protein-bound magnesium in donkeys compared to horses. Nutritional secondary hyperparathyroidism may be seen in donkeys consuming diets with low calcium or high phosphorus content but also occurs with the ingestion of oxalate-rich plants. Clinical signs (facial swelling, lameness, upper airway stridor, and neurological signs), diagnosis, and treatment are similar to horses.

3. Pharmacology

Nonsteroidal Anti-Inflammatory Drugs (NSAIDs)

Most NSAIDs used in horses are metabolized faster in donkeys. Therefore, for many of these drugs to reach therapeutic levels, either the dose or dosing frequency will have to be increased. Phenylbutazone mean residence time (MRT) is shorter and clearance is faster in donkeys compared to horses. Miniature donkeys clear phenylbutazone even faster than average sized donkeys. Miniature donkeys will need more frequent dosing as higher doses could potentially lead to renal and gastrointestinal injury. Flunixin meglumine area under the curve (AUC) and MRT were lower, but clearance was higher in donkeys compared to horses. Flunixin meglumine appears to have faster tissue penetration in donkeys than horses. Flunixin meglumine has less side effects than phenylbutazone. Meloxicam, firocoxib, and cimicoxib are selective COX-2 inhibitors with lower AUC and MRT, shorter half-life, and faster clearance in donkeys compared to horses. This indicates that more frequent dosing will be required to achieve adequate analgesia with these drugs. Meloxicam reduces the systemic inflammatory response from endotoxemia in donkeys and could be a safer alternative to flunixin meglumine. In contrast to most NSAIDs, intravenous carprofen has a slower clearance and larger AUC in donkeys, while intravenous ketoprofen has faster clearance in donkeys than horses. Except for flunixin meglumine, NSAID pharmacokinetic studies in mules are lacking.

Sedatives, Analgesics, and Anesthetics

Donkeys and mules are considered stoic animals, and their response to pain may be subtle or go unnoticed, which could have negative consequences (e.g., hyperlipemia, laminitis). For this reason, analgesia is very important to ameliorate the pain-stress cycle. Sedatives and tranquilizers used in horses are routinely administered to donkeys and mules. The dose of α2-adrenergic agonists (e.g., xylazine) often needs to be increased by 50% to achieve good analgesia with these drugs. However, analgesia from xylazine is less intense and of shorter duration than for detomidine and romifidine. For these reasons, detomidine and romifidine have been proposed as better options for pain management in donkeys. Acepromazine provides satisfactory tranquilization in donkeys, although some donkeys may require twice the acepromazine dose used in horses to achieve good tranquilization. Acepromazine is not an effective analgesic. Butorphanol also enhances the sedative and hypoalgesic effects of α2-adrenergic agonist in donkeys. tramadol is an effective analgesic in donkeys. Transdermal fentanyl patches provide good pain relief in donkeys. Metamizole (dipyrone) has a shorter half-life in donkeys than horses. In donkeys, ketamine has a shorter half-life and faster clearance than in mules and horses. Guaifenesin (glyceryl guaiacolate) has a longer half-life in donkeys than horses, and recovery time could be prolonged, requiring close supervision in donkeys. Intravenous propofol is a safe option for anesthesia induction and maintenance. Propofol can be combined with ketamine to lower the dose, and this combination produces longer anesthesia time, better muscle relaxation, and smoother recoveries than ketamine alone. By reducing the dose, the propofol and ketamine or guaifenesin, ketamine, and xylazine
combinations can be used to maintain anesthesia in donkeys.\textsuperscript{63,64} Thioental is a good induction agent in donkeys and mules but is rarely used.\textsuperscript{63} Induction time is faster with thiopental, but recovery is quicker and better with propofol.\textsuperscript{65} Alfaxalone with midazolam time to induction is faster compared to ketamine and midazolam, but recovery time is shorter with the ketamine and midazolam combination.\textsuperscript{66} Induction and recovery with either combination is acceptable after sedation with xylazine.\textsuperscript{66} The combination xylazine, diazepam, and ketamine is effective and safe in donkeys and mules.\textsuperscript{63} Inhaled anesthetics such as halothane, isoflurane, or sevoflurane have similar properties in donkeys and horses.\textsuperscript{37} Epidural lidocaine provides good regional analgesia to donkeys, but morphine is less effective.\textsuperscript{37} Local anesthetics are used in a similar manner and appear to be equally effective in donkeys, mules, and horses.

\textbf{Antimicrobials}

Similar to NSAIDs, compared to horses, donkeys and mules have a faster metabolism for a number of antimicrobial drugs, with faster clearances, lower MRTs, and shorter half-lives.\textsuperscript{37,49} For most antimicrobials, donkeys and mules require higher doses or shorter dosing intervals.\textsuperscript{37,49} Aminoglycosides (amikacin and gentamicin) have similar pharmacology between horses and donkeys.\textsuperscript{40} Fluoroquinolones (enrofloxacin and marbofloxacin) are cleared slower in donkeys, and once-a-day dosing interval is preferred.\textsuperscript{47} For many other antimicrobials used in equine practice, information in donkeys and mules is lacking but necessary for better treatments. In addition to appropriate criteria for antimicrobial selection in donkeys is the implementation of antimicrobial stewardship practices to reduce bacterial resistance. Similar to other equids, donkeys are susceptible to the toxic effects of ionophores.

\textbf{Antiparasitic Drugs}

Caution must be taken when using macrocyclic lactones (ivermectin, moxidectin, abamectin, eprinomectin) in donkeys, particularly those in poor body condition due to the higher risk of neurotoxicity compared to horses.\textsuperscript{37} Imidocarb dipropionate is an effective treatment for piroplasmosis. Of interest, donkeys and mules are overly sensitive to this drug, and hepatic side effects can be observed.\textsuperscript{67} Doses for donkeys and mules should be lower than for horses. Imidocarb dipropionate toxicity results from acetylcholinesterase inhibition and premedication with anticholinergic drugs such as glycopyrrolate (0.0025 mg/kg/IV) or N-butylscopolammonium bromide (0.1–0.2 mg/kg/IV) 5 to 10 minutes prior to imidocarb administration is recommended.\textsuperscript{37} Fenbendazole is a benzimidazole commonly used against strongyls in horses, donkeys, and mules. Oral fenbendazole is not very effective at eliminating lungworms in donkeys and horses.\textsuperscript{40} Moxidectin (400 μg/kg, PO) is the treatment of choice for lungworms in donkeys.\textsuperscript{37} Pyrantel pamoate in paste and granule formulations have poor intestinal absorption in donkeys but good efficacy (> 95%) against intestinal strongylidae. Psoroptic and chorioptic mange is treated similar to horses (pyrethroids, sulfur, ivermectin, moxidectin, eprinomectin).

\textbf{Other Drugs}

Prokinetic drugs such as lidocaine, metoclopramide, and cisapride are occasionally used in donkeys under protocols similar to horses, but pharmacological information, including efficacy studies, is lacking.

\textbf{4. Summary}

The evolution of the donkey occurred under environmentally harsh conditions, leading to adaptations for energy conservation, including the ability to digest poor-quality feedstuff, a unique capacity to accumulate adipose tissue, and efficient fat mobilization under increased energy demands or food scarcity. These features also predispose donkeys to obesity, dyslipidemias, insulin dysregulation, metabolic syndrome, pituitary pars intermedia dysfunction, and endocrinopathic laminitis. Endocrine and metabolic diseases are frequent in donkeys. Differences in hormone dynamics and testing protocols for endocrinopathies between donkeys and horses support species-specific approaches for appropriate diagnosis and treatment. In addition, donkeys and mules have distinct pharmacological features leading to differences in the way they metabolize and respond to drugs used in equine practice. Most of these drugs work well and are safe for donkeys and mules; however, some when used under equine protocols can lead to subdosing, overdosing, toxicities, and even death. Therefore, a basic knowledge of drugs used in horses, donkeys, and mules is important.

\textbf{Acknowledgments}

\textit{Declaration of Ethics}

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

\textit{Conflict of Interest}

The Authors have no conflicts of interest.

\textbf{References and Footnotes}


*Medoza FJ (personal communication), 2021.

"Immulite, Siemens, Munich, Germany."
Applying Current Knowledge of Clinical Pathology to Donkeys and Mules in Practice

Erin Goodrich, DVM, DACVPM*; and Erica Behling-Kelly, DVM, PhD, DACVP

1. Introduction
To appreciate the clinical importance of objective parameters for assessing donkey and mule health, such as established reference intervals (RIs) for hematologic and serum biochemistry values, one must first understand the behavior of equids. Donkeys and their hybrid counterparts, namely, mules and hinnies, pose unique challenges to the veterinarian due to their subtle expressions of pain and illness as compared to horses. It is imperative that veterinarians rely heavily on history provided by observant caretakers or owners to determine when a donkey or mule is in need of veterinary care. Very slight changes in behavior, such as a donkey or mule not coming up to feed as readily, not spending as much time with the group or a pasture-mate, or lying down more frequently or at unusual times, may be the only signals that the equid is unwell. Typical colic signs seen in a donkey may contrast very sharply to those seen in a horse, and even severe forms of colic may only result in dullness, isolation, reduced appetite (and sometimes sham eating), or recumbency. Other subtle pain behaviors in donkeys can be detected by astute observation of ear position, eye and nostril shape, and tension, as well as head, neck, and tail movement. Due to their innate ability to conceal signs of discomfort, or to display them in a more subtle manner than horses, it is imperative that objective means to assess the health status of equids is used whenever possible. Physiologic parameters should be assessed as part of every physical examination, including heart rate, respiratory rate, gastrointestinal sounds, and body temperature. These findings should be interpreted with donkey- and mule-specific intervals in mind, as they differ from those of horses. Subjective findings should then be combined with objective means of assessing the health status of the equid, such as clinical pathology parameters, which are exceedingly important in light of their stoic nature.

2. Sample Collection and Storage
Although not specific to donkeys, the results of diagnostic tests are directly affected by the quality and the integrity of the samples collected, stored, and submitted to the diagnostic lab. All of the steps taken from the point of diagnostic sample collection to the analysis are classified broadly as the preanalytical phase of clinical pathology. Several biological and nonbiological preanalytical factors can have effects on hematologic values. In human hematology testing, preanalytical factors account for 50% to 75% of the errors seen. Biological components, which are often
out of our control, may include the influence of species, breed, age, environmental factors and geographical location, animal handling, physical activity, nutrition, body condition, physiologic state, concurrent disease processes, and exposures to exogenous compounds (e.g., medications and toxins). It is imperative to document any of these factors that are known and include that information in the history provided to the diagnostic lab where the testing will take place, as they can have profound effects on the final diagnostic report and interpretation of the test results. Also, several technical factors can affect clinical pathology test results, including a host of nonbiological preanalytical factors associated with sample collection, handling, and storage. For instance, filling ethylenediaminetetraacetic acid (EDTA) blood tubes to maintain an ideal ratio between anticoagulant and blood is an important consideration. If an EDTA tube is not filled to the appropriate level, excess EDTA can result in red blood cell (RBC) shrinkage, falsely decreasing the mean corpuscular volume (MCV) and increasing the mean corpuscular hemoglobin concentration (MCHC). Also, clots or microclots may form within the blood tube if it is not properly admixed by gentle inversion of the tube immediately after blood collection. These clots can cause errors in the hematology results and can also clog automated analyzer equipment. Storage temperature and time to analysis can also have significant effects on hematology values. Although the effects of sample storage time and temperature on all hematology analytes across all species are not available, at least one study found that donkey blood should be analyzed within 12 hours of collection, with storage at 4°C, because storage for greater than 24 hours and at 24°C or more can cause pseudotoxic changes. If shipment to a laboratory is required, the EDTA-whole blood sample should be sent in an insulated box with a frozen ice pack. Including 2–3 air-dried, unstained, blood smears prepared using the EDTA blood sample is always beneficial because cellular changes may otherwise alter the results. The slides should be packaged carefully in a sturdy slide mailer and protected from direct exposure to ice packs, which can otherwise result in condensation and cell lysis. Because many of the world’s donkeys are located in hot, remote areas that are not favorable for sample integrity, paying particular attention to sample handling for clinical pathology assays of these equids is exceedingly important. Serum or heparin plasma may be used for biochemical analysis, although they can each be expected to result in some predictable differences when specific analytes such as potassium, phosphate, lactate dehydrogenase, protein, and globulin are measured. Heparin tubes may be advised for use when expedited analysis is required, as they can be centrifuged and analyzed immediately, rather than waiting for clot formation, as is the case when analyzing serum from a red-top blood tube. Similar filling and inverting measures should be taken when using heparin tubes as described above for EDTA tubes. The serum or plasma should be separated from the cells as soon as possible (ideally within 2 hours of collection) to avoid preanalytical errors. Likewise, when serum separator tubes are used, the serum should still promptly be removed and placed in a separate plain tube. Importantly, because the appearance of each (once separated) is otherwise indistinguishable, they must be labeled appropriately for an accurate interpretation of the results (heparinized plasma versus serum). The separated serum or plasma can be maintained at refrigeration temperatures for about 48 hours or frozen at −20°C for longer storage. If shipment to a laboratory is required, samples should be packed carefully in an insulated box with frozen ice packs for overnight delivery.

3. Analysis and Interpretation

To use objective clinical pathology assays for determining the health status of an equid, knowledge of the parameters that are considered normal for the species of interest must be relied upon. This requires the use of population-based RIs that best represent the animal in question. It is important to remember that equipment and procedures vary among laboratories, so results obtained from the patient in question must be compared to those from other studies with extreme caution. It is best practice for a laboratory to develop RIs for specific populations being tested, following American Society of Veterinary Clinical Pathology (ASVCP) guidelines. When this is not possible due to the population of interest being too small or resources being limited, ASVCP also provides guidelines for transference validation of established RIs to the population of interest. The wide array of donkey breeds, the low numbers of donkeys within most of those breed categories, and the typically harsh environments where many donkeys and mules reside have made the generation of accurate clinical pathology RIs for equids extremely challenging. Literature focused specifically on clinical pathology values of mules and hinnies is rare. The authors are aware of one study that compared hematologic and biochemical values of healthy mules, hinnies, horses, and donkeys, but more studies involving larger populations of hybrids are critically important. Donkey studies are slightly more numerous but often include low numbers, specific breeds, or donkeys from very specific geographic regions or in certain physiologic states, which may not provide the most accurate RIs for the clinician wishing to assess the health status of a domestic donkey encountered in private practice in the United States. Also, many studies lack descriptions of procedural details, such as timeframe from specimen collection to serum separation or validation information necessary to confidently extrapolate the results to the patient. In addition, the lack of widely available RIs for donkeys and mules often means that many veterinarians turn to RIs established for horses as a substitute, which can lead to erroneous diagnoses due to many physiological disparities between the
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<td>1.5-5.2 mmol/L (n=136)</td>
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<td>11.4-48.6 mg/dl (n=1219)</td>
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<td>1.7±1.42 μmol/L</td>
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<td>Mean+/-SD</td>
<td>64±43 U/L (n=108)</td>
<td>64±43 U/L (n=108)</td>
<td>195±104 U/L (n=97)</td>
<td>214±104 U/L (n=209)</td>
<td>195±104 U/L (n=97)</td>
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<td>2.5%–97.5%</td>
<td>128-525 U/L (n=137)</td>
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<td>15-149 U/L (n=4218)</td>
<td>171-567 U/L (n=137)</td>
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<td>Mean+/-SD</td>
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<td>0.84±0.37 mmol/L (n=97)</td>
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<tr>
<td>2.5%–97.5%</td>
<td>0.6-2.8 mmol/L (n=138)</td>
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<td>17.7-300.9 mg/dl (n=3176)</td>
<td>14.6-2.3 mmol/L (n=3176)</td>
<td>1.12 mmol/L (0.87–1.49 mg/dl)</td>
<td>55 mg/dl (40–62 mg/dl)</td>
<td>0.47 mmol/L (0.37–0.65 mg/dl)</td>
<td>42 mg/dl (33–58 mg/dl)</td>
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<td>Mean+/-SD</td>
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<td>2.79±0.76 mmol/L (n=215)</td>
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<td>2.5%–97.5%</td>
<td>1.4-2.9 mmol/L (n=137)</td>
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<td>71.11±26.3 mg/dl (n=97)</td>
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Reference ranges are given as Mean + standard deviation, 2.5%–97.5% percentiles or median with upper and lower quartiles.

*The analyzed sample size is included in parentheses. *, serum total protein; ‡, plasma total protein; #, unknown if serum or plasma; **, reference intervals established at the Animal Health Diagnostic Center of Cornell University (from at least 120 adult healthy horses).
Table 2. Comparison of Coagulation Parameters from Healthy Donkey and Horse Populations

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Andalusian Donkeys (Mendoza et al\textsuperscript{20})</th>
<th>Andalusian Horses (Mendoza et al\textsuperscript{20})</th>
<th>Andalusian and Crossbred Donkeys (Perez-Ecija et al\textsuperscript{21})</th>
<th>Andalusian and Crossbred Horses (Perez-Ecija et al\textsuperscript{21})</th>
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</thead>
<tbody>
<tr>
<td>Platelet count ($\times 10^9$/μL)</td>
<td>Mean (range)</td>
<td>190.8 (105–458)</td>
<td>193.9 (112–376)</td>
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<td>Mean ± SD (95% CI)</td>
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<td>177 ± 94 (118–250)</td>
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<td>Fibrinogen (mg/dL)</td>
<td>Mean (range)</td>
<td>462.1 (100–600)</td>
<td>389.6 (100–700)</td>
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<td></td>
<td>Mean ± SD (95% CI)</td>
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<td>406 ± 130 (332–480)</td>
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<td>Prothrombin time (seconds)</td>
<td>Mean (range)</td>
<td>8.71 (7.4–9.7)</td>
<td>9.09 (7.9–11.7)</td>
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<td>Mean ± SD (95% CI)</td>
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<td>12.1 ± 1.0 (11.5–12.2)</td>
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<td>Activated partial thromboplastin time (seconds)</td>
<td>Mean (range)</td>
<td>31.90 (23.2–37.7)</td>
<td>44.53 (37–51.9)</td>
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<td>Mean ± SD (95% CI)</td>
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<td>33.4 ± 5.2 (32.3–34.6)</td>
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species.\textsuperscript{11} One study obtained hematologic and biochemical values from 138 domestic donkeys in the United Kingdom and found that none of the 15 hematologic values and only 4 of the 20 biochemical values were transferable between this population of donkeys and the RIs established for a population of non-Thoroughbred horses, highlighting the need for caution when comparing values between the two species.\textsuperscript{11} The donkey hematologic and biochemical results from that study, as well as those of other donkey, mule, and hinny populations, appear alongside the horse RI values used at the Animal Health Diagnostic Center (AHDC) at Cornell University in Table 1.\textsuperscript{8,11–15} Caution must always be taken when comparing values from two different species and for tests performed at different laboratories and with different analyzers, but the table highlights some of the possible trends in hematologic and biochemical differences that may be seen between equid species and across various populations.

4. Parameters of Particular Interest to Equid Health

Some key analytes stand out as particularly important in the context of sick, inappetent equids due to their propensity for developing metabolic disease and hyperlipidemia. Serum triglyceride concentrations are most often used as an aid in diagnosing hyperlipidemia in equids. This can be difficult, though, due to discrepancies in the RI for this parameter across various studies, likely due to variation across donkey breeds as well as effects of environment, nutrition, body mass index, neck score, and management factors on this measurement.\textsuperscript{8,16} Cholesterol concentration may also aid in the diagnosis of hyperlipidemia but, similar to triglyceride concentrations, shows wide variability across studies (Table 1).\textsuperscript{8} Similar to equine metabolic syndrome, donkey or asinine metabolic syndrome is linked to insulin dysregulation (ID), recurrent bouts of laminitis, and obesity.\textsuperscript{17} As with those analytes used to diagnose hyperlipidemia, diagnosing ID relies on metabolic parameters that vary widely across sex, age, and study. Basal insulin concentrations and dynamic tests may be useful, but the interpretation of results is difficult. There is evidence to suggest that donkeys with ID often have higher plasma insulin concentrations than horses with ID.\textsuperscript{17} Measurements of glucose to aid in the diagnosis of ID must be cautiously interpreted because published donkey glucose RIs are highly variable, likely due to the sensitivity of that parameter to management practices, stress in the donkey, and preanalytical effects.\textsuperscript{5} In addition, when a dynamic test is applied to a donkey for the purposes of identifying ID, donkey-specific protocols should be followed to arrive at the most accurate diagnosis.\textsuperscript{17,18} Importantly, donkeys should not be fasted for testing. Rather, a flake of hay should be provided to the donkey the night before the dynamic metabolic test (and the test should be performed in the morning) to minimize the risk of hyperlipidemia.\textsuperscript{18} In addition, interpretation of both the intravenous glucose tolerance test and the combined glucose-insulin test requires knowledge of the fact that the glucose curves are right-shifted for donkeys when compared with horses.\textsuperscript{18} Mendoza et al\textsuperscript{18} summarizes the protocols and the timing required for each but also clearly states that insulin cut-off values have not yet been established for donkeys with these diagnostic protocols. Future work is needed to evaluate the use of the oral glucose tolerance test by using corn syrup in donkeys. Additional work to further define donkey and mule clinical pathology values indicative of disease states for other samples, such as body cavity fluids, respiratory system samples, and cerebral spinal fluid (CSF), is needed. One study evaluating serum and CSF from the lumbosacral space in 20 clinically normal miniature donkeys obtained results similar to those of horses for the analytes measured.\textsuperscript{19} A couple of recent studies have highlighted relevant differences in coagulation testing between donkeys and horses.\textsuperscript{20,21} One of those studies found that both activated partial thromboplastin
and prothrombin time were significantly shorter in the donkey than the horse, suggesting that the use of horse RIs may lead to the underdiagnosis of coagulopathies in donkeys.\textsuperscript{8,11} Some of the pertinent coagulation values from those two studies are summarized in Table 2.\textsuperscript{20,21}

5. Conclusions

Donkeys, mules, and hinnies prove time and again that they differ significantly from horses in their anatomy, behavior, nutritional requirements, pharmacokinetics, and much more.\textsuperscript{1,15,22} With the most current literature highlighting these important distinctions, it is not surprising to learn that the limited donkey- and hybrid-specific clinical pathology studies likewise demonstrate differences across the species.\textsuperscript{8,11} Reliance on clinical pathology testing of equids in veterinary medicine is exceedingly important due to their stoic nature and propensity to conceal ailments. For this reason, further work is necessary in this capacity to aid the veterinarian in elevating the health and welfare status of equids.

Acknowledgments

Declaration of Ethics

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

Conflict of Interest

The Authors have no conflicts of interest

References and Footnote

2. Taylor TS, Matthews NS. Mammoth asses—selected behavioural considerations for the veterinarian. \emph{Appl Anim Behav Sci} 1998;60:283–289.

\textsuperscript{a}Corvac, Coviden. https://us.vwr.com/store/product/4648903/monojecttm-blood-collection-tubes-coviden
Practical Aspects of Donkey Breeding Management

Igor F. Canisso, DVM, MS, PhD, DACT, DECAR (Equine Reproduction)*; Humberto B. Magalhaes, DVM, MS; and Lorenzo G.T.M. Segabinazzi, DVM, MS, PhD

1. Introduction

Donkeys are bred worldwide for a variety of uses. From the production standpoint, donkey milk has become a delicacy particularly useful for infants and those allergic to cow or goat milk and the cosmetic industry.1 In Asia, the renewed interest in donkeys for ejiao, a product of traditional Chinese medicine, has generated a higher demand for the production of donkeys.1 However, in many countries, donkeys are bred primarily to produce hybrids with horses (i.e., mules and hinnies).2 Hybrid animals are used for agricultural work, pleasure, Western sports, and herding cattle worldwide.2 In North and South America, mule showing and pleasure rides attract thousands of enthusiasts to specific events such as the Mule Day at the Shawnee National Forest in Herod, Illinois, and Mule Days in Bishop, California. Production of mules for showing, pleasure riding, and racing are reasons practitioners are contacted to breed horse mares with jack semen. In addition, practitioners are typically asked to breed seedstock jennies in the American continent to obtain quality jacks, and less commonly for pets or conservation efforts. Donkeys are classified as miniature, small standard, standard, and large breeds. Therefore, it is paramount that equine practitioners be prepared to provide reproductive services for donkey and mule breeders. Thus, this manuscript discusses recent advances in donkey breeding management and the authors’ clinical experiences with the species.

2. Female (Jenny) Features

Estrous Cycle and Sexual Behavior

The interovulatory interval is longer in donkeys (23–26 days) than in mares (~21 days; Table 1).2 Duration of the estrous cycle varies with breeds, body condition scores (BCS), geographical location, and time of the year.2 Estrus usually lasts about 6 days, but it varies from 4 to 10 days, and diestrus lasts from 15 to 19.6,7 Ovulation takes place 1 day before ceasing signs of estrus.7,8 In the spring and summer, the estrus is shorter than in the fall and winter.6,9,10 Well-fed donkeys do not display seasonal anestrus; under tundra weather, some jennies experience one prolonged interovulatory interval of 40 to 50 days during the deep winter and then resume regular interovulatory intervals.2 Also, a recent study described that older jennies display longer interovulatory intervals, a slower growth rate of the dominant follicle.6 In addition, the BCS affects the duration of the interovulatory intervals.6 Specifically, higher BCS (>6/9) lengthened the interovulatory intervals.

NOTES
due to longer diestrus; however, the follicle growth rate and the dominant follicle diameter were independent of the BCS in the same study. Multiple ovulation was positively associated with BCS and had no effect on the interovulatory interval. Signs of estrus in jennies include standing to be mounted, a lowered head with ears back against the neck, clitoral winking, urination, mouth clapping, and tail raising (Fig. 1). It is worth noting that the mouth clapping is the most typical and also the first and last estrus sign in jennies. Jennies kept on pastures will congregate into a sexually active group similar to cows. In the sexually active group, the female in estrus is mounted by other females coming in or out of estrus. Inhaling urogenital secretions from one another followed by the Flehmen’s response is a typical feature of sexually active groups. This jack-like behavior is a normal species-specific behavior in jennies, and it should not be confused with intersex and those with granulosa-theca cell tumors; jennies in diestrus typically do not stand to be mounted and may kick at the jack.

Folliculogenesis and Reproductive Hormones in Jennies

Follicular deviation (i.e., diameter when the dominant follicle starts growing faster than subordinate follicles) occurs 8 to 9 days before ovulation at an approximately 18-mm follicle for small-frame breeds and ~20 mm for large donkey breeds. Generally, jennies have one or two follicular waves per estrous cycle. In small-frame donkeys, the dominant follicle grows 2 to 3 mm/day from deviation to ovulation, while in large donkey breeds, the dominant follicle grows up to 4 mm/day. Thus, the diameter of the periovulatory follicle seems to be associated with the jenny’s body frame, and follicle size varies from 30 to 48 mm. Concentrations of estradiol-17β increase from 10 pg/mL during early estrus to peak around 40 to 60 pg/mL at 24 hours before ovulation. After ovulation, progesterone concentrations slowly increase for 4 to 6 days, reaching a peak by 10 days and a plateau by 14 to 16 days postovulation. Luteolysis starts by 14 to 15 days postovulation, lasting up to 2 to 3 days for progesterone concentration to nadir (<1 ng/mL; Fig. 2). Multiple ovulations seem to be higher in donkeys than in mares; it varies from 5% to 70% in jennies across studies. Some breeds have a high incidence of multiple ovulations, such as the Portuguese breed Miranda (48%), the Chinese black donkey Dezhou (13%), the Spanish breed Catalan (42%), and the American Mammoth (61%). The high incidence of multiple ovulations in donkeys makes a twin pregnancy more likely in jennies than in mares. At the same time, donkeys have an extremely efficient placenta rendering a high probability for the delivery of live twins. However, twin pregnancies have an increased risk for pregnancy loss, and live donkey twins are of a smaller frame and weaker than singletons; also, there is a high risk of dystocia (authors’ unpublished observations). Therefore, it is still advisable to perform manual reduction (as done in horses) by transrectally crushing one of the embryonic vesicles around 14 to 17 days postovulation.

### Table 1. Duration of Estrous Cycles, Estrus, and Diestrus in Various Donkey Breeds

<table>
<thead>
<tr>
<th>Breeds</th>
<th>Country</th>
<th>Estrous Cycles (n)</th>
<th>Estrus (d)</th>
<th>Diestrus (d)</th>
<th>IOI (d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Miranda</td>
<td>Portugal</td>
<td>33</td>
<td>6.6 ± 0.3</td>
<td>17.9 ± 0.5</td>
<td>23.8 ± 0.5</td>
</tr>
<tr>
<td>Pega</td>
<td>Brazil</td>
<td>13</td>
<td>8.0 ± 2.5</td>
<td>18.0 ± 2.3</td>
<td>26.0 ± 2.7</td>
</tr>
<tr>
<td>Burro Mexicano</td>
<td>Mexico</td>
<td>27</td>
<td>6.9 ± 0.7</td>
<td>17.9 ± 0.5</td>
<td>23.8 ± 0.4</td>
</tr>
<tr>
<td>Crossbreed</td>
<td>USA</td>
<td>19</td>
<td>6.4 ± 0.6</td>
<td>19.3 ± 0.6</td>
<td>25.7 ± 0.7</td>
</tr>
<tr>
<td>Catalanian</td>
<td>Spain</td>
<td>10</td>
<td>5.6 ± 0.2</td>
<td>20.0 ± 0.4</td>
<td>25.0 ± 0.3</td>
</tr>
</tbody>
</table>

IOI, interovulatory interval. Adapted from a review by Canisso et al. (2019) and numerous authors cited in the table.

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![Fig. 1. Signs of estrus in jennies. A, Baudet du Poitou displaying mouth clapping and ears lowered back while being teased by a mature jack. B, Tail lifting, clitoral eversion, and urination in a Northeast Brazilian jenny while being teased to a mature jack (not shown).](image-url)
Estrous Cycle Monitoring

Transrectal palpation and ultrasonography are the most widely useful approaches for the breeding management of jennies. To safely perform this examination, jennies can be restrained in horse stocks, placed in a doorway of a stall, or have a lip twitch placed and palpated with no minimal restraining (Fig. 3). Sedation is rarely necessary to palpate jennies. While the reproductive tract is proportionally larger in jennies than mares, rectally palpating small-frame jennies can be challenging due to the reduced anus and rectum sizes. In addition, some jennies may strain excessively during transrectal palpation and ultrasonography. Lidocaine mixed with lubricant (20 mL of 2% lidocaine in 80 mL of carboxymethylcellulose) alone or in combination with N-butylscoloploummonium bromide (10–20 mg/animal, IV) may be necessary to safely and successfully perform a transrectal examination in jennies. Miniature donkeys cannot typically be palpated transrectally without significant risk for rectal tears. The ultrasonographic appearance of jennies’ reproductive tract resembles mares; however, jennies are well-known to present a less pronounced endometrial edema, particularly in fat donkeys. Therefore, if the clinician waits to induce ovulation or breed jennies using the mare endometrial edema scores, ovulations will be missed. Close to ovulation, the periovulatory follicle changes from spherical to an oval or ellipsoid shape, and the follicular wall becomes irregular and thicker. Of interest, the vascularization beneath the follicular wall increases up to 24 hours before ovulation (Fig. 5). After ovulation, jennies can present homogeneous appearance and two other distinct corpus luteum (CL) echogenic appearances. One appearance has a white central hyperechoic line leading up to luteolysis; the second morphologic type has a nonechogenic central lacuna that gradually reduces in size until luteolysis (Fig. 6). On the first day of diestrus, the size of CL represents 77% of preovulatory follicle diameter. The vascularization of the CL rises gradually until 4 to 6 days after ovulation, peaks around 11 days, and starts to decline 2 to 3 days after luteolysis (14-15 days postovulation; Fig. 7).

Hormone Therapy for Jenny Estrus Cycle Manipulation

Prostaglandin F2alpha (PGF2α) analogs can be used to bring jennies back to estrus. Jennies typically return to estrus between 2 and 5 days after luteolytic agent administration (Table 2). Side effects such as sweating, abdominal pain, and loose manure can be seen with all luteolytic drugs. For small-frame donkeys, dinoprost tromethamine should be reduced to 2.5 mg to minimize the side effects caused by standard doses used in the horse (i.e., 5-7.5 mg). In general, luteolytic drugs are used >5 days after ovulation; however, some authors suggest giving a luteolytic agent 3 days after ovulation, with the efficacy varying from 17% to 100% (Table 2). The authors’ preference is to use dinoprostone (2.5-5 mg) or cloprostenol (0.075 mg) at least 5 days postovulation. Baudet du Poitou jennies may take up to 10 to 12 days to return into estrus after standard PGF2α administration 5 days postovulation. Induction of ovulation in jennies is typically carried out with human choric gonadotropin (hCG) or gonadotropin-releasing hormones (GnRH) analogs (e.g., lecirelin, buserelin, histrelin acetate, deslorelin acetate). The dose, route, size of the dominant follicle, and ovulation rates for each
therapy are highlighted in Table 3. The ideal time for induction of ovulation in jennies varies with BCS, age, and body size. As previously described, jennies do not consistently present a marked endometrial pattern before ovulation as mares do (Fig. 4),\(^{10,18}\) therefore, the endometrial pattern should not be considered

Fig. 4. Endometrial edema of jennies assessed via transrectal ultrasonography. A, Absent; B, Mild; C, Moderate; D, Pronounced.

Fig. 5. Sequential B-mode and power doppler ultrasonograms of a preovulatory follicle at 36 hours (A and D), 24 hours (B and E), and 1 hour (C and F) before ovulation. Over time, there is an increase in an anechoic line (asterisks) beneath the follicular wall, a loss of the spherical shape, and development of the stigma (apical area with brackets), and a thickening of the granulosa layer (arrows) can be noticed upon impending ovulation. Also, there is an increased vascularization beneath the preovulatory follicle. A-C, Power Doppler; D-F, B-mode.
the sole criteria to induce ovulation in jennies.\(^2\) Small-size jennies (200–250 lb body weight) usually can be induced when periovulatory follicles reach 28 to 32 mm in diameter. Small standard and standard jennies (300-400 lb body weight) can be induced when follicles measure 35 to 40 mm, and larger breeds (500-800 lb body weight) such as Mammoth and Baudet du Poitou follicles are induced when attaining 40 to 45 mm in diameter.\(^a\) Well-fed animals tend to grow follicles more rapidly and ovulate follicles with a smaller diameter for the expected body frame.

Estrus Synchronization Protocols

Hormones commonly administered to jennies to synchronize estrus include PGF2\(_\alpha\),\(^15\) progesterone or progestin,\(^15,28\) hCG, and GnRH analogs (Fig. 8).\(^15,28,29\) Single or double doses of PGF2\(_\alpha\) with 16-day intervals can be used. Administration of GnRH 7 days before the first PGF2\(_\alpha\) ensures ovulation, creating a CL that will be responsive to the second PGF2\(_\alpha\) administration.\(^29\) Progesterone (intravaginal device or intramuscular) coupled with PGF2\(_\alpha\) can also be used in jennies. Combining progesterone with estrogen for 10 days provides better synchrony and synchronous ovulation in 73% (8/11) of jennies; however, it is still not as efficient as double PGF injections 16 days apart (100%, 10/10).\(^15\)

Artificial Insemination

Artificial insemination (AI) in jennies is performed similarly to mares (Fig. 9).\(^2\) However, jennies have a narrowed and tortuous cervix, with the vaginal portion of the cervix assuming variable appearance from straight to C, D, and V appearance.\(^2\) Thus, particularly for some jennies, trans-passing the cervix for routine intrauterine procedures can be a daunting task.\(^2\) In addition, some jennies present excessive straining during intravaginal procedures, and the reduced lumen of the vagina and vulva can further complicate routine procedures. Thus, in this case, the authors may perform caudal epidural anesthesia with 2 to 4 mL of lidocaine 2%. Alternatively, transcervical AI can be performed using Wilsher and Allen’s forceps described for the embryo transfer technique (Fig. 10).\(^30\) A vaginal speculum is introduced, and the cervix is then grasped with the forceps and retracted caudally to facilitate the passage of the AI pipette through the cervix. This reduces the effects of manipulation and the risk of introducing contamination during AI. Semen can be deposited into the uterine body or guided to the uterine horn ipsilateral to the preovulatory follicle/ovulation. Deep-horn AIs are appropriated for small semen volumes (2-4 mL) to avoid reflux of semen, while larger volumes can be deposited into the uterine body. Of interest, a higher sperm dose should be used to breed jennies. Jennies inseminated with at least 1 billion motile sperm achieve similar pregnancy rates compared with mares inseminated with 500 million motile sperm.\(^31\) Overall, jennies and mares have satisfactory fertility when bred with raw or freshly extended donkey semen.\(^2\) Although frozen donkey semen typically presents excellent post-thaw quality and achieves satisfactory fertility rates in mares (~50%),\(^32\) it does not necessarily translate into satisfactory fertility in

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**Fig. 6.** The normal echogenic appearance of jenny corpus luteum. A, Homogeneous echogenic appearance. B, Nonechogenic central lacuna. C, Hyperechoic central line.

**Fig. 7.** Ultrasonography image of the corpus luteum and corresponding progesterone concentrations during luteolysis at 10 (A), 12 (B), 14 (C), and 18 (D) days postovulation. Images were obtained via power Doppler ultrasonography; progesterone was determined via radioimmunoassay.
Historically, pregnancy rates in jennies inseminated with frozen-thawed donkey semen varied from 0 to 30%. Replacement of the cryoprotectant such as glycerol to other cryoprotectants such as dimethyl sulfoxide (DMSO), glutamine, dimethylformamide, and dimethylacetamide or rediluting the semen after thawing with extender containing no glycerol improved post-thaw semen quality; however, the conception rates remained low. Since jennies have a more pronounced postbreeding inflammatory response than mares, an association between this acute inflammatory response and the poor conception rates when frozen donkey semen is used may be speculated in jennies. Dexamethasone is a widely used therapy to lessen the postbreeding inflammatory response in susceptible mares. However, many donkeys are insulin-resistant animals as an adaptive response to the lack of energy; therefore, donkeys can be more susceptible to laminitis, and corticosteroids should be used with caution in these animals. The authors typically use 10 mg/jenny before or after breeding with frozen semen. Authors have proposed uterine lavage and infusion with donkey seminal plasma (v:v, 1:2, frozen-thawed semen: seminal plasma). The addition of donkey seminal plasma seems to reduce the endometrial inflammatory response; the authors suggested that post-thawed semen resuspended in seminal plasma tended to increase the conception rates. Seminal plasma reduces the postbreeding endometrial inflammatory response and the polymorphonuclear cells bound sperm. A recent study concluded that donkey seminal plasma, not sperm-intrinsic factors, are able to trigger NETosis (the process that culminates in the release of neutrophil extracellular traps) in both a time- and sperm-concentration-dependent manner and suggests that NETosis could represent a mechanism by which the female reproductive tract selects sperm. Uterine lavage after insemination (at least 4 hours after insemination) can also be used to mitigate the postbreeding uterine reaction in jennies. The authors of the present report recommend that increasing the breeding dose coupled with deep horn insemination and uterine lavage 4 to 6 hours after breeding can be used to enhance pregnancy rates in jennies. A recent study out of China, utilizing a timed-AI protocol to synchronize ovulation in jennies, obtained >70% of conception rate on deep horn insemination with frozen-thawed jack semen (500 × 10⁶) at 28 and 40 hours after hormone (luteinizing hormone [LH] or hCG) injection. This study warrants testing in practice to see if these results can be consistently achieved. Also, the LH used in that study is not available in the United States. The authors use ecbolics extensively postbreeding in jennies; doses vary from 10 to 20 units/jenny, q6-12h.

### Table 2. Induction of Luteolysis in Jennies Using Prostaglandin F2 Analogs

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose (IM, mg)</th>
<th>Cycles (n)</th>
<th>Days Postovulation at PGF2α</th>
<th>Estrus Detection or to Progesterone Decline (&lt; 1 ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dinoprostone¹⁵</td>
<td>5</td>
<td>58</td>
<td>–</td>
<td>44/58 (76%)</td>
</tr>
<tr>
<td>Cloprostenol²²</td>
<td>0.075</td>
<td>10</td>
<td>3</td>
<td>10/10 (100%)</td>
</tr>
<tr>
<td>Cloprostenol²³</td>
<td>0.075</td>
<td>22</td>
<td>3</td>
<td>21/22 (96%)</td>
</tr>
<tr>
<td>Luprostiol²⁴</td>
<td>7.5</td>
<td>169</td>
<td>Diestrus</td>
<td>NS</td>
</tr>
<tr>
<td>Alphaprostol²⁵</td>
<td>3</td>
<td>6</td>
<td>3</td>
<td>1/6 (17%)</td>
</tr>
</tbody>
</table>

IM, intramuscularly; NS, not specified. Content adapted from review by Canisso et al. 2019 and numerous other authors cited above.

### Table 3. Induction of Ovulation in Jennies Using hCG and GnRH Analogs

<table>
<thead>
<tr>
<th>Hormones</th>
<th>N of Cycles</th>
<th>Dose (route)</th>
<th>Preovulatory Follicle Size (mm)</th>
<th>Ovulation Rates at 48 h after Induction</th>
<th>Induction-Ovulation Interval (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>hCG²⁶</td>
<td>12</td>
<td>2500 IU (IV)</td>
<td>30–35</td>
<td>11/12 (91.7%)</td>
<td>4.2 ± 13</td>
</tr>
<tr>
<td>hCG²⁶</td>
<td>15</td>
<td>2500 IU (IV)</td>
<td>36–40</td>
<td>15/15 (100%)</td>
<td>4.2 ± 13</td>
</tr>
<tr>
<td>Lecirelin²⁶</td>
<td>23</td>
<td>100 ug (IV)</td>
<td>30–35</td>
<td>19/23 (82.6%)</td>
<td>43.8 ± 14</td>
</tr>
<tr>
<td>Lecirelin²⁶</td>
<td>20</td>
<td>100 ug (IV)</td>
<td>36–40</td>
<td>16/20 (80.0%)</td>
<td>43.8 ± 14</td>
</tr>
<tr>
<td>Buserelin²⁷</td>
<td>103</td>
<td>3.3-0.4 mg (SC)</td>
<td>32–34</td>
<td>72/103 (69.9%)</td>
<td>49.1 ± 25.9</td>
</tr>
<tr>
<td>Histrelin¹⁸</td>
<td>10</td>
<td>250 ug (IM)</td>
<td>25–28</td>
<td>50% (5/10)</td>
<td>209.8 ± 48</td>
</tr>
<tr>
<td>Histrelin¹⁸</td>
<td>10</td>
<td>250 ug (IM)</td>
<td>29–32</td>
<td>100% (10/10)</td>
<td>145.2 ± 34.6</td>
</tr>
<tr>
<td>Histrelin¹⁸</td>
<td>10</td>
<td>250 ug (IM)</td>
<td>33–36</td>
<td>60% (6/10)</td>
<td>183.3 ± 33.9</td>
</tr>
</tbody>
</table>

GnRH, gonadotropin-releasing hormones; hCG, human chorionic gonadotropin; IM, intramuscularly; IV, intravenously; SC, subcutaneously; ND, nondisclosure. Adapted from a review by Canisso et al. (2019).
Fig. 8. Estrus synchronization protocols for jennies. A, Double PGF2α doses 16 days apart.\textsuperscript{15} B, Progesterone and 17-β estradiol preceded by PGF2α 10 days apart from the steroid administration.\textsuperscript{15} C, Double PGF2α 17 days apart.\textsuperscript{15} D, Serial PGF2α administrations.\textsuperscript{60} E, Continual progesterone administration for 10 days with PGF2α on day 8.\textsuperscript{60} F, Double PGF2α-GnRH and timed artificial insemination.\textsuperscript{29} G, Consecutive PGF2α-GnRH-PGF2α-GnRH and timed artificial insemination (AI).\textsuperscript{29} H, GnRH-PGF2α-GnRH and timed AI.\textsuperscript{29}
is our impression that oxytocin works as effectively as it does in mares. However, up to date, there are no studies assessing the efficacy of oxytocin in jennies. Guidelines for treating uterine fluid in jennies are currently lacking; however, the authors have used equine guidelines to determine when to flush jennies. For instance, jennies with a large amount of anechoic or moderate to large amount of intrauterine fluid accumulation (Fig. 11) are typically flushed with a crystalloid solution such as lactated Ringer’s solution.

Pregnancy Diagnosis

Although transrectal palpation can be used as a diagnostic tool in jennies, this method requires experience and skill for a reliable diagnosis, and feasibility is determined by the type and size of the jenny along with the stage of gestation. Therefore, pregnancy diagnosis by transrectal ultrasonography minimizes the risk of false-negative diagnosis and aids in the identification and management of twins. Pregnancy diagnosis can be performed by transrectal ultrasonography 9 to 10 days postovulation, when the embryo vesicle is $2.3 \pm 1.1 \text{ mm}$ in diameter (Fig. 12A); however, the accuracy of detecting an embryonic vesicle pregnancy this early in gestation is only 25%. The first pregnancy diagnosis is ideally performed between 12 and 15 days after ovulation when the embryonic vesicle measures between $6 \pm 2.3 \text{ mm}$ and $17.9 \pm 3.8 \text{ mm}$ (Figs. 12B and C). The early embryonic features in jennies are highlighted in Table 4. The embryonic vesicle retains its spherical shape from the first detection (10 days) to fixation (18.5 ± 1.4 days), and the vesicle grows continually 3.1 ± 2.7 mm/day. After fixation, the embryonic vesicle becomes irregularly shaped. The embryo proper appears at the ventral pole of the embryonic vesicle around days 19 to 21 postovulation (Fig. 12D), beginning the transition from the yolk sac to the allantoic sac phase (Fig. 12E). By day 24 to 25 of pregnancy, an embryo heartbeat can be detected.

3. Male (Jack) Features

Jack Sexual Behavior and Semen Collection

Jacks are generally regarded as challenging to collect semen using estrus jennies, mares, or a dummy mount. Strategies to optimize semen collection are described elsewhere in details. Except for a few high-libido donkeys, most jacks can take up to 60 minutes to collect semen or may not collect unless conditions are optimized. Jacks are attracted to jennies, and some can be conditioned to mares; unfortunately, most mares are not receptive to jacks (60%), and several react violently to jacks during teasing or mounting. Thus, in mares not conditioned to jacks, the authors elect to sedate the mare heavily with alpha-2-agonists, place breeding hobbles, and lip twitch (Fig. 13). Additionally, breeding stocks with or without a pit in the center can aid in restraining the mounting mare being used for live cover or semen collection (Fig. 13). Mares exposed to donkeys tend to become used to jacks, and after repeated uses, they become more receptive to them when in estrus. Some jacks present extremely low libido in the presence of an estrus mare; patience and incorporating mimicked elements of donkey courtship with jennies while working with mares can be extremely helpful. In equids, washing the penis immediately before collection, particularly in sexually rested animals, is paramount to decrease bacterial contamination and debris in semen. Teasing by proximity to the teaser or allowing the jack to interact with the mare or jenny should result in penile exposure and erection (Fig. 14). While washing, untrained
donkeys can benefit from cleaning the penis with wet cotton rather than throwing water with a cup as water splashing might be enough negative stimuli for a donkey to lose an erection.² Anecdotally, PGF2α analogs (dinoprost 2.5–5 mg/animal, IM, or sodium cloprostenol 125–250 mg, IM) have been used to enhance semen collection of donkeys failing to attain an erection in clinical practice.² A recent study demonstrated that administration of cloprostenol reduces the interval for semen collection without affecting semen quality in donkeys.⁴⁸ In the authors’ practice, some donkeys receive PGF2α analog throughout the breeding season three times/week without any apparent detrimental effect, and jacks do not seem to become refractory. Jacks can have semen collected in an artificial vagina while mounting an estrus mare, jenny, or a dummy mount (Fig. 15). All artificial vagina (AV) models (e.g., Missouri, Botucatu, and Hanover) used for horses can be successfully used to collect semen in donkeys.² Despite donkeys having a penis significantly longer than stallions, a medium-size AV (e.g., regular length 22 inches) can be used to collect jack semen (Fig. 15).² The water temperature of the AV is similar to horses and ranges from 49 to 55°C while filling the AV.² To filter the semen, practitioners may use horse filters or well-folded gauze. Filters can be coupled with the collection bottle, or semen filtration can be carried out right after collection.² The gel fraction is absent in most ejaculates, and when it is present, it will be in a much smaller proportion than typically seen in stallions.² Since some jacks may take a while to attain a sustained erection, the AV can be kept in a Styrofoam box or a warm incubator once ready. Currently, protocols used for chemical induction of ejaculation do not work in donkeys.² One study administered oral imipramine (3 mg/kg) followed by xylazine (0.66 mg/kg, IV) or a single dose of butorphanol (0.02 mg/kg, IV) and xylazine (0.33 mg/kg, IV),⁴⁹ which resulted in no ejaculation. A second study, combining

Fig. 11. Cross-section ultrasonographic images of the uterine horns in jennies. A, Moderate echogenic intrauterine fluid accumulation. B, Hyperechogenic intrauterine fluid accumulation (*).

Fig. 12. Transrectal ultrasonographic images of embryo development in pregnant jennies. Embryonic vesicle at 9 (A), 12 (B), 16 (C), 21 (D), and 27 (E) days after ovulation. White rows indicate the embryonic vesicle (A, B, C). EP, embryo proper; AS, allantoic sac; YS, yolk sac.
Imipramine (2 or 3 mg/kg, orally) and xylazine (0.44, 0.66, or 0.7 mg/kg) 1 or 2 hours later, resulted in only one animal ejaculating, even though 74.5% (41/55) of the animals displayed erection.2,50

Semen Processing and Evaluation

Donkey semen is processed similarly to stallions.2 It is worth noting that donkey semen has colors varying from white, light grey, to yellow,1 and the latter should not be confused with urospermia (Fig. 16). Gel-free semen volume can be assessed by direct measurement or more accurately by determining the weight of the semen (1 g = 1 mL). Sperm concentration can be evaluated with a hemocytometer, spectrophotometerb or nucleocounterc with horse settings.2 Donkeys are well-known to have larger testes, a shortened length of spermatogenesis, and the highest spermatogenic efficiency of domesticated animals per gram of testicular parenchyma.51,52 Therefore, concentration and the total number of sperm are greater in donkeys than horses, and younger donkeys produce ejaculates with lower volume and greater concentration than older jacks.47 Young donkeys tend to produce gel-free ejaculates of 30 to 50 mL, and older donkeys tend to produce gel-free ejaculates of 60 to 90 mL.2,47 Typically, sperm concentrations for donkeys vary from 250 to 500 million sperm/mL in donkeys collected two to three times/week.2,47,48 The typical total number of sperm ejaculates vary from 20 to 40 billion in donkeys collected once or twice a week.5,47,48 Donkey motility parameters, including velocity and progressive motility, are also higher than stallions.53 Jacks often have ejaculates with 80% to 90% of total and progressive sperm motility. Morphology is rarely associated with infertility in donkeys, with most donkeys presenting < 15% of morphologic defects.54 Therefore, sperm morphology evaluation is not typically carried out for the breeding unless there is a history of subfertility or poor semen quality in serial semen collections.

Semen Cooling and Shipping

Cooled-shipped semen in donkeys can be carried out using horse recommendations, with some exceptions.2 Passive cooling semen containers, such as those used for horses4,4 can keep donkey semen refrigerated at 5 to 8°C.2,55 Noncentrifuged semen should target 25 to 50 million sperm/mL, and it needs to be extended at least one part of semen to three parts of extender.2 If a milk-based extender is used to ship donkey semen, the seminal plasma should be removed via centrifugation or semen filter6 or the milk-based extender should be enriched with a source of cholesterol such as egg yolk to maintain motility and fertility.2 Alternatively, milk-based extenders containing cholesterol loaded in cyclodextrinb,i result in better semen cooling and fertility than milk-based extenders with no additional source of cholesterol.55,56 Commercially available semen-freezing egg-yolk-based extendersl can be used to cool donkey semen up to 24 hours at 5°C with excellent semen parameters and fertility in horse mares.32,34,36 However, up to now, the fertility of cooled donkey semen extended in egg-yolk based extendersl has not been tested in jennies. Satisfactory pregnancy rates (73%) can be obtained when jennies are bred with at least 1 billion progressively motile fresh sperm.31 In the authors’ practice, mares and jennies are bred with at least 2 billion progressively motile sperm, the uterus is flushed 6 hours

Table 4. Early Embryo Features in 8 Jenny Conceptuses

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Days of Gestation</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Detection of EV</td>
<td>11.8 ± 1.3</td>
<td>10–14</td>
<td>6.5 ± 1.9</td>
</tr>
<tr>
<td>Fixation of EV</td>
<td>18.5 ± 1.4</td>
<td>16–20</td>
<td></td>
</tr>
<tr>
<td>Loss of spherical shape</td>
<td>18.8 ± 1</td>
<td>18–20</td>
<td></td>
</tr>
<tr>
<td>Detection of embryo</td>
<td>22 ± 1.1</td>
<td>20–24</td>
<td></td>
</tr>
<tr>
<td>Detection of HB</td>
<td>25 ± 1.1</td>
<td>24–26</td>
<td></td>
</tr>
<tr>
<td>Detection of AS</td>
<td>27.3 ± 1</td>
<td>26–28</td>
<td></td>
</tr>
</tbody>
</table>

EV, embryonic vesicle; HB, heartbeat; AS, allantois sac. Data obtained from Crisci et al. (2014).51

Fig. 13. Restraining mounting females for donkey semen collection. A, Breeding hobbles and twitch used to restrain a jenny in estrus while being teased by a donkey. B, Estrus mare with breeding hobbles in place while heavily sedated with detomidine before donkey semen collection.
postbreeding, and ecbolics are given to aid uterine evacuation if needed. Barren mares or jennies with signs of endometritis (intrauterine fluid accumulation or infertility despite optimal breeding management with fertile semen) may require additional therapy as described elsewhere.

Semen Freezing

Protocols used to process donkey semen for freezing have been adapted from stallions. After collection, semen is extended 1:1 in a temperature-matched cooling semen extender, centrifuged at 600 \( \times g \) for 10 minutes, or cushion-centrifuged for 1000 \( \times g \) for 20 minutes. Thereafter, the supernatant is discarded, and the pellet is resuspended in a horse semen-freezing extender (or homemade extender). The semen is extended at 100 to 200 million sperm/mL and then loaded in 0.5 mL straws. Cooling and stabilizing semen can be carried out in a freezing control machine or a domestic refrigerator for 20 to 60 minutes, depending on the type of extender. Thereafter, the semen can be kept in the freezing machine or transferred 3 to 6 cm over liquid nitrogen vapor for 15 to 20 minutes and then plunged into liquid nitrogen. Semen frozen in 0.5-mL straws are typically thawed at 37 to 38°C for 30 to 60 seconds. The straws can be used for AI via deep-horn insemination with a flexible pipette coupled with a stylet or a couple with an inner catheter. If the practitioner is not familiar with deep-horn AI, semen can be thawed and deposited in the uterine body using a standard AI pipette. Pregnancy rates with frozen semen vary drastically with the protocol used and between species (mares vs. jennies). Mares bred with donkey frozen-thawed semen present pregnancy rates around 50% to 70% in commercial breeding programs, while jennies have much lower pregnancy rates, averaging 20% to 30% per cycle. Results are jack dependent, with some males demonstrating satisfactory pregnancy rates, while others obtain extremely poor pregnancy rates despite apparently satisfactory sperm motility.

Donkey Epididymal Transport and Epididymal Sperm Recovery and Processing

Upon unexpected death, castration, or euthanasia, donkey epididymal sperm can be harvested and cryopreserved using similar techniques described for stallions. The testes and epididymides should be harvested in bloc, and ductus deferens should be ligated to prevent semen wastage.
transportation, the epididymis or the whole testis should be submerged in some sort of liquid (i.e., lactated Ringer’s solution, 0.9% saline solution, or a semen-cooling extender) and stored in a passive cooling system at 4 to 5°C. Upon arrival in the laboratory, either immediately after removal from the live animal or postmortem, or after transportation, the epididymides need to be rinsed with room-temperature sterile lactated Ringer’s solution and wiped with gauze to remove any contamination from blood or debris. Then, the epididymides are dissected from the testes. Dissection should be performed well enough to be able to stretch the epididymis and allow for a good sperm recovery. Following dissection, semen is harvested by retrograde flushing or flotation/float-up technique. For retrograde flushing, the tail of the epididymis is transected with a straight-bladed Mayo scissor, and the ductus deferens is catheterized with an intravenous catheter, a Tomcat, or less ideally with a needle.

The epididymis is then flushed with semen extender until the fluid containing the semen is allowed to outflow into a 50-mL conical tube. The epididymis can be flushed with milk-based cooling extenders with volumes from 5 to 30 mL or can be flushed with a semen-freezing extender with volumes from 2 to 10 mL. In the authors’ clinical practice, the preferred technique is directly flushing the epididymides with freezing extender. Then, concentration is adjusted to 200 million sperm/mL, and semen is cryopreserved as described above. If poor recovery is obtained with the retrograde flushing, or if the ductus deferens or epididymis are inadvertently perforated, the float-up technique can be used. Sperm yield is approximately 40% to 60% less with the float-up technique than retrograde flushing. Up to now, the fertility of donkey epididymal frozen-thawed sperm has not been tested in mares or jennies.

4. Conclusion

In conclusion, reproductive features and protocols available to manage donkeys in breeding programs have been described. It is worth noting that despite the similarities between donkeys and horses, some reproductive features differ remarkably. Therefore, not all horse guidelines are useful for the donkey species. Knowledge of the reproductive features of the donkey is useful to implement strategies to improve the reproductive efficiency of this species.

Acknowledgments

Declaration of Ethics

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

Conflict of Interest

The Authors have no conflicts of interest.

References and Footnotes


Updates on Diagnostic Approach and Treatment of Select Donkey and Mule Diseases

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Donkeys and mules have their own physiology with species-specific reference ranges, drug dosages, and management that cannot be extrapolated from horse medicine. Authors’ address: School of Biosciences and Veterinary Medicine, University of Camerino, Matelica (MC), Italy; e-mail: fulvio.laus@unicam.it. *Corresponding and presenting author. © 2021 AAEP.

1. Introduction

The estimated global equine population totals 117 million animals, including 58 million horses, 50 million donkeys, and 9 million mules.1 Donkeys are mainly reared for milk production in several countries such as Italy2 because, thanks to its special properties, donkey milk is suitable for infants who cannot be breastfed and for people suffering from cow’s milk protein allergies.3–5 Currently, donkeys are also used as pets for recreational purposes and pet therapy rather than draught or burden animals, according to different countries. On the contrary, mule population has decreased in Europe and the Mediterranean European countries in recent years, while in developing countries, these animals are still widely used as beasts of burden or working equids for draft purposes.1,6

The diagnostic approach and treatment of some diseases including tick-borne disease, gastric ulcer syndrome, intestinal parasite infections, and ocular disease in donkeys and mules are herein discussed on the basis of current knowledge, updated publications, and personal experience. This paper focuses on those diseases capable of disrupting the normal physiology of donkeys and mules even in the absence of, or with minimal, clinical signs. Because of their stoicism, donkeys and mules tend to show only mild clinical symptoms, exhibiting signs of discomfort and pain differently from horses. Behavioral, anatomical, and physiological differences among horses, donkeys, and mules have a strong impact on clinical management and must therefore be known and understood when approaching these equids.

2. Tick-Borne Diseases in Donkeys

Among the infections transmitted by ticks that may affect donkeys, Theileria equi and Babesia caballi represent the most common etiological agents; however, Anaplasma phagocytophilum and Borrelia burgdorferi s.l. are also reported to be possible sources of infection.7,8 In horses, tick-borne diseases are commonly described, while only one paper is available on...
clinical signs in donkeys, this reporting important differences. In 2014, an epidemiological survey assessed the seroprevalence of tick-borne pathogens affecting donkeys in Italy.8 A total of 122 adult donkeys were tested for antibodies against T. equi, B. caballi, A. phagocytophilum, and B. burgdorferi s.l. by indirect immunofluorescent antibody test (IFAT), while the presence of pathogens was verified by using specific PCR protocols. According to IFAT results, 48/122 (39.3%) animals were positive for T. equi, 58/122 animals (47.5%) were positive for B. caballi, and 9/122 (7.4%) animals were positive for A. phagocytophilum, whereas no specific antibodies for B. burgdorferi s.l. were found in studied donkeys. Considering PCR results, 23/122 (18.8%) donkeys were positive for Babesia/Theileria spp., while no subject was positive for A. phagocytophilum or B. burgdorferi on PCR assays. On the basis of the survey by Veronesi and colleagues,8 a high exposure of Italian donkeys to piroplasm protozoans was found, thus confirming the results obtained in previous studies performed in donkey populations from other countries, including Turkey, Spain, and China, that showed a seroprevalence ranging from 17% to 93% for B. caballi and from 10% to 47% for T. equi.9-11 According to the study by Sgorbini and colleagues,12 the risk factors associated with piroplasmosis included advanced age and outdoor housing. These results agree with previous studies conducted on horses and are consistent with a high exposure to tick bites.12 Another study by Piantedosi and colleagues7 obtained similar results in donkeys tested for piroplasm infection by IFAT and found no evidence of symptomatic donkeys, as also observed by Veronesi and colleagues.8 Equine piroplasmosis is endemic in tropical and temperate areas and can occur as acute, subacute, chronic, and subclinical presentations, with subclinical being the most frequently observed. Animals surviving the acute phase may remain seropositive carriers with low levels of parasitemia, especially following T. equi infections,13 which is often responsible for overt clinical forms of piroplasmosis. On the contrary, B. caballi infections usually remain latent, although some animals can exhibit anemia, inappetence, pyrexia, edema, hemoglobinuria, reduced work efficiency, weight loss, and abortion in mares.13-15 Piroplasmosis in donkeys is an underestimated disease because of a natural resistance of these equids to infection and a prevalence of chronic asymptomatic forms typical of animals living in endemic areas. However, whenever observed, the clinical signs include anorexia, lethargy, disorders of intestinal motility (especially constipation), and splenomegaly.16 A study by Laus and colleagues17 investigated IFAT to detect immunoglobulin G antibodies against T. equi and B. caballi and PCR to detect Babesia spp. and Theileria spp. DNA in a donkey population in Central Italy. Clinical examination findings, hematological analyses, and serum bilirubin evaluations were compared to positive or negative immunologic status. A seroprevalence of 40.6% and 47.8% was found for T. equi and B. caballi, respectively. PCR results showed that 17.4% of the animals were found to be positive for T. equi and 3.6% for B. caballi by PCR analysis. Clinical examinations revealed 8.7% of the donkey population had only slight clinical signs consistent with chronic forms of the disease. Animals showed various degrees of anorexia, depression, pale mucus membranes, and poor body condition scores. The only symptom related to piroplasmosis was slight icterus, detected in 6 donkeys (Table 1). In contrast to a previous study performed on horses in Italy,14 no acute form of the disease was found. Despite the lack of specific clinical presentation, 58/138 donkeys had hematological and serum bilirubin alterations, and 96.6% of them were IFAT and/or PCR positive. Changes in erythrocyte number, packed cell volume, hemoglobin concentration, mean corpuscular hemoglobin, platelet number, and total bilirubin were significantly associated with test-positive animals even though they were asymptomatic. Therefore, piroplasm protozoans mostly cause subclinical infections in donkeys that potentially interfere with animal welfare and health other than productive and working abilities.17 The unique acute form of piroplasmosis in donkeys has been reported in an adult jack in Italy.7 This important difference between horses and donkeys should be taken into account when evaluating donkeys living in endemic areas. Current therapies, mainly based on the administration of imidocarb dihydrochloride, are derived from equine medicine and are not optimized for donkeys. Drug biodistribution appears to be different between donkeys and horses depending on the differences in plasma volumes that could result in increased kidney and liver toxicity in donkeys.16 Moreover, the severe side effects associated with the anticholinergic effect of the carbanilide derivates, like intense abdominal pain, diarrhea, and dyspnea, are much more marked in donkeys than in horses. Prophylaxis is based on the use of topical antiparasitic agents that are not specifically registered for donkey species. Medications registered for horses, bovines, and sometimes companion animals (i.e., dogs) like synthetic pyrethroids are sometimes used as they have wide safety/handling margins, high repelling power, and high residuals lasting about 2 weeks for the pour-on formulations. Among pyrethroids, permethrin is the most popular; however, sprays and pour-on formulations based on deltamethrin, cyfluthrin, alphacypermethrin, and flumethrin are commercially available as well.

3. Intestinal Cyathostomin Infections in Donkeys and Mules

Worldwide, gastrointestinal parasite infestations represent a major challenge for equids including mules and donkeys. Clinical signs typically include diarrhea, ventral abdominal edema, pyrexia, colic,
weight loss, and poor body condition. Equids are usually coinfected with different nematode species. Cyathostomins (small strongyles) are considered as the most important intestinal parasite group in wild and domestic equids for their pathogenic potential at both larval and adult stages to negatively impact wellness. Therefore, these nematodes, characterized by a widespread distribution, are of major concern, and efficient control based on the appropriate management, diagnosis, and use of anthelmintic products is mandatory.

In past years, control of gastrointestinal parasites was based on regular and frequent administration of anthelmintic drugs as preventive treatment strategy. However, in light of the increasing evidence of anthelmintic resistance and changes in parasite distribution, a re-examination of recommendations for parasite control has been necessary. In response to this need, the American Association of Equine Practitioners (AAEP) has formed a task force charged with producing a comprehensive set of recommendations for helping veterinarians to improve strategies and programs for parasite control in horses. The most significant change has been the development of a new way of thinking with the goal of limiting parasite infections rather than eradicating all parasites from the equine gastrointestinal tract (GIT). Therefore, equine clinicians should adopt suitable strategies in order to maintain limited parasite

<table>
<thead>
<tr>
<th>Donkeys</th>
<th>IFAT/PCR Positivity</th>
<th>Clinical Signs</th>
<th>Clinical Pathology</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Double IFAT</td>
<td>Depression</td>
<td>RGB, Hb, PCV, MCH, MCHC, Neutrophils, Eosinophils</td>
</tr>
<tr>
<td></td>
<td></td>
<td>BCS &lt; 2</td>
<td>Inappetence</td>
</tr>
<tr>
<td>2</td>
<td>Double IFAT</td>
<td>Depression</td>
<td>RGB, Hb, PCV, MCHC</td>
</tr>
<tr>
<td></td>
<td></td>
<td>BCS &lt; 2</td>
<td>Inappetence</td>
</tr>
<tr>
<td>3</td>
<td>Double IFAT</td>
<td>Depression</td>
<td>RGB, Hb, PCV, PLT</td>
</tr>
<tr>
<td></td>
<td></td>
<td>BCS &lt; 2</td>
<td>Icterus</td>
</tr>
<tr>
<td>4</td>
<td>Double IFAT</td>
<td>Depression</td>
<td>RGB, Hb, PCV, Neutrophils, PLT, TB</td>
</tr>
<tr>
<td></td>
<td></td>
<td>BCS &lt; 2</td>
<td>Icterus</td>
</tr>
<tr>
<td>5</td>
<td>Double IFAT</td>
<td>Depression</td>
<td>RGB, Hb, PCV, PLT, TB</td>
</tr>
<tr>
<td></td>
<td></td>
<td>BCS &lt; 2</td>
<td>Icterus</td>
</tr>
<tr>
<td>6</td>
<td>Double IFAT</td>
<td>Depression</td>
<td>RGB, Hb, PCV, Neutrophils, PLT, TB</td>
</tr>
<tr>
<td></td>
<td></td>
<td>BCS &lt; 2</td>
<td>Icterus</td>
</tr>
<tr>
<td>7</td>
<td>Double IFAT</td>
<td>Depression</td>
<td>RGB, Hb, PLT, TB</td>
</tr>
<tr>
<td>8</td>
<td>PCR T. equi</td>
<td>Depression</td>
<td>RGB, Hb, PCV, MCH, MCHC, TB</td>
</tr>
<tr>
<td></td>
<td></td>
<td>BCS &lt; 2</td>
<td>Icterus</td>
</tr>
<tr>
<td>9</td>
<td>PCR T. equi</td>
<td>Depression</td>
<td>RGB, Hb, PCV, PLT, TB</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pale MM</td>
<td>Icterus</td>
</tr>
<tr>
<td>10</td>
<td>PCR and IFAT (T. equi)</td>
<td>Depression</td>
<td>RGB, Hb, PCV, MCH, MCHC, WBC, PLT, TB</td>
</tr>
<tr>
<td></td>
<td></td>
<td>BCS &lt; 2</td>
<td>Pale MM</td>
</tr>
<tr>
<td>11</td>
<td>PCR and IFAT (T. equi)</td>
<td>Depression</td>
<td>RGB, Hb, PCV, MCH, MCHC, PLT, TB</td>
</tr>
<tr>
<td></td>
<td></td>
<td>BCS &lt; 2</td>
<td>Icterus</td>
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<tr>
<td>12</td>
<td>PCR and IFAT (T. equi)</td>
<td>Pale MM</td>
<td>RGB, Hb, TB</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Icterus</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: IFAT, indirect immunofluorescent antibody test; PCR, polymerase chain reaction; BCS, body condition score; RGB, red globe cells; WBC, white blood cells; PLT, platelets; Hb, hemoglobin; PCV, packet cell volume; MCV, mean corpuscular volume; MCHC, mean corpuscular hemoglobin concentration; TB, total bilirubin.
infection and egg shedding and guarantee animal welfare by minimizing the risk of parasitic disease. By doing this, there is a higher chance of maintaining efficacious drugs avoiding further development of anthelmintic resistance. To achieve these goals, it is fundamental to know the magnitude of egg shedding of individual horses by performing periodic fecal egg count surveillance.\textsuperscript{23} The modified McMaster fecal egg count procedure is the most extensively used technique; however, the technique\textsuperscript{a} is a new technique that has been developed by the University of Naples (Italy) and validated in several scientific papers as easy to use in clinical practice. The current guidelines established by the World Association for Advancement of Veterinary Parasitology\textsuperscript{24} and the AAEP suggest the assessment of the fecal egg count reduction (FERC) to know the percentage of efficacy of an anthelmintic substance. FERC can be calculated according to the formula FERC (\%) = 100(C – T)/C, where C is the geometric mean of eggs per gram (EPG) before the treatment and T is the geometric mean of EPG after the treatment. The geometric mean was calculated by averaging the log counts (x + 1) of the single EPG values, taking the antilogarithm, and then subtracting 1. At the present time, none of the three anthelmintic drug classes registered for horses (benzimidazoles, pyrimidines, and macrocyclic lactones) are currently available for treatment of parasites in mules, and only a few drugs are specifically licensed for use in donkeys.\textsuperscript{25–28} Therefore, mules and donkeys are usually treated with anthelmintic drugs at the same dosage, route, and intervals licensed for horses, despite the lack of scientific reports evaluating pharmacokinetics and efficacy related to their use in these animals.\textsuperscript{2,25,29}

Among anthelmintic compounds registered for equine species, ivermectin is a macrocyclic lactone commonly used due to its broad spectrum of activity against both endo- and ectoparasites.\textsuperscript{25,30} A recent study by Bazzano and colleagues\textsuperscript{31} investigated both pharmacokinetics and efficacy of ivermectin orally administered in mules at 200 \mu g/kg body weight (BW). Mules were found to have intermediate pharmacokinetic parameters between horses and donkeys, and these compounds seemed to be efficacious against cyathostomins (Table 2). Among anthelmintic drugs, macrocyclic lactones are characterized by a very long egg reappearance period, but recent reports have documented them shortened to just 4 to 5 weeks for both ivermectin and moxidectin on farms with high treatment frequencies.\textsuperscript{23} This is interpreted as emerging resistance in cyathostomins to this class. Thus, for macrocyclic lactones, it can be of value to run one set of posttreatment egg counts at around 4–6 weeks posttreatment to gauge the egg reappearance period status on the selected farm.\textsuperscript{23} The recurring onset of anthelmintic resistance together with the restrictions in the use of drugs in food-producing animals have enforced the search for sustainable alternative approaches for parasite control.\textsuperscript{32} Among the nutritional supplements used for the control of internal parasites in equine husbandry, promising results have been gained with the employment of plant-derived compounds.\textsuperscript{33,34} Although many plants have been listed as having anthelmintic activity in animals\textsuperscript{3–25} and the use of plant-derived anthelmintics would be preferable to synthetic drugs in dairy farming, scientific data demonstrating the real efficacy of these compounds against gastrointestinal parasites are scarce. In a recent study by Arfuso and colleagues,\textsuperscript{36} dairy donkeys were treated with the commercially available phytotherapeutic product.\textsuperscript{38} According to the manufacturer’s instructions, the product was administered two times at 2-week intervals (i.e., day 1, day 14) using the dose of one syringe (50 g) per donkey. Two administrations of phytotherapeutic supplement at fortnight interval were successful in reducing 56.9% intestinal strongyle egg shedding in naturally infected donkeys, causing no adverse reaction in treated animals throughout the experimental period. The FERC value obtained following phytotherapeutic supplementation was lower compared with other anthelmintic drugs tested in donkeys, such as ivermectin (96%)\textsuperscript{37} and epirinomectin (99%).\textsuperscript{38} The value was also lower when compared to the suggested cutoff values for interpreting results of strongyle FERC in horses.

<table>
<thead>
<tr>
<th>Species (Number of animals)</th>
<th>Parameter (Unit)</th>
<th>Parameter (Unit)</th>
<th>Parameter (Unit)</th>
<th>Parameter (Unit)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( t_{1/2a} ) (Day)</td>
<td>( T_{\text{max}} ) (h)</td>
<td>( C_{\text{max}} ) (ng/mL)</td>
<td>( \text{AUC}_{0-t} ) (ng × Day/mL)</td>
</tr>
<tr>
<td>Mule (( n = 5 ))</td>
<td>2.74 ± 0.02</td>
<td>16.8 ± 9.96</td>
<td>42.31 ± 10.20</td>
<td>135.56 ± 43.71</td>
</tr>
<tr>
<td>Horse (( n = 6 ))</td>
<td>6.53 ± 0.92</td>
<td>4.08 ± 2.16</td>
<td>61.28 ± 10.73</td>
<td>164.96 ± 30.07</td>
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<tr>
<td>Horse (( n = 5 ))</td>
<td>4.25 ± 0.24 (a)</td>
<td>9.22 ± 5.71</td>
<td>44.0 ± 23.1</td>
<td>132.7 ± 47.3</td>
</tr>
<tr>
<td>Horse (( n = 5 ))</td>
<td>2.93 ± 0.4 (a)</td>
<td>3.60 ± 0.96</td>
<td>51.3 ± 6.1</td>
<td>137.1 ± 35.9</td>
</tr>
<tr>
<td>Horse (( n = 3 ))</td>
<td>2.76 ± 0.2</td>
<td>3.3 ± 0.7</td>
<td>82.3 ± 12.4</td>
<td>200.92 ± 22.67</td>
</tr>
<tr>
<td>Donkey (( n = 3 ))</td>
<td>7.4 ± 2</td>
<td>24.0 ± 0.0</td>
<td>23.6 ± 4.4</td>
<td>119.3 ± 12.3</td>
</tr>
</tbody>
</table>

Abbreviations: (a), terminal half-life resulted from triexponential equation; \( \text{AUC}_{0-t} \), area under serum concentration-time curve from zero to the last concentration \( \geq \text{LOQ} \); \( \text{AUC}_{0-\infty} \), area under serum concentration-time curve from time zero to infinity; \( C_{\text{max}} \), maximum concentration observed; MRT, mean residence time; \( T_{\text{max}} \), time of maximum concentration observed; \( t_{1/2a} \), terminal half-life; \( ^{±} \) harmonic mean; \( ^{*} \) pseudo standard deviation.
4. Equine Gastric Ulcer Syndrome in Donkeys

The definition of equine gastric ulcer syndrome (EGUS) embraces different conditions characterized by ulcers in four typical localizations: terminal portion of the esophagus, proximal (squamous) and distal (glandular) parts of the stomach, and proximal part of the duodenum. This syndrome has been rarely described in donkeys, and reports are limited to post-mortem studies on animals submitted for necropsy. In a previous study by Sgorbini and colleagues, the prevalence, anatomical distribution, and effect of age and sex of EGUS were investigated in live adult donkeys. In that report, 39 donkeys underwent gastroscopic examination, and gastric lesions were present in 20/39 (51.3%) donkeys. In particular, 19/39 (48.7%) were affected by equine squamous gastric disease (ESGD), while 1/39 (2.6%) showed both ESGD and equine glandular gastric disease. Thus, 95% of donkeys affected by EGUS showed lesions located in the nonglandular mucosa. The ESGD grade was 0/4 in 19/39 (48.7%), 1/4 in 5/39 (12.8%), 2/4 in 10/39 (26.6%), 3/4 in 4/39 (10.3%), and 4/4 in 1/39 (2.6%) donkeys. In donkeys affected by EGUS, ESGD was primarily observed with lesions located around the cardia and along the lesser curvature. The equine glandular gastric disease lesion was a mild depression in the ventral glandular fundus. Despite endoscopic findings, no animal showed clinical signs of EGUS, and no significant correlation with sex, age, or breed was found.

5. Ophthalmology in Donkeys and Mules

Donkeys and mules can suffer from similar ocular diseases to horses but with a different prevalence. The incidence of ocular ulcer in a 3-year period in donkeys was 9% at the Veterinary Teaching Hospital of Camerino University (Italy; personal author data), much lower than that reported in horses (up to 76%).39 However, the egg shedding reduction was over 50%, revealing a great potential of phytotherapeutic products as useful tools for the control of intestinal strongyles in dairy donkeys under the multimodal integrated approach.36

 lif the upper eyelid is more difficult and sometimes impossible to perform, especially in the donkey. This is also due to the stronger palpebral muscles.42 As a result, during the ultrasound examination of the eye, it is sometimes not possible to visualize the retrobulbar region due to the obstacle caused by this portion of the frontal bone when approaching the supraorbital acoustic window. It follows that careful clinical inspection of the eye in donkeys and mules often requires sedation, nerve blocks, and local anesthesia, which are not necessarily required in horses in similar conditions. Prolonged ocular topical treatment is also challenging in donkeys and mules. The difficulty opening the eye, their small size, and less experience with being handled makes prolonged ocular topical treatment challenging in donkey and mules. For this reason, the application of a subpalpebral lavage system (SPL) in donkeys and mules is more often indicated than in horses. Furthermore, since sedation, nerve blocks (to overcome the strong palpebral muscles), and topical anesthesia are usually necessary for examination, SPL can be directly applied after diagnosis, if indicated. The technique for application of SPL in donkeys and mules is the same as in horses: the zygomatic process is slightly larger as in horses: the zygomatic process is slightly larger in donkeys and mules, and this should be taken into account when this part of the bone is palpated while looking for the supraorbital foramen for anesthetic injection.

6. Conclusions

In the present paper, the main features of piroplasmosis, equine gastric ulcer syndrome and ocular diseases in donkeys, have been highlighted. Epidemiology and clinical presentation of piroplasmosis and other tick-borne diseases show some differences between horses and donkeys, the latter having less severe symptoms despite high exposure to the parasites. Nonspecific clinical presentation or
asymptomatic forms of B. caballi and T. equi infections seem to be common in donkeys, and several clinical pathology alterations persist after natural infection. Therefore, even if clinically healthy, donkeys can be subjected to severe clinical pathological alterations. Acute presentations are very seldom observed in donkeys. Clinical monitoring of those donkeys that are chronically infected should be recommended because they represent a risk for transmission to other animals (as inapparent carriers) and their health status and productive performances could be altered. Decades of frequent anthelmintic use have selected for high levels of anthelmintic drug resistance in several parasite families like cyathostomin and Parascaris spp. populations, which emphasizes that the traditional approaches for parasite control are not sustainable anymore and that new strategies are needed. Preventive treatment should never be used, and a specific etiological diagnosis should be conducted before every treatment. Together with the recurring onset of anthelmintic resistance in equids, the increased public awareness of drug residues in animal products compels the scientific community to investigate novel strategies to control parasitic diseases in domestic animals. The results of investigations highlight a high prevalence of EGUS in a population of nonworking donkeys with no clinical signs of this disease. Furthermore, the squamous portion of the stomach was found to be much more frequently affected than the glandular region, as already reported for horses. This suggests that EGUS may be common in donkeys, but it is routinely overlooked. This suggests that subclinical forms of this syndrome could be present in apparently healthy donkeys. Thus, the effect of subclinical EGUS in donkeys needs to be thoroughly investigated in the future to exhaustively verify the impact on work or production performances. Several peculiarities of donkeys are present when compared to horses. Disease presentation can be very different, and the epidemiology of infectious and noninfectious diseases also needs to be specifically studied for donkeys to identify control plans to decrease the incidence and consequences of subclinical diseases.

Acknowledgments

Declaration of Ethics

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

Conflict of Interest

The Authors have no conflicts of interest.

References and Footnotes


*Mini-FLOTAC®, University of Naples Federico II, Corso Umberto I, 40, Napoli, Italy.
*PARAXITEBIO®, BIOEQUIPE SRL, Lombardy, Italy. Composition includes standardized extracts of Cardus mariano, Eucalyptus globulus, Gentiana lutea, Urtica urens, and Mallotus philippinensis and analytical components including crude protein (0.62%), crude fat (0.22%), crude fiber (0.09%), crude ash (0.32%), moisture (91.32%), and nitrogenous extracts (7.43%).
Improving the Understanding of Normal and Pain-Related Donkey and Mule Behavior

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1. Introduction

Donkeys, mules, and hinnies are likely the most misunderstood equines in terms of their behavior. They have served humans for thousands of years, and yet there is still very little known about them from a scientific or clinical point of view.1,2 The increasing popularity of donkeys as companion animals and mules as performance and recreational animals has led to an increase in cases being presented at clinics. Oftentimes when donkeys and mules are finally brought to the clinic, they are in advanced stages of a disease due to their stoic nature masking any signs of discomfort or pain.1,2 The natural behavior of donkeys and mules is more a freeze or flight behavior than flight, which has been reported to partially (around 20%) depend on a genetic donkey-specific basis.3 When compared to horses, studies have shown that mules especially have a keen sense of reasoning and donkeys have a high IQ.4,5 Keeping this in mind, the donkey, in particular, will continue to fight through the pain and discomfort by continuing to eat, drink, and appear, from a behavioral standpoint, that nothing is abnormal. Mules and hinnies (the reciprocal cross, where the sire is a stallion and the dam is a jenny, female donkey) may show clinical signs earlier than donkeys, but the disease may well be more advanced than they show. The other challenge to working with both mules and donkeys, from a behavioral standpoint, is getting to know the animal before performing routine procedures, such as taking temperature or performing a physical exam. Mules and donkeys tend to have a greater range of motion for kicking; hence, a practitioner needs to proceed with more caution than when working on a horse. A practitioner’s approach to getting to know a mule or donkey is essential to proceed. Time spent on this stage will enhance the scenario for both the patient and the practitioner. This paper will focus on several tips a clinician can implement when approaching a mule or donkey for the first time, how to safely and effectively restrain a donkey or mule for further examination or procedures, and how to notice subtle differences in behavior related to disease or pain.

Approach and Handling

When approaching mules and hinnies, research has found with over 900 mules, hinnies, and donkeys in many countries, including Egypt, Mexico, Peru,
Spain, and the United States, one should first approach the head of the animal.\textsuperscript{2,6} Research has revealed that oftentimes it is easier for a familiar person (e.g., owner or handler) to interact with mules and donkeys compared to an unfamiliar person (Fig. 1).\textsuperscript{1,2,6}

Practitioners should bear this in mind when they approach mules and hinnies, in particular, or when administering medication or drawing blood, among other actions, as the success in the application of a certain, even routine, action or procedure may strongly depend on the trust of the animal toward the operator or on the strength of the bond between a

![Fig. 1. Approach tests have shown that mules and hinnies are more willing to allow a familiar person to approach their forehead (green) and left side of their neck (blue) and touch their ears (yellow) than an unfamiliar person.](image)

![Fig. 2. Dr. Angie Varnum administering a vaccine at the Peru Equitariian Initiative Project. Notice how calm the mule is with its handler holding him for the vaccine injection.](image)

![Fig. 3. Dr. Farid Shkwy, approaching a mule in the Helwan brick kilns in Egypt. Notice the posture of the head and neck, that the ears are forward, and that nostrils have some flare but is not extensive.](image)
donkey or mule and the operator performing it. Conclusively, it may be helpful or more successful to have the familiar person assist (e.g., to pick up a leg or hold during radiographs compared to an unfamiliar person such as a veterinary technician; Fig. 2).

Fig. 6. An example of a handler using voice and touch while the practitioner takes the hinny's temperature in Antiqua, Colombia. Dr. Miguel Nova is familiar with these mules, but notice the mule is also tied during this procedure.

Fig. 7. This hinny has its head and neck leaning away as the practitioner approaches and has worked her hand backward. She is continuing to be calm and providing tactile support to quiet the animal (Equitarian Initiative Peru Project, 2016).
Reading Body Language

Approach the equine slowly, extending a hand for a gentle pet or rub on the forehead and move slowly and with patience. The long-eared equid will allow you to proceed if it drops its head and neck or extends its nose forward (Fig. 3). Even if donkeys tend to be less timid or resistant when meeting unfamiliar people than mules and hinnies, caution must be paid when approaching donkeys or horse-donkey hybrids. If the donkey, mule, or hinny remains alarmed, a difference will be noticed in body posture and stance, along with ears facing forward, enlarged nostrils, and a swishing tail, especially with mules and hinnies (Fig. 4). As aforementioned, this may make attempting routine veterinary or husbandry procedures more challenging when treatment is being provided by an unfamiliar person. Furthermore, the specific knowledge of operators on the particular stoic nature and of the pertinent cautions that approaching donkeys and their hybrid congeners require have been reported to condition the misinterpretation of body signs. A nervous or scared donkey may turn their ears to the side and clamp their tails. Previous studies have found that donkeys, mules, and hinnies tend to respond well to voice and touch. A soothing tone of voice appears to provide some comfort to the animal, likely allowing the practitioner to proceed (Fig. 5). Once the practitioner has made it past the equid’s forehead, the next step is to slowly work up its neck; if planning to use a...
stethoscope and listen to the heart, lungs or gastrointestinal sounds without incident, get to know the animal first.\textsuperscript{2,8,9} Gaining the trust of a mule or donkey is essential for performing a clinical exam (Figs. 5 and 6). When taking their temperature, make sure and stand to the animal’s side or simply place them in stocks if they are available. In some cases, the practitioner may have to apply a restraint to modify the equid’s behavior for both the practitioner’s and the animal’s safety. Many mules, hinnies, and donkeys are quite resistant to rectal thermometers; injections can also present challenges (Fig. 6). Based on how adept they are at using their neck for leverage, particularly when trying to evade an uncomfortable procedure, mules, hinnies, and donkeys seem to have a knack for pulling away from handlers. Mules and donkeys are less likely than horses to panic when tied. Consequently, practitioners may need to restrain the patient in this way if a stock is not available to apply more restraints. In these regards, restraints (e.g., a twitch) used in a proactive manner can be quite useful. The strength of mules and donkeys in their head and neck is unlike any horse many have dealt with, and they will slowly and easily move where they turn their heads or necks to evade any uncomfortable procedures.\textsuperscript{1} When lifting a leg, the action of mules and donkeys to lean into the operator instead of away from him/her is not an uncommon reaction. As a result, it may be necessary to ask the handler to lift the patient’s leg when performing a lameness exam and/or hold up a leg when examining another leg. Among all the body parts that can be informative about the behavioral status of a certain animal, donkeys and mules will tell a lot about their behavior by their ear posture, nostril flare, head and neck position, and tail movement (Figs. 7, 8A and B, 9A and B).\textsuperscript{1,2,8,11}

Other methods to proactively work with mules and donkeys is by offering a food reward if allowed by the owner or handler.\textsuperscript{3} In some cases, a mule or donkey may prove to be calmer and less resistant when in the presence of a familiar equid. If mules or donkeys are kept in the hospital, it is not uncommon for mules to be restless in stalls and express vocalization. If possible, place the mule where it can see a horse (or other equid) or has horses on either side. This will generally decrease their restless behavior, which can sometimes be counterproductive depending on the procedure or surgery that has been performed.

\textsuperscript{1,2,12} Twitches, when used judiciously, have shown to calm mules and hinnies that do not tolerate routine procedures (twitches at Idaho Equine, Nampa, ID). The twitch may actually decrease the stress on the mule or hinny when it is resisting a procedure.\textsuperscript{1,2,12}
<table>
<thead>
<tr>
<th>Phase</th>
<th>Drug</th>
<th>Dose</th>
<th>Route of Administration</th>
<th>Expected Duration</th>
<th>Duration is Dose Dependent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sedation</td>
<td>Xylazine</td>
<td>0.8 mg/kg (0.3–1.0 mg/kg)</td>
<td>IV, IM</td>
<td>15–20 mins</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td>Romifidine</td>
<td>0.08 mg/kg (0.05–0.1 mg/kg)</td>
<td>IV, IM</td>
<td>30 mins to 2 hrs</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>Detomidine</td>
<td>0.01 mg/kg (0.005–0.04 mg/kg)</td>
<td>IV, IM, sublingual</td>
<td>30–40 mins</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>Dexamethasone</td>
<td>0.005 mg/kg (0.0025–0.01 mg/kg)</td>
<td>IV, IM</td>
<td>30 mins to 2 hrs</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>Acepromazine</td>
<td>0.03 mg/kg (0.02–0.05 mg/kg)</td>
<td>IV, IM, sublingual</td>
<td>30 mins to 2 hrs</td>
<td>No</td>
</tr>
<tr>
<td>Induction</td>
<td>Ketamine</td>
<td>2.5 mg/kg</td>
<td>IV</td>
<td>15–20 mins</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td>Diazepam/midazolam</td>
<td>0.05 mg/kg (0.02–0.08 mg/kg)</td>
<td>IV</td>
<td>15–20 mins</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td>Propofol</td>
<td>2.0 mg/kg</td>
<td>IV</td>
<td>15–20 mins</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td>Alfaxalone</td>
<td>2.0 mg/kg</td>
<td>IV</td>
<td>15–20 mins</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td>Thiopental</td>
<td>8.0 mg/kg</td>
<td>IV</td>
<td>20 mins</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td>Telazol</td>
<td>1.0 mg/kg</td>
<td>IV</td>
<td>20 mins</td>
<td>No</td>
</tr>
<tr>
<td>Maintenance</td>
<td>Triple drip</td>
<td>12.5 g guaifenesin 500 mg ketamine 150 mg xylazine (combined in a 500-mL bag of LRS or 0.9% NaCl)</td>
<td>IV (requires infusion via IV catheter) Administering a bolus often results in apnea.</td>
<td>45 mins</td>
<td>No</td>
</tr>
<tr>
<td>Analgesics</td>
<td>Butorphanol</td>
<td>0.03 mg/kg (0.02–0.05 mg/kg)</td>
<td>IV, IM</td>
<td>30–50 mins</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td>Buprenorphine</td>
<td>0.006 mg/kg</td>
<td>IV, IM, sublingual</td>
<td>6 hrs</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td>Morphine</td>
<td>0.01 mg/kg</td>
<td>IV, IM</td>
<td>3 hrs</td>
<td>No</td>
</tr>
</tbody>
</table>

Abbreviations: IV, intravenous; IM, intramuscular; LRS, lactated Ringer's solution.
performed. Donkeys that stay in the hospital tend to do best if they can have a donkey mate or, again, a familiar animal with them.

Digestive Behavior

Both donkeys and mules may not drink well in the clinic and additional sweeteners may need to be added to the water to prevent dehydration and other complications like colic. The specific habits of the owner in regard to feeding and water provision must be also considered as particularly donkeys may display unexpected avoidance to patterns or practices to which they may not be accustomed.\(^\text{19}\)

Reproductive Behavior

When working with breeding jacks in the clinic, recognize that they may be very shy breeders, will need a quiet area to be collected, and again, will need to get to know the handlers and gain trust before being collected. Many jacks are very slow to be collected compared to horses, and the slightest change in their environment can decrease reproductive behavior. Also, be aware that jacks can be very strong when being handled so consider a stud chain across the nose. In some cases, they may show excessive signs of aggression toward mares or jennies and a muzzle may be needed.\(^\text{7}\)

Restraint

To perform a clinical exam, the mule or donkey must remain calm and still. This can be a challenge considering the abovementioned methods of introducing oneself to a mule. To reduce the amount of stress on the animal, handler, and practitioner, it is best to begin with the animal being calm. If a routine health task cannot be safely performed, nose twitches can be an effective form of restraint, especially a twitch with a long handle to hold and string twitch (Fig. 10). Blindfolds used to cover the mule’s and hinny’s eyes have also proven to be effective methods of calming an animal to receive treatment ranging from deworming to vaccinating to farrier work (Fig. 11). Another form of restraint that may work, but must be done by someone trained in the safe application of the procedure, is the lifting of an opposite leg and tying the leg up with a safety knot to a loop made around the neck of the mule that does not slip. Last but not least, chemical restraint has been reported to work the mule that does not slip. Last but not least, chemical restraint has been reported to work when aiming to administer an injection.\(^\text{8,12}\)

Pharmacological restraint can decrease the level of fear and resistance for routine procedures. Sedation with \(\alpha_2\)-adrenoreceptor agonists (xylazine and detomidine) alone or in combination with opioids (e.g., butorphanol) can be used as ancillary chemical restraint methods.\(^\text{2}\) When using xylazine in mules, studies have shown a shorter half-life, and a typical horse dose of 0.6 mg/kg intravenously (IV) should be increased 50% for mules, but this is not required for donkeys.\(^\text{12,13}\) Detomidine oral gel may also be useful for mules and donkeys if obtaining intramuscular or intravenous access is difficult.\(^\text{12,14}\) One should allow for at least 40 minutes of sedation for donkeys when using the typical horse dose.\(^\text{12}\) Currently, no reports have been given for doses and level of sedation when using detomidine oral gel in mules.\(^\text{13}\) Often, analgesic agents may be metabolized at a higher rate and need to be read ministered to produce the same effect in mules (refer to Table 1 for doses).\(^\text{11,12,13,15}\)

Pay close attention to the level of reaction and alertness when the mule is sedated to determine if more sedatives should be administered (Figs. 12, A and B).

Subtle Signs of Pain

Both donkeys and mules generally show subtle signs of pain.\(^\text{12,12}\) Lameness may be evidenced when donkeys, mules, or hinnies shift weight on all four limbs and occasionally rest or take weight off one hind limb. Still, the signs can generally go unnoticed. Contextually, owners and handlers who are very familiar with their donkeys and mules may be their best advocates and will often pick up on signs more quickly than anyone else. Example signs might be a slight decrease in appetite, shifting weight, slight twitch of the tail, standing in different places than usual, increased tension in the orbital area/nostrils, and ear positioning down or back, along with changes in their eyes from bright and alert to glossy or glazed. Any or all may suggest that the donkey or mule is showing signs of discomfort. Attempts to define facial and body assessments related to pain in donkeys were recently published by the authors (Figs. 13-16).\(^\text{11}\) Current ongoing research by the authors is investigating the use of sensor technology (SmartHalter) to assist in detecting pain or discomfort in mules and donkeys to better assist with detecting slight changes in physiological signs such as heart rate and respiration paired with behavioral signs such as recumbency or rolling.
2. Summary

A key to donkey and mule behavior is being patient and working to gain their trust. To safely work with mules and donkeys, reading their body language and preventing them from evading the procedure are necessary to perform a clinical exam. It may be necessary to have the owner or handler stand by to assist with holding or comforting the animal prior to beginning the procedure. Physical restraints, such as nose twitches are very effective in mules and somewhat in donkeys. Chemical restraint is likely the safest form of restraint, but make sure to use the proper dosage for a donkey or mule (Table 1).12,14,15 Both will metabolize medications more quickly than horses of the same size. When attempting to define pain or signs of discomfort in mules and donkeys, start by examining the face and take in the overall body stance and posture. Keep in mind both donkeys and mules will likely mask pain and signs of pain due to their fight or freeze nature, but one can still notice signs by observing facial action units, body posture, and tail movement.1,7 If a mule or donkey appears healthy but the owner is describing a difference in their behavior, the animal is likely sick or in pain, so consider their information and remember both donkeys and mules are generally in a more advanced stage of distress but will likely show fewer signs. Suggest to owners who are raising mules to handle their foals from day one so that routine procedures such as taking a temperature, using a stethoscope, picking up hooves, or administering an injection are not complicated and stressful.1,7,8

Fig. 13. Images A-I show examples of ear position and how it may relate to discomfort and pain. Notice the ear position in images C, D, F, and H, these positions can be associated with discomfort.15
and donkeys that have been handled from birth are much easier to interact with and better tolerate routine procedures. Additional information on mule and donkey medicine can be found in the Vet Clinics of North America 2019 special issue dedicated to mules and donkeys.

**Fig. 14.** A, slight change in nostrils expressing more flare such as B, may indicate discomfort or pain.\(^{11}\)

**Acknowledgments**

The Authors would like to acknowledge the owners and handlers that participated in the many studies included in this paper, the veterinarians and volunteers of the Equitarian Initiative projects, the Egyptian Society for Protection of Working Animals, and others who contributed to the research and collection of data.

**Fig. 15.** The eyes of donkeys and mules are expressive as shown in illustrations A–E. Changes in the shape and orbital tightening may indicate pain. Images A, B, C, and E suggest the donkey is in pain. These images were captured postcastration. Orbital tightening is observed as an indicator of pain in images B and C.\(^{11}\)
and the staff and practitioners at Idaho Equine Hospital, Nampa, Idaho.

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

Conflict of Interest
All Authors have no actual or potential conflicts of interest including any financial, personal, or other relationships with other people or organizations that could inappropriately influence, or be perceived to influence, their work.

References
How to Decide Which Heart Murmurs Are Relevant in Performance Horses

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1. Introduction

Given that the cardiovascular system is central to the delivery of oxygen and nutrients to the peripheral tissues, any cardiac abnormality that affects cardiac output could result in decreased athletic capacity, which is seen clinically as poor performance.1,2 The equine cardiovascular system, however, is one of tremendous reserve capacity; therefore, it can take quite considerable dysfunction to result in a measurable effect on performance. In addition, it should be considered that there are cardiac conditions that do not affect overall performance per se but could result in a sudden and dramatic adverse event during exercise (e.g., weakness, collapse, or sudden cardiac death).3 One of the difficulties practitioners face is trying to determine the cause of a horse’s reduced performance. There can be a variety of abnormalities detected on physical examination, but deciding if a single problem alone or, much more commonly, if a combination of problems are acting together to reduce the exercise capacity of an individual animal can be challenging. The breed of horse and the type of exercise (e.g., high-speed racehorse, endurance, or short duration high-intensity stock handling) will also influence how the clinical signs of poor performance are manifested. In addition, it is important to note if the performance of the horse has suddenly changed in recent times, if this problem is longer standing, or if, in fact, the horse has always performed below the owner/trainer’s expectations. Cardiac murmurs are common findings on physical examination of horses.4–6 Trying to decide if the murmur is clinically relevant requires experience, interpretation of other clinical findings, the addition of further diagnostic investigations, including echocardiography, resting and exercising electrocardiography (ECG) analysis, and in some cases stress testing under exercising conditions. Any reported change in murmur intensity or development of a new murmur should be evaluated particularly closely.

2. Recognizing and Describing Murmurs on Clinical Examination

The first step when evaluating a horse with poor performance is to perform a thorough clinical examination, including careful auscultation of the heart from both sides of the thorax and assessment of the entire cardiovascular system.7 The normal heart sounds should be identified, as seen in Fig. 1. S1 (closing of the atrio-ventricular valves) and S2 (closing of the semi-lunar valves) should be appreciated in all healthy horses. S4 is appreciated in many but not all horses and can become more prominent in older horses (as a result of a more active atrial contribution to ventricular filling in late diastole). S3 is usually quite subtle and dull and more commonly
heard in younger horses (due to a more active early ventricular filling phase); it can become very prominent in the rare cases of heart failure. Murmurs are sounds that occur between the normal heart sounds, either caused by the physiological flow of blood or from turbulence created by pathological abnormalities. Murmurs should be described using the criteria in Table 1. Some cardiac murmurs remain similar over a long period, whereas others can change in intensity and character over time (some murmurs increase in grade, whereas others become

Table 1. Criteria for Describing and Reporting on Cardiac Murmurs

<table>
<thead>
<tr>
<th>Criterion</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Timing</strong></td>
<td>Within the cardiac cycle</td>
</tr>
<tr>
<td>• Systolic, diastolic, or continuous</td>
<td></td>
</tr>
<tr>
<td>• early, mid, or late</td>
<td></td>
</tr>
<tr>
<td>• Holosystolic/holodiastolic (whole duration of systole/diastole but S1/S2 still audible)</td>
<td></td>
</tr>
<tr>
<td>• Pansystolic/pandiastolic (whole duration of systole/diastole, S1/S2 or both no longer audible)</td>
<td></td>
</tr>
<tr>
<td>• Diastolic murmurs tend to vary in duration depending on heart rate (longer at lower heart rates, shorter at higher heart rates), and systolic murmurs are similar duration regardless of heart rate</td>
<td></td>
</tr>
<tr>
<td><strong>Point of maximal intensity (PMI)</strong></td>
<td>Where is the murmur loudest</td>
</tr>
<tr>
<td>• Left or right thorax</td>
<td></td>
</tr>
<tr>
<td>• Base (near shoulder level) or apex (olecranon level)</td>
<td></td>
</tr>
<tr>
<td>• Over which valve (mitral, aortic, pulmonic, or tricuspid)</td>
<td></td>
</tr>
<tr>
<td><strong>Grade</strong></td>
<td>Intensity or loudness of the murmur (note that this can but not necessarily relate to the severity of the problem)</td>
</tr>
<tr>
<td>1. Very quiet and localized, often labile or variable</td>
<td></td>
</tr>
<tr>
<td>2. Quiet, localized but clear when over the PMI</td>
<td></td>
</tr>
<tr>
<td>3. Obvious, less loud than S1/S2</td>
<td></td>
</tr>
<tr>
<td>4. Obvious, louder than S1/S2, radiating in some cases to the other side of the thorax</td>
<td></td>
</tr>
<tr>
<td>5. Loud, radiating, palpable thrill on the chest wall</td>
<td></td>
</tr>
<tr>
<td>6. Audible with the stethoscope off the chest wall</td>
<td></td>
</tr>
<tr>
<td><strong>Character and shape</strong></td>
<td>Descriptive terms, typically including</td>
</tr>
<tr>
<td>• Soft</td>
<td></td>
</tr>
<tr>
<td>• Blowing</td>
<td></td>
</tr>
<tr>
<td>• Harsh</td>
<td></td>
</tr>
<tr>
<td>• Whooing</td>
<td></td>
</tr>
<tr>
<td>• Musical</td>
<td></td>
</tr>
<tr>
<td>• Dive-bomber</td>
<td></td>
</tr>
<tr>
<td>• Vibrant</td>
<td></td>
</tr>
<tr>
<td>• Buzzing</td>
<td></td>
</tr>
<tr>
<td>• Squeak</td>
<td></td>
</tr>
<tr>
<td>• Honk</td>
<td></td>
</tr>
<tr>
<td><strong>Shape</strong></td>
<td></td>
</tr>
<tr>
<td>• Band-plateau (even throughout the murmur)</td>
<td></td>
</tr>
<tr>
<td>• Crescendo (increasing in intensity)</td>
<td></td>
</tr>
<tr>
<td>• Decrescendo (decreasing in intensity)</td>
<td></td>
</tr>
<tr>
<td><strong>Effect of exercise</strong></td>
<td>It is important to auscultate the heart before and after exercise</td>
</tr>
<tr>
<td>• Some flow murmurs and mitral regurgitation murmurs can increase in intensity after exercise</td>
<td></td>
</tr>
<tr>
<td>• Most diastolic murmurs decrease in duration and intensity at higher heart rates, as seen after exercise</td>
<td></td>
</tr>
</tbody>
</table>
becomes stiffer and more when nodular degeneration on the aortic cusp mechanics (e.g., as seen with aortic regurgitation pensatory mechanisms fail) or different vibration intra-cardiac gradients (pressure equalization as com-

Fig. 2. Coronal view of the location of the heart valves. The great vessels can be seen located cranially on the left thorax, and the mitral valve is quite caudal on the left. The tricuspid valve is appreciated on the right. The most common location of a ventricular septal defect (VSD) is identified with the black arrow. Illustration by Matthias Haab, University of Zurich, modified by the author.

quieter). These changes can reflect alterations in intra-cardiac gradients (pressure equalization as compensatory mechanisms fail) or different vibration mechanics (e.g., as seen with aortic regurgitation when nodular degeneration on the aortic cusp becomes stiffer and more fibrotic vibrating less or a sudden honking vibratory murmur in a horse with long-standing mitral regurgitation [MR], indicating chordal rupture). A sudden change in murmur quality is an indication for re-evaluation with echocardiography. It is important to clearly describe the findings of the auscultation in the medical record and when discussing the case with colleagues, to ensure accurate information is recorded. The use of a high-quality analogue stethoscope is invaluable for clear auscultation, whereas the auscultation conditions (e.g., quiet barn, no external noise, and horse standing still) are extremely important for a thorough cardiovascular evaluation. The use of a digital amplifier on an analogue stethoscope or the use of a fully digital stethoscope can provide additional information about the timing and duration of the murmur and allow recording of the murmur to be included for teaching purposes and as part of the medical record.

3. Physiological Flow Murmurs
In some horses, particularly younger horses with lower body condition or a thin chest wall, the flow of blood in the great vessels can be appreciated. These murmurs are often referred to as “ejection murmurs,” corresponding with the ejection of blood into the great vessels in early systole. It is important to recognize that these physiological murmurs are short in duration (early systolic), quiet (grade 2 or less), and audible only cranially on the left, over the area of the great vessels (Fig. 2).7 Occasionally, physiological diastolic filling murmurs are detected, particularly in young horses. Some of these murmurs are described as an early-mid diastolic “squeak” or “rub” and are considered normal.

4. Pathological Murmurs
Caused by the turbulent flow of blood, these pathological murmurs are most commonly the result of valvular regurgitation, although occasionally they are caused by abnormal communications between chambers and/or vessels.1,4,7 Valvular stenosis is exceedingly rare in horses, compared with other species, and is usually only seen in combination with a complex congenital malformation. Rarely in horses, additional sounds on auscultation superimposing the “normal” heart sounds can be caused by pericardial disease; these pericardial friction rubs are caused by motion of the heart against the pericardium and are often highly variable in intensity, location, and duration. Valvular regurgitation, causing a backflow of blood across the valve, is a common finding, particularly in athletic horses.4,6 The difficulty for practitioners is determining the hemodynamic effects of any regurgitation and correlating that to the reported abnormalities of reduced or poor performance. Some valvular regurgitations are known to increase with athletic training, and it is important to recognize these “typical” regurgitations from those with pathological consequences. It is also fundamental to recognize that the regurgitation per se is not the biggest issue but rather the cardiac remodeling that occurs in the “receiving chamber,” the cardiac chamber that receives the regurgitant blood. The degree of cardiac remodeling cannot be easily determined based on physical examination alone; therefore, echocardiography and ECG analysis are important parts of the diagnostic evaluation. The common murmurs and their consequences are summarized in Table 2.

5. Echocardiographic Assessment and ECG Analysis
Although the clinical examination is critical to alert the practitioner to a problem, it is the additional diagnostic evaluation (echocardiography and ECG analysis) that will provide information about the severity, hemodynamic relevance, and likely rate of progression. A standardized approach to echocardiography and ECG analysis18,14 is recommended, in order to fully assess all the cardiac structures. The specific indications for echocardiography and ECG analysis are listed in Table 3.15 Still images and cineloops should be stored and standardized measurements performed, which will allow a comparison of structures over time, which is particularly important when evaluating the rate of disease progression. These data on cardiac chamber size, systolic and diastolic function, valvular and chamber morphology, and the presence of any concurrent arrhythmias assist in the evaluation of identified cardiac abnormalities as a likely cause of
<table>
<thead>
<tr>
<th>Problem</th>
<th>Murmur</th>
<th>Consequence</th>
<th>Comments</th>
<th>Clinical Signs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mitral regurgitation</td>
<td>Systolic, loudest over</td>
<td>Receiving chamber: left atrium (LA). Left atrial enlargement can predispose</td>
<td>MR is a common finding in athletic animals. Compensation: normal to increased LA contractile function. Decompression: increasing LA pressures, pulmonary congestion, pulmonary hypertension (PH), pulmonary edema (particularly in cases of acute MR).</td>
<td>If AF develops, reduced athletic capacity and poor performance are common. With acute MR or in cases of chronic MR with severe LA enlargement and decompensation, exercise intolerance, respiratory signs (tachypnea, dyspnea, frothy nasal discharge), and congestive (usually biventricular) heart failure can develop. In more chronic cases, the signs of right-sided heart failure can predominate.</td>
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<td></td>
<td>the mitral valve on the</td>
<td>to atrial arrhythmias (particularly atrial fibrillation, AF).</td>
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<tr>
<td></td>
<td>left side (often</td>
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<td></td>
<td>radiating caudodorsally)</td>
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<td></td>
</tr>
<tr>
<td>Tricuspid regurgitation</td>
<td>Systolic, loudest over</td>
<td>Receiving chamber: right atrium (RA). Right atrial enlargement can predispose</td>
<td>TR is a common finding in athletic animals and can worsen with training; this is usually harmless and often considered “normal”. TR can also develop secondary to PH, leading to more severe consequences. Compensation: normal to increased RA contractile function. Decompression: increasing RA pressures, signs of right-sided congestive heart failure.</td>
<td>If AF develops, reduced athletic capacity and poor performance are common. If severe RA enlargement and decompensation, exercise intolerance and congestive heart failure can develop (jugular vein distension/pulsation, ventral and limb edema, ascites).</td>
</tr>
<tr>
<td></td>
<td>the tricuspid valve on</td>
<td>to atrial arrhythmias (particularly AF).</td>
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<tr>
<td></td>
<td>the right side.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aortic regurgitation</td>
<td>Diastolic, left sided,</td>
<td>Receiving chamber: left ventricle (LV). Left ventricular enlargement can</td>
<td>AR is a common finding, particularly in older animals; however, AR in a young horse is rare and should be immediately investigated. Compensation: normal to increased LV function. Decompression: decreased LV function leads to increased LV volume and pressures, often development of MR results in volume overload of the LA in addition. Ventricular arrhythmias pose the biggest concern over safety and as a cause of poor performance in horses with AR. LV enlargement and compensatory LV hyperkinesis are associated with increased pulse pressure (difference between SAP and DAP) that can easily be felt as a “hyperkinetic pulse” during physical examination; this is a prognostic indicator that indicates potential risk for ventricular arrhythmias and can be used to monitor for disease progression. If the LV decompensates, then exercise intolerance, respiratory signs, and congestive heart failure can develop.</td>
<td>Ventricle...</td>
</tr>
<tr>
<td>Problem</td>
<td>Murmur</td>
<td>Consequence</td>
<td>Comments</td>
<td>Clinical Signs</td>
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</tr>
<tr>
<td>Pulmonary regurgitation (PR)</td>
<td>Diastolic, left sided, cranial (over the pulmonary valve), typically quiet and difficult to hear. Louder if PH is present.</td>
<td>PR is a common echocardiographic finding but is rarely associated with clinical disease unless PH is present.</td>
<td>Clinical relevant PR is found in cases of PH. Clinical signs of PH include exercise intolerance, respiratory signs (tachypnea, dyspnea) and delayed recovery after exercise.</td>
<td></td>
</tr>
</tbody>
</table>
| Ventricular septal defect (VSD) | Holosystolic, right sided but often radiating to the left side, harsh/coarse. In the rare cases of subpulmonic VSD location, the murmur can be loudest very cranial on the left, over the pulmonary valve. | There are several things that influence the clinical relevance of a VSD:  
1. Size of the defect (relative to the size of the heart)  
2. Location of the defect (paramembranous/muscular/subpulmonic)  
3. Pressure gradient between the LV and RV (and pulmonary circulation)  
4. Abnormalities of aortic root alignment (e.g., dextro-positioned/overriding aorta)  
5. Involvement of the aortic cusps and tricuspid valve in the defect  
6. Presence of concurrent congenital anomalies | The clinical signs of a VSD will depend on the degree of cardiac remodeling present. Horses with small, restrictive VSDs can have normal performance and normal life expectancy. Horses with clinically relevant VSDs can have reduced athletic capacity and poor performance, respiratory signs, and weakness and are predisposed to arrhythmias that can cause reduced performance or have safety implications. |
Table 2. (Continued)

<table>
<thead>
<tr>
<th>Problem</th>
<th>Murmur</th>
<th>Consequence</th>
<th>Comments</th>
<th>Clinical Signs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complex congenital malformation</td>
<td>Typically, these have a VSD included in the malformation; therefore, the predominant murmur is usually a holosystolic, right-sided, coarse/harsh murmur that radiates often to the left heart. Depending on the malformation, other murmurs may also be present.</td>
<td>Depending on the malformation, both the right and left heart can be affected; severity is associated with the degree of shunting across the defects.</td>
<td>The clinical relevance of the malformation is largely dependent on the combination and severity of the defects. Most of these defects will result in exercise intolerance and many will be life threatening. Frequent clinical signs include respiratory signs (tachypnea, dyspnea) and congestive heart failure (tachycardia, jugular distension/pulses, ventral and limb edema and ascites).</td>
<td></td>
</tr>
<tr>
<td>Aortic-cardiac (ACF) / aortic-pulmonary fistulas (APF)</td>
<td>ACF: diastolic-systolic (or continuous) right-sided murmur, frequently associated with arrhythmia and colic-like signs. APF: diastolic-systolic (or continuous) cranial left sided murmur (these are often difficult to auscultate). Receiving chamber (ACF): These typically affect the aortic root, interventricular septum (IVS), right ventricle +/- pericardium. They can cause right-sided or bilateral cardiac enlargement depending on the severity of shunting across the defect. Receiving chamber (APF): These typically affect the aorta and pulmonary artery at the level of the ligamentum arteriosum. They can cause right-sided or bilateral cardiac enlargement depending on the severity of shunting across the defect.</td>
<td></td>
<td>Although uncommon, these defects can cause severe clinical signs, potentially during exercise. Exercise intolerance, weakness, collapse, or sudden cardiac death have been reported, typically as a result of malignant ventricular arrhythmia or massive extra-cardiac hemorrhage. The clinical presentation of an ACF is a new onset right-sided murmur, with colic, tachycardia, and arrhythmia, and APFs are frequently associated with tachycardia and colic-like signs.</td>
<td></td>
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</tbody>
</table>
6. Summary

In most cases, the common causes of cardiac murmurs, namely, valvular regurgitations, are well tolerated, and most horses with cardiac disease have completely normal exercise tolerance, performance, and life expectancy. In these horses, a thorough investigation of the other organ systems is required to determine the cause of their poor performance (e.g., musculoskeletal, respiratory, neurological, and behavioral).\(^1\),\(^2\),\(^16\) It should be noted that diastolic murmurs compatible with aortic regurgitation (especially in horses with increased pulse pressure or a “hyperkinetic” pulse) are generally more worrisome due to the potential consequences (left ventricle [LV] dilation, malignant ventricular arrhythmias, and sudden cardiac death) than systolic murmurs of mitral and tricuspid regurgitation (causing atrial dilation and atrial fibrillation). The combination of a murmur and development of a pathological arrhythmia like atrial fibrillation certainly might be very relevant when it comes to performance limitation and should definitely be considered when monitoring for disease progression. Far less commonly, a rapidly progressing, severe valvular regurgitation can become hemodynamically relevant, resulting in reduced exercise capacity, poor performance, and decrease life expectancy.\(^3\),\(^7\),\(^12\),\(^16\) Most (but not all) congenital defects are identified earlier in a horse’s life, and depending on the severity of the defect, some horses can perform and have a normal life expectancy, whereas others will develop severe clinical signs and present with exercise intolerance or signs of congestive heart failure.\(^10\) As practitioners, the key is to recognize cardiac abnormalities on a thorough clinical examination, investigate those meeting the criteria for additional diagnostic evaluation, and then closely monitor the horse for any change in performance. From a safety perspective, early recognition and close monitoring of horses with aortic regurgitation are considered particularly important.

Acknowledgments

Declaration of Ethics

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

Conflict of Interest

The Author has no conflicts of interest.

References

How to Diagnose Mild and Moderate Equine Asthma as a Cause of Poor Performance in Sport Horses

Jean-Pierre Lavoie, DMV, DACVIM

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1. Equine Asthma—Name Change
The chronic, non-infectious lower airway diseases of horses have been known under different names over the years and cause great confusion in both the equine veterinary scientific and lay communities. For these reasons, the medical term “equine asthma” is now being used to foster better communication between all stakeholders.1–3 Based on severity, the term “severe equine asthma” is now used to describe the condition previously known as heaves or recurrent airway obstruction (RAO; equine COPD, chronic bronchitis, equine emphysema, etc.), while “mild and moderate equine asthma” are used to describe what was known as “inflammatory airway disease (IAD).” “Moderate equine asthma” describes the condition of horses with clinical signs (e.g., cough) or clinical findings (abnormal lung sounds) of a lung disease but without the presence, or a history, of labored breathing at rest. The term “mild equine asthma” is used for horses presenting exercise intolerance and a lower airway inflammation or obstruction but without obvious clinical signs suggestive of lung diseases. Equine asthma may further be defined based on the triggering factors (barn/hay and pasture) and the inflammatory cells in bronchoalveolar fluid cytology (neutrophilic, mastocytic, eosinophilic, mixed granulocytic, and pangranulocytic). Other phenotypes (clinical findings) and endotypes (mechanistic pathways) are emerging in equine asthma and may eventually lead to personalized therapy.4

2. Main Features
Inhalation of the offending antigens, especially those present in hay and bedding during stabling, causes inflammation and obstruction of the airways. This in turn is responsible for the development of the clinical signs observed in equine asthma, which include exercise intolerance and, for the more severe syndrome, cough, increased respiratory rate, and labored breathing at rest. While the severity of the clinical signs may progress in genetically susceptible horses, the condition may be transient or intermittent in some horses or even persist without further progression in mild and moderate equine asthma. The time between exposure and the development of clinical signs is variable, as is the severity of airway hyperreactivity and inflammation. While strong epidemiological studies are lacking, it has been proposed that up to 75-80% of horses will develop milder forms of equine asthma in their lifetime.3 Recent findings suggest that inflammation-induced airway structural changes (remodeling) may be responsible for the progression of equine asthma in some horses.5

NOTES
3. How to Diagnose Moderate Equine Asthma

The diagnosis of moderate equine asthma is usually straightforward and is based on the presence of a chronic cough in an otherwise healthy horse. It is confirmed by demonstrating inflammation using bronchoalveolar lavage fluid (BALF) cytology. Exercise intolerance and tachypnea after exercise may also be present. Bloodwork, thoracic ultrasound, and radiographs are normal. Clinical signs include intermittent or frequent coughing episodes at rest, when eating, or during exercise. Physical examination may reveal a bilateral, intermittent, or persistent serous or seromucous nasal discharge. A tachypnea at rest, but without nasal flaring or increased abdominal excursions as in severe asthma, may be present. Thoracic auscultation may reveal localized or generalized crackles and wheezes when using a rebreathing bag. Physical examination is otherwise unremarkable. These findings indicate the presence of a lower airway condition and should raise suspicion for equine asthma. BALF cytology revealing increased percentages of neutrophils, mast cells, or eosinophils is confirmatory for the diagnosis of moderate equine asthma when combined with the presence of respiratory signs. In absence of BALF cytology, increased amount of tracheal mucus on endoscopy combined with a positive response to therapy is supportive of the diagnosis.

4. How to Diagnose Mild Equine Asthma

Mild equine asthma is a diagnostic challenge as decreased exercise tolerance or poor performance with a prolonged recovery period after exercise are the only presenting clinical signs in athletic horses. Because of the absence of clinical signs suggestive of a lung condition, it is a largely overlooked cause of exercise intolerance in high performance horses. The diagnosis is based on the presence of exercise intolerance and lower airway inflammation documented by an abnormal BALF cytology, combined with the exclusion of other common causes of exercise intolerance (upper airway obstruction, lameness, myopathy, cardiac pathology, arrhythmia, etc.). History, physical examination, and CBC and blood biochemistry are otherwise unremarkable. When present, crackles or wheezes on thoracic auscultation using a rebreathing bag or increased tracheal mucus on endoscopy will lead the clinician to investigate the lungs, but these findings are inconsistent. The response to therapy (decreased inhaled antigens and noxious gas, corticosteroids, bronchodilators) will often be required to confirm the diagnosis.

5. Diagnostic Aids for Equine Asthma

Bronchoalveolar Lavage Fluid Cytology

The different techniques used to collect BALF (type, volume, and temperature of the fluids, how it is infused and aspirated from the lower airways—syringe, pump, or tube of videodendoscopy—the method for the preparation—cytospins, sedimentation, smears—and staining of the cytopreparation, how the differential cell count is made, as well as the experience of the cytologist) may influence the BALF differential cell counts. For these reasons, there are no universal “normal values” for BALF cytology in horses. Of note, lower airway inflammation may have numerous causes and represents a normal response to inhalation of irritants, noxious gas, and various microbes. For these reasons, lower airway inflammation without the presence of abnormal clinical signs is not considered sufficient for a diagnosis of equine asthma. Nevertheless, the recent report that increased percentages of mast cells, and to a lesser extent, neutrophils, in BALF cytology of Thoroughbred racehorses was associated with decreased racing speed suggests that lower airway inflammation of any causes may lead to impaired performance. Inflammation documented from tracheal washes or aspirates is not considered appropriate for the diagnosis of equine asthma.

Tracheal Mucus on Endoscopy

The presence of tracheal mucus has been associated with decreased performance in sport horses. Excess tracheal mucus (score >1/5 for racehorses and >2/5 for sports/pleasure horses) is common in horses with mild and moderate asthma, but mucus accumulation may have many other causes. Therefore it has low sensitivity and specificity for diagnosis of severe equine asthma, but its presence is suggestive of the condition.

Airway Obstruction

The poor performance caused by equine asthma results from a decreased gas exchange in the alveoli due to airflow impairment. The asthma diagnosis would therefore be based ideally on the documentation of lower airway obstruction. Different methods have been developed to measure the airway function of horses. Bronchoprovocation with histamine or methacholine, lung mechanics detected using a rebreathing method, airflow measured after forced expiration, and PaO2 and decreased VO2 peak during exercise have been used for the diagnosis of mild and moderate equine asthma. However, their use is currently limited by the specialized equipment and expertise they require, lack or portability, or commercial availability.

Blood Biomarkers

Blood biomarkers may facilitate the diagnosis of equine asthma in clinical practice as there is evidence of systemic inflammation in affected horses. While more work is needed, the combination of acute phase proteins including serum amyloid A (SAA), haptoglobin, and C-reactive protein (CRP), with lung specific biomarkers such as surfactant protein (SP)-D and secretoglobin, appear promising. Of note, high concentrations of SAA and other acute phase proteins may be indicative of infection rather than equine asthma.
Performance Testing

Performance testing is increasingly available at referral centers or to individual practitioners and may help identify the cause of exercise intolerance. Diagnostics may include dynamic videoendoscopy with structured exercise testing and exercising heart rate, VO₂ peak, ECG, arterial blood gases, and stress echocardiography.

Endobronchial Biopsies

The presence of a remodeling of the epithelium, the lamina propria, and the bronchial smooth muscle layer in the central and peripheral airways of horses with severe asthma is well documented. These structural changes correlate with decreased lung function in affected horses. Using endobronchial biopsies, which are easy to collect and are well tolerated by horses during bronchoscopy, it was recently observed that a remodeling of the airways is also present in the milder forms of asthma. Follow-up studies would be required to determine whether these tissue changes allow prognosticating on the outcome or are useful for the implementation of specific therapy (precision medicine).

6. Conclusion

Mild and moderate asthma are common and under-diagnosed causes of exercise intolerance in performance horses. The diagnosis is based on the presence of compatible clinical signs, lower airway inflammation documented by BALF cytology, and the exclusion of other causes of exercise intolerance. Response to therapy may be required to confirm the diagnosis.

Acknowledgments

Declaration of Ethics

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Conflict of Interest

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References

How to Diagnose and Manage Gastric Ulcers as a Medical Reason for Poor Performance

Frank M. Andrews, DVM, MS, DACVIM (LAIM)

Poor athletic performance in horses is often multifactorial, and a comprehensive examination is needed to determine the cause. A comprehensive examination should include endoscopic examination of the stomach to determine the presence of equine squamous gastric disease and/or equine glandular gastric disease, as they might be causing or contributing to the poor athletic performance. If gastric ulcers are present, then appropriate antiulcer treatment could improve stomach health and athletic performance. Author’s address: Equine Health Studies Program, Veterinary Clinical Sciences, Louisiana State University School of Veterinary Medicine, Skip Bertman Drive, Baton Rouge, LA 70803; e-mail: fandrews@lsu.edu. © 2021 AAEP.

1. Introduction

Poor athletic performance in horses can be multifactorial, and it is difficult to determine the effects of one component on athletic performance. Thus, a comprehensive clinical evaluation, at rest and during exercise, is needed to determine causes and prescribe effective interventions. The gastrointestinal (GI) tract has largely been overlooked as a cause of poor athletic performance in horses. Nearly 60% of human athletes complain of upper GI symptoms while exercising, which likely affects performance. Several reports have implicated equine gastric ulcer syndrome (EGUS) as a cause of poor athletic performance in horses, but only one of these studies collected objective data.

EGUS involves ulcerative lesions in the squamous mucosa (equine squamous gastric disease [ESGD]) and glandular mucosa (equine glandular gastric disease [EGGD]; Fig. 1). ESGD is similar to gastroesophageal reflux disease (GERD) in humans, for which stomach acids are refluxed onto the sensitive esophagus squamous mucosa. In horses, the esophageal tissue (squamous epithelium) is present in the proximal one-third of the stomach and is not protected by the lower esophageal sphincter and, therefore, is constantly exposed to gastric acids (hydrochloric, volatile fatty, and bile acids). The condition of GERD is common in human athletes, and a number of provocative physiological events, including increased gastric acid secretion, impaired gastric emptying, and increased internal gastric pressure, occur during exercise that exacerbate esophageal reflux. In fact, published epidemiological data on GERD indicate that upper GI symptoms occur in 58% of surveyed athletes, and these symptoms are proportional to exercise intensity. As exercise intensity increases and blood flow shifts away from the GI tract to working skeletal muscles, GI motor activity is modified and gastric emptying is delayed. Factors
that could explain exercise-induced gastroesophageal reflux in humans include the following: body position, which delays esophageal clearance as subjects shift away from the upright position (e.g., sprinter); increased abdominal pressure generated by the Valsalva maneuver (i.e., weightlifters); or the bent-over racing position maintained by most cyclists. All of these activities can increase the abdominal component of the vectorial force, pushing gastric contents up against the lower esophageal sphincter. This scenario might be similar in horses with ESGD during exercise. Previously, Lorenzo-Figueras and Merritt showed that intragastric pressure in horses sharply increased at a trot and gallop, which was due to increased intra-abdominal pressure related to the exercise. Increased abdominal pressure led to increased gastric pressure, refluxing acid stomach contents as measured by a pH probe inserted in the stomach just distal to the lower esophageal sphincter. Reflux of acids on the squamous mucosa might explain the high prevalence of ESGD in racehorses. It is also consistent with the following observations:

- Squamous lesions regress or disappear when horses are taken out of training or are treated with acid-suppressing drugs.
- Lesions are most often found in the squamous mucosa near the margo plicatus on the lesser curvature, where acids are in constant contact with gastric mucosa.

This “how to” article discusses the specific approach for determining if EGUS is causing or contributing to poor athletic performance. The impact of EGGD on performance remains to be determined.

**Comprehensive Approach**

A comprehensive examination to determine poor athletic performance should include a general physical examination, upper respiratory endoscopy examination, endoscopy, blood work (hematology and biochemical panel), and an endoscopic examination of the distal esophagus, stomach, and proximal duodenum.

**Clinical Signs**

Because the prevalence of EGUS is high in performance horses and clinical signs are often vague, signs of EGUS are often subclinical and missed by trainers and owners. Clinical signs of EGUS typically include poor body and coat condition, weight loss, behavioral changes, mild colic, discomfort after eating, and reluctance to train. However, in a previous study in 4 horses with gastric ulcers as a cause of poor athletic performance, clinical signs were absent and there was no evidence of abdominal pain, which makes it difficult to determine GI causes of poor performance.

**Endoscopic Examination of the Distal Esophagus, Stomach, and Proximal Duodenum**

A thorough endoscopic examination of the distal esophagus, stomach, and proximal duodenum should be performed as part of a comprehensive athletic performance examination. The preparation, sedation, endoscopy procedure, and care postendoscopy are available online (https://www.youtube.com/watch?v=l_ZAxnxmE-0).

Briefly, gastroscopy can be performed in the standing and sedated horse or a horse under general anesthesia. To perform standing gastroscopy in the horse, feed, but not water, should be withheld for at least 8–12 hours prior to the procedure. A muzzle should be placed on the horse during the feed-deprivation period to prevent ingestion of fecal material or residual feed in the stall. All bedding and feed material should be removed from the stall during the feed-deprivation period. Three people might be needed to perform a thorough standing endoscopic examination of the stomach; one person restrains the horse, one passes the endoscope, and one drives the endoscope. Xylazine (0.5 mg/kg bodyweight [bwt],
IV) or detomidine (0.01–0.02 mg/kg bwt, IV), and/or butorphanol (0.02 mg/kg bwt, IV) can be administered prior to the procedure, and the horse is left undisturbed until sedation is observed. A twitch might be applied to the horse’s muzzle on the opposite side from which the endoscope is to be passed. Endoscopy of the stomach should be performed using a 3 m or longer endoscope, as shorter endoscopes do not provide a complete view of the stomach and proximal duodenum. To improve visualization of the stomach and enable observation of the squamous mucosa (fundus ventriculi), margo plicatus, and glandular mucosa (corpus ventriculi), the stomach can be insufflated using the air-feed directly from the endoscope or an air compressor attached to the endoscope biopsy channel (until the rugae or stomach folds disappear). Mucosa should be rinsed of adherent feed material and mucus using tap water flushed through the endoscope biopsy channel using 60-ml syringes or fluid pump. Ulcers might be seen under adherent feed material and mucus and might be obscured by debris. When horses are difficult to handle, are under general anesthesia for abdominal surgery, or when a complete examination of the stomach is not possible standing, endoscopy can be performed under general anesthesia. To perform endoscopy under general anesthesia, it is best to place the horse in right lateral recumbency so that the stomach is up. The horse can be rolled up on its back and then to the left side so that all of the stomach can be viewed. Orienting oneself to the anatomy can be more challenging than in the standing horse. Care must be taken not to oversufflate the stomach, as this could result in air escaping into the small and large intestine, resulting in bloating and abdominal pain during recovery. Residual air should be removed from the stomach after the procedure to prevent colic or other complications.9 Ulcerations, hyperkeratosis, and hyperemia should be recorded, and a scoring system can be used to keep track of severity. Several scoring systems are available for use and include the one recommended by the EGUS Council and European College of Equine Internal Medicine (Table 1)10,11 that evaluates the size of ulcers. Another scoring system evaluating lesion number and severity can also be used for both squamous and glandular regions of the stomach (Table 2).5 In addition, EGGD can be described using another system (Table 3).11 The advantage of recording ulcer scores is to assess improvement or worsening on follow-up examinations. The presence of gastric ulcers on endoscopic examination does not confirm cause and effect, as ulcers could be secondary to other causes of poor performance.

Measuring Gastric Juice pH

Upon endoscope entry into the stomach and before insufflation or flushing with tap water, one can aspirate gastric fluid from the biopsy channel and measure pH within 2 hours by using a benchtop pH meter, pH paper, or a urine dipstick, with pH disk. Measuring gastric juice pH, especially in horses treated with antiulcer medications, is important to determine if treatment with acid-suppressing drugs is effective. A pH of ≥4.0 is desirable, and it has been shown that a pH <4.0 can damage the squamous stomach mucosa.12

Other Diagnostic Testing

Hematology

There are no clinicopathologic parameters that are pathognomonic for EGUS as a cause of poor athletic performance. However, hematology, at the

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Description</th>
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<tbody>
<tr>
<td>Lesion Number Score</td>
<td>0: No lesions; 1: 1–2 localized lesions; 2: 3–5 localized lesions; 3: 6–10 localized lesions; 4: &gt;10 lesions or diffuse (very large) lesions</td>
</tr>
<tr>
<td>Lesion Severity Score</td>
<td>0: No lesions; 1: Appears superficial (only mucosa missing); 2: Deeper structures involved (&gt;depth than number 1); 3: Multiple lesions and variable severity, (1, 2, and/or 4); 4: Deeper structures involved (&gt;depth than number 1) and has active appearance (hyperaemic and/or darkened); 5: Same as number 4 plus hemorrhage or blood clot</td>
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Table 1. EGUS Scoring System for ESGD5-10

<table>
<thead>
<tr>
<th>Score</th>
<th>Explanation</th>
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<tbody>
<tr>
<td>0</td>
<td>The mucosa is intact and there is no appearance of hyperemia</td>
</tr>
<tr>
<td>1</td>
<td>The mucosa is intact, but there are areas of reddening</td>
</tr>
<tr>
<td>2</td>
<td>Small single or multifocal superficial lesions (&lt;5)</td>
</tr>
<tr>
<td>3</td>
<td>Large single or multifocal lesions or extensive superficial lesions (≥5)</td>
</tr>
<tr>
<td>4</td>
<td>Extensive lesions with areas of apparent deep ulceration</td>
</tr>
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</table>

Table 2. Number and Severity Scoring System for Squamous and EGGD5

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minimum, should be performed in horses presenting for poor athletic performance. In one report, 3 of 4 horses presenting for poor performance due to EGUS had hemoglobin concentrations below the reference range. In another study, horses with EGUS had significantly lower red blood cell counts and hemoglobin concentrations than horses without EGUS. Unfortunately, these values were not outside reference ranges. In addition, previous epidemiological studies have failed to find an association between hematological parameters and poor athletic performance in populations of racehorses.

**Fecal Occult Blood Testing**

It is probably worth mentioning the use of fecal occult blood to determine horses with EGUS. A guaiac-based fecal occult blood test was used in one study and showed high specificity, low sensitivity, and high false-negative results for determining the location of ulceration (stomach or colon). Also, in a recent study, there was a high number of false-negative tests, and no significant correlation was found between results of a new fecal blood test and gastric ulcer scores in horses undergoing stall confinement and bolus feeding.

### 2. Treatment

The gold standard for the treatment of horses with EGUS is omeprazole. However, other pharmacologic agents have been used for the treatment and prevention of EGUS with variable success (Table 4). In the study by Franklin et al., all horses presenting with poor performance due to EGUS had severe ESGD and mild-to-moderate EGGD. All horses were treated with omeprazole paste (4 mg/kg bwt, orally daily) and one horse received sucralfate (20 mg/kg bwt, orally twice daily), and all returned to racing after treatment. Two of the 4 horses in that study had follow-up gastroscopy and the squamous ulcers healed, whereas the glandular mucosa still had hyperemia in 1 horse and was healed in the other horse. Improvement in athletic performance was documented by an increase in mean Raceform rating and increased earnings after treatment. It should be noted that omeprazole has no performance-enhancing properties by itself, as confirmed by a previous study. A recent study in human runners addressed the effect of GERD and treatment with antisecretory agents on poor performance. In that study, runners with frequent GERD had a significantly decreased time to exhaustion compared to runners without reflux. However, prophylactic rabeprazole (omeprazole-like proton-pump inhibitor) administration did not improve performance in the runners with GERD. Therefore, treatment in these human athletes did not result in a significant effect on performance. The authors speculated that decreased performance in human athletes with GERD might be caused by esophageal pain and not stomach pain. In contrast, because the squamous mucosa is located in the stomach, stomach pain (due to acid reflux and resultant ESGD) might be the cause of poor athletic performance in horses.

<table>
<thead>
<tr>
<th>Severity</th>
<th>Distribution</th>
<th>Appearance</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mild</td>
<td>Focal</td>
<td>Flat and hemorrhagic</td>
<td>Cardia</td>
</tr>
<tr>
<td>Moderate</td>
<td>Multifocal</td>
<td>Flat and fibrinansuppurative</td>
<td>Fundus</td>
</tr>
<tr>
<td>Severe</td>
<td>Diffuse</td>
<td>Raised and hemorrhagic</td>
<td>Antrum</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Raised and fibrinansuppurative</td>
<td>Pylorus</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Depressed ± blood clot</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Depressed and fibrinansuppurative</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Other (describe)</td>
<td></td>
</tr>
</tbody>
</table>

### Table 4. Commonly Used Therapeutic Agents and Doses for Treatment and Prevention of EGUS

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose</th>
<th>Dosing Interval (h)</th>
<th>Route of Administration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ranitidine</td>
<td>6.6</td>
<td>q 6–8</td>
<td>PO</td>
</tr>
<tr>
<td>Ranitidine</td>
<td>1.5</td>
<td>q 6</td>
<td>IV, IM</td>
</tr>
<tr>
<td>Omeprazole</td>
<td>4 (treatment)</td>
<td>q 24</td>
<td>PO</td>
</tr>
<tr>
<td>Omeprazole</td>
<td>1–2 (prevention)</td>
<td>q 24</td>
<td>PO</td>
</tr>
<tr>
<td>Omeprazole</td>
<td>0.5–1.0</td>
<td>q 24</td>
<td>IV</td>
</tr>
<tr>
<td>Esomeprazole</td>
<td>1.0</td>
<td>q 24</td>
<td>IV</td>
</tr>
<tr>
<td>Esomeprazole</td>
<td>1.0–2.0</td>
<td>q 24</td>
<td>PO</td>
</tr>
<tr>
<td>Pantoprazole</td>
<td>1.0–1.5</td>
<td>q 24</td>
<td>IV or PO</td>
</tr>
<tr>
<td>Sucralfate</td>
<td>12–20</td>
<td>q 8</td>
<td>PO</td>
</tr>
<tr>
<td>Al- or Mg hydroxide</td>
<td>0.5 mL/kg</td>
<td>q 4–6</td>
<td>PO</td>
</tr>
<tr>
<td>Misoprostol, prostaglandin analogues</td>
<td>1–5 μg</td>
<td>q 8-12</td>
<td>PO</td>
</tr>
</tbody>
</table>

*Units are (mg/kg) unless otherwise indicated.*
performance in horses. Recent investigations have shown a reduced efficacy in omeprazole-treated horses with EGGD, when compared with ESGD. Only 36% of EGGD cases healed when treated with omeprazole (4 mg/kg, orally every 24 hours) compared with a 78% healing rate in horses with diagnosed ESGD. The reason for this finding is not known but is likely a reflection of the different pathogenesis causing EGGD, and therefore, EGGD is considered a related but distinct disease from ESGD. Because of the poor response of EGGD to omeprazole administration, sucralfate has been recommended for treatment. It should be noted that sucralfate, as a monotherapy, was shown to be ineffective in improving foals with ESGD lesions but has efficacy in treatment of EGGD. Sucralfate binds to stomach ulcers and promotes healing. Sucralfate is an aluminum complex sulfated polysaccharide (α-D-glucopyranoside, β-D-fructofuranosyl-α-D-fructofuranosyl, octakis-[hydrogen sulfate]), in combination with octasulfate and aluminum hydroxide. Its mechanism of action involves adherence to ulcerated mucosa, forming a proteinaceous bandage, and stimulating prostaglandin E1 synthesis and mucus secretion. Sucralfate also inactivates pepsin and adsorbs bile acids. Omeprazole plus sucralfate (12 mg/kg bwt, orally, q6h-q12h) led to healing in 63% of horses with EGGD grade ≥2 and improvement of at least 1 grade in 83% of horses with EGGD grade ≥2.

The synthetic prostaglandin E2 analogue misoprostol has been recommended for use in treating EUGS. Misoprostol is a methylester analogue of prostaglandin, and it showed a time-dependent increase in the basal gastric pH in horses. Misoprostol enhances mucosal protection by stimulating mucus and bicarbonate production and might aid in the treatment and prevention of EGGD. A recent report showed that misoprostol significantly inhibited inflammatory mediators (tumor necrosis factor alpha, interleukin-1β, interleukin-6, and superoxide) produced ex vivo from equine leukocytes exposed to lipopolysaccharide. This study showed that misoprostol has anti-inflammatory effects that might be effective for treating EGGD because lesions in the glandular mucosa are frequently inflammatory. In more recent studies, misoprostol (5 μg/kg bwt, orally every 12 hours) was found to be superior to combined omeprazole (4 mg/kg, orally every 24 hours) and sucralfate (12 mg/kg, orally every 12 hours) therapy in horses with EGGD. Misoprostol might cause diarrhea and/or colic signs and should not be used in pregnant mares because it might induce parturition and termination of the pregnancy.

3. Antibiotic Treatment of Chronic ESGD and EGGD

*Helicobacter pylori* and other *Helicobacter* spp. have not been shown to cause ESGD or EGGD, although *Helicobacter* DNA has been isolated from the stomach and feces of horses. Instead, other resident, acid-tolerant bacteria (*Escherichia coli*, *Lactobacillus* spp., and *Streptococcus* spp.) are suspected to contribute to the worsening of squamous ulcers. In that study, bacteria were isolated from the gastric contents of horses with spontaneous ESGD that were fed a high-concentrate diet. It was found that trimethoprim sulfadimidine (15 mg/kg bwt, orally, once daily) decreased ESGD ulcer numbers and severity compared to untreated control horses. These data suggest that resident stomach bacteria are important for the maintenance and progression of ESGD. However, once-daily administration of trimethoprim-sulfadimidine did not improve healing of EGGD in horses receiving omeprazole. The author has used trimethoprim sulfadiazine (15–20 mg/kg bwt, orally, twice daily) for treatment of nonresponsive ESGD. Recently, doxycycline (10 mg/kg bwt, orally twice daily) has been used to some success to treat nonresponsive EGGD. Tetracycline compounds have antibiotic, anti-inflammatory, and antioxidant tissue-protective properties. Frequently, EGGD lesions are inflammatory in nature, and recently, it was shown that EGGD lesions might be colonized by resident bacteria. Antibiotics should be used responsibly and only when acid-suppressive therapy alone is not effective.

4. Prevention

Prevention of ulcer recurrence is important, as in one study squamous ulcers recurred within 8 days after discontinuing omeprazole treatment in horses engaged in light and heavy training regimes. The use of low-dose omeprazole (1 mg/kg bwt, orally, q24h) has been shown to prevent recurrence of ESGD. In a recent meta-analysis study, omeprazole prophylaxis in active training horses significantly reduced gastric ulceration compared with no prophylaxis (sham), with the absolute effect of 566 fewer ulcers per 1000 horses treated.

5. Management

Management changes may be more cost effective but difficult, especially in Thoroughbred racehorses housed in stalls. To reduce gastric acidity, horses can be provided access to pasture grazing, fed *ad libitum* forage, and provided smaller and more frequent concentrate feedings. Serum gastrin concentrations are high in horses fed high-concentrate (grain) diets. Large bolus grain feeding can increase soluble carbohydrates in the stomach, which are fermented by resident stomach bacteria (*Lactobacillus* spp.), resulting in the production of volatile fatty acids, which in the presence of low stomach pH (≤4), have been shown to cause acid damage to the squamous mucosa. A previous study, using an *in vitro* model, showed that feeding grain at greater than 0.5 kg/100 kg bwt every 5 hours resulted in volatile fatty acid concentrations above the threshold (20 mmol), which led to acid damage, as indicated by changes in
squamous tissue bioelectrical properties. Thus, smaller grain feedings every 5 to 6 hours may help in the prevention of EUGS. Regarding EGGD, when management factors associated with EGGD were evaluated, dietary factors were not included in the final multivariable models, suggesting that dietary factors may be less important for the control of EGGD than for the control of ESGD. However, decreased pasture turnout or increased grain concentrate frequency were retained in the univariate model and might be associated with EGGD. Therefore, increasing pasture turnout and decreasing grain concentrations might be useful management strategies for preventing EGGD. In the previous study, horses with EUGS as a cause of poor performance were managed with increased pasture grazing and showed improvement in EGGD. Another dietary strategy, feeding alfalfa hay, has been shown to decrease gastric ulcers in horses. A previous study showed that horses fed an alfalfa hay/grain diet had significantly higher stomach pH (for 5 hours after feeding) and fewer, less severe gastric ulcers than horses fed brome grass hay. In a more recent study, horses fed alfalfa hay and exercised showed decreased gastric ulcer scores compared to horses fed a coastal hay diet. Thus, feeding alfalfa, because of its superior buffering capacity (high calcium and protein concentrations), may protect the squamous mucosa from acid damage. There are no studies on the effect of alfalfa hay on EGGD. Intense exercise, racing, and race training have been shown to contribute to worsening of ESGD; thus, taking the horse out of training, reducing exercise intensity, and incorporating pasture turnout might improve ESGD. However, there are no studies showing that exercise intensity or exercise restriction has any effect on EGGD. In summary, gastric ulceration, part of EUGS, is common in racehorses throughout the world and has been linked to poor athletic performance. It should be emphasized that EUGS could be the sole cause of poor athletic performance, but it also could contribute to or be secondary to other causes of poor performance. Omeprazole is the treatment of choice; however, complementary pharmaceutical agents such as sucralfate and misoprostol might improve healing in horses with EGGD. Because ulcers will likely return after cessation of treatment, prophylaxis with a low dose of omeprazole (1–2 mg/kg bwt, orally daily) is beneficial for long-term prevention of recurrence. Management, such as increasing pasture turnout and decreasing dietary concentrate feeding, is suggested, but more research is needed to determine which management strategies are effective and which ones are of the greatest benefit for maintaining a high level of athletic performance.

Acknowledgments

Declaration of Ethics

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

Conflict of Interest

The Author has no conflicts of interest.

References and Footnote


*Gastrogard paste, Boehringer-Ingelheim Animal Health, Duluth, GA 30096.*
How to Diagnose Equine Degenerative Myeloencephalopathy in Sport Horses

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1. Background

Neuroaxonal degeneration in the brainstem and spinal cord of horses, termed equine degenerative myeloencephalopathy (EDM) or neuroaxonal dystrophy (NAD), has been reported sporadically in the equine veterinary literature for over 40 years. Eventually, consensus developed that these two conditions have such striking clinical and pathologic similarities that NAD should be considered a localized form of EDM in which degenerative changes in the spinal cord are subtle and easily overlooked.¹,² Disease has been described in equids of many different breeds, with familial or genetic causes suspected. The author’s impression is that NAD/EDM was a common pathologic diagnosis when the disease was first described and then less commonly recognized, only to resurge in the last decade. Whether this resurgence is because of true changes in disease incidence or because of changes in clinical suspicion or knowledge of the disease is unclear. In the author’s practice (New Bolton Center [NBC]), there is an increasing diagnosis of NAD/EDM, and affected horses have different clinical presentations from those in earlier literature. These proceedings will focus on clinical observations from recent years, with a brief review of existing literature. For simplicity, the disease will be referred to as EDM.

2. Prevalence and Signalment

The first report of EDM described young horses of several breeds as well as a zebra.³ After its recognition, EDM was considered a common neurologic disease of horses, representing 23/96 (24%) of horses with spinal cord disease evaluated at Cornell from 1974 to 1976.⁴ A later report indicated that EDM remained the most common cause of spinal cord disease in horses at Cornell University; it was diagnosed clinically in 171/383 (45%) horses and histologically in 140/287 (49%) horses from 1977 to 1987.⁵ Early reports of EDM described the onset of clinical signs from birth to 1.2 years of age, with a mean onset at 0.4 years.⁴ Likewise, out of 43 horses with EDM reported at Cornell from 1978 to 1987, owners reported that approximately 80% showed clinical signs by 14 months of age. However, a few outliers were identified, with 3 horses reported by owners to develop signs at 5, 7, and 12 years of age.⁶ As time went on, familial predisposition to EDM was documented in multiple breeds of equids, including Appaloosa, Arabian, Thoroughbred, Standardbred, Morgan, Paso Fino, captive Grant zebras, and Przewalski horses.⁷ Approximately 90 horses clinically evaluated at NBC from 2016 to 2020 had a final post-mortem diagnosis of EDM, with the number increasing each year (2016, 4 horses; 2017, 6 horses; 2018, 12 horses; 2019, 28 horses; 2020, 40 horses). Contrary to previous reports, most of the
Sport horses diagnosed by the author are between 5 and 15 years of age, with young to middle-aged Warmbloods making up the bulk of cases diagnosed at NBC. However, Quarter Horses, Standardbreds, Thoroughbreds, Arabsians, and horses of many other breeds have also been diagnosed. It is possible that EDM has a delayed onset in Warmbloods, but it is also possible that the perceived later onset is due to management and training differences, causing subtle abnormalities to be missed early in life that only becomes evident when workload and level of performance increase. Many of the Warmblood sport horses diagnosed with EDM have been imported from Europe, but they are not from a consistent region, of a consistent age group, or breed. Importantly, American-bred horses, both Warmbloods and many other breeds, are also affected by this condition.

3. History
Obtaining a thorough history from people familiar with the horse, including owner, trainer, rider, and manager, is very helpful for diagnosing EDM. Although not observed in every case, behavior changes are a very common clinical feature that should alert the veterinarian to the possibility of EDM. The behavior changes cannot always be detected by people less familiar with the horse’s “normal” behavior, and hence, veterinarians should listen carefully to the observations of those who know the horse well.

“Personality” changes are common, including changes in the horse’s general level of awareness, reaction to stimuli, and interactions with people, animals, and the environment. Many horses eventually diagnosed with EDM are purchased because they are initially perceived to be calm and quiet. Some horses become increasingly dull and lethargic, to the point of appearing sedated, and they seem to lose interest in social interactions with people and other horses. This overly dull/sedate behavior might be interspersed with periods of uncharacteristic anxiety, spookiness, and aggression toward horses, other animals, or people. Other horses appear to be persistently anxious or on edge, often becoming cranky or aggressive. Owners frequently comment “he’s not the same horse he used to be.”

Bad behavior under saddle is frequently the first sign detected. Horses often start spooking unpredictably and severely. This spookiness might lead clients to suspect eye problems, but ophthalmic exam is usually normal. Many of these horses were considered unusually calm before the onset of uncharacteristic spooking. Even professional riders can rarely predict (or recognize in hindsight) the stimulus for the spook. Although some horses become very reactive to sound and others reactive to visual stimuli, many spook for no apparent reason, often after they have gone past an area uneventfully several times. For example, a horse might canter over a line of fences three times and then on the fourth spook violently in between fences. In addition to excessive and unpredictable spookiness, other bad behaviors are common, including bucking, bolting, rearing, spinning, refusing fences, and becoming unwilling to stand at the mounting block. Importantly, these bad behaviors are a marked change from the horse’s normal behavior and are usually unprovoked and unpredictable. Owners frequently use descriptions such as “it was like a switch flipped” or “it was like he didn’t know where he was or what happened – he had a glazed/vacant expression” when they describe the episodes. Sometimes clients detect signs that might indicate ataxia and sometimes they do not. It is more common for behavior changes to be the primary reason for evaluation, but suspected lameness or loss of coordination can be the primary complaint. Horses might start knocking rails, struggle with lateral movements, have difficulty holding leads at a canter or changing leads, or feel weak. Tripping with the thoracic limbs and “falling out behind” (tripping, stumbling, or buckling with the pelvic limbs) are common observations. Additional client observations include topline musculature that seems underdeveloped or atrophied and haircoat changes such as a long or dull haircoat. These changes can be reminiscent of a horse with pituitary pars intermedia dysfunction (PPID) and sometimes prompt endocrine testing despite the relatively young age of the horse. Other clients have observed changes in behavior that might result from sensory abnormalities, including abnormal reactions to grooming or being sprayed with water, a perceived inability to sense temperature changes, and abnormal reactions to pressure when asked to move away from the hand on the ground or the leg under saddle.

4. Clinical Signs
Initial descriptions of EDM reported symmetric tetraparesis and ataxia, generally noticed between birth and 1 year of age. These signs reflected upper motor neuron and general proprioceptive tract lesions, with no evidence of cranial nerve, cerebral, or cerebellar involvement. Hypogalgesia, hypotonia, hyporeflexia, or muscle atrophy were not observed. These signs were considered indistinguishable from those caused by other focal, multifocal, or diffuse myelopathies affecting the cervical spinal cord. However, most focal myelopathies cause deficits that are slightly more severe in the pelvic limbs than in the thoracic limbs, whereas horses with EDM were often observed to have deficits in the thoracic limbs that were either similar in severity to those in the pelvic limbs or much less severe than those in the pelvic limbs, with a bigger disparity than would be expected for a focal compressive lesion. Curiously, a subsequent report described similar tetraparesis and ataxia but with significant hyporeflexia over the neck and trunk, particularly in horses with a longer duration of clinical signs. This hyporeflexia was evident during assessment of cervical, cervicofacial, laryngeal adductor (slap), and cutaneous trunci reflexes. In the
author’s experience, horses with EDM as the sole problem usually appear to be systemically healthy and often are well-conditioned or overconditioned, and some have a phenotype consistent with equine metabolic syndrome. Additionally, some relatively young horses diagnosed with EDM have phenotypic characteristics more commonly associated with PPID, including topline muscle wasting and a long, dull haircoat. Even though most owners and trainers observe convincing behavior changes, the horse might appear normal in terms of mental status and behavior during veterinary examination. However, some horses appear unusually dull or sedate during examination. Other horses appear overly anxious and inappropriately spooky when considering their age, breed, and previous experiences. Cranial nerve assessment is usually normal. Rarely, horses will have an inconsistent menace response or hyperreactive menace response with rapid repeated (clonic) blinking. Spinal reflexes are usually normal, although the cervicofacial reflex might be subjectively decreased. Cervical range of motion is typically normal unless concurrent cervical arthritis is present. Dynamic gait evaluation typically reveals proprioceptive deficits and signs of mild-to-moderate ataxia and paresis consistent with a cervical or diffuse myelopathy. Most horses are graded 1 to 2/5 on the modified Mayhew ataxia scale at the time of first evaluation, although occasionally horses show more severe deficits (2.5 to 3/5). Repeated evaluations over months might show a progression of neurologic disease. Evaluation at the walk most frequently reveals a normal-to-long strided gait, sometimes with a “floaty” or toe-flipping appearance. Horses might show irregular foot placement, occasional limb interference, or forging. These signs might be exacerbated by walking with head elevation. Circling the horse in hand tightly in both directions is usually the most helpful test to detect proprioceptive deficits. Horses often show delayed protraction, such that their body leans to the side, but they are slow to initiate limb movement—their feet never seem to catch up with their center of gravity. Limb interference might be seen from the level of the hocks/carpi down to the hooves. The toe of the outside limb might scuff or drag on the ground as it is widely and spastically swung into position. The inside foot might pivot for an abnormally long time on the ground. When the horse is stopped suddenly or asked to rapidly change direction, excessive truncal sway and loss of balance might be observed. Horses often spontaneously place their limbs in unusual positions relative to their center of gravity and then fail to replace them in a more normal position. Deficits are often more obvious in the pelvic limbs than thoracic limbs. When walked down a hill, particularly with head elevation, the horse might overreach or search for the ground with its front feet while scuffing, sinking, or buckling with its pelvic limbs. When observed at the trot, the horse might appear more bouncy or spastic than expected, with difficulty moving in a straight line and a tendency to drift in either direction.

5. Differential Diagnoses

Horses with EDM might be presented for bad behavior under saddle, poor performance, or poor movement and suspicion of lameness. The list of problems that can cause these types of complaints is extensive. The author strongly believes that most horses are quite willing and eager to please and that development of bad behavior in a horse that previously performed well is almost always related to a medical problem. Marked behavior changes in a horse that has undergone no major environmental or training changes are almost always due to pain or brain disease. Therefore, if the primary complaint is abnormal behavior, the owner should be carefully questioned and the horse should be carefully observed for evidence of neck, back, limb, or abdominal pain. The author finds 24-hour video monitoring with behavioral analysis to be helpful with many cases; horses sometimes show very demonstrative and localizing pain behaviors when barns are quiet and no one is interacting with them. If the horse is presented for a vaguer complaint of poor performance or poor movement, careful and potentially repeated orthopedic and neurologic examinations are necessary to determine whether the poor performance and abnormal gait are due to orthopedic disease alone, neurologic disease alone, or a combination of both. Lameness diagnostics such as local anesthesia can help confirm an orthopedic source and narrow down differential diagnoses. If the gait abnormality is not severe enough to assess the effects of local anesthesia (not “blockable”), a phenylbutazone trial might yield helpful information. If the horse’s performance improves on phenylbutazone, orthopedic and potentially other painful problems should be investigated. If signs of proprioceptive deficits and ataxia are clearly present, neurologic causes of poor performance and behavior changes should be considered. Horses with EDM typically have a chronic course of disease and often have not been right for months. Ataxia typically is symmetric and affects all four limbs, although the pelvic limbs might be more obviously affected. In the author’s practice, most horses with symmetric proprioceptive ataxia of a duration of multiple weeks have one of three following categories of disease: (1) compressive myelopathy, usually due to cervical vertebral stenotic myelopathy (CVSM); (2) infectious myelitis, usually due to equine protozoal myeloencephalitis (EPM) caused by Sarcocystis neurona; or (3) degenerative myeloencephalopathy (EDM/NAD). Therefore, diagnostic testing is aimed at differentiating between these three types of diseases.
6. Laboratory Results

Standard hematologic tests, including complete blood cell count, serum amyloid A, fibrinogen, and chemistry panel, generally yield unremarkable results unless comorbidities exist. Cerebrospinal fluid (CSF) collection is strongly recommended for horses with neurologic disease, and results can confirm or refute the presence of infectious neurologic disease, with EPM being the infectious disease of most concern. Horses with EDM usually have a normal CSF nucleated cell count (NCC; <5 cells/μL) and normal cytology (very low number of mononuclear cells, without neutrophils, eosinophils, or other abnormal cells). CSF total protein can be normal (<90 mg/dL) or mildly increased; these mild increases are typically in the 90- to 120-mg/dL range. If CSF cytology is abnormal, with increased NCC indicating meningitis, infectious viral and bacterial diseases should be strongly considered. EPM is the most common infectious disease of North America, and specific testing should be considered in most areas of the United States.9 Horses with EDM might be negative or positive on EPM serologic tests, depending on exposure status. Generally, negative serologic results have a high negative predictive value and rule out EPM. Rare exceptions exist in horses with immunodeficiencies or recent infection, and confirmation with CSF testing should be considered if CSF is available. Positive serologic results have a low positive predictive value but indicate the need for further testing, and more accurate assessment of EPM status should be pursued, ideally by using quantitative antibody testing on paired serum and CSF samples to detect intrathecal antibody production. Although a rare cause of neurologic disease in horses, Lyme disease is a frequent concern of clients and theoretically could cause both behavior changes and ataxia.10 Horses with EDM might be negative or positive on Lyme serology, depending on exposure status. Diagnosis of neuroborreliosis is very challenging in horses, and antibody levels are not necessarily predictive.11 Because most true neuroborreliosis cases have abnormal CSF cytology, with neutrophilic or lymphocytic pleocytosis, the author generally discounts Lyme disease as the primary cause of neurologic deficits if CSF cytology is normal, regardless of serum and CSF antibody levels against Borrelia burgdorferi. Vitamin E deficiency has been associated with EDM, and vitamin E concentration should be assessed in suspect cases.7 Low vitamin E concentration (less than 2 ppm or 200 μg/dL) is supportive of EDM diagnosis, particularly when other causes of neurologic disease have been excluded. However, adequate vitamin E status does not preclude the diagnosis of EDM. Many horses diagnosed by the author have vitamin E concentrations well within the normal range at the time of diagnosis. It is possible that these horses were deficient in utero or during early stages of their lives, contributing to disease development. The utility of biomarkers such as phosphorylated neurofilament heavy (pNF-H) for diagnosis of equine neurologic disease, including EDM, is under investigation.12 Current evidence suggests that abnormally high concentrations of pNF-H in serum and CSF are suggestive of EDM if diseases such as EPM have been excluded. However, pNF-H testing has a low sensitivity for EDM diagnosis, and many confirmed cases have normal pNF-H concentrations in blood, spinal fluid, or both.

7. Imaging Results

Survey cervical radiography, cervical myelography, and sometimes cervical myelography-computed tomography are used to assess for CVSM. One of the major challenges in EDM diagnosis is that affected horses might also have cervical arthritis, CVSM, or both. Normal survey cervical radiographs and/or negative cervical myelography make CVSM highly unlikely and increase clinical suspicion for EDM, assuming infectious causes of disease are also excluded. Unfortunately, abnormal cervical radiographs and even positive cervical myelography do not exclude the possibility of EDM because false-positive myelograms are possible and horses can be affected by both diseases simultaneously. With increasing recognition of EDM, the author has become more cautious in recommending surgical cervical fusion for horses with equivocal (“gray zone”) myelograms and horses with positive myelograms but behavior changes suggestive of EDM.

8. Postmortem Results

EDM diagnosis currently requires post-mortem examination, but the characteristic degenerative changes can be missed if the pathologist is inexperienced or the brainstem is not carefully evaluated. A diffuse degenerative myeloneuropathy was initially described by Mayhew et al.,3 with the most pronounced degeneration in thoracic segments of the spinal cord. Ventral and dorsolateral funiculi, particularly the dorsal spinocerebellar tract, were considered most severely affected, and dorsal funiculi were considered least severely affected. Axonal swelling (dystrophy) and abnormal neuronal cell bodies were seen in the gray matter of the spinal cord and in some brainstem nuclei; the most prominent changes were observed in the spinal cord proprioceptive nucleus (nucleus of the dorsal spinocerebellar tract) and the caudal medullary proprioceptive nuclei (gracile and cuneate, particularly the lateral cuneate nuclei). Mayhew et al.2 included the summary description “neuronal fiber degeneration throughout the spinal cord (particularly in the dorsolateral and ventromedial tracts), and NAD, lipofuscin-like pigment accumulation, astrogliosis and necrobiosis of neuronal cell bodies in specific brain stem and spinal cord nuclear areas.” Around the same time as the above description, Beech13 described NAD in Morgan
horses, which had similar degenerative changes (NAD, gliosis, vacuoles, and pigment) as horses with EDM; however, these changes were localized to the accessory (lateral) cuneate nuclei, without substantial microscopic spinal cord changes. In the author’s experience with sport horses, the brainstem changes are most consistent, particularly in the lateral cuneate nuclei, whereas spinal cord lesions are minimal or difficult to detect.

9. Summary
EDM remains a common cause of neurologic disease in the horse but is almost certainly underdiagnosed due to the challenges in antemortem diagnosis. Practitioners should maintain a high degree of suspicion for this disease in horses with behavior changes and mild-to-moderate ataxia or in any horse with relatively symmetric ataxia that lacks evidence for infectious disease or spinal cord compression. Diagnosis in the living horse is based primarily on exclusion of other potential differential diagnoses through CSF analysis and appropriate imaging. Post-mortem diagnosis requires an experienced pathologist and careful attention to the brainstem, where degenerative changes are most evident.

Acknowledgments

Declaration of Ethics
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Conflict of Interest
The Author has no conflicts of interest.

References
Diagnosis and Management of Myofibrillar Myopathy in Warmblood Performance Horses

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1. Introduction
Exertional muscle disorders cause muscle pain and/or weakness that impair athletic performance. One subset of exertional myopathies, exertional rhabdomyolysis, is characterized by muscle degeneration and elevations in serum creatine kinase (CK) and aspartate transaminase (AST) activities. Another subset of exertional myopathies that includes type 2 polysaccharide storage myopathy (PSSM2) and myofibrillar myopathy (MFM) in Warmbloods is characterized by exercise intolerance without elevations in serum CK and AST activities.1,2 The lack of a readily available blood test to screen these horses for a myopathy and the overlap in clinical signs with other disorders necessitate a detailed diagnostic work up to rule out other more common causes of exercise intolerance before proceeding to a muscle biopsy.

2. What is MFM?
Myofibrils contain myofilaments aligned within contractile units called sarcomeres that are bordered by Z discs. Z discs provide structural support as well as mechanosignaling whereby tension during muscle contractions sends Z disc proteins to the nucleus that activate genes which initiate training adaptations.3 Desmin is a cytoskeletal protein located at the Z disc that aligns adjacent sarcomeres. Horses with MFM have disrupted myofibrillar alignment within the sarcomere, Z disc disruption, and ectopic accumulation of desmin in select type 2A (fast-twitch oxidative) muscle fibers.2 Muscle samples from MFM horses may be characterized by cytoplasmic aggregates of glycogen, likely because pools of glycogen form within disrupted myofibrils.2 Transcriptomic and proteomic studies of muscle from MFM Warmblood suggest that the basis for MFM could be aberrant gene and protein expression that impact molecular signaling, the alignment of contractile proteins, mitochondrial function, and oxidative stress.4 This hypothesis, however, requires further investigation.

3. Are PSSM2 and MFM the Same Disease in Warmbloods?
Type 1 polysaccharide storage myopathy (PSSM1) refers to horses with excessive muscle glycogen concentrations, abnormal amylase-resistant polysaccharide in muscle histology, and a mutation in the glycogen synthase 1 gene (GYS1).5,6 The term PSSM2 is applied to horses with abnormal appearing glycogen aggregates in muscle biopsies that do not possess the GYS1 mutation.7 PSSM2 in essence represents a
Histologic description of glycogen aggregates in horses with exercise intolerance rather than a specific disease. To determine if PSSM2, like PSSM1, is associated with excessive glycogen storage, glycogen concentrations recently have been measured in a variety of breeds diagnosed with PSSM2. Mean glycogen concentrations in Quarter Horses diagnosed with PSSM2 (n = 67, 130 ± 60 mmol/kg) were found to be lower than those of PSSM1 Quarter Horses (n = 20, 175 ± 39 mmol/kg) and significantly higher than control Quarter Horses (n = 185, 80 ± 27 mmol/kg).8 Glycogen concentrations in Warmblood and Arabian horses with PSSM2, however, were found to be similar to those in control horses.8–10 Thus, the term PSSM2 is a histologic descriptor that may encompass different diseases in different breeds. Further research into PSSM2 found that some PSSM2 Warmbloods have aggregates of desmin, myofibrillar disarray, and Z disc streaming resembling a condition described in humans as MFM.2 Because clinical signs of exercise intolerance are very similar between Warmblood horses with PSSM2 and MFM, and horses with MFM can have glycogen aggregates resembling PSSM2, it is suspected that PSSM2 in Warmbloods potentially represents an earlier stage of MFM.1,2 However, more research is required to state this definitively. The aggregates of amylase-sensitive glycogen used to diagnose PSSM2 in Warmbloods could occur because of pooling of glycogen in breaks within the myofibrils seen in electron microscopy of MFM horses.2

4. Clinical Signs of PSSM2 and MFM

Warmblood horses diagnosed with PSSM2 and MFM have an insidious onset of exercise intolerance notable by 6 to 8 years of age characterized by a lack of stamina, unwillingness to go forward, inability to collect, abnormal canter transitions, and inability to sustain a normal canter.1,4 Stiffness, muscle pain, and rarely an episode of exertional rhabdomyolysis are reported with PSSM2 and MFM as well as a hindlimb lameness that remains unresolved after a thorough orthopedic assessment.1 Serum CK and AST activities are usually within normal limits unless samples are taken in conjunction with rare episodes of exertional rhabdomyolysis.

5. Diagnosis

A physical examination that includes a full lameness and neurologic examination is strongly recommended prior to pursuing a diagnosis of PSSM2 or MFM (Fig. 1).11 This is because other causes of poor performance are much more common than PSSM2 and MFM and because the sensitivity and specificity of muscle

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**Fig. 1.** A decision tree for the diagnosis and management of PSSM2 and MFM in Warmblood horses with clinical signs of exercise intolerance with normal to mildly increased serum creatine kinase (CK) activity. *These are suggested recommendations based on current research in normal horses and anecdotal reports from the field and have not yet been fully tested on horses with PSSM2/MFM.
biopsy histopathology leave room for false-positive and false-negative diagnoses. Tack and saddle fit should be inspected, and the horse should be observed under-saddle to determine if there are potential rider, training, or behavioral issues that are primary contributors to poor performance. Other potential diagnostics include endoscopy to rule out gastric ulcers, diagnostic joint/nerve blocks for associated lameness, radiography, ultrasonography, and scintigraphy. In the absence of other causes of reluctance to go forward and engage the hind quarters, a treatment trial of management changes or a muscle biopsy are reasonable next steps (Fig. 1). One potential complication of a treatment trial is that, unlike PSSM1, aggregates of polysaccharide and desmin appear to decrease with the prescribed management, leading to a potential false-negative diagnosis if biopsies are pursued after initiating appropriate management.

Muscle Biopsy

Either the gluteal muscle or the semimembranosus/tendinosis muscles are sampled to diagnose PSSM2 and MFM. A sample that provides a 2-cm square of tissue in cross-section is recommended in order to provide enough muscle fibers to identify the limited number of type 2A fibers that contain glycogen or desmin aggregates. It is recommended that both fresh and formalin-fixed samples be evaluated with a minimum of hematoxylin-eosin, periodic acid Schiff’s (PAS), amylase-PAS, and desmin stains. Handling of muscle tissue is extremely important. It is recommended that 1) the sample be handled with forceps at only one end, 2) the sample is not squeezed with forceps, 3) the samples be placed in slightly damp gauze in a hard container (urine cup) packed with enough gauze to prevent movement during shipping, 4) the samples be kept chilled on ice packs until shipping, and 5) the samples should be shipped overnight to arrive in the laboratory within 24 hours. Squeezing of muscle creates abnormal glycogen (false positives) and delays in chilling or shipping result in glycogen and desmin depletion (false negatives) as well as artifactual cracks within muscle cells that stain positively with PAS (false positives). Prior to placing samples in formalin, best practices suggest the sample be allowed to sit in air for 3 to 5 minutes to fully contract. The diagnostic criteria for PSSM2 include the presence of aggregates of cytoplasmic glycogen in PAS stains and a negative GYS1 test. The aggregates of glycogen are usually amylase sensitive (similar to normal glycogen), in contrast to amylase-resistant aggregates seen in PSSM1. Because amylase-sensitive glycogen is a normal feature of muscle histology, the interpretation of the appearance of glycogen is a subjective diagnosis. The diagnostic criterion for MFM is the presence of aggregates of cytoplasmic desmin that are at least one-half the width of myonuclei in size and found in mature (not regenerating) muscle fibers. It is the author’s clinical impression that these desmin aggregates are not concurrent with the initial clinical signs of exercise intolerance, and thus it is possible to have a false-negative diagnosis of MFM in early stages.

Genetic Testing

Mutations in 16 genes are known to cause MFM in humans. A comparison of the sequences of these 16 genes (including MYOT and FLNC) between horses diagnosed with MFM by muscle biopsy (n = 8) and healthy Warmblood horses (N = 8) did not identify any mutations associated with MFM in the horses studied. A commercial company offers genetic testing for variants (single nucleotide polymorphisms) in the genes myotilin MYOT (P2), filamin C FLNC (P3), and myozin MYOZ3 (P4) and purports that they are causative of PSSM2 and MFM (http://equiseq.com/buy_pssm2). The prevalence of these variants in Warmblood horses with a muscle biopsy diagnosis of PSSM2 (n = 54) and MFM (n = 68) and no histopathology (n = 54) as well as in 205 horses in publicly available genetic databases has recently been determined. No statistical association was found between any of the P variants, or combinations of P variants, and the presence of either PSSM2 or MFM considered as one diagnosis, or when PSSM2 or MFM were considered separately. Only 38% of Warmbloods with MFM possessed a P variant and 29% of Warmbloods appear to have at least one P variant regardless of whether they have a muscle disease or not. Thus, there does not appear to be evidence to support that the P variants are themselves causative or diagnostic of a muscle disease in the horse.

6. Management

Management of PSSM2 and MFM requires both altering diet and exercise regimes and is based largely on retrospective studies or clinical impressions rather than controlled diet trials. More information needs to be collected from owners of horses who use the management approaches described below to determine their efficacy.

Diet

A low nonstructural carbohydrate (NSC), high-fat diet was previously recommended for PSSM2 based on the assumption that PSSM2 was a glycogen storage disorder similar to PSSM1. In Warmbloods, however, it is now clear there is not excessive glycogen storage in skeletal muscle based on biochemical analysis of glycogen concentrations. In addition, a survey of owners found that 80% of PSSM2 Warmbloods improved on the low NSC, high-fat diet; however, 53% of Warmbloods did not advance as expected in training, with reluctance to go forward and lack of collection persisting in approximately one-third of horses. A revised diet was developed based on transcriptomic and proteomic analyses of muscle from horses with MFM and based on controlled diet trials in healthy Thoroughbreds. The revised diet has not been evaluated in controlled diet trials of MFM horses,
however. The diet was formulated based on indicators that aberrations in cysteine-based antioxidants, protein degradation, oxidative stress, and the mitochondrial respiratory chain are key signatures of MFM.4,15

**MFM Diet**

A nutritionally balanced diet with appropriate caloric intake and adequate protein, vitamins, and minerals is essential, and the formulation may require the assistance of a nutritionist. The diet provided below is summarized from Pagan and Valberg.17 Designing a diet requires determination of 1) daily nutrient requirements, 2) the type and amount of forage, 3) the type of concentrate to supply additional energy, and 4) the need for supplements to supply additionally required nutrients not provided by forage and concentrate.

**Forage.** MFM horses will typically consume 1.5 to 2% of body weight per day of hay. Good quality grass or grass-legume mixed hays (55-65% neutral detergent fiber [NDF], 10-12% crude protein [CP], and 10-17% NSC) are preferable.17

**Concentrates.** Recommended concentrates for PSSM2 and MFM Warmbloods are those with moderate levels of NSC (20-30%) and fat (4-8%) and higher levels of protein (12-14% CP) containing high-quality amino acids. Amino acids such as lysine, methionine, and threonine are important for muscle repair and generation of cysteine-based antioxidants. Leucine stimulates protein synthesis in the muscle postexercise,18 which would be beneficial to MFM horses.

**Supplements**

**Amino acids.** Whey-based proteins or supplements containing N-acetyl cysteine are recommended for horses with MFM because they are rich in cysteine. Cysteine is a key component of many antioxidants. MFM horses may have an increased cysteine requirement based on alterations in genes involved in conversion of methionine to cysteine found in Arabian horses with MFM following exercise.15 A supplement designed for MFM horses that contains N-acetyl cysteine and branched chain amino acids has recently become commercially available.

**Antioxidants.** Coenzyme Q10 (CoQ10) is a key component of the first step in the mitochondrial electron transport chain, and Warmblood horses with MFM have a decreased expression of proteins involved in the first two steps in electron transport.4 When fed to healthy horses, N-acetyl cysteine and coenzyme Q104 were found to increase mitochondrial proteins.16 CoQ10 is now being trialed as a supplement for MFM horses.

**Exercise**

**Exercise Schedule**

An equally important part of the management of PSSM2 and MFM is an exercise regime. PSSM2 horses were previously recommended to exercise every day; however, a recent survey of owners suggests that PSSM2 and MFM Warmbloods require days off training during the week to recover.10,18 The number of days per week to ride varies with each horse. Many owners of PSSM2 and MFM horses have found that 3 days of work followed by 2 days off works best.a For some horses, exercise more frequently than 3 days in a row or rest for more than 2 days in a row results in a stiff horse in the subsequent ride.

**Warm-Up**

A warm-up on the lunge-line in both directions in a long, low frame for 10 to 15 minutes is reported by owners to improve ridden work. During the warm-up, engagement of the hindquarters is not the aim, but rather, muscle relaxation, releasing at the base of the neck, and rounding and lifting of the back are desirable. Aids that help create a long, low frame on the lunge-line include Vienna reins, Pessoa lunging system®e, or neck-stretchers. Initially, exercise is performed at a walk and trot. Strides at a canter can gradually be added looking for the horse to release at the base of the neck. When transitioning down to trot, the horse should begin to move forward with more impulsion.

**Riding**

A long and low warm-up at the trot and canter with adequate stretching is recommended before any collected work. Rest periods that allow the horse to relax and stretch their muscles between 2- to 5-minute periods of collection under saddle may be of benefit. Total ride times in horses with MFM appear to be most productive if kept between 30 and 45 minutes, with horses just starting out having rides between 15 and 20 minutes (including walking).a

**Strengthening Exercises**

Hill work, cavaletti, and small jumps can be used to build strength. Aids5 and core strengthening exercises such as those found in Activating Your Horse’s Core could also be beneficial.19

**Turnout**

As much daily turnout as possible in an area where the horse is encouraged to move about is recommended.18

**Professional Help**

Some Warmblood horses have strong, reactive temperaments and can develop behavioral issues such as refusing to go forward under saddle and bucking even after painful conditions have been resolved. In these cases, groundwork that reestablishes leadership and the help of a professional trainer experienced with difficult horses may be required to safely ensure “forward” means forward without protestation.

**Acknowledgments**

This research was supported by the endowment of the Mary Anne McPhail Dressage Chair in Equine
Sports Medicine. The text was not reviewed by the endowment sponsor prior to submission.

Declaration of Ethics

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

Conflict of Interest

The Author has research collaborations with Kentucky Equine Research, Versailles, KY, and receives financial support for research projects from the company. The Author receives royalties from the PSSM1 genetic test. The Author runs the Neuromuscular Diagnostic Laboratory at Michigan State University. She does not receive any personal remuneration from the Diagnostic Laboratory.

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How to Use Serum Chemistries in the Evaluation of Poor Performance Thoroughbred and Standardbred Racehorses

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1. Introduction

Poor performance in racehorses is a common complaint that equine practitioners are often asked to evaluate. The poor performance might be a documented decline in performance, in which case clinical problems are plausible, or the poor performance might instead be a result of the horse never achieving owner and trainer expectations wherein searches for medical causes may be elusive. The evaluation of a poorly performing racehorse should begin with a complete history, including race records if available, and a thorough clinical examination. Endoscopic (airways and stomach) and ultrasound (thorax) examinations are often required. If the cause of the poor performance cannot be determined at this point, laboratory testing may be the next diagnostic step, and both complete blood counts (CBCs) and serum chemistry panels are recommended. The white blood cell count may provide information suggestive of an inflammatory disease, and the red blood cell evaluation provides an assessment of red cell volume and hemoglobin content, although resting equine red cell values have a wide normal range and do not reflect the values found during racing. Serum or plasma chemistry measurements should be a routine part of the poor performance evaluation in racehorses unless the history and clinical exam have already revealed an answer for the poor performance. The chemistry analytes that most often provide important diagnostic information in the evaluation of the poor performance horse are aspartate aminotransferase (AST), creatine kinase (CK), and γ-glutamyl transferase (GGT). Measurements of serum or plasma proteins, bilirubin, total carbon dioxide (CO₂), and creatinine concentrations, in addition to glutamate dehydrogenase (GLDH) and sorbitol dehydrogenase (SDH) activity, may also provide important diagnostic information. The diagnostic value of each analyte is discussed below.

2. Aspartate Aminotransferase and Creatine Kinase

Abnormally high serum or plasma AST activity in racehorses is most often a result of muscle disease, although liver disease can also cause increased AST activity. If CK activity is also increased in the sample, this strongly suggests that the AST increase is a result of muscle injury. However, the absence of elevated serum CK activity in a horse with a high serum AST activity does not rule out muscle disease as a cause for the high AST because the approximate half-lives of the 2 enzymes are very different (AST half-life is approximately 7-8 days, whereas CK is approximately 2 hours).¹ For example, if a horse worked on Monday and a transient muscle injury occurred, the initial increase in CK activity might...
be in a normal reference range by Wednesday, whereas AST activity would likely remain elevated for several days. Postexercise increases in serum CK and/or AST activity are most common in horses with recurrent exertional rhabdomyolysis (RER), a disease that affects approximately 5% to 7% of Standardbred and Thoroughbred racehorses. Female horses are at a significantly greater risk for RER than males. Although RER horses can have persistently high serum AST activity, likely due to continuing subclinical episodes of rhabdomyolysis, this finding may not be predictive of performance. A combination of clinical signs (stiffness, lameness, and poor recovery following exercise) suggestive of a muscle disorder along with markedly increased serum or plasma CK activity 4 to 6 hours following a race or moderate elevations in AST activity even several days later support RER as a cause of poor performance. The timing of blood collection following exercise for the measurement of serum muscle enzyme activity is important, as serum CK activity generally peaks at 4 to 6 hours and AST activity peaks at 12 to 24 hours following transient muscle injury. Submaximal exercise tests that cause a 3-fold or greater increase in CK activity 4 hours after exercise can help confirm RER, but this may not be predictive of clinical expression of RER at racing speeds. Regardless, elevations in CK and AST activity following exercise warrant consideration of changes in both diet and training/racing protocols in hopes of reducing further muscle injury. Mild increases in muscle-derived enzymes in the serum may also be caused by a lack of fitness or overtraining.

3. Gamma-Glutamyl Transferase

Another common serum biochemical abnormality detected in racehorses (both Thoroughbreds and Standardbreds) is an increase in serum or plasma GGT activity above normal reference ranges, sometimes referred to by trainers and veterinarians as the “GGT syndrome”. Although the serum GGT activity in horses with this syndrome is most commonly 2 to 5 times above the maximal reference range, other hepatic-specific enzymes such as SDH and GLDH and the nonspecific hepatic enzymes AST or lactate dehydrogenase (LDH) are either within the normal reference range or only mildly increased. The 2- to 5-fold increase in GGT activity often persists as long as the horse continues in full work. Studies have shown that mild-to-moderate increases in GGT activity are associated with racing frequency and cumulative training load and that values return to normal when horses are removed from work. This has led to suggestions that the high GGT is caused by overtraining or maladaptation to training; the author prefers the latter term. The prevalence of the syndrome is not well documented and varies with the trainer. A recent prospective study involving three Thoroughbred stables with samples collected monthly for 5 months found the prevalence of horses that at some time point had a GGT value of >50 IU/L was nearly 15%. In that study, GGT values were as high as 127 IU/L. When high GGT activity was initially detected, the value of GGT was generally of similar or higher activity in the next monthly sample. In the author’s experience, Standardbred stables seem to have a higher prevalence of high GGTs than Thoroughbred stables. Although the increased GGT activity in affected horses is certainly abnormal, its association with poor racing performance is not well documented, and some horses with mild-to-moderate-increases in GGT activity will win races. It has been suggested that GGT levels approaching 100 IU/L or greater are likely to be associated with a decline in performance. The association between high serum GGT activity and poor performance is likely increased if the horse has recently experienced any of the following: decreased weight in spite of continued normal appetite, an increase in resting heart rate, and increased time needed to cool out following training. The etiology of the GGT syndrome is unproven, but a recent case-controlled study found evidence that supported both oxidative stress and cholestasis as being involved in the syndrome. There are no controlled treatment studies to allow specific treatment recommendations, but based on a recent case-controlled study, treatments to decrease oxidative stress and improve bile flow might be reasonable. Although there is no statistical association with the equine hepatitis viruses (equine parvovirus-hepatitis [EqPV-H] and equine hepacivirus [EqHV]) and the GGT syndrome, infection with either of these viruses should be considered as a cause for poor performance when multiple hepatic enzymes (GGT, SDH, GLDH, and AST), and especially bile acids, are increased in serum or plasma. With either virus infection, hepatitis may occur at the time of both peak viremia and increasing antibody production, suggesting that the liver disease is caused by immune attempts to clear the viruses from the liver. Chronic infection with either virus can occur, but chronic infection is unlikely to cause disease or be associated with poor performance. Paired serum qPCR viral testing 4 weeks apart will help sort out recent infection and chronic persistent infection. A decline in viremia over 4 weeks suggests that the infection is more acute and likely causative of the increased liver enzymes. Horses with hepatitis following recent infection with either virus could have a decline in performance and should not be heavily worked until the serum GLDH activity has returned to normal and the GGT is normal or approaching the normal range. If hepatic disease is being monitored by sending overnight samples to laboratories, monitoring of GLDH activity is preferred over SDH, as the latter is a less stable enzyme. When serum samples from healthy racehorses are analyzed within a few hours of racing, it is common to find mild elevations in SDH activity, and the clinical significance of this is unknown. LDH activity increases may occur with liver, skeletal muscle, or cardiac muscle.
damage. Measurement of LDH activity is included in several commercial chemistry profiles, but the low tissue specificity and its short half-life limit its diagnostic value.

4. Bilirubin
Abnormally high concentrations of serum or plasma bilirubin in horses may be caused by hemolysis, decreased appetite, or liver dysfunction. It should also be noted that a small percentage of healthy horses will have indirect and total bilirubin values well above the normal reference range without any of the above known causes. The hyperbilirubinemia in those horses may be as high as 11 mg/dl and is thought to be due to a familiar deficiency of hepatic uridine diphosphate-glucuronyl transferase. A few horses with the GGT syndrome will have mildly elevated bilirubin, but this is an inconsistent finding.

5. Serum Proteins
High serum total proteins and globulins with concur- rent mild decreases in albumin concentration in horses are strongly suggestive of a subacute-to-chronic inflammatory disease (e.g., lung abscess); localizing the site of inflammation can usually be accomplished by clinical exam and ancillary testing such as ultrasound exam. Decreases in albumin and total protein generally indicate an intestinal disorder. When serum protein abnormalities are found on the chemistry panel, clinical signs such as weight loss and decreased appetite are often noted along with the decline in rac- ing performance. Although not part of a routine bio- chemistry panel, serum amyloid A (SAA) is a commonly used stall-side test used to detect acute-to- subacute inflammation in horses. Significant in- creases in SAA were not observed in healthy Thoroughbreds or Standardbreds following racing; if SAA is increased in a horse with poor performance, then an acute or subacute inflammatory disease should be suspected. SAA values might be normal if the disease process is chronic, and in one study, measurement of SAA was not helpful in the diagnosis of mild asthma in racehorses.

6. Bicarbonate, Total CO₂ and Lactate
Bicarbonate values on a chemistry panel provide an assessment of metabolic acid-base status. Some laboratories report total CO₂ (TCO₂) concentration rather than bicarbonate concentration. TCO₂ concentrations are generally 1 to 2 mEq/L higher than bicarbonate. In racehorses, pre-race TCO₂ is measured to monitor bicarbonate ("milkshakes") administration, and pre-race values greater than 37 mEq/L (or mmol/l) suggest recent bicarbonate administration. Drug administration or medical disorders that decrease serum chloride or albumin concentrations and increase strong ion differences may also raise bicarbonate values. Furosemide administration can cause a 1- to 1.5-mEq/L increase in bicarbonate. Excessive sweating in horses causing a loss of chloride in excess of sodium can also cause an increase in bicarbonate and TCO₂. Increases in bicarbonate could play a physiologic role in the development of synchronous diaphrag- matic flutter ("thumps"). Bicarbonate decreases most commonly occur secondary to increases in lactate production. When compared to well-performing race- horses, fit racehorses with asthma, pulmonary hemor- rhage, and poor performance had lower bicarbonate and higher blood lactate concentrations 5 and 15 minutes following a standardized treadmill study. L-lactate values were approximately 2-fold greater in the poor performing horses, with mean values at 5 minutes postexercise of 10.5 to 12.2 mmol/L for the poor performance horses. Higher than expected lactate values are likely caused by cardiopul- monary disorders and increased anaerobic gly- colysis. Lactate testing using whole blood is pre- ferred over serum or plasma sample submission to a laboratory, and point-of-care tests are readily available for on-site measurements. Unfortunately, there can be considerable overlap of post-race lactate values between poor- and well-performing racehorses, which diminishes its practical diagnostic value in evaluating the poor performance horse. Lactate testing might be better suited for controlled treadmill performance evaluations. Any organ system failure would likely cause poor performance in a racehorse, but in those cases, the horses would likely demonstrate weight loss and decreased appetite. On rare occasion, a poor performance horse will have chronic renal failure and a serum creatinine in the 2.5- to 3.5-mg/dl range. Horses with creatinine values above 3.5 mg/dl usually have weight loss and decreased appetite.

7. Sampling Conditions
The following several factors must always be consid- ered when interpreting serum chemistry results: the timing of the sample, any unusual delay that may have occurred in submission or testing of the sample, and adverse storage conditions prior to testing (the so-called "dashboard" effect). For example, if blood for serum chemistry analysis is collected immediately af- ter a race, one would normally expect serum sodium and total proteins to be slightly increased and bicar- bonate decreased due to loss of plasma water and increase in blood lactate, respectively. Low plasma glucose and high potassium concentrations are two common abnormalities that occur with improper or prolonged sample storage prior to testing. Also, consider that, on rare occasions, laboratory results could be erroneous due to laboratory error, so if the results do not fit the patient, be sure to retest.

8. Summary
Determining the cause of poor performance in race- horses can be a diagnostic challenge. History and a complete clinical examination should always be the initial diagnostic steps. Serum chemistry analysis
should be part of the diagnostic workup when history and clinical examination do not reveal a cause. The most common abnormalities noted on chemistry analysis of poor performance horses are increased activity of muscle enzymes (CK and AST) and increased GGT activity. The time of sample collection following exercise should be considered when evaluating CK and AST values, whereas samples for GGT measurements can be taken at any time.

Acknowledgments

Funding for the investigation of the pathogenesis of high gamma-glutamyl transferase activity in the serum of Thoroughbred racehorses was kindly provided by the Grayson Jockey Club Research Foundation.

Declaration of Ethics

The Author has adhered to the principles of Veterinary Medical Ethics of the AVMA.

Conflict of Interest

Dr. Divers is Emeritus Professor at Cornell University where polymerase chain reaction (PCR) testing for EqHV-H and EqPV can be performed for a fee in the New York State Diagnostic Laboratory.

References

How to Assess the Suitability of Rider Size—Height, Morphology, and Weight—for Optimal Horse Welfare and Performance: A Review

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1. Introduction

As the human population gets larger, there has been growing debate about relative rider-horse sizes and the effect that a large rider may have on equine welfare and performance. The issue of rider weight was highlighted as a priority for research at the 2nd International Saddle Research Trust Workshop.

The following represents a synopsis of recent work that has investigated the potential effects of rider size on equine welfare and performance in horses ridden in English saddles. It is not exhaustive but provides some evidence-based information on which professional advice can be given concerning saddle fit for horses and riders and the size of a rider relative to a horse. In this context, the term rider size is more pertinent than rider weight because the suitability of a rider for a horse is influenced not only by their weight but also by their height and the morphology of the rider’s body. Moreover, rider size is potentially a less sensitive and emotive term, particularly when body mass index of the rider is not in itself a problem. Studies investigating rider weight have been performed, but few have satisfactorily addressed this issue in a typical riding situation. They have, for example, utilized lead weights to alter the total load carried by horses—which does not address the potential differences in physique and balance in riders of differing weights or their position in the saddle—and treadmill exercise, which does not necessarily equate with overground exercise and does not include turns and circles. In addition, several of the previous studies have utilized very high total load:horse bodyweight ratios, or been restricted to walk and trot, and therefore this does not permit determination of whether changes in equine performance may occur at lower rider:horse bodyweight ratios, during all gaits and activities, or permit extrapolation to real-life situations. Therefore, although it is widely recognized that excessive rider size has welfare implications for horses, there has been a lack of reliable scientific evidence on which to base guidelines. This is a multifactorial issue with many interrelated aspects, including the horse’s age; its fitness and muscle development; the length of the thoracolumbar region; the presence or absence of lameness or other musculoskeletal problems; the type, speed, and duration of work; the rider’s skill, fitness, balance, and

NOTES
coordination; the ability of the rider to sit straight; the fit of the saddle to both the horse and rider; and the terrain over which the horse is ridden.5

2. The Effect of Rider Size and Ridden Horse Gait and Behavior

Two recent studies in more typical riding situations reached somewhat conflicting results on what proportion of a horse’s bodyweight a horse can carry without potential welfare implications.13,14 Study A (Dyson et al.), in which four experienced riders, of variable size but of similar ability, rode six horses in random order in a cross-over design, demonstrated that there were adverse effects on both gait and behavior for the two larger riders, ≥ 17% of the horses’ bodyweight.13 Study B (Christensen et al.) showed no such effects when 20 horses were ridden by their usual riders, without and with additional lead weights strapped to the riders’ torsos, up to 23% of the horses’ bodyweight.14 This is likely to reflect the differences in methodology employed: Study B added dead weight, which is not the same as mimicking the real-world scenario of Study A,13 when the distribution of the weight of a large rider (tall and/or heavy) was more difficult to control. Moreover, the duration of riding was substantially different, 5 minutes in Study B versus 30 minutes in Study A.13 In addition, in Study B, there was a wide spectrum of baseline rider: Horse bodyweight percentages (including tack, 15.3% ± 0.4; range 12–19%) and the average maximum with added lead weights was 18.5% ± 0.5 (range 15–23%).14 Study A compared the same six horses ridden by four riders (without tack) of 10–12% (L, light), > 12 ≤ 15% (M, moderate), > 15 ≤ 18% (H, heavy), and > 20% (VH, very heavy) of each of the horse’s bodyweight.13 In both studies A13 and B,14 there was no significant effect of rider size on heart rate variables or salivary cortisol concentrations. However, spontaneous blink rate, defined as full occlusions of the left eye by the eyelid, recorded for 15 minutes, was significantly increased postexercise for rider H (p = 0.03; t = 0, df = 5, paired t test; Fig. 1) in Study A.15 Spontaneous blink rate is an indicator of dopamine function, and an increase reflects a stress response. A Ridden Horse Pain Ethogram (RHpE) has been developed, comprising 24 behaviors, the majority of which are at least 10 times more likely to be seen in a lame horse than a nonlame horse.16 An RHpE score of ≥ 8 of the 24 behaviors is likely to reflect the presence of musculoskeletal pain,16–20 although some lame horses score < 8. Reduction in the RHpE scores after abolition of lameness using diagnostic anesthesia proves a causal relationship between these behaviors and musculoskeletal pain.17 The RHpE scores can also be influenced by rider weight distribution and saddle fit for the horse (specifically tight tree points of the saddle).21 In Study A,13 there was a significant difference in RHpE scores according to riders (Fig. 2; analysis of variance [ANOVA], Bonferroni: M to H, p < 0.01; L and M to VH, p < 0.001; H to VH, p < 0.05) and a linear positive correlation between rider weight and the RHpE score (R = 0.4, p < 0.01, Spearman; Fig. 3).22 The number of behavioral markers reflecting head position (ANOVA, p < 0.001, F = 17.27; Fig. 4) and facial expression (ANOVA, p < 0.001, F = 18.72; Fig. 5) significantly increased with the H and VH riders.
whereas body markers (head and tail movement) and gait markers had nonsignificant increases. However, the total RHpE scores were less than 8 for the majority of tests. There were predefined criteria for test abandonment: 1. Development of lameness grade ≥ 3/8 in one limb or grade ≥ 2/8 in ≥ 2 limbs; 2. Exacerbation of pre-existing grade-1 lameness by ≥ 2 grades (≥ 8); and 3. An RHpE score ≥ 10/24.13 One test for the heavy rider was halted prematurely because of the display of a total of 10 behaviors of the RHpE by the conclusion of the first canter and all the other tests of the heavy rider, and all tests for the very heavy rider were abandoned prematurely because of the development of transient forelimb or hindlimb lameness. In Study B, a narrower range of behaviors was assessed, which were less specifically defined.14 No effect of rider weight on behavior was seen, but the large variation in the frequency of so-called “conflict behaviors” (e.g., mouth open, tail swishing, head tossing) among the horses before addition of extra weight and the high frequency of tail swishing may have concealed any effect of rider weight. In a third study, there were effects of additional weight on gait, although overt lameness was not observed.11 Eight Icelandic horses carried 20%, 25%, 30%, and 35% of their own bodyweight (a single professional rider plus lead) at the tölt on a 321-m oval track. Kinematics were measured using a high-speed camera in an incremental exercise test (5 × 642 m). Although there were no measurable changes in regularity of rhythm or symmetry, increasing weight was associated with decreased stride length, increased stride frequency, and increased duty factor (the proportion of the stride time during which a limb is in the stance phase) in all limbs, factors that were not assessed in the previous studies. Force is the product of mass and acceleration, so any effect of added weight is likely to be greater at faster than at slower speeds. These observations emphasize the need to consider the expected stride and gait characteristics for the type of horse that is being ridden.

3. Rider Size and Weight Distribution

Rider size, both height (also influenced by limb length versus trunk length) and seat size, and the design, fit, and balance of a saddle determine how a rider sits in a saddle. For optimal weight and force distribution, the rider should sit in the middle of a saddle to enable them to be in balance with the horse and as close as
possible to the horse’s center of gravity. In a pilot study, the seat of the rider was too large for the saddle in 41% of 34 horse-rider combinations. In a more recent study, the seat of the rider was too large for the saddle in 40% of 193 horse-rider combinations, and 51% of riders sat on the back rather than in the middle of the saddle. This may have been because the saddle was too small relative either to the rider’s leg length or to the size of their seat. Alternatively, it could have been the result of the saddle being the wrong shape for the rider, the stirrup bars being in an inappropriate position, or the rider being in an inherently poor position. In Study A, the heavy and very heavy riders sat on the caudal third of the saddle (Figs. 6, 7). This was in part related to the tall height and long leg length of the heavy rider. It is notable that the usual rider of Horse 2 was of similar weight (91 kg) to the heavy rider (92 kg) but was considerably shorter. When ridden by the usual rider, who sat in the middle of the saddle, the horse performed completely normally; when ridden by the tall rider (H), who sat on the back of the same saddle, the horse showed transient lameness and the RHpE score increased. It was objectively demonstrated in Study A that the larger riders (H and VH) who sat on the back of the saddle increased forces transmitted through the caudal half of the saddle. Pressures were significantly higher under the caudal aspect of the saddle compared with cranially for rider VH in walk ($p < 0.05$, ANOVA, Bonferroni). At rising, trot pressures were higher cranially for riders L, M, and H ($p < 0.05$, ANOVA, Bonferroni) but were similar cranially and caudally for rider VH. The highest maximum peak pressure was recorded for rider VH in canter. For the longitudinal (craniocaudal) center of pressure (COP), rider VH had a median COP significantly more toward the caudal aspect of the saddle compared with all other riders ($p < 0.01$, ANOVA). Alteration in the craniocaudal distribution of pressure has the potential to adversely affect thoracolumbar movement and hindlimb gait. In a small study that compared a standard-fitting saddle to one with panels that were 10 cm shorter, there was increased pressure under the middle and caudal thirds of the saddle panels and caudal displacement of the COP with the shorter saddle. This was associated with reduced range of motion in the caudal thoracic and lumbar regions and reduced hindlimb protraction. These effects could be potentially compounded if other saddle-fitting problems were also present. In a study of 191 horse and rider combinations, 49% of riders were observed to be out of balance; 19% of saddle seats tipped backwards, and many of the saddles moved excessively during ridden exercise (dorsosventral 47%, side to side 48%, saddle slip 45%). In a related study comprising a subset of 148 of the horses, there was a significant positive association between RHpE scores for horses ridden by riders who sat on the caudal third of the saddle compared with those in the middle of the saddle.

4. Rider Size, Epaxial Muscle Tonicity and Pain, and Thoracic Dimensions

In Study A, the thoracolumbar region was palpated systematically before and after each ridden test, and the presence and location of epaxial muscle hypertonicity or pain were recorded. Increased muscle tension was defined as muscle stiffness with little or no
development and function and may result in atrophy of longissimus dorsi in the thoracolumbar region. These factors could be compounded if the rider sat crookedly, was not in synchrony with the horse’s movement, or if the saddle did not fit the horse ideally, which is a common finding. Ill-fitting saddles were identified in 78% of 193 sports and leisure horses in the United Kingdom, with tight tree points (67%) being the most common problem. In a previous United Kingdom study, ill-fitting saddles were identified in 43% of 205 sports and leisure horses. In Switzerland, 74% of 237 sports and leisure horses had ill-fitting saddles.

5. The Fit of the Saddle to Both the Rider and the Horse

It is generally accepted that the tree of the saddle should not extend beyond the 18th thoracic vertebra (T18), or last rib, although the ability of saddle fitters to identify these landmarks reliably was limited, and agreement among saddle fitters about appropriateness of saddle length was poor. The scientific basis for the principle of the tree not extending beyond T18 is limited, and the Society of Master Saddlers has advised that the panels of the saddle can extend to the first lumbar vertebra. However, if the rider’s weight is centered caudal to the 13th thoracic vertebra, the rider will be positioned behind the horse’s center of gravity, which potentially influences the rider’s ability to be in balance with the horse. There is a significant body of evidence that demonstrates that if a rider is sitting on the back of the saddle, rather than in the middle, this is potentially detrimental to the horse. With some riders, it may be possible to alter the rider’s position by using a saddle that is better suited to their size and that also fits the horse. The seat of the saddle and the panels can be lengthened without increasing the length of the tree. The position of the bars of the saddle, from which the stirrup leathers and stirrups are suspended, can be altered. The shape, position, and size of both the saddle flaps and the knee blocks can be adjusted. However, with some horse-rider combinations, it may be impossible to have a saddle that fits both the horse and the rider optimally (Figs. 9A, B).

6. Overall Considerations: How to Assess Rider Suitability for a Horse

There is sufficient evidence to indicate that a rider who is too large for a horse has the potential to have an adverse effect on the horse’s gait, performance, muscle development, and long-term musculoskeletal health. However, rider size cannot be considered in isolation. There are many other factors to consider—for example, the skill, core strength, balance, and fitness of the rider; the physical well-being of the horse; its fitness and musculoskeletal strength, and its coordination; the duration and intensity of work; and the terrain and footing. Although it has been suggested...
that fit, appropriately conditioned Arab endurance horses are able to carry 20–30% of their bodyweight for 160 km, there is currently insufficient knowledge to give a categorical upper rider:horse weight ratio, beyond which riding is unacceptable. Increasing horse body weight to reduce the ratio is not an appropriate solution. There are, however, some broad guidelines to consider with respect to rider size based on the scientific data presented. The horse’s thoracolumbar length must be able to accommodate a saddle that allows the rider to sit in the center of the saddle, with their shoulder, “hip,” and heel in vertical alignment, and to ride in balance with the horse. An equine veterinarian should be able to evaluate thoracolumbosacral epaxial muscle development, tonicity, and pain, both before and after ridden exercise; to assess static and dynamic saddle fit for the horse and rider; to understand the basic concepts of correct rider position, straightness, and balance; to observe and understand the behavior of horses during tacking-up, mounting, and ridden exercise; to pay particular attention to head and neck position and facial expressions; and to recognize alterations in gait in trot and canter, including shortening of the stride. Comparison of the horse’s behavior and the quality of the movement (step length, limb flight, suspension, rhythm, speed ± lameness) in trot and canter on the lunge without the rider and when ridden (Fig. 9, A and B) may be valuable. During ridden exercise, the horse should be viewed from the side, behind, and in front to assess not only the horse’s movement but also any side-to-side oscillation of the saddle, dorsi-ventral movement of the back of the saddle (“bouncing”), or saddle slip to one side and whether the rider is crooked. It may be helpful to acquire video recordings so that the observations can be discussed with the rider, together with the implications of those findings with respect to the long-term musculoskeletal health of the horse. When discussing the biomechanical implications of an oversized rider, it should be borne in mind that at walk, the maximum forces transmitted through the panels of the saddle are continuous, whereas at trot they are biphasic. In the three-beat canter, not only are peak forces higher because force is the product of mass and acceleration, but they are also asymmetrical, reflecting the movement of the thoracolumbar region. If the horse is used for jumping, it should be pointed out that the forces under the front of the saddle at landing are considerably greater than those experienced at walk, trot, and canter. Use of a force mat to quantify the distribution, magnitude, and symmetry of forces under the saddle provides some limited objective information. There is a positive association between rider weight and magnitude of force, although it is not a linear relationship. However, it must be borne in mind that what looks wrong probably is wrong (Fig. 9, A and B), and the potential consequences need careful discussion with the rider. Knowledge of the scientific evidence supporting the potentially adverse effects of a rider who is too big for a horse provides a foundation on which to base discussions with an owner. Involvement of an experienced human physical therapist, as an independent expert who is able to assess rider static and dynamic symmetry, balance, and coordination, is sometimes also helpful.

7. Conclusions
Veterinarians, riders, and trainers should be more aware of the potentially deleterious effects of a rider who is too large for a horse, with respect to gait, thoracolumbar muscle function, and development, and the
potential risk of musculoskeletal injury. They should also recognize that behavioral abnormalities that are often considered as “normal horse behavior” may reflect musculoskeletal pain. Observation of a horse’s behavior during tacking-up and mounting and during ridden exercise may be key indicators that the horse is uncomfortable during ridden exercise.\textsuperscript{25,42} Comparison of spontaneous blink rate before and after exercise may also be helpful because an increased rate after exercise is an indicator of stress. There is no doubt that for some horse and rider combinations, it may be impossible to find a saddle that is suitable for both the horse and the rider, and in these cases, this means that the rider is likely to compromise the horse’s long-term welfare if they continue to ride. There is a moral responsibility for the equine veterinary profession to advise such riders that they are too large for the horse, however difficult that discussion may be; to fail to do so would jeopardize the horse’s short-term and long-term welfare.

Acknowledgments
The colleagues in the cited studies conducted in association with the Author: Jeannine Berger, Anne Bondi, Cheryl Chan, Janet Douglas, Andrea Ellis, Line Greve, Russell Guire, Pat Harris, Andrew Hemmings, Rachel Murray, Catherine Morris, Jessica Mullard, Danica Pollard, Laura Quiney, Alice Roberts, Linda Roost, Jenny Routh, Katy Thomson, and Jan Van Dyk.

Support for Research Performed
Sources of funding include World Horse Welfare, the Saddle Research Trust, Frank Dyson, British Equestrian Federation, British Horse Society, Pony Club, Polocross, The Showing Council, The Showing Register, The Society of Master Saddlers, Riding for the Disabled, British Eventing, British Dressage, the British Horse Foundation, the Worshipful Company of Saddlers, and Endurance GB.

Declaration of Ethics
Studies performed by the Author and colleagues were approved by the Clinical Ethical Review Committee of the Animal Health Trust. The horse owners gave informed consent for inclusion of their horses in the studies. Principles of Veterinary Medical Ethics of the AVMA were adhered to.

Conflict of Interest
The Author has no conflicts of interest.

References


A Review of How to Recognize Signs of Abnormal Equine Behavior During Tacking-Up and Mounting and to Understand Their Potential Clinical Significance

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1. Introduction

In the absence of environmental stimuli, the majority of healthy, stabled horses, managed under good welfare conditions, normally exhibit relaxed, quiet, and passive postures and behaviors.1 These normal behaviors should be maintained during tacking-up and mounting, with no agitation, anxiety, or nonphysiological postures or movements. However, some horses display a wide range of abnormal behaviors and postures when tacked-up or mounted that deviate from their normal patterns of behavior. These include, for example, threatening behaviors, fidgeting, and odd postures; indeed, the term “cold-backed” behavior is often used to describe extreme abnormal behavior when a horse is tacked-up or mounted.2 Understanding the reasons for abnormal behavior during tacking-up and/or mounting and managing the horses accordingly may help to optimize horse welfare and ultimately improve athletic performance. Suggested reasons for abnormal behavior during tacking-up include ill-fitting tack, girth region hypersensitivity, or anticipation of musculoskeletal pain when ridden.3-6 Previous studies have identified that a large percentage of horses have ill-fitting tack7-9 and that a large proportion of sports horses considered lame by their owners are lame or have other pain-related gait abnormalities.9-14 Although many horses with gastric ulceration do not show girth aversion behavior,15 an association between gastric ulceration and girth aversion behavior (“girthy” behavior, “girthiness”) was observed in a small number of horses.6 Some riders of horses with extreme cold-backed behavior or those that buck immediately after mounting2 may seek veterinary advice. However, it has been observed that more subtle abnormal behaviors during tacking-up or mounting are largely ignored by riders or are not recognized as abnormal. Additionally, there is a failure of riders to see these abnormal behaviors, or the rider becomes habituated to them. Riders sometimes report grumpy behavior when their horse is tacked-up but
say that this behavior is usual for their horse, assuming that it is a normal equine characteristic. However, it has previously been observed in a limited number of horses that had shown markedly abnormal behavior when tacked-up that when underlying musculoskeletal pain was successfully managed long term by addressing all problems, behavior during tacking-up improved dramatically. Improvement in a horse’s demeanor after successful treatment of musculoskeletal pain has anecdotally been noticed by riders, despite their previous failure to notice the presence of abnormal behavior. The objectives of this paper are to review recent studies that provide evidence-based information concerning tacking-up and mounting behavior, to highlight the reasons why it is important to assess this behavior, and to provide guidance about how to assess such behavior.

2. How Is Abnormal Behavior During Tacking-Up and Mounting Defined?

In order to define what may represent abnormal behavior during tacking-up and mounting, a large-scale observational study was performed on horses at rest and during tacking-up and mounting. A purpose-designed protocol for documenting facial expressions and behavior during each phase of tacking-up (approaching the horse, putting on the bridle, placing the saddle, doing up the girth) and mounting and moving forward after mounting was developed. This was adapted from previous publications relating to tack, the Ridden Horse Pain Ethogram (RHpE), and observations of horses’ behavior, including posture and movement. In the final protocol, a total of 64 behaviors for tacking-up and a total of 30 behaviors for mounting and moving off were documented. Additional observations were documented freehand. The study horses (n = 193) represented a convenience sample of sports and leisure horses, ranging from equine college horses to elite competition horses, ridden by amateurs or professionals. The horses were in full work and assumed by their riders to be working comfortably. To establish the normal behavior baseline patterns for individual horses at rest, the horses were observed undisturbed for approximately 8 minutes during acquisition of predefined information from the riders. The riders were then asked to tack-up and mount as they would normally do. Abnormal behavior, defined as changes that deviated from an individual horse’s normal baseline behaviors, were then recorded during bridling, placement of the saddle, girthing, and mounting. The majority of horses (67%) were bridled first. The median sum of abnormal behaviors during tacking-up was 10/64 (interquartile range 7–13, range 0-33). There was an equal frequency of abnormal behaviors during bridling and saddling in 52% of horses, 34% of horses showed more abnormal behaviors during saddling than bridling, and 15% of horses showed more abnormal behaviors during bridling than saddling. The duration of abnormal behaviors related to total tacking-up time was 25% to 75% in 51% of horses. There was a positive relationship between the sum of abnormal behaviors and the duration of abnormal behaviors (p = 0.0001). Repeatedly chomping on the bit occurred most frequently during bridling (67%). Ears back (Figs. 1B; 2C; 3A and B; 5A-C; 57-65%) and an intense stare (Figs. 1B and C; 2C; 4A; 5A and C; 54-62%) were similar in all phases. Fidgeting was more common during saddle placement (32%) and girthing (21%) than bridling (9%). Tail swishing was more frequent during girthing (34%) and saddle placement (20%) than bridling (10%). Turning the head to the girth (Figs. 2B; 5D) was only seen during saddle placement and girthing (11% and 40%, respectively) and rubbing the nose (Fig 2A; 8% and 21%, respectively). The median sum of abnormal behaviors during mounting was 1/30 (interquartile range 1-3, range 0-12; Figs. 6-8). The majority of riders (90%) mounted from a mounting block; 17% of horses were reluctant to approach the block or moved away from it before mounting. The most frequent abnormal behaviors observed during or immediately after
mounting included fidgeting (26%; Figs. 6 and 7), tail swishing (17%), chomping on the bit (17%), extension of the thoracolumbar region (14%; Fig. 8), yanking down on the reins (12%), and tossing the head (11%). The most common behavior observed when moving forward after mounting was the horse showing reduced range of motion of the thoracolumbar region (12%). Many of the behaviors observed during tacking-up were typical of those previously attributed to stereotypical behavior (for example, head tossing, tongue out, nose rubbing, licking), which is frequently stress associated.21–26 Biting and kicking have been considered as aggressive behaviors.27 Some behaviors may be a reflection of pain (for example, ears back, intense stare, tail swishing).21,28–31

3. Do Horse Riders Recognize Signs of Abnormal Behavior During Tacking-Up and Mounting?

In order to determine whether riders recognize signs of abnormal behavior during tacking-up and mounting, prior to tacking-up, the riders were asked if they recalled whether their horse showed abnormal behavior when tacked-up or mounted (yes/no).32 They were subsequently asked if their horse showed specific behaviors (yes/no) during approach to the horse with the tack (n = 2), bridling (n = 13), saddling (n = 22), girthing (n = 21), mounting (n = 19), and moving off after mounting (n = 13). Each horse was observed during tacking-up and mounting by one veterinarian who recorded the occurrence of each behavior. Agreement between the riders and the veterinarian was evaluated using intraclass correlation (ICC) coefficients with 95% confidence intervals (CI). ICC values less than 0.40 are indicative of poor agreement, values between 0.40 and 0.59 indicate fair agreement, values between 0.60 and 0.74 indicate good agreement, and values ≥ 0.75 indicate excellent agreement.33 The 95% CIs should be included in the interpretation of cutoffs for qualitative rating of agreement.34 Overall, 34.2% (66/193) of riders reported that their horse showed behavioral abnormalities during tacking-up or mounting,32 whereas abnormal behavior was observed by the veterinarian for ≥ 25% of the total tacking-up time in 66% of horses.16 For attempts to bite, there was fair to excellent agreement during saddling (ICC 0.67, CI 0.56-0.75) and good to excellent agreement during girthing (ICC 0.73, CI 0.64-0.79). There was poor to good agreement between the riders and veterinarian for horses putting their head up to avoid bridling (ICC 0.53, CI 0.37-0.64) and being reluctant to open their mouth for the bit (ICC 0.52, CI 0.36-0.64). There was
poor to fair agreement for evading noseband tightening (ICC 0.41, CI 0.21-0.56), elevating the head (ICC 0.24, CI 0.00-0.43), and teeth grinding (ICC 0.23, CI 0.00-0.42). Results for some behaviors suggested potential systematic disagreement between the veterinarian and riders. The majority of riders were unaware that their horses showed behavioral abnormalities during tacking-up or mounting. The notable exceptions were attempting to bite during saddle placement or girthing and kicking at the abdomen during saddle placement. This recognition may be because such behaviors can be classed as dangerous. There were common abnormal behaviors, which were either interpreted as normal or not recognized by riders, including chomping on the bit, ears back, and an intense stare during bridling, saddling, and girthing; tail swishing during saddling, girthing, and mounting; fidgeting during girthing and mounting; head tossing during girthing; yanking down on the reins immediately after mounting; and spontaneously walking forward before a cue from the rider (Figs. 6B, 7). These data highlight the importance of not relying on owner recall when acquiring the history of a horse; personal assessment by the veterinarian is essential.

4. What Do These Behaviors Mean?

The behavioral abnormalities during tacking-up or mounting were related to the presence of epaxial muscle hypertonicity or pain, girth region hypersensitivity, ill-fitting tack, rider position and balance, or equine musculoskeletal pain manifest as lameness in hand or when ridden or gait abnormalities in canter. Lameness was graded on a 0-8 scale. The RHpE was applied retrospectively to video recordings of the horses acquired during ridden exercise. Multivariable negative binomial regression modeling was used to assess the relationship between the sum of tacking-up and mounting behaviors and horse, rider, and tack-fit variables. Equine college horses comprised only 12% of the sample population but had higher rates of abnormal behaviors during both tacking-up ($p < 0.0001$) and mounting ($p = 0.007$) compared with general purpose horses. The rate of abnormal behaviors during tacking-up for horses with moderate lameness (defined as lameness $\geq$ grade 3 $< $ grade 5/8, or RHpE score $\geq$ 8 behaviors and lameness $\leq$ grade 2/8, to include symmetrical gait abnormality, e.g., lack of hindlimb impulsion) or severe lameness (defined as $\geq$ grade 5/8) was 1.4 times higher ($p = 0.02$) than for nonlame horses. Horses

Fig. 4. A, The horse has tilted its head during bridling. The mouth is wide open with separation of the teeth. The left eye has a glazed expression, and the eyelids are partially closed. B, The horse has lowered the head and neck and turned toward the rider during noseband tightening.

Fig. 5. A, The horse has its ears back behind a vertical position and an intense stare in the left eye during tightening of the noseband. B, Bridling is complete; the eyelids of the left eye are closed and the ears back behind a vertical position. C, During girthing, the ears remain back behind a vertical position, the lips are separated, and there is an intense stare in the left eye. D, During tightening of the girth on the right side, the horse has turned its head and neck to the girth region on the right side.
with lameness in hand or ridden had 1.5 times higher rates of abnormal behavior during mounting than nonlame horses. Tight tree points of the saddle \((p = 0.03)\) and epaxial muscle pain \((p < 0.001)\) were associated with higher abnormal behavior scores during tacking-up. Higher static saddle-fit scores were associated with higher abnormal behavior scores during mounting. The significant relationship between epaxial muscle pain, lameness, and tight tree points and the behavior scores of horses observed during tacking-up suggest that such behaviors are in anticipation of pain, either caused directly by the tack or reflecting musculoskeletal pain during ridden exercise.

5. Why, When, and How Should Behavior During Tacking-Up and Mounting Be Observed?

These observations indicate that the display of abnormal behaviors during tacking-up and mounting is likely to be of potential clinical significance. Therefore, when assessing a horse as part of a prepurchase examination, as a routine maintenance assessment, or when performing an investigation of poor performance, it is important to include an evaluation of the horse’s behavior initially at rest and then during tacking-up and mounting. This should be combined with systematic palpation of the thoracolumbosacral and girth regions, an oral examination, evaluation of the fit of the bridle, and assessment of static and dynamic saddle fit for both the horse and the rider to include the saddle pad(s), numnah(s), and girth. Many horses show a transient increase in the frequency of partial eyelid closure, blinking, eye closure, and putting the ears back as the headpiece of the bridle is placed behind the ears. Many horses transiently mouth the bit several times immediately after the bridle is put on. These behaviors are not considered to be abnormal. The horse should be observed for several minutes prior to tacking-up in order to appreciate its normal posture and behavior. Events occur rapidly during tacking-up and mounting and as the horse moves forward after mounting, so focused attention is required. The use of a checklist (Table 1), which the examiner has become familiar with in advance, is recommended. This includes assessment of the horse’s reaction to the approach of the rider with the tack, evaluation of facial expressions (Figs. 1A-C; 2C; 3A and B; 4A; 5A-C; 6A and B; 7; 8A and B), head and neck posture and movement (Figs. 1C; 2A and B; 3B; 4A and B; 5D; 6A and B; 7; 8A and B), limb (Fig 2C) and tail movements, and whether the horse stands still or moves repeatedly. To put the findings into context, it is also necessary to consider the skill of the rider’s handling of the horse, the suitability of their size for the horse (Fig 7), and the method that is used to mount the horse (from the ground, via a leg-up, or using a mounting block; Figs. 6-8). As with any clinical skill, the ability of an examiner to recognize all the behaviors exhibited is
Table 1. Ethogram Checklist for Abnormal Behaviors Observed During Tacking-Up, Mounting, and Moving Off in Comparison With Pre-Tacking-Up Assessment

<table>
<thead>
<tr>
<th>Behavior</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Approach the horse with tack</td>
<td>Horse avoids being caught</td>
</tr>
<tr>
<td>Bridling, placing saddle, girding</td>
<td>Takes ≥ 5 s to put bit in mouth</td>
</tr>
<tr>
<td>Puts head up to avoid bridle being put on</td>
<td>As the bridle is being placed</td>
</tr>
<tr>
<td>Elevates head above normal resting position</td>
<td>At any stage during tacking-up</td>
</tr>
<tr>
<td>Lowers head below normal resting position</td>
<td>At any stage during tacking-up</td>
</tr>
<tr>
<td>Tosses head up and down</td>
<td>At any stage during tacking-up</td>
</tr>
<tr>
<td>Tosses head up and down or opens mouth</td>
<td>Audible bruxism</td>
</tr>
<tr>
<td>As the bridle is being placed</td>
<td>Separates the maxilla and mandible and opens the buccal cavity</td>
</tr>
<tr>
<td>In any stage during tacking-up</td>
<td>Ears back behind a vertical position ≥ 5 s</td>
</tr>
<tr>
<td>Elevates head above normal resting position</td>
<td>Repeatedly</td>
</tr>
<tr>
<td>Elevates head above normal resting position</td>
<td>Glazed expression ≥ 5 s</td>
</tr>
<tr>
<td>Lowers head below normal resting position</td>
<td>Tongue hangs out of oral cavity or repeatedly moves in and out</td>
</tr>
<tr>
<td>Elevates head above normal resting position</td>
<td>Increased frequency of partial eyelid closure, full eyelid closure, or transient eye closure</td>
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<tr>
<td>As the bridle is being placed</td>
<td>Opening of the mouth repeatedly and excessively</td>
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<tr>
<td>As the bridle is being placed</td>
<td>Separates the maxilla and mandible and opens the buccal cavity</td>
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<td>As the bridle is being placed</td>
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<tr>
<td>As the bridle is being placed</td>
<td>Separates the maxilla and mandible and opens the buccal cavity</td>
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</tbody>
</table>

(continued on next page)
likely to increase with practice. It is also clear that riders need education about the recognition of abnormal behaviors during tacking-up and mounting because such behaviors are likely to reflect an underlying problem with tack fit, musculoskeletal pain, or other pain (for example, gastric ulceration). However, it is acknowledged that a small proportion of the observations, such as reluctance to open the mouth to accept the bit or walking forward after mounting, may reflect inadequate or inappropriate training.

### 6. If Abnormal Behavior Is Observed During Tacking-Up or Mounting, What Should Be Done Next?

It is beyond the scope of this paper to provide a comprehensive review of how to investigate further. However, key areas for assessment include the fit of the bridle, saddle, girth, pads, and additional equipment such as breastplates and martingales. The saddle must fit both the horse and the rider, enabling the rider to be in optimal balance with the horse. A comprehensive evaluation of the oral cavity and the musculoskeletal system should be carried out, with particular attention paid to the presence of girth region sensitivity, epaxial muscle hypertonicity, or pain. The horse should be observed during ridden exercise to evaluate any signs of lameness or modifications of canter. Gastroscopy should be considered, and modifications to evaluate any signs of lameness or modifications of canter. Gastric ulcers may develop in mind that gastric ulceration is usually associated with a history of colic or inappropriate feeding practices, and the identification of the primary cause(s) of underlying problems, following the identification of the primary cause(s) of underlying problems, should be considered when assessing the problem. The skill of the rider’s handling techniques and the method of the rider’s handling techniques may be assessed, and modifications may be required. Detailed reviews of approaches to handling techniques may be required. Detailed reviews of approaches to handling techniques may be required. Detailed reviews of approaches to handling techniques may be required. Detailed reviews of approaches to handling techniques may be required.

### 7. Conclusions

Assessment of behavior during tacking-up and mounting is considered to be an essential part of the diagnostic armamentarium for an equine veterinarian in order to optimize both equine welfare and performance. There is an association between the number of behaviors exhibited during tacking-up and mounting and the presence of lameness and/or abnormal behavior. Some behaviors can be learned—such as failure to stand still while being mounted—retraining may also be required.

### Table 1. (Continued)

<table>
<thead>
<tr>
<th>Behavior</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>As rider mounts or immediately after mounting</td>
<td>Restlessness/flighting</td>
</tr>
<tr>
<td></td>
<td>Chomps on the bit</td>
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<tr>
<td></td>
<td>Grinds the teeth</td>
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<tr>
<td></td>
<td>Tosses the head</td>
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<tr>
<td></td>
<td>Yanks down on the reins</td>
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<tr>
<td></td>
<td>Pawsing the ground with a forelimb</td>
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<tr>
<td></td>
<td>Kicks out with a hindlimb</td>
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<tr>
<td></td>
<td>Tail swishes</td>
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<tr>
<td></td>
<td>Attempts to bite</td>
</tr>
<tr>
<td></td>
<td>Yawning</td>
</tr>
<tr>
<td></td>
<td>Extends thoracolumbar region on mounting</td>
</tr>
<tr>
<td></td>
<td>Flexes thoracolumbar region on mounting</td>
</tr>
<tr>
<td></td>
<td>Reluctant to walk forward</td>
</tr>
<tr>
<td></td>
<td>Walks with short steps</td>
</tr>
<tr>
<td></td>
<td>Holds thoracolumbar region stiffly</td>
</tr>
<tr>
<td></td>
<td>Flexes TLS region</td>
</tr>
<tr>
<td></td>
<td>Jogs, trots, or canters away</td>
</tr>
<tr>
<td></td>
<td>Bucks</td>
</tr>
<tr>
<td></td>
<td>Reverses</td>
</tr>
<tr>
<td>Moving forward after mounting</td>
<td>Does not stand still; walks forward, sideways, or backward</td>
</tr>
<tr>
<td></td>
<td>Repeatedly</td>
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<tr>
<td></td>
<td>Dips back</td>
</tr>
<tr>
<td></td>
<td>Hunches back</td>
</tr>
<tr>
<td></td>
<td>Walks very slowly; has to be encouraged to walk</td>
</tr>
<tr>
<td></td>
<td>Reduced range of motion</td>
</tr>
<tr>
<td></td>
<td>Hunches back</td>
</tr>
</tbody>
</table>

TLS, thoracolumbosacral.
paramount importance to raise their awareness of abnormal equine behavior and what it might mean.

Acknowledgments

Declaration of Ethics

Studies performed by the Authors were approved by The Clinical Ethical Review Committee of the Animal Health Trust. The horse owners gave informed consent for inclusion of their horses in the studies. Principles of Veterinary Medical Ethics of the AVMA were adhered to.

Conflict of Interest

The Authors have no conflicts of interest.

References and Footnote


*Dyson S., unpublished data, 2020.*
Review of Hysteroscopy in the Mare: A Video Perspective

Patrick McCue, DVM, PhD, DACT*; and Charles Scoggin, DVM, MS, DACT

Videendoscopic examination of the cervix and uterus (hysteroscopy) is a valuable procedure for reproductive tract issues that cannot be detected or resolved by other means. Authors’ addresses: Colorado State University, 3101 Rampart Road, Fort Collins, CO 80521 (McCue); Rood and Riddle Equine Hospital, 2150 Georgetown Road, Lexington, KY 40511 (Scoggin); e-mail: patrick.mccue@colostate.edu. © 2021 AAEP.

1. Introduction
A majority of equine uterine problems can be diagnosed using assessment of reproductive history and diagnostic techniques such as transrectal ultrasonography, vaginal speculum examination, uterine culture, uterine cytology, and endometrial biopsy.1–3 A videendoscopic examination of the uterus, or hysteroscopy, can also be a valuable procedure to detect or confirm suspected uterine abnormalities, to obtain uterine samples for subsequent analysis, to be used as a therapeutic delivery modality, or to deposit semen at the uterotubular junction. Hysteroscopy is most valuable in the detection of pathologic abnormalities within the uterine lumen or endometrial surface that cannot be diagnosed by traditional diagnostic techniques. Abnormalities may include intrauterine adhesions, retained endometrial cups, localized lesions, and focal sites of infection. Pioneer studies on the use of hysteroscopy in the mare began in the late 1960s and continued through the early 1990s.4–8 Several recent reviews have described the endoscopy equipment, mare preparation, and general procedures for hysteroscopy.9–11 In general, a videendoscope or gastroscope with a working length of 1 to 1.5 meters, an external diameter of 9.8 to 12.8 mm, and a biopsy channel size of 2.8 to 3.8 mm is recommended. The endoscope should be cold sterilized with 2.4% glutaraldehyde and subsequently rinsed with 0.9% sterile saline prior to use. The mare should be restrained in examination stocks and sedated (i.e., with a combination of detomidine hydrochloride and butorphanol tartrate), and a thorough cleansing of the perineum should be performed prior to the procedure. Insufflation of the uterine lumen is required for optimal visualization during the examination. The goal of this review is to describe how hysteroscopy can be utilized in equine practice as an adjunct diagnostic or therapeutic modality.

2. Detection of Intrauterine Adhesions
Adhesions across the lumen of the uterus are uncommon and occur secondary to dystocia or obstetrical procedures used to resolve a dystocia.12–15 Intrauterine adhesions are difficult to
diagnose on transrectal ultrasound but may become visible if fluid (i.e., sterile saline or lactated Ringer’s solution) is infused into the lumen to distend the uterus. The best method for detection of intrauterine adhesions is videendoscopy (Fig. 1).5,8

3. Detection of Uterine Trauma

Trauma to the uterus can occur during an apparently normal foaling, during a dystocia with or without obstetrical manipulations, or as a consequence of a surgical procedure such as a cesarean or ovariec-tomy.16–18 Diagnosis of uterine trauma may be based on reproductive history, clinical signs, transrectal and/or transabdominal ultrasonography, laparoscopy, abdominocentesis, manual evaluation of the uterine lumen, and videendoscopic evaluation of the uterine lumen (Figs. 2 and 3). Obtaining an accurate diagnosis is key to formulation of a therapeutic plan and prognosis for recovery and future fertility.


Primary uterine tumors are rare in the horse and would include leiomyomas, leiomyosarcomas, lymphosarcomas, adenocarcinoma, and other
tumors. Videoendoscopy can be used to visualize intralumenal and intramural masses, obtain small biopsies, facilitate accurate location for collection of a larger biopsy sample, or facilitate removal of pedunculated masses (Fig. 4).

5. Detection of Focal Areas of Infection or Inflammation

A general presumption is that a uterine infection is evenly distributed throughout the uterus. However, endometritis may be associated with localized, discrete adherent plaques or regional areas of bacterial or fungal infection. Videoendoscopy can help identify areas of infection and allow for sample collection (culture, cytology, biopsy) from affected areas (Figs. 5 and 6).

6. Evaluation of a Mare with Pyometra

The term pyometra, as used in horses and cattle, refers to the accumulation of a large volume of inflammatory fluid associated with a chronic infection. An open pyometra is associated with a patent cervix and a chronic or intermittent vaginal discharge. In contrast, a closed pyometra implies that the cervix is not patent (i.e., closed or adhered shut), in which case there is no vaginal discharge despite accumulation of a large quantity of fluid in the uterus. A videoendoscopic examination can be used to evaluate patency of the cervix (Fig. 7), the presence of vaginal or cervical adhesions (Fig. 8), or...
accumulation of cloudy fluid within the uterus (Fig. 9) in affected mares.

7. Detection of Persistent Endometrial Cups
Persistence of endometrial cups has been described in mares with an early termination of pregnancy, such as fetal loss or abortion, and mares that carried a normal pregnancy to term.\textsuperscript{8,30,31} Persistent endometrial cups have been associated with failure to show behavioral estrus, complete ovarian inactivity, sporadic follicular growth, and luteinization of partly developed follicles. Diagnosis is based on observation of endometrial cups on ultrasonography per rectum, videoendoscopic evaluation of the uterine lumen (Fig. 10), and/or measurement of equine chorionic gonadotropin in peripheral blood. The functional life span of persistent cups has been reported to be 6 to 30 months.

8. Detection and Removal of Foreign Bodies
Occasionally, a foreign body will be located within the uterine lumen of a mare.\textsuperscript{32–34} Foreign bodies or substances found within the uterine lumen of mares include a mumified fetus or fetal parts, the tip of a uterine culture instrument (Fig. 11) that fractured off during sample collection, a marble intentionally placed in the uterus in an attempt to block behavioral estrus (Fig. 12), and residues or precipitates from intrauterine medications. Initial detection may be made by recognition of an echogenic object casting a shadow during transrectal ultrasonography. Videoendoscopy can subsequently be used to confirm the diagnosis and facilitate removal using a grasping instrument or basket passed down the biopsy channel (Figs. 13 and 14).
9. Detection and Removal of Fetal Remnants

Death of an embryo or fetus early in gestation usually results in complete resorption or expulsion of the fluids and tissues. However, in some instances, fetal tissues may remain in the uterine lumen.8 Fetal death later in gestation usually results in abortion of the fetus, but on rare occasions, retention and subsequent mummification of the fetus may occur.34 Initial indication of the retention of fetal tissue may be obtained by transrectal ultrasound visualization of an echogenic mass casting a shadow, and videoendoscopy can be used to confirm the diagnosis.35 Smaller fetal remnants can be removed using either a grasping instrument or basket passed down the biopsy channel of a videoendoscope (Fig. 15).

10. Detection and Removal of Endometrial Lymphatic Cysts

Endometrial lymphatic cysts develop secondary to deposition of fibrosis around endometrial lymphatic ducts and decreased myometrial contractility normally responsible for movement of lymphatic fluid through the duct system.7,8,38 Lymphatic cysts may be solitary or occur in clusters and range in size from a few millimeters to greater than 5 cm. The incidence of lymphatic cysts in mares increases with advancing age.7,36,37 Clinical diagnosis is made by transrectal ultrasonography, but endoscopic evaluation of the uterine lumen can also be used to detect endometrial cysts (Fig. 16) as well as facilitate temporary deflation (Fig. 17) or more long-term destruction (Fig. 18) of the cysts by laser therapy.8,39–42
11. Confirmation of the Presence of Urine in the Uterus

Vesico-vaginal reflux, or urine pooling, in the cranial vagina may lead to contamination of the uterus with urine during estrus when the cervix is open and relaxed onto the floor of the vagina. Urine contamination of the uterus leads to a chemical-induced endometritis and results in decreased pregnancy rate or decreased ability to maintain a pregnancy. Urine in the uterus may be recognized on transrectal ultrasound as a turbid, highly echogenic fluid usually at the base of a uterine horn or in the uterine body. Confirmation of urine contamination of the vagina and uterus may be made by vaginal speculum examination and videoendoscopy of the uterus, respectively (Fig. 19).

12. Hydrotubation of the Utero-Tubular Junction

The oviduct or uterine tube is an important link from the ovary to the uterus and is the site of fertilization in vivo. The equine oviduct may become obstructed by masses of collagen, fibroblast cells, and other debris, which may lead to a reduction in fertility. Diagnosis of uterine tube pathology is usually made by exclusion once other potential causes of subfertility have been ruled out. A procedure termed “hydrotubation” has recently been developed in which the uterine papillae is cannulated and the uterine tube flushed in a retrograde fashion. The hydrotubation procedure has been used clinically as a technique to remove presumptive oviductal masses and restore fertility potential in mares with unexplained infertility (Fig. 20).
13. Detection of Congenital Uterine Abnormalities

Congenital abnormalities of the equine cervix and uterus are very rare. Abnormalities may include segmental aplasia of the uterus, uterus didelphys, uterus unicornis, double cervix connected to separate uterine horns (uterus bicornor bicollis), and neoplasia or metaplasia. Disorders of sexual development may be associated with an underdeveloped reproductive tract, a complete absence of reproductive tissue of Mullerian duct origin, or incomplete development of the reproductive tract. Congenital neoplastic or metaplastic conditions are also highly uncommon but should be considered in maiden mares with detectable abnormalities in their reproductive tract. Videoendoscopic examination is an important diagnostic procedure to evaluate the integrity of the reproductive tract of affected mares.

14. Deep Horn, Low-Dose Insemination

Traditional artificial insemination with fresh semen involves deposition of 500 million progressively motile spermatozoa into the uterine body to achieve maximum reproductive efficiency. Insemination of a small volume of semen containing a low number of spermatozoa has been reported to yield a higher pregnancy rate if deposited onto or adjacent to the uterotubular junction (UTJ) ipsilateral to the preovulatory follicle. A low dose of semen can be deposited using a manual transrectally guided deep horn approach or using a videoendoscope to visualize and deposit semen directly onto the UTJ via a catheter passed through the biopsy channel (Fig. 21).

15. Evaluation of Endometrial Health

Videoendoscopy can be used to visualize the entire surface of the mucosal or luminal surface of the equine uterus, allowing for gross evaluation of coloration, vascular patterns, focal sites of infection, localized lesions, scarring, and other surface abnormalities. Videoendoscopic evaluation of small arteries visible under the endometrium was noted to be correlated with age-related sclerotic changes of the endometrial vasculature determined by histopathology. It was suggested that endoscopic evaluation of the uterus could be used as a noninvasive technique to estimate the degree of endometrosis and provide a prediction for reproductive ability of mares. In addition, narrow-band imaging through a videoendoscope was reported to allow for recognition of an endometrium affected by endometrosis and vessel degeneration subsequently confirmed by histopathology.

16. Summary

Hysteroscopy is a procedure that can be used in the diagnosis and treatment of uterine abnormalities. Videoendoscopy of the uterus can be used diagnostically for detection of uterine issues such as intralumenal adhesions and persistent endometrial cups, as well as directed biopsy of focal uterine lesions or masses. Therapeutically, hysteroscopy can be used in the identification and removal of foreign bodies, temporary ablation of lymphatic cysts, hydrotubation to alleviate oviductal blockage, and low-dose insemination at the utero-tubular junction. It should be noted that the procedure can be associated with iatrogenic infection or inflammation.

Acknowledgments

Declaration of Ethics

The Authors have adhered to the Principles of Veterinary Medical Ethics of the AVMA.
Conflict of Interest
The Authors have no conflicts of interest.

References


How to Identify Common Bacterial Pathogens from an Equine Uterine Sample in Clinical Practice

Christina Divine, DVM†; and Patrick McCue, DVM, PhD, DACT*

A basic microbiology laboratory can be used by an equine veterinary practice to efficiently and accurately detect and identify common equine uterine pathogens. Authors’ addresses: Department of Clinical Sciences, College of Veterinary Medicine and Biomedical Sciences (Divine), 3101 Rampart Road (McCue), Colorado State University, Fort Collins, CO 80521; e-mails: christina.divine@colostate.edu, patrick.mccue@colostate.edu. *Corresponding author; †presenting author. © 2021 AAEP.

1. Introduction

Bacterial endometritis is one of the most common causes of reduced fertility in broodmares. Identification of infectious endometritis is based on a combination of reproductive history, transrectal ultrasonography, vaginal speculum examination, as well as microbial culture and cytologic evaluation of a uterine sample and possibly histologic evaluation of a uterine biopsy.1–6 Uterine samples for culture may be collected by guarded swab, low-volume lavage, or biopsy. The most common bacterial pathogens of the equine uterus include Streptococcus equi subspecies zooepidemicus, Escherichia coli, Pseudomonas aeruginosa, and Klebsiella pneumoniae. Options for microbial culture in clinical practice include performing the analysis in-house or sending samples to a diagnostic laboratory. Advantages of an in-house culture system include a more rapid result, which leads to improved patient care and reproductive management and financial savings by not paying an outside laboratory. Disadvantages include the need for equipment and supplies, a dedicated laboratory space, as well as training and availability of personnel.

2. Material and Methods

Equipment and supplies needed to set up a basic microbiology laboratory include an incubator, a refrigerator, a biohazard waste container, microbial agar plates, disposable inoculating loops, disposable nitrile exam gloves, and a form for recording results. Once a uterine sample has been collected, an agar plate is removed from the refrigerator and allowed to equilibrate to room temperature and labeled prior to use. The “quad plates” used in the authors’ clinical microbiology laboratory contain four different microbial agars (tryptic soy agar with 5% sheep blood, MacConkey II agar, Gram-positive chromogenic agar, and Gram-negative chromogenic agar; Table 1).
individual colonies can be identified and selected for subsequent analysis if needed. The labeled plate is incubated agar side up at 37°C and evaluated for microbial growth at 24, 48, and 72 hours. At each observation period, the presence or absence of microbial growth, amount of growth (very light growth, light growth, moderate growth, and heavy growth), morphological characteristics of microbial colonies (circular, irregular, pinpoint or punctiform, raised, flat), and presence of more than one colony type are recorded for each agar type. In addition, the presence or absence of hemolysis is noted for colonies present on tryptic soy agar blood agar. Finally, the presence of potential bacterial contaminants is noted. Pure growth of a single pathogenic organism on the primary streak and/or secondary streak is clinically significant. In contrast, growth of a single colony, growth outside of the primary or secondary streaks, and/or growth of an aberrant colony type likely represents a contaminating organism that is not clinically significant. In addition, if the organisms are contaminants of sample collection and/or processing, one would expect an absence of inflammatory cells on uterine cytology and an absence of fluid in the uterine lumen on ultrasonography. However, it has been noted that bacterial endometritis can be present in the absence of uterine inflammation.\(^7\)

3. Results

Microbial growth characteristics for each of the four primary equine uterine pathogens on each agar type of the quad plate are described in Table 2 and presented in Figs. 1 to 4.

4. Discussion

The first description of the use of chromogenic agar for equine uterine culture was by Beehan and McKinnon in 2009.\(^2\) A recent study by Ferris and colleagues noted that chromogenic agar allowed correct identification of 96% of equine uterine pathogens when compared to identification acquired by DNA sequencing.\(^6\) In contrast, another group evaluated a different type of chromogenic agar\(^6\) originally marketed for detection and identification of microorganisms associated with urinary tract infections in people.\(^8\) Results of the latter study indicated that...
only 50% of equine uterine bacterial isolates were correctly identified based on color characteristics using the urinary tract infection agar. The difference in results in the two studies emphasizes the fact that not all chromogenic microbial agars are the same and not all are suitable for identification of equine uterine pathogens. In addition to use of the quad plate microbial agar system, there are several other techniques that can be used to help identify microbial organisms, including a catalase test, urease test, and Gram stain. A Kirby-Bauer disk diffusion system can be used to obtain antimicrobial susceptibility results and direct appropriate treatment of uterine health.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Gram Stain</th>
<th>Morphology</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Streptococcus equi</em> subsp. <em>zooepidemicus</em></td>
<td>Positive</td>
<td>Cocci (ovoid, chains)</td>
<td>Pinpoint blue colonies on Gram-positive chromogenic agar, (β-hemolysis)</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>Negative</td>
<td>Rods</td>
<td>Circular or irregular pink colonies on MacConkey agar, Pink mucoid colonies</td>
</tr>
<tr>
<td><em>Klebsiella pneumoniae</em></td>
<td>Negative</td>
<td>Rods</td>
<td>Circular or irregular pink colonies on Chromogenic agar, Circular dark blue colonies (± slight pink halo)</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>Negative</td>
<td>Rods</td>
<td>Irregular, flat, metallic blue or gray colonies (β-hemolysis)</td>
</tr>
</tbody>
</table>

Fig. 1. Culture of *Streptococcus equi* subsp. *zooepidemicus* on a quad plate. Note the growth of small white colonies with beta hemolysis on blood agar (1), lack of growth on MacConkey agar (2), blue colonies on Gram-positive chromogenic agar (3), and no growth on Gram-negative chromogenic agar (4).

Fig. 2. Culture of *Escherichia coli* on a quad plate. Note the growth of cream-colored colonies without hemolysis on blood agar (1), medium-sized pink colonies on MacConkey agar (2), no growth on Gram-positive chromogenic agar (3); and pink to red colonies on Gram-negative chromogenic agar (4).
infections. These techniques, although valuable, are not covered within the scope of this paper. In the event that microbial growth characteristics are not consistent with a known bacterial organism, it is recommended that the initial culture swab or culture plate be submitted to a diagnostic laboratory for further evaluation using advanced biochemical analysis, such as matrix-assisted laser desorption/ionization time of flight spectrometry, or polymerase chain reaction analysis. In summary, a basic microbiology laboratory can be set up in any veterinary clinic or breeding farm. The techniques described can be used in equine veterinary practice to efficiently obtain microbial culture results, which would allow for an accurate early diagnosis and subsequent implementation of a therapeutic protocol.

Acknowledgments

Declaration of Ethics

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

Conflict of Interest

The Authors have no conflicts of interest.

References and Footnotes


Fig. 3. Culture of *Klebsiella pneumoniae* on a quad plate. Note the growth of gray mucoid colonies without hemolysis on blood agar (1), pink mucoid colonies on MacConkey agar (2), no growth on Gram-positive chromogenic agar (3), and growth of blue colonies on Gram-negative chromogenic agar (4).

Fig. 4. Culture of *Pseudomonas aeruginosa* on a quad plate. Note the growth of flat metallic blue-gray colonies on blood agar (1), pale greenish colonies on MacConkey agar (2), no growth on Gram-positive chromogenic agar (3), and growth of transparent green colonies on Gram-negative chromogenic agar (4).
How to Prepare Platelet-Rich Plasma for Use in Reproductive Practices with Mares

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1. Introduction

Infusion of platelet-rich plasma (PRP) has recently gained popularity in reproductive practice with mares1 to mitigate postbreeding inflammatory response and consequently improve the fertility of barren and embryo donor mares with persistent-breeding-induced endometritis (PBIE).1–6 The PRP consists of a plasma sample with high platelet concentrations; greater platelet concentrations result in the enrichment of growth factors (GFs, e.g., hepatocyte growth factor), cytokines (e.g., transforming growth factor β, CXCL8, and IL1β) released from platelets after activation,7,8 and natural antimicrobial peptides (e.g., RANTES and platelet factor 4) that collectively modulate the uterine immune response and bacterial infections.9–11 Currently, there are various methods available to prepare PRP. Automatized commercial1,3 and manual protocols have been described for intrauterine infusion in mares. Practitioners wanting to start using PRP or already using it in reproductive practice with mares may be confused with the numerous options available and not be able to understand all protocols available consistently. Commercial protocols offer the advantage of being standardized and less human dependent; however, the high cost of acquiring the machines and supplies may discourage practitioners to routinely use PRP. Conversely, manual methods do not require specialized equipment and can be an affordable alternative to produce PRP. In addition, the majority of protocols are able to produce a small volume of PRP (~2 to 5 mL) for intra-articular or intratendinous injection. However, for intrauterine infusion, a higher volume (~10 to 60 mL) is presumably needed to reach the entire endometrial surface. This manuscript is an overview of how to prepare PRP for intrauterine infusion in mares and how each available protocol can be useful for broodmare’s practice. The most manual methods and automated systems to prepare PRP are discussed onward. In addition, the authors’ clinical and research experiences preparing PRP are also incorporated in the review.

2. Methods

Regardless of the method used to prepare PRP, whole blood (WB) has to be aseptically collected by venipuncture of the jugular vein. The authors typically scrub the overlying skin with three rounds of povidone iodine or chlorhexidine scrub followed by three rounds with 70% isopropyl alcohol. The placement of an intravenous catheter is not mandatory but may be placed in needle-shy horses or in case the practitioner...
is not comfortable drawing a large amount of blood off a needle. Also, the authors typically use 1.5-inch 18-gauge needles to avoid platelet damage and activation during the blood drawing. Following collection, the blood can be left at room temperature (−22°C) for 2-3 hours until processing with no apparent detrimental effects to PRP quality. Protocols to prepare PRP can be classified as manual or automated. The first type has the advantage of requiring minimal equipment and being low cost but is more time consuming, whereas the latter has the advantage of being standardized and requiring less labor but demands acquisition of expensive processing systems. The non-commercial methods are described herein, and the step-by-step of commercial systems can be found on the manufacturers’ webpages.

Noncommercial Methods

**Single Centrifugation in Vacutainer Tubes**

Blood is collected in 4.5-mL vacutainer tubes containing 3.2% sodium citrate and immediately gently homogenized. Blood tubes are then centrifuged at 120 x g for 10 minutes. In each tube after centrifugation, the top third layer of the plasma is discarded, while the remaining plasma adjacent to the Buffy coat is recovered as PRP (Fig. 1).

**Double Centrifugation in Vacutainer Tubes**

Blood is harvested in 4.5-mL vacutainer tubes containing 3.2% sodium citrate. Citrated blood should be slowly homogenized and centrifuged at 120 x g for 10 minutes. After the first centrifugation, the plasma fraction is recovered and transferred into 15-mL conical tubes. This fraction is submitted to centrifugation at 1000 x g for 10 minutes. After the second centrifugation, 2.5 mL of plasma at the bottom of each tube is considered as platelet-poor plasma (PPP), whereas the remaining sedimented plasma can be recovered as PRP (Fig. 3).

**Sedimentation**

Blood is collected using an 18-gauge needle into a 60-mL syringe prefilled with 7 mL of anticoagulant (CPD-A). Right after collection, each syringe should be wrapped in aluminum foil and placed in an upright position at room temperature. Four hours later, the top 10 mL of plasma can be discarded as platelet-poor plasma (PPP), whereas the remaining sedimented plasma can be recovered as PRP (Fig. 4). For this, the syringe is kept in an upright position, a 21-gauge butterfly catheter can be connected to the syringe, and PRP and PPP are recovered by applying steady pressure to the syringe’s plunger.14

3. Discussion

The cell composition, as well as soluble factors reported in the PRP produced by different methods, are highlighted in Table 1. All methods, but one, were reported to be able to improve platelet concentration in PRP compared with the WB. Although the cell composition cannot be compared among results from different studies, it is important to note that there is a discrepancy in the volume of blood harvested to produce PRP, the final volume of PRP, and the number of platelets and leukocytes in PRP from different studies, which can impact for intrauterine infusion in mares. Also, the concentration of erythrocytes and GFs are not available for all methods. Of interest, one system produces 10 mL of PRP, however, up to date, there is no report of the cell composition of the PRP produced by this system. The methods described in the present study, with the exception of the four commercially available products and sedimentation, have been tested in broodmare’s practice and used to mitigate postbreeding uterine inflammation (i.e., PMN counts in cytology or endometrial biopsy, intrauterine fluid accumulation, uterine inflammatory cytokines [IL1, IL6, IL8, IL10], endometrial inflammatory markers [COX-2]) and
improve fertility rates of mares susceptible to endometritis, barren mares, or mares inseminated with frozen semen that do not become pregnant in the first cycle (Table 2). Also, there were differences in the time and protocols of treatment and volume of PRP among studies. A summary of protocols used to obtain PRP, the respective platelet concentration, volume of plasma, time of uterine infusion, endometrial inflammatory markers, and fertility of mares is highlighted in Table 2. One of the first reports using PRP in mares described the postbreeding expression of inflammatory cytokines in the endometrium of barren mares treated with intrauterine PRP. The protocol used in that study to produce PRP applied a specialized blood fractionated machine. Although the cell composition of the PRP was not described and the PRP was mixed with PPP for intrauterine infusion, the treatment reduced endometrial expression of proinflammatory cytokines (IL1β, IL6, and CXCL8) in this group of mares. Later, another study using the same technique to produce PRP and treating barren mares showed that intrauterine PRP therapy response lowered intrauterine fluid accumulation and improved fertility when compared to the untreated cycle. Although in these studies the cell composition of PRP was not reported, another study using the same
method produced PRP with platelet concentration two-to three-folds greater than the whole blood. Later, one study using a semiautomated method to produce PRP by double centrifugation in vacutainer tubes also reported a reduction in postbreeding inflammatory reaction in mares with chronic degenerative endometritis after intrauterine PRP therapy. The mares were treated 4 hours after insemination with the intrauterine infusion of 20 mL of PRP and had lower uterine fluid and PMN counts in endometrial cytology postbreeding. In that study, 100 mL of WB was harvested in vacutainer tubes and then centrifuged at 120 x g for 10 minutes, then after the first centrifugation, the lower 70% of the plasma was further centrifuged at 240 x g for 10 minutes. This protocol yielded 20 mL of PRP containing ≥250 x 10^3 of platelets/μL and 5 x 10^9 platelets per treatment. Later, the same group compared the previously published treatment times by infusing PRP, either 24 hours before or 4 hours after insemination, and demonstrated that both time points reduced endometrial PMN counts and COX2 expression in mares susceptible to PBIE, as well as improved pregnancy rates of mares. In that study, PRP was produced by single centrifugation in vacutainer tubes. For this, 45 mL of WB was collected in 4.5-mL sodium citrate tubes and centrifuged once at 120 x g for 10 minutes; the protocol yielded 20 mL of PRP with platelet concentration of 354 ± 17 x 10^9 of platelets/μL and 7 ± 0.3 x 10^9 platelets per treatment. Most recently, PRP produced by another commercially available system was reported in a clinical trial to treat mares that did not become pregnant after insemination with frozen semen. Similar to the early studies, the authors did not provide the cell composition of PRP used for treatment; however, 61% of the mares treated with PRP became pregnant after insemination with frozen semen. In a most recent study, the effect of PRP and PPP was tested on postbreeding endometrial inflammation and fertility. PRP and PPP were obtained after the collection of WB (450 mL) in a blood transfusion bag and double centrifuged. The mean number of platelets infused in the uterus of mares was 24.9 ± 1.2 x 10^9 platelets in PRP and 1.4 ± 0.2 x 10^9 platelets in PPP. Embryo donor mares susceptible to PBIE were treated four times (two before and two after insemination) during the estrous cycle with 40 mL of one of the treatments. Interestingly, both plasma therapies reduced the postbreeding...
Table 1. Protocols for Preparation of Platelet-Rich Plasma for Use in Mare’s Reproductive Practice

<table>
<thead>
<tr>
<th>Method</th>
<th>Amount of Blood Necessary</th>
<th>Volume of PRP Recovered</th>
<th>Platelet Concentration (10^9/mL)</th>
<th>Leukocyte Concentration (10^9/mL)</th>
<th>Erythrocyte Concentration (10^12/mL)</th>
<th>Growth factors (PDGF and TGFβ)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Angiox®</td>
<td>180 mL ACD-A</td>
<td>320 ± 198.1</td>
<td>182.3 ± 39.7</td>
<td>892.3 ± 354.7</td>
<td>32.5 ± 62.2</td>
<td>Not available</td>
<td>Geburk et al., 2015¹²</td>
</tr>
<tr>
<td>Angel®</td>
<td>60 mL ACD-A</td>
<td>70 ± 240</td>
<td>4.6 ± 19</td>
<td>6 ± 19</td>
<td>6 ± 54</td>
<td>0.008 ± 0.07</td>
<td>Hessel et al., 2015¹²</td>
</tr>
<tr>
<td>Restigen PRP®</td>
<td>6 mL</td>
<td>3.9 ± 40.6</td>
<td>0.05 ± 0.3</td>
<td>Not available</td>
<td>Not available</td>
<td>Geburk et al., 2015¹²</td>
<td></td>
</tr>
<tr>
<td>Osteokine®</td>
<td>60 mL ACD-A</td>
<td>354.7 ± 14.1</td>
<td>14.1 ± 7</td>
<td>Not available</td>
<td>Not available</td>
<td>Geburk et al., 2015¹²</td>
<td></td>
</tr>
<tr>
<td>Arthrex ACP®</td>
<td>60 mL ACD-A</td>
<td>20 mL</td>
<td>354.7 ± 14.1</td>
<td>14.1 ± 7</td>
<td>Not available</td>
<td>Geburk et al., 2015¹²</td>
<td></td>
</tr>
<tr>
<td>CLINICAL PERSPECTIVES ON MANAGING EQUINE UTERINE HEALTH</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

inflammation (PMN counts, uterine fluid, and inflammatory cytokines); however, PRP apparently had additional immunomodulatory properties. In addition, the results of the study suggested that PRP may have antimicrobial properties as PRP therapy reduced the number of uterine infections after breeding in embryo donor mares susceptible to PBIE.⁶ It is important to note that many of the commercially available techniques produce a much-reduced volume of PRP than the manual methods. In the early studies applying a specialized blood fractionated machine,²,³ the PRP was diluted in PPP to reach the volume for intrauterine infusion. It is an important factor that should be considered in broodmare’s practice as it is assumed that higher volume (e.g., 10–60 mL) is required to reach the entire uterus.¹⁶ It is not yet clear the optimal cell composition of PRP for uterine infusion or whether there is a synergism between the beneficial effects of PRP and platelet concentration. Also, the effects of white and red blood cells in PRP are not well known. The PRP’s efficacy in mitigating PBIE has been attributed to the release of GFs, cytokines, and active platelet metabolites.⁸,¹⁷ It has been suggested that the greater number of platelets would release higher levels of desirable molecules and presumably produce better clinical response;¹⁸,¹⁹ however, this suggestion did not seem to be supported by a study testing different protocols to produce PRP in horses as there was no correlation between platelet counts and release of GFs in that study.¹² Although the immunomodulatory properties have been described in plasma and PRP with lower platelet concentrations,²⁰ in a recent study, the PRP’s antimicrobial properties were associated with the platelet concentration as no mares treated with PRP therapy developed positive aerobic endometrial culture 3 and 9 days postbreeding,⁶ and 30% of these mares had positive bacterial cultures at the same time points when treated with PPP. The antimicrobial activity of PRP has been described in humans and horses against Staphylococcus aureus, Escherichia coli, and Klebsiella pneumoniae,¹⁰,¹¹,²¹,²² which are known causes of endometritis in mares.²³ However, further studies are needed to determine whether the PRP’s properties could be amplified by increasing platelet concentrations and its clinical efficacy. Worth noting, intrauterine infusion of PRP is often carried out without platelet activation. Presumably, activation happens once the platelets interact with the uterine lumen; however, this has yet to be confirmed. Of interest, in vitro PRP is associated with sustained release of GFs over a minimum of 4 days when relying on endogenous activation only.¹⁹ Intra-articular infusion of PRP is also carried out without platelet activation under the same assumption that platelets get activated when exposed to the lumen of a joint.²⁴ In topical use, PRP is activated, and it acquires a gel consistency, which is desirable.
Table 2. Uterine Inflammatory Markers and Fertility Rates of Mares Treated with Different Protocols of Platelet-Rich Plasma

<table>
<thead>
<tr>
<th>Method</th>
<th>Volume of PRP per Treatment</th>
<th>Amount of Platelet per Treatment</th>
<th>Time of Treatment</th>
<th>Type of AI</th>
<th>Mares</th>
<th>Inflammatory Markers</th>
<th>Fertility</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Angel™</td>
<td>10 mL (PRP+ PPP)</td>
<td>Not available</td>
<td>24–36 h before AI</td>
<td>Not available</td>
<td>Barren mares</td>
<td>PRP reduced IUF and expression of IL1β, IL6, CXCL8, and iNOS</td>
<td>Control: 19% (3/19) PRP: 67% (16/24)</td>
<td>Metcalf et al., 2012; Metcalf, 2014</td>
</tr>
<tr>
<td>Restigen PRP®</td>
<td>15 mL (6 mL PRP + 9 mL PPP)</td>
<td>Not available</td>
<td>44 h before AI</td>
<td>Frozen semen</td>
<td>One negative cycle</td>
<td>No difference in IUF</td>
<td>Control: 0% (0/18) PRP: 61% (11/18)</td>
<td>Pasch et al., 2021</td>
</tr>
<tr>
<td>Single centrifugation in vacutainer tubes</td>
<td>20 mL PRP</td>
<td>$7 \times 10^9$ platelets</td>
<td>24 h before or 4 h after AI</td>
<td>Fresh semen</td>
<td>Susceptible to PBIE</td>
<td>PRP reduced PMN counts and endometrial expression of COX2</td>
<td>Control: 31% (3/13) PRP 24 before AI: 69% (9/13) PRP 4 h after AI: 58% (8/13)</td>
<td>Segabinazzi et al., 2017</td>
</tr>
<tr>
<td>Double centrifugation in vacutainer tubes</td>
<td>20 mL PRP</td>
<td>$\sim 5 \times 10^9$ platelets</td>
<td>4 h after AI</td>
<td>Fresh semen</td>
<td>Mares with CDE</td>
<td>PRP reduced PMN counts and IUF</td>
<td>Not available</td>
<td>Reghini et al., 2016</td>
</tr>
<tr>
<td>Double centrifugation in blood transfusion bag</td>
<td>40 mL PRP</td>
<td>$24.9 \pm 1.2 \times 10^9$ platelets</td>
<td>48 and 24 h before, and 6 and 24 h after AI</td>
<td>Fresh semen</td>
<td>Susceptible to PBIE</td>
<td>PRP reduced PMN counts, IUF, and IL1β and CXCL8. Plasma P4 concentrations were increased</td>
<td>Control: 33% (4/12) PRP: 83% (10/12)</td>
<td>Segabinazzi et al., 2020</td>
</tr>
</tbody>
</table>

Abbreviations: PRP, platelet-rich plasma; PPP, platelet-poor plasma; IUF, intrauterine fluid; PMN, polymorphonuclear neutrophil; COX2, cyclooxygenase-2; IL1β, interleukin-1β; IL6, interleukin-6; CXCL8, chemokine ligand 8 (interleukin-8); iNOS, nitric oxide synthase; AI, artificial insemination; PBIE, post-breeding-induced endometritis; CDE, chronic degenerative endometritis; P4, progesterone.
as PRP becomes sticky and adheres to the skin. Another factor that has been discussed in PRP practice is the amount of leukocytes in PRP samples. Some studies suggest that leukocytes are undesirable cells in the PRP for treating joints and tendons. High amounts of leukocytes can intensify the undesirable effects of inflammatory response, induce cellular catabolism, decrease extracellular matrix synthesis in tissues, and increase the release of proinflammatory cytokines, which may cause tissue damage.

Neutrophils are the body’s first line of defense and the most critical cell in uterine defense. These cells are already in the uterus about 30 minutes after semen contact with the endometrium and have a peak inflammatory reaction between 6 and 12 hours, being eliminated up to 48 hours in mares with a competent immune system. Postbreeding therapies such as uterine lavage aimed to prevent an excess of neutrophils and inflammatory molecules in the uterus of mares susceptible to PBIE as this is thought to be detrimental for a controlled immune response.

However, one study suggested that intrauterine infusion of white blood cells (WBCs) could faster eliminate bacterial contamination from the uterus of mares susceptible to PBIE. In addition, removal of red blood cells seems beneficial as an excess of blood can be detrimental to sperm, limiting its use prebreeding. Controversy exists on whether fresh WBCs and red blood cells from blood could affect the uterine incompatibility reaction, induce cellular catabolism, decrease extracellular matrix synthesis in tissues, and produce PRP with higher WBC concentration than other methods. V-PET consists of two blood fractions which are isolated, and a filter is isolated, and a filter by a complex interation is back flushed through the filter to recover the PRP. This protocol also has been described to yield PRP with great platelet concentration and higher WBCs than baseline. Therefore, in vivo studies are warranted to assess the clinical efficacy of PRP obtained by these three methods in mitigating PBIE in mares. Of interest, the anti-coagulants reported for processing PRP differ among protocols. The acid citrate dextrose solution (ACD-A) is the most cited in the experiments, followed by sodium citrate and citrate-phosphate-dextrose with adenine (CPD-A). Some protocols described in the present study have the kit provided by the manufacturer and use ACD-A as an anticoagulant. There are two studies that suggested that ACD-A has an inferior capacity to maintain platelet viability compared to CPD-A and sodium citrate. However, it should be clarified in the methods presented in the current study if changing the anticoagulant in these protocols can affect platelet viability and PRP composition. In conclusion, the methods described here are available to practitioners to produce PRP for intrauterine infusion in mares. It is important to note that PRP is not a standardized medical product. Therefore, variations can be observed between practitioners, methods, and animals. Some of the techniques presented in this manuscript require additional clinical trials to prove their efficacy. PRP is a safe and easy product for use in broodmare’s practice.

Acknowledgments

The Authors would like to thank FAPESP (Fundacao de Apoio a Pesquisa no Estado de Sao Paulo) and USDA Animal Health Hatch funds for funding some of the key studies described in this manuscript.

Declaration of Ethics

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

Conflict of Interest

The Authors have no conflicts of interest.

References and Footnotes


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€V-PET™, Pall Corporation, Port Washington, NY 11050.

²Osteokine®, Orthogen, Düsseldorf, Germany.
How to Use N-Acetylcysteine to Enhance Diagnosis of Bacterial Endometritis in Barren Mares

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1. Introduction

Endometritis is a leading cause of reproductive inefficiency in broodmares. Accurate diagnosis is critical in formulating an effective treatment plan. Multiple methods for obtaining endometrial samples for culture and cytology exist, with a range of associated sensitivity and specificity patterns. Endometrial sampling by low-volume lavage has been heralded for good-quality results and offers an advantage over swab/brush techniques through sampling of a greater surface area of the endometrium. This may be of particular advantage in mares with a history of subfertility. The presence of mucus within the equine uterus has been considered and explored as a factor influencing a mare’s reproductive soundness. N-acetylcysteine (NAC), a mucolytic that acts through disruption of disulfide bonds linking mucin, has been harnessed in equine reproductive practice as an antifilm agent and has been demonstrated to improve reproductive performance. Operating under the hypothesis that endometrial mucus may harbor bacteria and prevent their diagnosis through traditional sampling methods such as swab/brush or low-volume lavage, the impact of NAC infusion on uterine culture and cytology results was investigated.

2. Materials and Methods

Fifty-nine mares (58 Thoroughbreds and 1 Warmblood), barren from the 2020 breeding season, were evaluated between August and December 2020. Mares included were examined during the course of routine clinical practice in Lexington, KY.

Day 0

On day 0, 1-L of sterile lactated Ringer’s solution was instilled into the uterus and immediately collected back into the original bottle. Following this 1-L lavage, each mare was infused with 3.3% NAC (20 mL 20% N-acetylcysteine with 100 mL sterile 0.9% saline). A neat sample of lavage fluid (approximately 3 mL) was retained for turbidity analysis. The sample of neat lavage fluid was analyzed using a densimeter to obtain an objective measure of turbidity. A cuvette of water was used to zero the machine reading. Then, 1.8 mL of lavage fluid was added to a new cuvette and the
sample turbidity measured. The remaining uterine lavage fluid was submitted the same day for standard processing for culture and cytology. The 1-L bottle was allowed to rest undisturbed for one hour to encourage sediment formation at the bottom of the bottle. The sediment was aspirated using a pipette and divided into two 50-mL conical tubes for centrifugation. One centrifuged pellet was plated on Tryptic Soy Agar with 5% Sheep Blood, Columbia CNA Agar with 5% Sheep Blood, and MacConkey Agar, with the other prepared on a microscope slide and stained with Diff-Quik and Gram stain for cytologic evaluation. Bacterial growth was reported as no growth, scant (< 10 colony forming units), light (growth on one quadrant of plate only), moderate (growth on two quadrants of plate), or heavy (growth on three quadrants of plate). While the cytology results were reported in more detail than white blood cell evaluation alone, cytology results were compared between patient samples based on the number of white blood cells present (none, rare: 0-1 per high-power fields, few: 1-3 per high-power field, moderate: 6-10 per high-power field, many: > 10 per high-power field). A negative cytology was defined as no or rare white blood cells present, with all others defined as a positive cytology result. To minimize operator variation, all cytology slides for this project were read by one of two trained laboratory technicians.

Day 1
The following day, each mare had the above lavage culture procedure repeated in an identical fashion (instillation of 1-L sterile lactated Ringer’s solution, collected immediately back into the original bottle). Laboratory sampling and processing procedures were performed as above.

Statistical Analysis
Statistical analyses were performed using SAS® 9.4 using varying models depending on the data. Quantitative/continuous data means were analyzed utilizing an independent-group T test to make comparisons on means. Qualitative/categorical data were analyzed utilizing a chi-squared test for comparisons of proportions. Significance was set to \( P < 0.05 \).

3. Results
On day 0, 81% (48/59) of samples were cytologically negative, with 27% (16/59) cytologically negative the following day. This was not unexpected as any intraluminal manipulation of the uterus is likely to incite an inflammatory response. On day 0, 63% (37/59) of samples had no bacterial growth, 20% (12/59) had scant bacterial growth, 10% (6/59) had light bacterial growth, 7% (4/59) had moderate bacterial growth, and none had heavy bacterial growth. The following day, these bacterial growth profiles changed to 31% (18/59) no growth, 25% (15/59) scant growth, 15% (9/59) light growth, 24% (14/59) moderate growth, and 5% (3/59) heavy growth. Forty-eight mares had a negative cytology and no or scant bacterial growth on day 0. These 48 mares would reasonably have been designated “clean” by a practitioner based on these results. However, following NAC infusion, 17 of these mares (35%) displayed an inflammatory cytology and an increase in bacterial growth to light (5 mares), moderate (10 mares), or heavy (2 mares). Turbidity values were reported in million cells/mL and ranged from 0 to 135 for day-0 samples and 0 to 946 for day-1 samples. A threshold value of 50 million cells/mL was established as a cutoff for negative (below 50 million cells/mL) or positive (above 50 million cells/mL) densimeter results. Ninety-two percent (54/59) of day-0 samples were negative, and 75% (44/59) of day-1 samples were negative. Densimeter result was significantly associated with culture result, with 100% of those mares displaying an increase in densimeter reading from negative to positive also having an increase in bacterial culture grade \( (p < 0.01) \). However, if densimeter reading remained negative on day 0 and day 1, only 36% of those samples had an increase in bacterial culture grade \( (p < 0.05) \).

4. Discussion
The inclusion of NAC infusion prior to uterine lavage for culture and cytology has facilitated the diagnosis of a notable number of mares with bacterial endometritis that may otherwise have gone undetected by standard diagnostic methods. It is presumed that some mares produce a layer of mucus that acts to protect bacteria and prevent their detection, and only by disrupting this mucus layer are they able to be accurately diagnosed. Objective assessment of lavage fluid turbidity via densimeter was included in an attempt to relate the gross appearance of lavage efflux with laboratory findings. A very strong association between gross turbidity of a lavage and its likelihood of growing bacteria was found, making this an exciting potential adjunct diagnostic tool, especially in clinical settings that may have limited opportunities for performing cytology and must be selective in which samples are submitted for culture due to budgetary concerns. By no means is this diagnostic method proposed as a replacement for cytology and culture.

As this project was undertaken during clinical practice, there are inherent limitations to design and interpretation. First and foremost, no control groups were available to rigorously test the hypothesis. Control groups would have allowed for assessment of the influence of the low-volume lavage process itself on the following day’s results, as well as shed light on the potential role of any vaginal contamination during the lavage and infusion processes. Despite this drawback, the authors opine that this procedure has greatly enhanced the ability to diagnose (and subsequently treat) endometritis in mares.
Acknowledgments

The Authors are deeply grateful for the efforts of the Hagyard Laboratory Staff, whose skills are matched only by their kindness and willingness to collaborate on clinical research.

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Conflict of Interest

The Authors have no conflicts of interest.

References and Footnote


Fetal Ultrasonography of the First Phalange: A New Tool to Assess Fetal Growth and Bone Development

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The first phalange (P1) of the equine fetus can be imaged in vivo using transrectal ultrasonography. P1 length correlates strongly with days of gestation, and the time of appearance of the secondary ossification centers as well as the time of epiphyseal closure can serve as a marker of bone maturation. Authors’ addresses: 1206 Deodara Street, Davis, CA 95618 (Renaudin); Veterinary Medical Teaching Hospital (Wensley), School of Veterinary Medicine (Morgan, Cassano, Spriet), University of California-Davis, Davis, CA 95616; e-mail: cdrenaudin@gmail.com. *Corresponding and presenting author. © 2021 AAEP.

1. Introduction
In Quarter Horses (QHs), fetal age can be predicted within 2 weeks prior to 200 days of gestation via ultrasound, but as pregnancy advances, accuracy decreases. In this study, P1 is evaluated for its length and for the time of appearance and closure of its secondary ossification centers after 240 days of gestation.

2. Materials and Methods
Eight healthy pregnant mares (7 Quarter Horses and 1 Thoroughbred carrying a QH fetus) with known artificial insemination and ovulation dates were used. Fetuses were ultrasonically examined transrectally every 2 weeks, from 9 months of gestation until parturition. At each examination, the length of the ossified portion of P1’s main ossification center was measured, and the presence or absence of the secondary ossification centers (proximal and distal) was documented.

3. Results
P1 was observed in most examinations. However, in 29.5% (18/61) of examinations, P1 was not seen due to fetal presentation (1 posterior presentation) or posture (carpal or fetlock flexion). In some mares, additional examinations were performed to obtain data. On few occasions (4/61) in late gestation, P1 from hind limbs were measured while front limbs could not be seen because they were located too deep in the uterus. P1 length correlated strongly with
days of gestation (P < 0.0001). A correlation equation was established: $y = 0.3668x - 64.43$ ($y$ = predicted value of P1 length; $x$ = gestational age). The proximal and distal ossification centers both appeared between 277 and 303 days of gestation (± 2 weeks). The proximal ossification center did not close as opposed to the distal one that closed between 313 and 333 days of gestation (± 2 weeks).

4. Discussion
P1 length can be used as a novel biometric parameter during late gestation, and the presence or absence of its ossification centers can serve as markers of bone maturation in QHs. Fetal ultrasonographic assessment of proper bone development may be used in the future in the decision-making process of inducing parturition in the mare.

Acknowledgments
This project was supported by the Center for Equine Health with funds provided by the State of California satellite wagering fund and contributions by private donors and by Fujifilm Sonosite who generously provided the ultrasound machine.

The Authors are very grateful to the UCDavis Animal Science Horse Barn and would like to thank Kelli Davis, Alyssa Ortega, and Curtis Lewis for their contributions to the study.

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

Conflict of Interest
The Authors have no conflicts of interest.
Equine Postmortem Oocyte Recovery: A Retrospective Analysis

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A fifteen year retrospective evaluation of oocyte recovery from postmortem ovaries to create in-vitro embryos within a clinical program provides insight into success and recommendations for equine veterinarians. Authors’ address: Equine Reproduction Laboratory, Colorado State University, 3101 Rampart Road, Fort Collins, CO 80521; e-mail: Jenn.Hatzel@colostate.edu. *Corresponding and presenting author. © 2021 AAEP.

1. Introduction
Collecting equine oocytes from deceased mares for in-vitro embryo production through the use of intracytoplasmic sperm injection (ICSI) has been commercially available since 2005 and is considered an equine emergency. This retrospective analysis provides unique insight into clinical data from inception.

2. Materials and Methods
Data was collected from January 2005 through December 2020 and evaluated: the total number of oocytes collected, the number of oocytes reaching metaphase II compared to the total number collected (maturation rate), and the number of cleaved embryos and blastocysts to injected oocytes (cleavage rate and blastocyst rate). Utilizing the development of an embryo to the blastocyst stage as an endpoint, statistical comparisons were made evaluating timing from death, ovarian temperature upon arrival, and semen type. Statistical analysis was performed utilizing one-way ANOVA tests and significance established at \( P \leq 0.05 \).

3. Results
In total, 168 sets of ovaries were evaluated yielding 1,524 oocytes. An overall maturation rate of 41% (620) was identified, cleavage rate of 40% (246), and blastocyst rate of 19% (117). There was a linear association between temperature on arrival of ovaries from deceased mares and the likelihood of obtaining an embryo that successfully develops to a blastocyst stage \( (P = 0.003) \). However, there was no association between transportation time and semen choice \( (P = 0.17 \) and \( P = 0.65, \) respectively).

4. Discussion
This is the first clinical retrospective analysis determining successful blastocyst development derived from equine postmortem ovary collections from a data set this large. Although often uncontrollable, referring veterinarians can improve successful outcomes by understanding the importance of variables such as temperature during transportation and make every effort to ship ovaries while maintaining temperatures between 15–25°C.
Acknowledgments

Declaration of Ethics

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

Conflict of Interest

The Authors have no conflicts of interest.
Understanding and Overcoming Impostor Syndrome

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Impostor syndrome is seen commonly in medical professionals and can adversely affect career success and satisfaction. The characteristics of impostor syndrome include: self-doubt; inability to accurately judge your own abilities; giving the credit of your success to other external factors; overachieving; constant fear of not standing up to the expectations of people; self-sabotage; fear of isolation, exposure, and rejection; rumination; and anxiety and depression.1 Overcoming this condition requires self-awareness and diligence in reshaping mindset. Authors’ addresses: PO Box 192, Virginia City, MT 59755 (Grice); 6954 Point Pleasant Pike, New Hope, PA 18938-9715 (Clark); 120 Reservoir Road, Pawling, NY 12564-1740 (Fish); PO Box 256, Belgrade, MT 59714 (Lindroth); Athletic Equine, Manorville, NY 11949 (Spillman); e-mail: amyvmdmba@gmail.com.

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1. Introduction

Impostor syndrome is defined by the Oxford Dictionary as “the persistent inability to believe that one’s success is deserved or has been legitimately achieved as a result of one’s own efforts or skills.” First described in a research paper in 1978,2 the syndrome has subsequently been found in people of all demographics and is common in the medical fields. A recent review of the literature published in 2020, utilizing 62 studies and over 14,000 subjects, found that 9%–82% of people experience impostor syndrome. The numbers vary depending on who participates in a study but revealed that impostor syndrome was common among both men and women and across a range of age groups (adolescents to late-stage professionals).2

2. Discussion

Psychologists first described and named impostor syndrome in 1978.2 The authors wrote, “In our clinical experience, we have found that the phenomenon occurs with much less frequency in men and that when it does occur, it is with much less intensity ... Attribution research findings imply that the impostor phenomenon would be found less frequently in men than in women... Unlike men, who tend to own success as attributable to a quality inherent in themselves, women are more likely either to project the cause of success outward to an external cause (luck) or to a temporary internal quality (effort) that they do not equate with inherent ability.”

Subsequent studies have found that this disorder affects people of all ages, races, genders, and career...
paths, though it is over-represented in highly educated professionals. According to Gill Corkindale, writing for the Harvard Business Review, “Imposter syndrome can be defined as a collection of feelings of inadequacy that persist despite evident success. ‘Imposters’ suffer from chronic self-doubt and a sense of intellectual fraudulence that override any feelings of success or external proof of their competence. They seem unable to internalize their accomplishments, however successful they are in their field. High achieving, highly successful people often suffer, so impostor syndrome doesn’t equate with low self-esteem or a lack of self-confidence. In fact, some researchers have linked it with perfectionism, especially in women and among academics.”

Pauline Rose Clance, one of the authors of the groundbreaking 1978 study, developed a validated test for the presence of impostor syndrome. This test can be accessed at: https://paulineroseclance.com/pdf/IPTestandscoring.pdf. Subsequently, other researchers created additional screening tests. A systematic review of the literature published in 2020 by Bravata et al. stated that clinicians lacked evidence on the prevalence, comorbidities, and best practices for diagnosing and treating impostor syndrome and that its actual effects on professional performance and burnout both among healthcare professionals and others were not known. Consequently, the purpose of their study was to “critically evaluate the published literature on impostor syndrome—specifically to evaluate the prevalence of impostor syndrome in employed populations, characterize its relationship to workplace performance and burnout, characterize common comorbidities, and determine the most effective treatments for populations suffering from impostor symptoms.”

Few of the studies reviewed by Bravata et al. were designed to assess the prevalence of impostor syndrome, which was found to vary widely from 9 to 82%, largely depending on the screening tool and cutoff used to assess symptoms. When considering gender, thirty-three of the sixty-two articles reviewed compared the rates of impostor syndrome by gender. Sixteen of these found that women reported statistically significantly higher rates of impostor feelings than men, and 17 studies found no difference in rates of impostor syndrome. However, a reviewed study by Hutchins et al. found that men and women cope differently with their impostor feelings. The Bravata review article included six studies that compared the rates of impostor syndrome by age, half of which reported that impostor symptoms declined with age, but the other studies did not have this finding. Many articles included in the review explored psychological issues that were found to frequently co-exist with impostor syndrome, including depression, anxiety, low self-esteem, physical symptoms, and social dysfunctions. Importantly, among high school students, a history of prior suicidal ideation, suicide attempts, and depression were correlated significantly with impostor syndrome. According to Dr. Colleen Best, “Imposter syndrome can be self-reinforcing; doubt in one’s ability in one area can spread to others. Sometimes that becomes a self-fulfilling prophecy as we begin to look for evidence that this negative self-impression is correct.” Because human survival has always depended on the ability to recognize danger, a negativity bias exists that causes negative experiences, emotions, and thoughts to dominate over the positive. By being aware of this bias, a person can look more objectively at his or her performance. Five types of behaviors common to those with impostor syndrome were categorized by Dr. Valerie Young. They include perfectionism, expertize, soloist, over-achieving, and natural genius.

Perfectionists set excessively high goals for themselves, and then, when they fail to meet their standards, they experience self-doubt and anxiety about measuring up. Because these people have a high need for control, they may feel like if they want something done right, they have to do it themselves. This often leads to micromanaging, failure to delegate, and excessive rumination about deviations from perfection. Because of these impossible standards, perfectionists always believe they could have performed better. By finding minor flaws, they exert negative pressure on themselves rather than looking at the positive aspects of their experience. This leads to dissatisfaction and unhappiness.

Those that exhibit expertise behavioral traits measure their competence based on their knowledge base and skillset. Because there is an ever growing body of knowledge, they believe they will never know enough and have a fear of being exposed as inadequately prepared or unknowledgeable. They frequently seek out training or certifications to improve their skills in order to prevent their lack of knowledge from being discovered. If or when they are termed an expert, they feel anxious rather than proud. This can be particularly daunting for doctors who graduated many years ago.

Soloists prefer working alone and feel asking for help is a sign of weakness. They have the need to accomplish things on their own without assistance. If forced to ask for assistance with something, they will frame the request in terms of the needs of the patient, typically, rather than their needs as a person.

The behavior of over-achievers is to always feel that they have not done enough. They will underrate their own worth and feel inadequate compared to their colleagues. Because of this, they often push themselves to work harder and harder to assuage their insecurities. These people may become stressed when they are not working because they interpret downtime as unproductive. As a result, they abandon hobbies and passions as they strive to feel worthy by sacrificing themselves at work. This need for external validation is common among those with impostor syndrome. Unfortunately, the excess workload may harm not only their own mental health, but also their relationships with others.
Those with natural genius behaviors have been nurtured to believe they have unusual talent, and as a result, their internal standards are set very high. They believe they must complete the work perfectly on the first attempt or they feel a sense of failure. Because they judge their competence based on ease and speed as opposed to the quality of their results, they may experience shame if they struggle to master something. Those that have excelled without much effort in the past and were told by their family of origin that they were “at the top” can struggle with shame when they encounter difficulty. Because these people feel they should be able to excel on their own, they may dislike the idea of mentors and avoid challenges because it is uncomfortable to try something new that may be hard to accomplish immediately.

In human medicine, LaDonna et al. reported on physicians’ struggles with impostor syndrome, stating “Mistakes are ubiquitous in medicine; when confronted by error, physicians may experience anxiety, guilt, and self-doubt. Feedback may be useful for navigating these feelings, but only if it matches a physician’s self-assessment; self-doubt and the impostor syndrome are examples of inaccurate self-assessments that may affect receptivity to feedback. The impact of real or imagined underperformance on seemingly competent physicians is poorly understood.” They concluded, “Despite identifying coping strategies, feelings of inadequacy persisted for several participants; medicine was perceived to be a culture with few opportunities to share these struggles. Participants described that self-doubt, and the impostor syndrome were taboo subjects; confiding or seeking help for these feelings equated to ‘an admission of perceived weakness’. Even consultant participants who had found it ‘very helpful, and probably validating’ for a senior colleague to speak openly about these feelings often did not share their experiences with their colleagues or with their learners.” In addition, the authors shared that “Many participants—even those at advanced career stages—questioned the validity of their achievements; progressive independence and career advancement were variably experienced as ‘rising to the level of your incompetence.’” They concluded that even positive feedback could not buffer participants’ insecurities, which participants rarely shared with their colleagues, self-doubt affects clinicians variably at all career stages, and medical culture must create space for physicians to share their struggles if this issue is to be effectively combated.

Imposter syndrome can affect a veterinary career in important ways. It may prevent a veterinarian from applying to a job they are well-qualified for or prevent them from marketing their abilities to clients, causing lowered earnings. Doctors may turn down job offers, refuse to see certain kinds of cases, or even decide to leave the position they have due to the stress they feel. Some practitioners with impostor syndrome refer cases quickly and before they have done the work up/treatment that they are capable of. When a promotion or partnership buy-in proposal arises, they may feel inadequate and turn down these offers. All of these situations lead to missed opportunities, including those of gaining experience and becoming recognized as an “expert” by clients, colleagues, and perhaps most importantly, by themselves. Lower financial gain and lower professional status (always the associate, never the boss—and not by desire) eventually may affect workplace morale, motivation, and happiness. The end result may be a change of careers or simply burnout. When a person thinks about being insecure or being a fraud, this stimulates the amygdala in the limbic system, which controls mood and instincts. The amygdala then sends signals to the regions of the frontal cortex that analyze and interpret data, where the brain evaluates whether this data is accurate. If the data is perceived as concerning, the adrenal gland produces a release of catecholamines, especially norepinephrine and epinephrine, bringing physiological stress. When partnered with the normal stresses of a veterinary career, impostor syndrome-related stress can be a factor in negative physical and mental health outcomes. Risk factors for developing impostor syndrome include new challenges, such as an educational opportunity, new job, or career success. The person may feel undeserving of the new position or worried that they will be unable to perform adequately. Family environment can set a person up for later difficulty—a person growing up alongside a “gifted” sibling may internalize feelings of inadequacy that are not justified. Conversely, a person who always found it easy to excel during childhood may experience doubts when faced with a task that is hard to achieve later in life. Research also suggests that people from marginalized population groups may be more at risk, as well as those that have depression. The syndrome is common in the high-stakes and evaluative culture of medicine. Overcoming the challenges of impostor syndrome begins with self-awareness and understanding its prevalence among high achievers. In order to achieve the goal of becoming a veterinarian, external validation in the form of excellent grades and recommendations from established practitioners was required. After entering practice, one’s self-worth may be reflected by the fullness of the work schedule or the competitive success of the horses one treats. “If external sources comprise the entirety of one’s self-worth, that is a precarious position to be in. However, the more one’s locus of self-worth is internal, the less one has to look to others for validation and the less susceptible one is to impostor syndrome.”

Working with a therapist can be helpful in alleviating the condition of impostor syndrome. Cognitive behavioral therapy (CBT), an evidence-based intervention commonly employed by mental health professionals, has resulted in positive outcomes by deconstructing pathologic belief systems and dismantling negative behaviors. One particular exercise...
calls for generating mock responses from superiors such as: “I did not give you an extra week of vacation because I think you are tired ... I rewarded you for your outstanding work,” or “I don’t like you negating my opinions about your work when I give you positive feedback.” In this manner, CBT challenges the individual’s perceptions surrounding feedback and performance to bring about recognition of his or her true abilities. Group therapy can be especially effective, as other participants’ negative self-perceptions can often be clearly seen as unrealistic, prompting recognition that their own feelings of inadequacy are not substantiated by reality either. Some veterinarians appear to be addicted to the validation that comes from working, not to the work itself. When a person becomes more attuned to internal validation and able to nurture their inner confidence that states that they are competent and skilled, it is much easier to feel how much work is reasonable. It is also important to internalize the idea that there is no shame in asking for help when needed, either to learn a new skill or when the workload is overwhelming. Mentoring interns or younger colleagues, or volunteering, can be a great way to discover strengths and become more comfortable being in a position of expertise. When a person shares their knowledge, it not only benefits others but also helps that person mitigate their fraudulent feelings. To move past feelings of being an impostor, people must see themselves as a work in progress. Lifelong learning and skill-building continue for everyone, even the most confident and learned people. If impossibly high standards are an obstacle, doctors must identify specific, changeable behaviors that they can improve over time. It is much more productive to focus on honing specific skills than to avoid performing certain types of services that cause anxiety. Individuals experiencing impostor syndrome often perceive themselves as being the only one having these feelings, resulting in even greater isolation. In human medicine, it is considered that “referral to group therapy in which peers/coworkers discuss their feelings of doubt and failure might be particularly therapeutic. Clinicians and other high-achieving professionals may be reluctant to participate in such groups unless they are carefully designed to normalize and destigmatize impostor feelings and provide a safe environment in which to share experiences openly.” Networking groups of peers that provide a psychologically safe environment can be instrumental in allowing professionals to see that they are not alone in these struggles. Young equine veterinarians are increasingly participating in groups of this nature.

For students and new graduates, it is important that they own their own success and expect to stretch as they gain confidence with their skills while remaining cognizant of when they need to ask for help. Veterinarians of all ages should know that they are not alone with impostor syndrome. Group discussions can normalize feelings of impostor syndrome and blunt their impact. Understanding that everyone has feelings of inadequacy at times may allow doctors to be kinder to themselves. By redefining “failures” as “learning opportunities”, forgiving themselves for mistakes, and noticing all the important things they got right, practitioners can start to overcome their feelings of inadequacy. It can help to remember that “Emotions aren’t facts,” “You can’t know everything, nobody does,” and “Asking for help or guidance does not mean you’ve failed.” Mentors can intentionally steer conversations in these directions and repeat these phrases to reset mentees’ self-perceptions. Self-talk can be remarkably valuable. Questions that should be asked include the following: What are my core strengths? Am I trying to impress others with perfection? How much does the approval of others matter to me? Who am I really disappointing? And why? Additional recommendations include avoiding comparison to others, sharing feelings with colleagues, and seeking professional help if needed. Keeping a list of “firsts” or “accomplishments” for the year, such as new procedures, clinical skills, or diagnoses, can be confidence building. Learning to accept compliments and stop crediting success to other factors can be accomplished with the repeated deliberate practice of simply saying “Thank you” in response. In mentor/mentee relationships, participants should create discussion with others about things they do not know to help establish the “I’m not alone” feeling. Lastly, adopting a growth mindset can change outcomes. Carol Dweck, a psychology researcher, identified two different theories describing how malleable one’s personal characteristics (e.g., intelligence, musical ability, sociability) are. The first is “fixed,” a belief that one has a set amount of capacity in a given area, and it is not changeable; that talent is the main contributor to one’s success. The second is “growth,” a belief that hard work and dedication can lead to increased ability and capacity, with one’s talent simply providing a foundation. Dweck’s research has shown that adopting a growth mindset improves resilience and willingness to persevere in the face of failure. If people believe that they can grow and change, then failure stops being a threat to their sense of self. This decreases the power that impostor syndrome has to create fear and paralysis.

In summary, knowledge is power. Understanding the broad prevalence of impostor syndrome among all ages and genders, especially in high achieving professionals, can assist veterinarians in diminishing its effects. Adopting strategies for limiting the associated negative thoughts and behaviors can help individuals be more successful and satisfied in their careers.

Acknowledgments

Declaration of Ethics

The Authors have adhered to the Principles of Veterinary Medical Ethics of the AVMA.
Conflict of Interest
The Authors have no conflicts of interest.

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Personal Finance for Vet Students and Recent (or Not-So-Recent) Grads

Mindy C. Smith, DVM

Financial stress is a major contributor to the attrition rate of equine practitioners. Personal finance education can help improve the well-being of all veterinarians. This article will review the basics of budgeting, increasing your savings rate, approaching student loan repayment, and investing. Author’s address: 26904 County Road 13, Elizabeth, CO 80107; e-mail: drsmith@ironhorseequine.net. © 2021 AAEP.

1. Introduction
Equine veterinarians are an endangered species. In recent years, only about 1% of new grads enter equine medicine and about half fail to renew their AAEP membership within 5 years, which is an approximation of those leaving equine practice. The reasons are multifactorial, but financial stress ranks highly, especially for those with high student loan debt. Many prospective equine students are discouraged from entering the field because the salary is much lower compared to small animal medicine. Little time is devoted to personal finance in the formal curriculum. Most information is centered on the available loan repayment plans. Unfortunately, choosing one of these repayment options does not create a comprehensive financial plan, and without the right strategy, one may still face uncertainty about which approach is the right one. It is incumbent upon the individual to self-educate about personal finance, but it can be overwhelming to know where to start. The objective of this article is to provide actionable advice on how to approach finances for a veterinary student or recent graduate, but any veterinarian may find this information useful. The article will cover strategies to maximize your savings rate, approach student loan repayment, and invest. The core tenet of wealth building is simple: spend less than you earn and invest the difference.

2. Solution
Part I: Spend Less Than You Earn
It may sound patronizing to start with such a simple instruction as spend less than you earn. The reason this article starts here is because there are only two ways to increase your wealth: spend less money or earn more money. Spending less is an attractive starting place for several reasons: It can be implemented immediately without additional time or capital investment, there are tax advantages due to marginal taxation, it decreases the risk of being unable to pay for the unexpected, and it decreases financial stress when expenses are kept under control. Without intentional and optimized spending, money will flow out of your bank account and it is easy to fall victim to lifestyle creep. In lifestyle creep, discretionary spending increases with higher income and...
luxury items are falsely identified as necessities. The real danger of lifestyle creep is that as monthly expenses increase, you perpetuate the paycheck-to-paycheck cycle and the subsequent financial stress leads to poor well-being and burnout.

**The Budget**

To avoid these pitfalls, a budget is needed. The author suggests a zero-based budgeting method like ynab.com (You Need a Budget), but the process is similar for most budgeting software or excel spreadsheets.

**Step 1**: Calculate annual or monthly income after taxes. This is your take-home pay.

**Step 2**: List expenses by category. These include essentials (housing, transportation, loan payments, groceries, cell phone, insurance, clothing, child care, pet care, etc.) and non-essentials (dining out, spending money, gifts, vacation, entertainment, gym membership, charity, etc.). Don’t forget non-recurring expenses like car registration, home/auto maintenance or medical bills. A great way to remember all of your expenses is to categorize each line item in your bank or credit card statements and average them over several years.

**Step 3**: For each category, divide the annual expense by 12 months. Fund these categories monthly, even for line items that don’t recur each month like holiday spending. This helps prevent your budget from getting derailed by unexpected expenses.

**Step 4**: Calculate the savings rate. This is your income minus expenses. Savings should be assigned a job to be sure your money is working toward your goals.

**Step 5**: Analyze spending. Look for areas of over-spending or consistent underfunding. Eliminate spending that does not provide value (e.g., redundant subscription services or an unused gym membership).

**Step 6**: Set realistic goals. Choose areas to improve spending habits or increase saving, but don’t give up after the first slip up. Recognize and reward any progress made and forgive yourself or past mistakes.

When analyzing spending, it is likely to find that the biggest recurring expenses are housing, transportation, and groceries. For veterinarians, student loan debt may also rank high on that list. Another common realization is that there is nothing left to save after the monthly obligations are met. You may be living paycheck-to-paycheck with a zero percent or, in the case of consumer debt, negative savings rate.

**The Savings Rate**

The savings rate is your path to wealth and ultimately, retirement. Conventional wisdom recommends 10% of income be invested in retirement accounts to retire by age 65. This is a reasonable starting place, but retirement is not the only goal for which one needs to save. A larger savings rate is required to allow you to develop a reasonable emergency fund, save money for a down payment on a home, prepare for the potential tax bomb for individuals pursuing loan forgiveness, and contribute to a child’s college fund or leave money to charity. Growing the gap between spending and earning is the single most powerful tool to eliminate financial stress and build wealth. Consider the following scenario in which Dr. A has a $60,000 income with a 10% savings rate, Dr. B has a 20% savings rate, and Dr. C has a 50% savings rate (Fig. 1). This demonstrates that after a 40-year working career (with no increase in salary for simplicity), Dr. A has saved $240,000 dollars, Dr. B has saved $480,000, and Dr. C has saved $1.2 million. If this money is invested (more on this later), instead of kept in a savings account, the earning potential is far greater, and its buying power will not be eroded by inflation. However, not every dollar has to be invested in the market to be beneficial. For many, having 3–6 months of living expenses set aside can have tremendous psychological benefit. It will only take Dr. C 3 months to save 3 months of living expenses, compared to the 9 months it will take for Dr. A. These savings can also be earmarked for a house down payment, practice acquisition/start up or investment in real estate.

**Spend Less**

A key strategy to decrease spending is to pay yourself first. This means, that with every paycheck, deposit a predetermined sum into a separate account before paying any other bills or making discretionary purchases. Common bills and expenses can be reduced through creativity and flexibility (Fig. 2). If one of the ideas below is intriguing, then dive in with more research. The more options you take advantage of, the faster your financial situation will improve. Student loans require some additional thought, but the finer details of the available loan repayment options are beyond the scope of this article. If feeling out of your depth, consider using a
service like www.studentloanplanner.com for debt consultation. Generally speaking, if your salary is more than half of your loan burden, the total cost of the loan will be lowest if it is refinanced and paid back as aggressively as possible. If refinancing, any eligibility for loan forgiveness options provided to borrowers with federal student loans will be lost. Even if your debt-to-income ratio falls in the above category, you may still prefer an income-driven repayment program. The reasons for this may include your monthly payment remains much lower, you or your partner’s current job is not stable, legislation may change to allow partial or full loan forgiveness with or without tax forgiveness, the opportunity cost for paying your loan aggressively interferes with plans to save for a home, start a family or invest earlier, or the financial stress of a large monthly student loan payment is psychologically damaging. It may be equally psychologically damaging for some to have an outstanding loan balance for decades, so aggressive pay down may be the best option for those individuals. At the end of the repayment period, any forgiven loan balance will be taxable income. Prepare for this tax bomb by investing a portion of income; the student loan calculator at www.vin.com is an excellent resource to estimate potential tax burden. In the potential case where the tax bomb is eliminated through future legislation, this money can be used elsewhere. If you choose not to pay loans aggressively, it may be comforting to know that high student loan debt will not disqualify you from buying a home or financing a practice purchase, assuming you have a good credit score and little consumer debt (i.e., credit cards, car loan). Credit cards are not evil. When used responsibly, credit cards have many benefits including sign up bonuses, cash back rewards, fraud protection, free credit score access, 30-day interest-free loan on all purchases, and a huge positive impact on one’s credit score. An effective budget will prevent overextending yourself on purchases made with credit cards. Any current credit card debt should be paid off as the highest financial priority.

**Earn More**

Cutting expenses is a key part of any financial plan to prevent lifestyle creep and optimize spending. There is, however, an upper limit to how much can be saved: 100% of your income. Conversely, there is no upper limit to how much can be earned. Even as an equine veterinarian, there are plenty of ways to increase take home pay within and without the veterinary field (Fig. 3). The potential for increasing your earning is linked to your creativity and drive. It can be a powerful tool to grow the gap and increase investing potential.

**Part II: Invest the Difference**

Investing in the stock market is intimidating. It also carries risk. Fortunately, with a relatively brief education, even the least financially-inclined veterinarian can utilize it successfully to build wealth. One of the best strategies to maximize long-term earning potential is also simple and minimizes risk.

**Investment Strategy**

This strategy is to buy low-cost, broad-based index funds and hold them for decades. A broad-based index fund is comprised of hundreds or even thousands of publicly traded companies. The S&P 500 contains the top 500 largest companies in the United States, while a total stock market index fund contains nearly all ~3,000 publicly traded companies. Buying and holding index funds allows you to match the annual market returns because it essentially buys all of the market. A conservative estimate of the stock market return is 6–8% based on historical averages, although this does not guarantee future market performance. A buy-and-hold strategy does not attempt to time when to enter and exit the market, it doesn’t rely on individual stock picking or day trading, and it doesn’t panic at market volatility. Hold on to these
investments through high and low points in the market (they will come) because, since its inception, the market has continued to climb doggedly upward. To illustrate the wealth building potential of the stock market and the benefits of compound interest, recall Drs. A, B, and C (Fig. 4). If these doctors invest every dollar saved, the compound return on their investment makes each a millionaire. Dr. A invested only $500 per month, Dr. B $1,000 per month, and Dr. C $2,500 per month.

**Investment Accounts**

Regardless of the investment strategy used, all stock purchases are made within investment accounts. The government allows tax-advantaged accounts for specific uses such as retirement, health savings, or funding college. A taxable brokerage account is an investment account without any of these tax advantages. You may need to use a taxable account after reaching contribution limits for the tax-advantaged accounts. Each type of account has access to similar funds, though may be limited by what is offered in an employer retirement plan. Retirement plans fall into two broad categories: pre-tax and after-tax (Fig. 5). Pre-tax retirement accounts save on income tax in the year of the contribution, because they lower taxable income. For example, if your salary is $90,000 and you contribute $10,000 to a pre-tax retirement account, you will only pay taxes on the remaining $80,000 and avoid the 24% tax bracket that would have applied to income >$86,375. Withdrawals made in retirement are subject to income tax. Examples of these accounts include the 401(k) and the traditional IRA, as well as self-employed accounts like the i401(k), solo 401(k), and SEP IRA. The 401(k) is typically set up through an employer, often with a contribution match up to a salary percentage. Always take full advantage of an employer match as this is tax-free income and does not count against your contribution limit. After-tax retirement accounts are taxed the year of contribution but can be withdrawn tax-free in retirement. These accounts are especially advantageous when income taxes are low due to a lower salary. Examples of these accounts include the Roth IRA and Roth 401(k). Another advantage of a Roth IRA is the ability to withdraw contributions tax- and penalty-free at any time (because this money has already been taxed). This may make it an attractive account to keep a savings or emergency fund. It will be making more money than in a bank account (standard savings account typically pay something like 0.01% return and even high-yield savings accounts are only paying 0.5% return at the time of writing), but investing this money in the market is riskier than using an FDIC insured bank account. Be aware that contribution limits for after-tax accounts are lower than pre-tax accounts. One additional tax-advantaged account is worth noting here, the Health Savings Account (HSA). To qualify for this account, at least one family member must be enrolled in a qualifying high deductible insurance plan (most of these plans will have HSA in the name). An HSA allows tax-free contributions, tax-free growth, and tax-free withdrawals. Withdrawals can be made at any time to reimburse medical expenses even for family members not in a qualifying plan. You can choose to reimburse yourself decades later if you pay out of pocket initially. This makes the HSA an incredibly powerful wealth building tool. To get started with investing, choose a company and create an account. Many companies exist, but
Vanguard, Fidelity, and Schwab are all great options. After verifying your information, set up a bank transfer to fund the settlement account. This is a holding place for your money before making a purchase.

**Index Funds**

Each brokerage company will have access to similar stocks, but recall that low-cost, broad-based index fund investing is one of the best options. (If you develop a keen interest in investing, you may prefer to diversify your portfolio beyond index funds.) Let’s explore the advantages of index funds further. First, they conveniently let you buy hundreds of highly successful companies in a single transaction. Second, they are self-cleansing. This means that companies that perform poorly and go out of business, with returns falling to 0%, will be removed from the fund. Conversely, a successful company can have returns of 100%, 200%, 1000% or more. Third, when purchasing U.S. index funds, you will get international exposure, since many are international businesses. Finally, and this point cannot be overstated, they are inexpensive to buy or “low cost.” Specifically, they have low expense ratios. An expense ratio is the fee to buy any stock, based on operating costs. Low fees are so important because they cut into returns. Dr. B from the previous example is considering two different funds to invest in, the first has a 0.4% expense ratio which seems low, but the second has 0.04% expense ratio. The difference after 40 years of investing is over $275,000 (Fig. 6). Put another way, with $1,000,000 at a 0.04% expense ratio, $400 are lost to fees annually. At 0.4%, that number increases to $4,000. If using a financial planner or other actively managed fund with a 2% fee for assets under management on top of this expense ratio, $20,400 is lost to fees annually. If you still aren’t convinced about index funds, consider Warren Buffet’s recommendations to the trustee of his estate: “My advice to the trustee couldn’t be more simple: Put 10% of the cash in short-term government bonds and 90% in a very low-cost S&P 500 index fund. (I suggest Vanguard’s.) I believe the trust’s long-term results from this policy will be superior to those attained by most investors—whether pension funds, institutions or individuals—who employ high-fee managers.”

All stocks and funds have a ticker symbol, which is used to search when buying. You can do a Google search for each company’s funds: “Vanguard S&P 500” is VOO, “Vanguard total stock market index fund” is VTSMX or VTSAX. Although similar funds, VTSAX has a lower expense ratio (0.04% compared to 0.14% for VTSMX) but requires a $3,000 minimum investment. If that seems unattainable right now and you want to start getting money into the market sooner, choose “Vanguard total stock market ETF”, VTI (expense ratio of 0.03%), which can be purchased as individual shares (trading at $207.40 at the time of writing). Similar mutual funds and ETFs are available from Fidelity and Charles Schwab (Fig. 7). Buy index funds offered by the brokerage with whom you invest to avoid transaction fees.

**Bonds**

With stocks, you are buying part ownership of a company; the risk is higher, so the returns are higher (~8–10%). With bonds, you are loaning money to a company (or the government, in the case of Mr. Buffet’s short-term government bonds). Here, the risk is lower, so the return is lower (~4–6%). Wealth building potential is much higher with stocks, but bonds are less volatile. The main benefit of bonds in a portfolio is to smooth the ride and help you sleep at night if your risk tolerance is lower. Risk tolerance is highly individual, so no single proportion of stocks/bonds is right for everyone. To provide a framework, 100% stocks are appropriate during the early decades of your working career, and as you near retirement, you can increase your bond exposure, landing somewhere near 75% stocks to 25% bonds. For bonds, you can also buy a total bond market index fund (e.g., VTBMX).

To avoid the need to rebalance your portfolio as you age, you can buy a Target Retirement Fund (e.g., “Vanguard Target Retirement 2055 Fund” which assumes you want to retire in Year 2055). This will automatically increase your bond exposure as you get closer to your retirement year, but the expense ratio is higher (0.15%).

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<th>Expense Ratio</th>
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<td>Fidelity</td>
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<td>SWTSX</td>
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Fig. 6. The cost of expense ratios.

Fig. 7. Comparison of index funds from three brokerage companies.
3. Results and Discussion

No financial plan is immune to life circumstances or setbacks. These events are predictable in their unpredictability. The stock market has had long stretches of plummeting returns, which can rattle the most resolute investor. The worst response is to sell at the bottom, only to watch it inevitably climb beyond the prerecession peak. The equine veterinary profession is also experiencing very real challenges. The current decline of new equine graduates and attrition rate threatens our ability to adequately care for patients if the trend continues. The strategies discussed in this article provide groundwork to address some of the financial stress associated with this career and help equine practitioners thrive and contribute to the rebound of our profession.

Acknowledgments

Declaration of Ethics

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

Conflict of Interest

The Author has no conflicts of interest.

References


Additional Resources

www.avma.org/resources-tools/personal-finance
www choosefi.com
www jlcollinsnh.com
www paulmerriman.com
www studentloanplanner.com
www vinfoundation.org
www ynab.com
The New Paradigms Needed in Equine Practice

Amy L. Grice, VMD, MBA

With fewer new graduates choosing a career in equine medicine (less than 2% for the last decade) and significant numbers of equine veterinarians transitioning to other sectors or retiring each year, many practices are struggling to fill openings for associates. New paradigms of equine practice are needed to change these trends. Finding our way forward as an industry will require innovative ideas, flexibility, family-friendly cultures, shorter work weeks, better boundaries, capturing all revenue, improved efficiency, and higher fees. In addition, improved internships, collaborative emergency services, better support and mentoring for new graduates, client education, and competitive salaries will be needed. Fostering connection, minimizing injury, and modifying restrictive covenants are also important. Together, we can build a better, more satisfying life for equine practitioners. Author’s address: PO Box 192, Virginia City, MT 59755; e-mail: amyvmdmba@gmail.com. © 2021 AAEP.

1. Introduction

Equine practices are widely varied in size and scope. According to AVMA statistics, equine veterinarians are a small fraction (5.6%) of the total number of private practice veterinarians, representing 4,151 of 74,554.1 Females have become the majority, comprising 55.5% of equine veterinarians. Veterinary students are now 85% female as an average. In the membership of the AAEP, 44% are over the age of 50 years, of which 17.4% are 51–60 years, and 26.6% are greater than 60 years of age.2 The AVMA estimates that there will be about 60 equine DVM retirements per year, growing 3% per year. According to the 2016 AVMA AAEP Economic Impact Study, 52.8% of equine practices have two or fewer full-time equivalent veterinarians, whereas just 20.6% have six or more.2 With these demographic changes come challenges. One of the most stressful and alienating aspects of equine practice is the need to provide emergency care 24/7/365. The 2012 AAEP Owner Trainer Survey showed that one of clients’ top priorities in choosing a veterinarian is the availability of on-the-farm emergency care.3 This need contributes to high attrition in the field, perhaps because new female entrants often have more family responsibilities leading to more difficulty in providing after hours care. In the last 20 years, about 50% of new graduates that were AAEP members have let their membership lapse within five years of graduation. The number of new graduates entering equine practice has also been decreasing. From a high of 5.7% in 2003, this number fell to 1.0% in 2019 before rising to 1.4% in 2020.4 There is currently a shortage of veterinarians to fill available jobs in all sectors. Sixty-six percent of job postings have 0 applicants, according to AVMA figures.5 The companion animal job to applicant ratio is 0.0–0.5 for more than 50% of states. Salaries, work hours, and emergency service requirements are more attractive to many young graduates in the companion animal sector than the equine sector. Both
unemployment (0.8%) and underemployment are currently very low for veterinarians. Veterinary employment is projected to grow 19% percent from 2016 to 2026, much faster than the average for other occupations. Geographic maldistribution is likely to remain a problem in the profession, which could leave some areas underserved. As the U.S. population increases, pet ownership percentage forecast to remain stable, so demand for veterinarians will continue to increase. However, pet horse population numbers have fallen 61% in the last 5 years, according to the 2017–2018 AVMA Pet Owner Demographic Survey. These statistics illustrate some of the factors in the recent difficulties in attracting and retaining equine veterinarians in equine practice. A recent study indicated that equine veterinarians leave equine practice primarily due to lifestyle and number of work hours required, emergency on-call duty, and mental health and stress. New paradigms are necessary to mitigate these factors.

2. Discussion

Innovative thinking is necessary to relieve the pain points that equine veterinarians experience. Embracing necessary change requires flexibility and the willingness to try things that may be outside of one’s comfort zone.

Family-Friendly Culture

When young women attend college followed by veterinary school in the usual temporal fashion, they graduate with their DVM/VMD at the age of 28. Biological imperatives imply that starting a family (if this is what they desire) will be most successful if undertaken within the next several years. Within human medicine, a geriatric pregnancy is one that occurs anytime a woman is over the age of 35. Unfortunately, this timing coincides with the important years of a new graduate’s career. Owners of equine practices often must invest profit for a year or so to augment a new hire’s salary until they grow their gross revenue production sufficiently to support their compensation. If a new hire fails to build a client base quickly enough, profitability of the practice suffers. Practicing while pregnant can be dangerous, and women may wish to avoid some of their usual duties. Disability of pregnancy and delivery must be treated like any other disability and, when uncomplicated, lasts 6–8 weeks. Some women desire to take additional maternity leave or prefer to return to work on a reduced schedule initially. All of these factors can create short-term challenges at a practice, but good communication, flexibility, and genuine caring can increase loyalty and job satisfaction. Thinking through and having a collaborative plan in place for maternity leaves is smart business. The alternative is often losing talented associates permanently. Veterinarians with young families generally cannot work long hours each day, 6–7 days per week, as has traditionally been the norm in equine practice. Having a “hard stop” to the day is often necessary to allow children to be picked up at daycare or from after school activities. Household necessities such as grocery shopping and doctors’ appointments must be attended to. Many female veterinarians are married, with spouses that are employed full time and often not available for these responsibilities. Many of these young families do not have relatives living nearby to provide help for these needs. Practices that offer shortened work-days and work weeks will attract and retain associates more successfully. A 4-day work week with no routine appointments scheduled to begin past 3 or 4 p.m. can help provide the time that current associates need. To cover the needs of the practice, more veterinarians may be required, efficiency must be improved, or other steps must be taken.

Boundaries

The Gallup’s State of the American Workplace poll in 2017 revealed that 53% of employees say that a greater work-life balance is “very important” to them. Workplace boundaries can help veterinarians achieve and safeguard work-life balance and physical as well as mental health. These limits may have to do with how much or what kind of work a veterinarian is willing or not willing to take on (e.g., Will that doctor collect stallions or obtain spinal fluid in the field?) or what kinds of relationships they are willing or not willing to establish with colleagues and clients from work. Creating boundaries in practice can be tricky because there is the real worry of being fired by clients or receiving negative feedback from colleagues or an employer. Yet with clear communication, practice, and preparation, it can be done successfully. It is generally easier to set boundaries when first starting a job or a practice. “Start as you intend to continue” is good advice. It is much harder to change in midstream. When a person believes in the validity of their own personal boundaries, others typically will, too. The adage “you teach what you tolerate” is a true statement. There are three major types of work boundaries:

- **Physical boundaries** refer to the rules that define personal space and touch (e.g., hugs vs. handshakes) in the workplace.
- **Emotional boundaries** may cause a doctor to decline certain tasks at work (e.g., euthanizing a young healthy horse), and this type of barrier will not allow others’ attitudes to have influence, because it is seated in personal values.
- **Mental boundaries** refer to thoughts on matters in the workplace. For example, an opinion on the ethics of certain behaviors at work will not be swayed by someone else’s ideas of how things should go.

When considering and defining boundaries for an equine practice, it is important to consider the
number of hours each veterinarian should plan to work; under what circumstances and conditions they will need to work after-hours; how they will be referred to by clients and staff, and to which clients, if any, they will give their cell phone number. Setting boundaries for the practice will protect team members from burning out and/or finding themselves in uncomfortable situations that could have been otherwise avoidable. Setting boundaries is important because without them practice members will likely start to feel undervalued, underappreciated, disrespected, or worse. Examples of workplace boundaries could include the following:

- Expecting to be called Doctor Last Name by colleagues, clients, and staff;
- Saying no to scheduling routine appointments on the weekends;
- Decreasing availability by teaching clients not to expect team members to continue to check emails, texts, and answer nonemergency communication after work hours;
- Not answering nonemergency emails, calls, and texts after hours;
- Saying no to opportunities that, although enticing monetarily, or because they would enhance status, do not suit lifestyle needs;
- Utilizing paid time off when sick, needing a mental health day, having a funeral to attend, or wanting to take a vacation or go to a horse show;
- Turning down tasks that are not within the practice’s current professional capabilities; and
- Staying professional with colleagues, even if some of them are unlikable.

Dr. Erin Denney-Jones, AAEP Board member and solo practitioner in Florida, stated, “Better boundaries are needed to decrease burnout. When clients encourage veterinarians to become a ‘member of the family’, this makes boundaries much more difficult. ‘Family’ tend to expect discounts, which can lead to disappointment and feelings of betrayal when these clients move on to a new veterinarian.” In addition, moving away from being addressed as “Doctor” leads to disrespect, and always being available and giving full access to clients downplays the respected role of a veterinarian. Dr. Denney-Jones added, “Does your physician give you their personal cell phone number? Do you call them with routine questions on Sunday evening?” To improve boundaries, Dr. Denney-Jones carries two cell phones, one personal, and one professional.

Capturing Revenue

Not capturing all the revenue for the work done can have a marked effect on practice financial health. Failing to account for all services and dispensed supplies on invoices is common. Discounting is widespread. When all the revenue earned is not invoiced and received to offset expenses, the funds needed to pay competitive salaries may simply not be there. All discounting is not bad, but much of it is. Planned discounting that incentivizes behaviors that flatten out the seasonality of equine practice is much different from reactionary discounting. When bills are reduced for clients who complain or harangue or threaten, morale falls. The “good” clients that are grateful for the service pay full price without discussion. The unfairness of rewarding bad behavior affects everyone working in the practice. Special deals are also sometimes made with clients who are friends, or who insinuate that their veterinarian is “like family.” Disharmony can result among partners when profit leaks away from this type of behavior. Clients begin to perceive that fees are negotiable. When an associate charges the “list” price, and the practice owner gives a discount, the negative possibilities multiply. Discounts bleed away profit, leaving less money for compensation. Forming clear practice financial policies and then abiding by them has the power to increase clients’ and associates’ respect for the profession and the practice.

Efficiency

Equine ambulatory practice generally involves many hours driving from client to client, or farm to farm. This is not billable time, and as service providers, veterinarians have only their time to sell. Utilization of an assistant can make these hours more efficient. If the employee is the driver, the veterinarian can be preparing laboratory submission forms, finalizing invoices, looking up history for the next call, scheduling appointments, or making callbacks. If the doctor prefers to drive, a well-trained assistant can accomplish most of these tasks. For those doctors that are solo in the truck, driving time can pivot to personal time by visiting on the phone with friends or family, or listening to a favorite podcast. When driving time is productive, the workday shortens because there is less to do at the end of the day. Scheduling calls in geographically segmented areas on an unvarying routine basis (e.g., north on Tuesday, south on Thursday) allows less driving time, and sets expectations for clients as to when you will be in their region. For this to work best, Dr. Jim Zeliff, AAEP Board member and managing partner of Allegheny Equine, suggests having a “float doctor” to do daytime emergencies each weekday in a multi-doctor practice. By helping to keep the other doctors on schedule, they can finish at the time they anticipated. When doctors stop operating as silos, share the work, and limit the length of days by not scheduling any routine calls after 3 or 4 p.m., scheduled workdays can have a “hard stop” when the doctor is not on emergency duty. This allows veterinarians with young children to pick them up at daycare or from school at a predetermined time and creates space for life outside of practice. In some areas of the country, trailer ownership is common, and haul-in
facilities are the rule rather than the exception. This can markedly increase efficiency and productivity, but the facility must be utilized regularly to offset the additional costs involved in maintaining a physical building. In areas where ambulatory service at the farm is the cultural expectation, re-training of clients to a haul-in model should focus on the benefits that out-patient appointments at a central location will bring to them. This may involve less cost for haul-in appointments than for farm visits (by raising farm trip fees), access to more specialized equipment, more comfortable temperature-controlled surroundings, and other advantages. Providing emergency service limited to haul-in can be less onerous for equine veterinarians, as they can work in a well-lit, heated space with their supplies and equipment close at hand, and no driving time. Dr. Erica Lacher, a practice owner from Gainesville, Florida, has recently taken a different approach to emergency service by offering coverage to other veterinarians in the area. In order to increase efficiency, the practice is moving to a model where horses come to the veterinarian rather than the veterinarian to the horses. This model requires training clients to purchase a trailer or develop a transportation plan before an emergency occurs.

Fee Increases

Salaries in the equine veterinary field are substantially less than in the companion animal sector. Part of the reason for this difference is the inherent efficiency of having small animal patients booked in a central facility every 15–20 min and use of veterinary technicians for many tasks. To have compensation increase for equine practitioners, gross revenue production must increase. This could occur through working more hours, performing services with higher yields, or charging more robust fees. As equine veterinarians that are leaving equine practice cite long work hours as a primary factor, working more hours is not a solution. Concentrating services in an area of high value can result in higher wages, but these opportunities are not available to all equine doctors. General practice is still the most prevalent practice type, and most horses primarily need wellness and low-level acute care, rather than high-level diagnostics or surgery. As a consequence, higher salaries will need to come at least in part from fee increases. As equine veterinarians become more scarce (decreased supply) and the number of horses remains stable (stable demand), this will put increased upward pressure on prices. To have service, horse owners will have to pay higher fees. When one considers the cost of having an appliance repair person come to the house to repair a stove, suddenly the prices of the professionally educated doctor do not seem so high. Dr. Zeliff of Allegheny Equine remarked, “Most equines are no longer herd animals—they are pets, not livestock, so every horse should get a physical exam with vaccines. Fees need to increase. Equine veterinarians should be able to earn gross revenue sufficient to support a $100,000 salary in a 4-day work week. This will require educating clients, collecting fees at the time of service, and minimizing accounts receivable.”

Internships

As the numbers of veterinary students with an equine interest have diminished, opportunities for learning basic skills have also been depleted. With an increase in private referral hospitals across the United States, many universities now see mostly complicated cases that are not representative of general practice. Students must work hard to find circumstances where they can develop the basic skills that are essential for an entry-level equine veterinarian. Many employers do not have the time or temperament to mentor a new graduate this intensely. In addition, clients’ expectations have become increasingly sophisticated. Well-constructed internships may offer the best way for new graduates to acquire the skills they need for a successful career in equine practice. Unfortunately, some practices that offer internships have a business model that utilizes interns as low-paid technicians and provides little formal skill acquisition and mentoring in return. For internships to be the bridge to a great career in equine practice, there must be a mutually beneficial exchange where the practice takes pride in the mentoring relationship and the intern emerges with the skillset and confidence of a much more experienced practitioner. Systems must emerge that rank internships by their success in keeping equine interns in equine practice. Students must begin to seek internships in practices that will best position them for the career they want, not choose simply on the basis of name recognition or size. Seeking externships at smaller practices with internship programs may reveal better learning opportunities for general practice skills. Practices should consider offering weekly case rounds, journal club, and radiology rounds for all doctors to attend. Mentoring interns is a two-way interaction, as the newest techniques and medication uses at universities can be learned from new graduates. The objective must be a mutually beneficial arrangement, where interns receive as much in mentoring, skill acquisition, and experience as they give in time, money (in the form of reduced salary) and effort.

Emergency Service

Another primary reason for the exodus of equine veterinarians from the field is the burden of emergency duty. Being on call to attend emergencies can be stressful and exhausting, especially for small or solo practices. With more than half of equine practices having two doctors or less, it is not uncommon for equine veterinarians to be on call 50–100% of the time. After the arrival of children, this increasingly can become untenable for female veterinarians. Among larger ambulatory
practices that do not share emergency duty with other practices, the increased radii of service and high numbers of clients often lead to very busy on-call shifts, even when those shifts are reduced in number. Another not uncommon situation in larger practices is the inequitable sharing of emergency duty, with owners opting out entirely, or doing minimal shifts. Other negatives associated with emergency duty include not receiving additional compensation for emergencies, not having control over last-minute coverage changes, and being scheduled for excessive shifts compared to veterinarians with more seniority. Emergency cooperatives are increasingly filling the need for minimal scheduled days of emergency coverage, with small practices in a region banding together to share the responsibility of urgent care. Veterinarians of more recent generations seem to find the collaboration, cooperation, and comradery a positive addition to their lives, with minimal competitive drives. Doctors in these cooperatives have covered periods of maternity leave for each other, as well as stepped up when a colleague is ill or injured. Those that meet regularly also provide a tribe that can add a sense of belonging. Changes that can help mitigate the negatives of emergency duty include paying 100% of any collected emergency fee to the attending doctor, creating an equitable schedule on a quarterly, half-year, or annual basis that rotates in a regular pattern, rotating holidays equitably, and joining an emergency cooperative if one exists in your region. Charging robust emergency fees, refusing emergency service to nonclients, and giving a compensatory day off in the week following a weekend on emergency call are additional ways to sweeten the taste of emergency service. Dr. Michelle Egli of Delmarva Equine in Dover, Delaware, heads a corporately owned practice of four equine veterinarians, working a rotating four-day week out of three trucks. The rotating on-call schedule is shared with two local solo practitioners, with a total of five doctors in the rotation. Each veterinarian has a rotating weekday (Monday–Friday) on call each week, and works on call one weekend (Saturday and Sunday) in five. “When you’re off, you’re off”, said Dr. Egli. The practice cell phone is left at the practice on days off. The clients are bonded to the practice, not to a particular veterinarian, although each veterinarian sees certain clients routinely. The practice does a lot of reproductive work, and this too is shared among whichever veterinarians are working that day. These changes to traditional equine work life can allow associates to be much happier in their work.

Mentoring and Support to Reduce Burnout

In many long-established equine practices, the unwritten rule is to work as much as possible, always seeking to grow the practice by saying “yes” to all client requests as the default expectation. If an associate pushes back against this culture, they are sometimes told without words that “you don’t deserve to be here.” Young veterinarians can burn out from never saying “no.” The culture at some practices to never say “no” is most often intended to increase revenue and business growth. Young equine veterinarians are generally full of grit and determination, and they usually look up to the senior doctors as icons and seek their approval. Because of their work ethic, they will usually soldier on until one day they may simply find they are exhausted, depressed, and have nothing left to give. Although sometimes the advice given for overcoming burnout is to become more involved in outside activities, or giving back by volunteering, this really means doing even more! “Just do more” may not be the answer. With the gender shift in the profession, the majority of equine veterinarians are women. The responsibilities of family, children, and household often are theirs, layered on top of their career responsibilities. Keeping these talented doctors in equine practice often requires them saying “no” more often and doing less rather than more. Moving to a 4-day work week with shared emergency duty is a first step in reducing the stress that leads to burnout. Having the practice leaders (those senior doctors that the younger doctors respect and seek to please) model the way is important in building real change in the practice culture. Showing support for associates’ needs can help keep them in the profession. Humans are hard-wired to “belong,” and when veterinarians’ needs conflict with belonging to the traditional equine veterinary culture, they suffer. Culture change is imperative.

Re-Educate Clients

Increasing horse owner regard for the value of equine veterinarians is needed. The use of first names, the liberal sharing of personal cell numbers, and the 24/7/365 availability without boundaries has eroded some clients’ respect for equine veterinarians. Lay practitioners are frequently consulted by many owners, despite a lack of credentials. Veterinarians often inhabit a culture of scarcity, anxious that if they charge appropriate prices or erect professional boundaries their clients will abandon them. As one veterinarian stated,

We need to create a culture where equine veterinarians are respected. Bills need to be collected, on time and in full. Clients need to set up appointments to have medical questions answered. Veterinarians need to stop allowing clients to dictate their medicine. And they need to deal with their fear of losing clients. A bad client that doesn’t pay you is worse than not servicing that client at all. There are clinics out there that have a statement written in contracts that allows them to not pay the associate if money has not been collected from the clients. But then they allow clients to avoid payment and don’t allow associates to refuse service or fire those clients. So who gets punished here?

Changing the expectations of clients will require ongoing education. The value of an equine veterinarian has been eroded by many factors. Striving for a
high degree of professionalism, having appropriate boundaries, providing a high value for a fair and robust fee, providing compassionate care and honest communication, and adhering to strong ethical and moral values can elevate our profession to its former position over time.

Compensation
With educational debt at higher levels than ever, the average debt to income ratio (DIR) of equine veterinarians in the last decade is more than twice what is considered reasonable. Equine-oriented students have less debt than other students at the time of graduation, but the substantially lower entry level salaries create this untenable ratio. Although about 70% of new equine veterinarians have educational debt, they persist in following their dream of being equine practitioners despite the challenges. New models of compensation are necessary to keep them in the equine sector. With the passage of the CARES Act in 2020, employers were given the opportunity to pay educational debt of employees as a nontaxable benefit directly to the lender up to the amount of $5,250 through 2024. This is a deductible expense for the practice and is a good additional compensation to consider for every associate with student loans. With equine jobs far exceeding applicants, starting salaries have increased. The total acceptable cost of employing an associate equine veterinarian, including salary, benefits, payroll taxes, licenses, memberships, and continuing education, has risen from 25% to 30% of their gross revenue production. This means that practices are lowering their practice profits by investing in increased salaries to compete for associates. This will likely be followed by fee increases over time. However, new models are essential in navigating this challenge. One innovative model takes the approach that it is a veterinary team that produces results, so the team doctors should share the commission on shared revenue. If a less experienced associate can stay busy with health maintenance, general practice and cover daytime emergencies, allowing a more experienced doctor to concentrate on more lucrative specialty work, perhaps the combined gross revenue is apportioned to the team, rather than the individual. By creating learning opportunities for the newer doctor to acquire more skills with each year that enhance revenue production, the rising tide then raises both boats. This approach can be extended to the entire practice team, with a certain percentage of the practice profit being set aside to share with them. Each employee could have three measurable, attainable annual goals. If, at their annual review, they achieved all three, they would receive 100% of their piece of the profit. If they achieved just two, they would receive 70%. If only one, their reward would be 30% of their possible total share. When the practice owner shares ideas of where profit leaks from the practice (missed charges, discounts, improperly priced invoices, etc.), employees typically apply diligence to corrections. The financial results of these efforts may more than compensate for the profit sharing. Another action to take in increasing compensation for younger equine veterinarians is in facilitating practice ownership. Increasing numbers of young equine practitioners are leaving associate positions to open their own practices. This often increases their income substantially. With increased choice and control, the ability to set boundaries according to their own values and needs, and experiencing collaborative emergency coverage, many young veterinarians find this path to be much more rewarding than being an employee. If established practices wish to keep young doctors, they may need to offer them the opportunity to buy a meaningful share of the practice fairly early in their tenure. A token 5% is unlikely to have the desired effect, as the associate typically understands that the gesture is simply to lock them in place at the practice. Veterinarians interested in practice ownership want decision-making capability, increased income, and the ability to shape the practice in new ways. When ownership is held out as a possibility and then repeatedly postponed, offered as a tiny percentage, or the practice is then sold to a corporate entity, bitterness and departure is often the result.

Connection
With many newer graduates experiencing different pressures than their employers did when they were new to practice, young equine veterinarians often yearn for connection with their peers. Mental health concerns due to feeling overwhelmed by the competing demands of practice and family may exacerbate these stressors. As achievers, veterinarians are often perfectionists, and may find they cannot perform perfectly in all or any of the areas of their life, leading to distress. Mentoring by older, more experienced practitioners can be helpful for career advice, difficult cases, and interpersonal difficulties with coworkers or clients, but networking with others of their cohort has value in helping them feel they are not isolated or alone in their struggles. Mentors are available through the AAEP Outrider Mentoring program, and networking through groups such as Decade One. Extending these types of connection to veterinary students interested in equine practice may help to support their retention.

Physical Demands
Equine practice is physically demanding and dangerous. The British Equine Veterinary Association reported, following their study in 2014, that being a horse vet in the United Kingdom appeared to carry the highest risk of injury of any civilian occupation in the United Kingdom. Some young veterinarians may feel pressure from their employers not to ever refuse to work on a horse, may feel guilty if they feel fear, and may get into dangerous situations as a result. Associates need to
advocate for themselves and make their safety a priority. Because about 40% of equine practitioners are in solo practice, unless they return to work swiftly after injury they risk losing clients and essential income. Returning to work against medical advice is currently the norm among equine practitioners and may be a cultural imperative at some practices. To decrease both acute and chronic injuries, practices should foster safe work practices, utilizing veterinary assistants or technicians whenever possible, using and advocating for adequate sedation and restraint, and not setting a bad example by allowing doctors to continue working with fractured limbs or with a head injury. Personal protective equipment should be available (e.g., helmets and vests in recovery or collecting stalls) and its use encouraged or mandated.

Restrictive Covenants

New graduates entering equine practice are overwhelmingly female. Many are married, and their spouses relocate and take positions where their veterinarian wives are working, or vice versa. With two careers to manage, excessive noncompete clauses in an employment contract can contribute to veterinarians leaving the equine sector in order to avoid the need for relocation. Corporate practices often have the resources to litigate even minimal breaches of restrictive covenants. Litigation is very expensive—even getting a declaratory judgment from a judge to rule if the noncompete as written is valid or not valid can be beyond most young doctors’ means. This can trap an equine veterinarian at a practice where they are unhappy if they want to continue treating horses. Frank Muggia, an attorney specializing in veterinary practices, feels that keeping equine associates productive and happy is more successful in retaining staff than an onerous noncompete clause. Although many states have abolished noncompetes, as a protection that is not typically available in the marketplace, if a practice is in a state allowed to have one, it will be more defensible if it names specific barns or current clients than a mileage radius. Time frames longer than 6 months may be dismissed, as the judge is likely to determine that reestablishing goodwill should take no longer than that. Job seekers should be careful to negotiate restrictive covenants with their future in mind, and not sign those that they could not keep without leaving their chosen career.

3. Conclusion

If our profession is to prosper in the future, we must attract and retain dedicated equine veterinarians. New paradigms must evolve that allow our changing workforce to have the flexibility and support that they need while still keeping practices financially healthy. We must all contribute to bringing about the changes that will keep the equine veterinary industry viable for the future.
How to Protect Against Prepurchase Exam Malpractice Complaints

Nina Mouledous, DVM

Buyer complaints related to allegations of negligence when veterinarians perform prepurchase exams can be minimized by following recommendations that will be presented in this lecture. Author's address: AVMA, PLIT, PO Box 1629, Chicago, IL 60690-9850; e-mail: nina.mouledous@avmaplit.com. © 2021 AAEP.

1. Introduction

The most common and costly equine malpractice complaints observed by the largest veterinary malpractice program involve buyer complaints related to prepurchase examinations (PPEs). PPE claims are much more likely to go into a suit faster than the other types of equine malpractice claims. In addition, the indemnity provided in PPE settled claims and plaintiff verdicts have higher costs than most of the other types of equine claims and lawsuits. The majority of the claims involve performance horses, particularly of the Hunter/Jumper, Dressage, and Western disciplines and the complaints are related mostly to undisclosed radiographic abnormalities. Other complaints have been associated with missed heart murmurs, missed ophthalmic disease, missed surgical scars, and failure to recommend genetic testing. Certain drivers have been identified. Analysis of past claims shows that a majority of the PPE closed claims deal with lameness issues after the sale. A small percentage of the PPE allegations are related to cardiac, ophthalmic, and other uncommon abnormalities. After years of collecting data from PPE claims, the author has created tips on avoiding complaints and allegations of malpractice related to PPE claims to help educate equine practitioners. This presentation will focus on how to prevent PPE malpractice claims and will be reinforced by actual closed claims to support the recommendations. The goal of this presentation is to help practitioners reduce the incident of PPE complaints by adhering to the recommendations. The PPE has developed into a highly detailed experience not only for the practitioner, but also for the horse, seller, and buyer in many cases. Because of the highly litigious atmosphere today, veterinarians must be extra diligent when providing PPEs for clients.

2. Solution

First and foremost, a veterinarian should acknowledge and understand their role when asked to perform a PPE. The role can be established by acknowledging who the veterinarian is working for, the seller or the buyer. Veterinarians can greatly reduce their risks of complaints by avoiding conflicts of interest. A definition of a conflict of interest is a
situation in which a person or organization is involved in multiple interests, financial or otherwise, and serving one interest could involve working against another. Veterinarians should have a clear sense of who they represent and who they are working for when providing this service. If the veterinarian has a relationship with the seller, this is considered a conflict of interest and the safest course of action would be to decline the PPE and recommend another veterinarian. If there is no option to decline the exam, documenting that all parties waive conflicts of interest may be helpful but not “sue” proof. When a conflict of interest arises, such as if the veterinarian has a veterinarian–client–patient relationship (VCPR) with the seller and is performing a PPE for a buyer, veterinarians should obtain permission to disclose complete medical histories and preexisting conditions if they have knowledge of the horse’s background or access to the horse’s medical records. Even when the examining veterinarian does not have a VCPR with the seller and horse, if the horse is a patient of the practice and under the care of an associate, this situation could still be perceived as a conflict of interest by attorneys. This recommendation is an important one for all PPEs even if a conflict does not exist. If the seller is not compliant with this request, documentation of this omission should be entered into the PPE report. Taking on a PPE lawsuit where there is a conflict of interest is a plaintiff attorney’s dream. Even when the buyer has been informed of the conflict and even in the face of signed consent forms related to acknowledging a conflict of interest, these types of claims and lawsuits can be difficult to defend. In summary, PPE malpractice claims where a conflict of interest exists can be challenging to defend and therefore it is recommended to avoid these situations.

Secondly, documentation cannot be emphasized enough and could be a veterinarian’s only defense in the face of allegations of negligence. It is recommended that everything is documented, including buyer declinations of procedures, such as radiographs and drug testing. Document all communications between all parties involved including the seller, buyer, agent, and trainer such as phone call conversations, electronic communications, and verbal conversations. If the buyer’s primary veterinarian (PDVM) is involved, documenting communication between the PPE veterinarian and the PDVM is important, especially when the ultimate opinion lies with the PDVM. Having buyers initial all declinations of recommended tests or specific exams can be helpful in resolving disputes later if a client alleges that a certain test or exam was not offered. Ultimately, when a complaint involves an issue where the buyer declines a test or radiographs and then later denies that they were offered, if the declination is initialized by the buyer, this will provide proof that the conversation and declination occurred.

Another important step in the exam is to attempt to positively identify the horse, especially if the buyer is not present. This may seem awkward at times, so asking for a copy of the Coggins Test may be a good way to accomplish this task. All attempts to ensure accuracy of the age should be done through interpreting tattoos, checking registration papers, dental arcade exam, or microchips. Document the steps taken to accurately determine the age of the horse. PPE malpractice claims related to misidentification and improper aging have been reported.

Providing good quality imaging with complete radiographic studies is necessary. If the buyer declines a full set of radiographs, this declination should be clearly recorded in the report. Even if the practitioner opines that a full set of radiographs may not be necessary, the veterinarian should at least offer a full set and let the client decide. In many PPE claims, it never fails that the one joint that was not radiographed was the one with a significant lesion. If possible, obtaining additional documented opinions of image interpretations is optimal, preferable by a boarded equine radiologist. The PPE imaging allegations are most often related to poor quality of images that can be difficult to interpret by experts, missed radiographic abnormalities and not disclosed to the buyer, missed diagnosis, lack of radiography, incomplete radiological studies, and lesions on PPE films that developed into significant pathology that were originally opined as not significant. Examples from previous PPE malpractice closed claims were lucencies that develop into fractures and bone spurs that developed into bone chips.

Providing a comprehensive form or report to the buyer in a time frame agreed upon between the veterinarian and the buyer is necessary for a complete and comprehensive PPE. Too often, buyers are seeking results and opinions before the veterinarian can provide a complete report. Therefore, informing the buyer before the exam when to expect a full and complete report is important to avoid any miscommunication later. The PPE report should indicate that all systems have been examined, including ophthalmic, respiratory, neurologic, integument, especially for scars and the reproductive tract, if applicable. The report should also include all imagery including radiographs and ultrasound images, videos, and opinion statements. This is especially important with absentee buyers. Consider sending a hard copy via certified mail to officially document receipt by the buyer. If the report is sent via email, be sure to ask for acknowledgment of receipt. Many PPE negligent allegations are related to delayed receipt of or failure to send a final PPE report to the buyer. Again, it cannot be stressed enough that thorough documentation is important and could be the only proof that an exam of an anatomical area was performed. Recommend drug testing for analgesics, anabolic steroids, behavior altering medications and
breed specific genetic or inheritable diseases (hyper-
Kalemic periodic paralysis, hereditary equine regional
dermal asthenia). Assure blood samples are drawn
prior to any treatments such as sedation for
radiographs. If the buyer declines a blood test, be
sure they initialize the declination on the PPE
report. Consider providing pre-sale photography or
videotaping for conformation, size, body condition,
and serviceability. This documentation is
especially important for PPEs involving absentee
buyers.

Not all malpractice policies are the same.
Understanding the policy limits on one’s malpractice
policy is important and should be in line with the
value of the veterinarians’ patients’ fair market
value. When veterinarians secure or are renewing
their malpractice insurance policy, it is important to
insure they have adequate coverage for the monetary
value of the horses they are examining for
PPEs. And lastly, consider the use of PPE liability
release agreements. As the cliché goes, contracts
make it difficult for a defense and
provide a false sense of security. However, the
author opines that it won’t hurt, could be helpful for
defense, and may remind the buyer that the veteri-

narian should not be held liable for future issues or
unforeseen problems. Here is a link for an example
of a PPE liability release agreement, equine PPE
liability release: https://avma.avmaplit.com/acton/
attachment/43421/f-45296470-1be5-40b0-8953-93b551
ff41c/1/-/-/-/-/PrePurchase%20Exam%20Release%20
Form.pdf.

3. Discussion

Allegations of negligence associated with PPEs are
the most common cause of equine claims and account
for almost twenty percent of the total equine claims
reported. Over the years of handling PPE com-
plaints, the author has provided the above tips for
equine practitioners to educate them on how to mini-
mize PPE claims and lawsuits. Keep in mind that
many of the allegations are determined to be frivolous
once the complaint goes through a review process
with a veterinarian's insurance carrier and the claim
may be denied. Often, the buyer will accept this
review opinion and drop the claim, especially if they
are unable to support the allegations through expert
opinions. On the other hand, the buyer may pursue
the claim and file a suit at which time the veterinar-
ian’s insurance carrier should respond by assigning
defense council to the veterinarian. One downfall
that is often discovered too late is insufficient coverage
for an alleged loss. As a reminder, veterinarians
should check their malpractice policy limits and con-
firm they have enough coverage for the monetary
value of the horses they are examining for PPEs. It
is worth mentioning that veterinarians should know
the value of the horse beforehand. This can be an
awkward situation, because PPE guidelines recom-
mend that the veterinarian not inquire about the
price and should not use any findings in the exam to
aid the buyer in negotiations. However, if a year
down the road, an allegation is made that a practi-
tioner was below the standard of care when perform-
ing a PPE on a $150,000 Olympic hopeful, it is
important that the policy that was in place at the
time of the incident is sufficient to cover the market
value of the horse in case the claim will need to
be paid. The author supports the AAEP PPE
Guidelines and encourages veterinarians to refer to
the guidelines when performing PPEs. The guide-
lines outline important areas veterinarians should
adhere to such as medical records, identification, doc-
umenting abnormalities, informing buyers of the cli-

cial significance of the findings, documenting buyer
declaration of further testing, retaining records and
contact information for all parties involved, including
witnesses and maintaining PPE records for appropri-
tate time periods.

In conclusion, by avoiding conflicts of interest,
requesting disclosure of past medical records, main-
taining complete PPE records, exercising good com-
munication and documentation with all parties
involved, documenting all declines, offering and doc-
umenting additional testing, videotaping the exam,
properly identifying the horse and its age, providing
good quality imaging, seeking additional opinions of
imaging and abiding by the AAEP PPE guidelines,
veterinarians can greatly reduce their risk of PPE
liability claims.

Acknowledgments

Declaration of Ethics

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

Conflict of Interest

The Author has no conflicts of interest.

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Equine Hepatitis Viruses

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1. Introduction
Since 2011, four viruses have been described in the context of equine hepatitis. Two of these, Equine pegivirus (Pegivirus E) and Theiler’s disease associated virus (Pegivirus D), have since been shown to be nonpathogenic and unrelated to liver disease.1 Equine parvovirus-hepatitis (EqPV-H) has been revealed as the cause of Theiler’s disease and mild acute hepatitis, and Equine hepacivirus (EqHV) has been implicated in cases of mild acute and severe chronic hepatitis.

2. Equine Parvovirus-Hepatitis and Theiler’s Disease

History of Theiler’s Disease
A highly fatal form of acute liver failure occurring weeks after administration of equine antiserum for African Horse Sickness was first described by Sir Arnold Theiler in the early 1900s.2 Horses demonstrated acute or peracute onset of liver failure, with hepatic encephalopathy and sudden death as predominant clinical presentations. The syndrome has been observed periodically since then in many countries. Theiler’s disease most often occurs 4 to 10 weeks after administration of an equine-origin biologic product, such as an antitoxin or plasma. Tetanus antitoxin is the most frequently implicated product, likely because it is the most frequently used equine-origin biologic product.3–10 There are also many cases that occur without any history of equine-origin biologic product administration. Sometimes these are horses on the same farm as a biologic-associated Theiler’s disease case, and sometimes there is no history of biologic product use on the farm.2,11 These nonbiologic cases tend to occur from May through November and are often in small outbreaks that span a few weeks.11

Virus Discovery
The natural history of Theiler’s disease demonstrated that the hepatitis was both transmissible and contagious, and a viral cause was suspected. A novel parvovirus, EqPV-H, was discovered by next-generation sequencing of liver from a horse that died of Theiler’s disease and was first reported in 2018.12 Prospective case series showed that this new virus was detectable by serum or liver PCR in 27 of 28 Theiler’s disease cases.3,11 Eighteen of those cases were associated with administration of an equine-origin biologic product, including tetanus antitoxin (n = 12), equine plasma (n = 3), and allogenic stem cells (n = 3).3 Aliquots of the administered products were available for 9 tetanus antitoxin cases and 1 stem cell case, and all were positive for EqPV-H, confirming the route of infection.3 Ten of the cases had no history of equine biologic product administration, and 9 of these were EqPV-H positive.11 Herd-mates of horses with Theiler’s disease also often showed infection and subclinical hepatitis.11 EqPV-H was subsequently demonstrated to be hepatotropic and to cause acute subclinical to mild clinical hepatitis after experimental inoculation.13
Clinical Manifestations
Based on the findings in clinical Theiler’s cases, their herd-mates, and experimentally infected horses, it appears that EqPV-H infection causes hepatitis with a spectrum of severity ranging from subclinical to fatal. What causes an individual horse to be more severely affected is a topic of ongoing investigation. Hypotheses include variations in individual immune responses, inoculation dose, and a two-hit model where a second insult, such as hepatotoxic plant ingestion, could enhance viral replication or exacerbate pathology.

Theiler’s Disease
Also known as acute hepatic necrosis or equine serum hepatitis, Theiler’s disease is infrequent but often fatal. Affected horses develop acute liver failure that clinically manifests as icterus, edema, hepatic encephalopathy, and often rapid progression to death (Table 1). Some horses are simply found dead without preceding signs. Clinical pathology indicates both hepatocellular injury, with high aspartate aminotransferase (AST), sorbitol dehydrogenase (SDH), and glutamate dehydrogenase (GLDH), and biliary damage or cholestasis, indicated by high gamma glutamyl transferase (GGT); Table 2. Theiler’s cases typically also show functional deficits, as evidenced by high bile acids. Histopathologic examination of affected horses shows diffuse centrilobular hepatocyte necrosis with variable inflammatory infiltrate, biliary reaction, and vacuolar change in surviving hepatocytes.5,9,10,14

Mild or Subclinical Hepatitis
Monitoring of herd-mates in Theiler’s disease outbreaks has shown that there are often subclinical or mild hepatitis cases alongside the overt Theiler’s disease cases.5,11,15 Some cases have also been identified as incidental findings on screening bloodwork or as mild clinical cases without more severely affected horses on the farm. Mild cases typically exhibit icterus, inappetence, and quiet or dull demeanor, although any signs on the spectrum between subclinical and fatal disease can be observed. Fever has not been observed in subclinical and mild clinical cases.13 Clinical pathology in these cases is a less severe version of what is seen in Theiler’s disease, with mixed increases in hepatocellular enzymes and induction enzymes (Table 2). Histopathology shows individual hepatocyte necrosis with variable lymphocytic infiltrate. In moderate cases, bile ductular reaction can also be observed.

Diagnosis
In classic Theiler’s cases of fulminant hepatic necrosis, a simple serum or liver PCR in combination with history, clinical signs, clinical pathology, and histopathology is generally sufficient to establish the diagnosis. In milder or subclinical cases, the diagnosis is not as straightforward. Experimental infections demonstrate that EqPV-H has a prolonged incubation phase of 5 to 8 (median 6.5) weeks, where the horse is viremic before the onset of hepatitis.13 Hepatitis coincides with peak viremia and a decline in viral titer; however, after the acute infection and hepatitis resolve, many horses remain persistently low-level serum PCR positive for months to years.12,13,15 Additionally, the prevalence of viremia in apparently healthy horses is around 13%.12 Therefore, there are many EqPV-H serum PCR positive horses where the virus is not actively causing hepatitis. Additionally, horses have seroconverted and often reached peak antibody titers by the time hepatitis develops, making paired serology unlikely to aid in diagnosis.13 This means that accurate diagnosis of EqPV-H as the cause of hepatitis presents similar diagnostic challenges as for equine protozoal myelopathy and Lyme disease, where a single positive serum test does not neces-

Table 1. Clinical Signs of Hepatitis

<table>
<thead>
<tr>
<th>Icterus</th>
<th>Pigmenturia (from direct bilirubin)</th>
<th>Weight loss</th>
<th>Photosensitization</th>
<th>Respiratory distress (laryngeal paresis)</th>
<th>Ventral edema</th>
<th>Colic, gastric impaction</th>
<th>Hepatic encephalopathy:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Yawning</td>
<td>Playing in water bucket</td>
<td>Central blindness</td>
<td>Head pressing</td>
<td>Ataxia</td>
<td>Circling</td>
<td>Yawning, Dullness, Seizures</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Playing in water bucket</td>
<td></td>
<td>Ataxia</td>
<td>Ataxia</td>
<td></td>
<td>Sudden death</td>
</tr>
</tbody>
</table>

Table 2. Clinical Pathology Values Observed in Horses with Equine Parvovirus-Hepatitis Infection, Which Developed Fulminant Hepatic Necrosis, Also Known as Theiler’s Disease, Associated with Administration of an Equine-Origin Biologic Product (Biologic), or without Such History (Nonbiologic), and in Horses Experimentally Infected with Equine Parvovirus-Hepatitis, Which Developed Subclinical or Mild Clinical Hepatitis

<table>
<thead>
<tr>
<th></th>
<th>Biologic3</th>
<th>Nonbiologic11</th>
<th>Experimental13</th>
<th>Reference intervals</th>
</tr>
</thead>
<tbody>
<tr>
<td>AST (U/L)</td>
<td>1,187 (770–3,426)</td>
<td>2,925 (1,239–6,177)</td>
<td>542 (310–1,068)</td>
<td>222–489</td>
</tr>
<tr>
<td>GGT (U/L)</td>
<td>134 (68–314)</td>
<td>116 (97–185)</td>
<td>49 (15–233)</td>
<td>8–33</td>
</tr>
<tr>
<td>Total bilirubin (mg/dl)</td>
<td>15.1 (9.6–24.3)</td>
<td>20.1 (8.7–21.7)</td>
<td>1.9 (1.4–4.3)</td>
<td>0.5–2.1</td>
</tr>
<tr>
<td>Bile acids (μmol/L)</td>
<td>128 (111–171)</td>
<td>90 (76–176)</td>
<td>11 (6–148)</td>
<td>2–10</td>
</tr>
</tbody>
</table>

Reference intervals shown are from the New York State Animal Health Diagnostic Center. Tests for the Theiler’s Cases were performed at multiple laboratories, and laboratory-specific reference intervals varied. Median and ranges are shown.
necessarily indicate the agent is actively causing disease. Approaches to improve diagnostic accuracy could include paired serum PCR to observe declining titers and advanced histopathologic imaging such as in situ hybridization to demonstrate viral infection in affected hepatocytes (Fig. 1), thereby linking the virus to the pathology.

Transmission
EqPV-H is known to be transmitted iatrogenically through equine-origin biologic products. Therefore, care should be taken when using and preparing products such as allogenic stem cells and whole blood transfusions to ensure that the donor animal is EqPV-H serum PCR negative. The United States Department of Agriculture has issued a notice requiring that all commercial equine serum and plasma companies ensure their products are free of EqPV-H. There is no evidence to date of vertical transmission.13 However, EqPV-H has moderately high population prevalence of 13% in Coggins samples,12 which can be much higher on individual farms.11 This high prevalence suggests a natural mode of horizontal transmission. Limited data suggests oral transmission is possible; however, a seasonal distribution in Theiler’s cases limited to the summer and fall suggests insect transmission is more likely.11,13 This is an open area of investigation.

Treatment and Prevention
As of now, there is no known treatment for EqPV-H, aside from supportive therapies for liver disease. Antiviral drugs targeting parvoviruses have not been developed, and therefore direct antiviral treatment is unlikely to be developed. Also, given the rapid progression of Theiler’s disease, and severity of necrosis already present at the time of diagnosis, it is unlikely that an antiviral therapy would be helpful. Therefore, efforts should focus on prevention. Equine-origin biologic products should be confirmed EqPV-H PCR negative before administration. Otherwise, there is no known strategy to prevent horizontal transmission at this time. Effective vaccines have been developed for other parvoviruses, e.g., canine parvovirus, and this could be a possibility in the future.

Summary
EqPV-H is known to be a cause of acute to subacute hepatitis that can range in severity from subclinical to fatal. Although serum can remain PCR positive for many months to years, chronic parvoviral hepatitis has not been observed. Serum and liver PCR and in situ hybridization can be used to diagnose infection, but results must be interpreted in light of other findings.

3. Equine Hepacivirus and Acute and Chronic Hepatitis

Virus Discovery
In 2011, a group discovered EqHV in samples from dogs by using unbiased high-throughput sequencing to characterize the viral flora of companion animals.16 The virus was initially named Nonprimate hepacivirus.16 Subsequent serologic screening of multiple species identified horses, rather than dogs, as the primary host species,17 and it has since been renamed Equine hepacivirus and classified as hepacivirus A.18 EqHV is genetically the closest homolog of human hepatitis C virus (HCV) discovered to date. Multiple experimental and natural infections have demonstrated that EqHV is hepatotropic and pathogenic in horses.19–24 EqHV is ubiquitous in horses, with 2% to 35% viremia and 22% to 84% seroprevalence in 7 countries across 6 continents.17,19,21,22,24–36

Clinical Manifestations
As seen with HCV in people, two outcomes are observed with EqHV infection: horses either clear the virus associated with mild hepatitis, or they become persistently infected and remain viremic for > 6 months.24,37 One horse has been documented to remain viremic for at least 12 years without hepatitis.24 There is some evidence that horses that are infected at < 8 months of age might be more likely to develop persistent infection.22,23

Acute Resolving Infections
Experimental infections result in biochemical and histopathologic evidence of hepatitis (Table 3), but disease is mild, and there have been no clinical signs observed in infected horses.20,23,37 Elevations in functional markers, such as direct bilirubin and bile acids, are rarely observed. Histopathologic findings include normal liver or lymphocytic infiltrate and
scattered individual hepatocyte necrosis. Ductular reaction is typically absent.

Chronic Hepatitis

Persistent infection with HCV in people is a major cause of chronic hepatitis, cirrhosis, and hepatocellular carcinoma.38 A form of chronic EqHV-associated hepatitis is less well documented; however, there is increasing evidence that this can occur. Recent case reports have demonstrated persistent EqHV infection associated with chronic hepatitis in two horses.39,40 One case presented with weight loss and the other with fever, anorexia, and weight loss. The first horse showed primarily an elevation in hepatocellular enzymes (AST) and survived at least 15 months in stable condition.39 The other showed more predominant elevation in GGT as well as increased bile acids and was euthanized at 15 months due to declining condition.40 Histopathology was performed, and the horse had severe micronodular cirrhosis with bile duct hyperplasia and portal inflammatory infiltrate.40 Further delineating the incidence and clinical findings of EqHV-associated chronic hepatitis cases is a field of ongoing investigation.

Diagnosis

As with EqPV-H, determining whether EqHV is the cause of a particular case of hepatitis presents diagnostic challenges and is an open area of investigation. Because of the high prevalence of EqHV viremia, and because horses can remain viremic for many months to years without hepatitis, a single positive serum PCR cannot determine whether EqHV is the cause of disease. In cases of mild acute hepatitis, clearance of viremia and resolution of the hepatitis within 1 to 4 months37 would be consistent with EqHV as the cause of disease. In cases of chronic hepatitis, ruling out other causes, such as bacterial infection or exposure to toxins, and demonstrating persistent EqHV viremia for at least 6 months would increase the likelihood that EqHV is the cause of disease. PCR findings should also be assessed in the context of liver histopathology.

Transmission

As with EqPV-H, EqHV can be iatrogenically transmitted through contaminated equine-origin biologic products, such as plasma.21,23 EqHV can also be vertically transmitted in utero.21,41 However, the high prevalence across many countries indicates an efficient method of horizontal transmission. For the related virus, HCV, mosquito transmission has been suggested,42 although it is not widely accepted as a major route of transmission. EqHV has not been detected in mosquitoes to date.33 This is an area of ongoing investigation.

Treatment and Prevention

There is no available treatment or prevention for EqHV at this time. Unlike for paroviruses, there are many effective direct acting antivirals that have been developed for HCV, which might have efficacy for EqHV. At least one, sofosbuvir, is predicted to bind EqHV by computer modeling.43 However, there will be significant development and cost burdens before these medications become available for horses as they are mostly all high cost and of limited availability, even for people. The best approach to prevent transmission currently is to ensure that equine-origin biologic products are PCR negative before administration; however, there is no regulation by the United States Department of Agriculture for this virus at this time. There is also no known means of stopping horizontal transmission since the route is unknown. Vaccines developed for HCV have had disappointing efficacy; therefore, a successful vaccine for EqHV might not be feasible, and efforts will more likely focus on treatments.

Summary

EqHV is a highly prevalent virus that apparently can cause both mild acute hepatitis and severe chronic hepatitis. The incidence and clinical characteristics of EqHV-associated chronic hepatitis need further study. Serum and liver PCR can be used to diagnose infection, but results must be interpreted in light of other findings.

4. Summary

There is a lot of work ongoing to further delineate the epidemiology, diagnosis, treatment, and prevention of both viruses. Based on current knowledge, EqPV-H and EqHV are causes of hepatitis in horses. Both viruses should be considered as differentials for cases of mild acute to subacute hepatitis with evidence of primarily hepatocellular or mixed hepatocellular and induction enzyme elevations and histopathologic findings of lymphocytic infiltrate and/or individual hepatocyte necrosis, with or without biliary ductular reaction. EqPV-H can also cause severe acute hepatic necrosis, also known as Theiler’s disease. EqHV might be a cause of chronic hepatitis characterized by persistent infection and persistent elevation in hepatocellular or mixed hepatocellular and induction enzyme elevations. Histopathologic findings of fibrosis and lymphocytic infiltrate could be consistent with chronic EqHV hepatitis.

Table 3. Clinical Pathology Values Observed in Horses with Acute Resolving Equine Hepatitis Virus (EqHV) Infection

<table>
<thead>
<tr>
<th></th>
<th>Experimental EqHV</th>
<th>Reference intervals</th>
</tr>
</thead>
<tbody>
<tr>
<td>AST (U/L)</td>
<td>511 (402–691)</td>
<td>222–489</td>
</tr>
<tr>
<td>GGT (U/L)</td>
<td>23 (19–51)</td>
<td>8–33</td>
</tr>
<tr>
<td>Total bilirubin (mg/dl)</td>
<td>1.8 (1.3–3.2)</td>
<td>0.5–2.1</td>
</tr>
<tr>
<td>Bile acids (μmol/L)</td>
<td>7 (5–12)</td>
<td>2–10</td>
</tr>
</tbody>
</table>

Horses remained subclinically affected. Median and ranges are shown.
Acknowledgments

The Author acknowledges Drs. Gerlinde Van De Walle, Thomas J. Divers, Charles M. Rice, Troels K. H. Scheel, Mason Jager, Brad Rosenberg, and Amit Kapoor and many co-authors for their mentorship and collaboration on these equine hepatitis virus studies.

Declaration of Ethics

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

Conflict of Interest

The Author has no conflicts of interest.

Support for Research Performed

The Author has received support from the following: Agriculture and Food Research Initiative Competitive Grants 2016-67015-24765 and 2020-67015-31297 from the USDA National Institute of Food and Agriculture, the Jack Lowe Equine Health Funds/Mollie Wilmot Equine Research Fund, the National Institute of Allergy and Infectious Diseases of the National Institutes of Health under Award K08AI141767, the Harry M. Zweig Memorial Fund for Equine Research, and Boehringer Ingleheim Vetmedica, Inc. 2016 Advancement in Equine Research Award. The text was not reviewed by any sponsor prior to submission.

References


EMERGING AND RE-EMERGING INFECTIOUS DISEASES

Equine Coronavirus

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1. Introduction
Coronaviruses are single-stranded, positive-sense, non-segmented, enveloped RNA viruses belonging to the Coronaviridae family and the following 4 genera defined based on serological cross-reactivity and genetic homology: Alphacoronavirus, Betacoronavirus, Deltacoronavirus, and Gammacoronavirus.1 Equine coronavirus (ECoV) is classified within the Betacoronavirus 1 genus, along with human coronaviruses OC43, 4408, and HKU1; bovine coronavirus (BCoV); porcine hemagglutinating encephalomyelitis virus; canine respiratory coronavirus; mouse hepatitis virus; bubaline coronavirus; and sialodacryoadenitis rat coronavirus.2 Horses appear to be susceptible to the human severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) based on the high homology to the ACE2 receptor;3 however, there are at the present time (January 2021) no data documenting antigen or antibody detection to SARS-CoV-2 in equids. The only data available are from the closely related Middle East respiratory syndrome coronavirus (MERS)-CoV. The various studies have shown conflicting results, with one study documenting high seroprevalence in equids from endemic areas and another study reporting that experimentally infected horses developed no clinical signs, showed minimal viral shedding, and did not seroconvert.4,5

2. Clinical Presentation
Almost 12 years ago, a research group investigated an unusual outbreak of fever and enteric signs in 2- to 4-year-old racing draft horses in Tokachi, Hokkaido, Japan.6 It is of interest to note that enteric signs were only reported in 10% of the horses, and a total of 132/600 horses (22%) became diseased. The same racing venue experienced one additional outbreak with similar signs three years following the first outbreak.7 Additional outbreaks have since been observed and reported in the United States and Europe.8–11 Collectively, these outbreaks have been able to refine the clinical presentation of ECoV, one that is still perplexing considering the inconsistent development of enteric signs. Clinical information collected from various outbreaks involving 406 horses showed that 122 horses (30%) showed clinical signs.8 The main clinical signs reported were anorexia (98%), lethargy (89%), and fever (84%). The rectal temperature of febrile horses ranged from 101.5 to 105.8°F (median 103.8°F). Changes in fecal character, ranging from soft-formed to watery consistency, and colic were observed in 25% and 18% of horses, respectively. Signs of encephalopathy, including circling, head pressing, ataxia, proprioceptive deficits, nystagmus, recumbency, and seizure, have occasionally been reported in ECoV-infected horses.8,12 Although clinical disease is apparent in most ECoV-infected horses, one needs to consider that some horses remain subclinical after infection. Subclinical infection is defined as a lack of clinical disease in a horse from which ECoV is detected in feces by quantitative PCR (qPCR).11,13
3. Laboratory Diagnosis

The ante-mortem diagnosis of ECoV relies on the presence of clinical signs compatible with ECoV infection, abnormal cell blood count, the exclusion of other infectious causes, and molecular detection of ECoV in feces. The consistently observed hematological abnormalities observed with ECoV infection are leukopenia due to neutropenia and/or lymphopenia. Additional, less consistent hematological abnormalities included the presence of band neutrophils and shifts in monocyte counts. Occasional rebound leukocytosis due to neutrophilia and monocytosis during the disease course and recovery can be observed as well. Complete blood cell count (CBC) abnormalities are expected to resolve within 5–7 days as long as no complications associated with the disruption of the gastrointestinal barrier occur. However, both the CBC and white cell differential can be unremarkable in clinically infected horses. Biochemical parameters may be unremarkable, but elevation of total and indirect bilirubin due to partial or complete anorexia, electrolyte changes consistent with enterocolitis, transient elevation of liver enzymes, and renal parameters suggested of pre-renal azotemia have been observed in some of the cases. It is judicious to measure blood ammonia in horses with suspected ECoV infection and concurrent signs of encephalopathy. Fielding and collaborators reported on a case of severe hyperammonemia (677 μmol/L; reference interval, ≤60 μmol/L) with encephalopathic signs that subsequently died. Hyperammonemia associated with ECoV infection is likely due to increased ammonia production within and absorption from the gastrointestinal tract due to gastrointestinal barrier breakdown. An increase in enteric ammonia production could also be the result of bacterial microbiome changes associated with ECoV infection. Historically, coronavirus detection in feces has been based on negative-stain electron microscopy (EM) and antigen-capture enzyme-linked immunosorbent assay (ELISA). However, the sensitivity and specificity of these diagnostic modalities have not been evaluated, and detection may be unsuccessful if viral particles are not present in sufficient numbers. Sensitive laboratory diagnosis of ECoV is through fecal qPCR. A recent study evaluated the overall accuracy of qPCR and determined 90% accuracy between clinical status and PCR detection of ECoV in various outbreak populations. The author has documented a few cases of ECoV infection that tested qPCR negative during early disease. These few horses ended up testing qPCR positive on a 24- to 48-hour recheck fecal sample. It is hypothesized that during peracute stages of infection or when diseased horses experience gastrointestinal stasis due to colic, there are not enough viral particles in the feces to be detected. Peak viral shedding is observed on day 3 to 4 after the development of clinical signs, and qPCR detection of ECoV in naturally infected horses generally lasts 3–9 days but can occasionally extend up to 25 days from onset of clinical disease. As with many viral infections, viral kinetics are likely influenced by viral strain, age of patient, and comorbidities. The role of subclinical shedding during an outbreak cannot be ignored, as 4% to 83% of healthy horses have been shown to test qPCR positive for ECoV in their feces. Such horses act as a source of infection and actively contribute to viral spread.

4. Treatment and Prevention

Most adult horses with clinical ECoV infection recover spontaneously in a few days without specific treatment. Horses with persistent elevated rectal temperature, anorexia, and lethargy are routinely treated with nonsteroidal anti-inflammatory drugs for 24 to 48 hours, as long as their hydration status is believed normal. Horses with colic, persistent lethargy and anorexia, and/or diarrhea have been treated more intensively with fluid and electrolyte per nasogastric intubation or intravenous administration of polyionic fluids until clinical signs have resolved. Additionally, antimicrobials and gastrointestinal protectants should be considered in horses developing signs of endotoxemia and/or septicemia secondary to disruption of the gastrointestinal barrier. Although hyperammonemia-associated encephalopathy only occurs in a small percentage of horses with ECoV infection, early recognition and treatment are associated with a positive outcome. Specific preventive measures are scarce, and there are yet no licensed vaccines against ECoV. Due to the close genetic homology of ECoV with BCoV, serological responses to BCoV vaccines have recently been investigated. One study used a killed-adjuvanted BCoV vaccine in six healthy yearling horses and reported a measurable serological response in all horses following the administration of two vaccines given 28 days apart. A second study investigated the safety, humoral response, and viral shedding in horses inoculated orally, intranasally, or intrarectally with a commercially available modified-live BCoV vaccine. The results of that study showed that the modified-live BCoV was safe to administer to horses via various routes, caused minimal virus shedding, and resulted in detectable antibodies to BCoV in 27% of the vaccinates. Collectively, these two BCoV vaccines, although showing measurable antibody responses to BCoV, cannot be recommended at this time due to the lack of efficacy data. The cornerstone of ECoV prevention resides in strict biosecurity measures aimed at reducing the risk of introducing and disseminating ECoV on equine premises. It is important to be vigilant when working up horses presenting with fever, anorexia, and lethargy, with or without concurrent enteric signs. Such horses should be isolated until ECoV, as well as other potential infectious pathogens, has been ruled in or out. ECoV qPCR-positive horses should be isolated and stable- or herd-mates closely monitored until the outcome of past exposure has been determined. Outbreaks of ECoV are
generally short lasting, especially when strict biosecurity measures have been followed, and quarantine can routinely be lifted 2–3 weeks after the resolution of clinical signs in the last affected horse. ECoV is susceptible to common disinfectants, including sodium hypochlorite, povidone iodine, chlorhexidine gluconate, phenols, quaternary ammonium compounds, accelerated hydrogen peroxide, and peroxynitrate compounds. However, it is still unknown how long ECoV remains infectious in the environment.

Acknowledgments

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

Conflict of Interest
The Author has no conflicts of interest.

References
Novel Rickettsial Species Causing Equine Neorickettsiosis

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1. Introduction

Neorickettsia spp. are Gram-negative, obligate intracellular bacteria. This bacterial species is widely distributed in nature and can be found in a variety of trematode species, including Fasciola hepatica, the liver fluke of sheep, cattle, and humans, and hosts of unknown trematodes worldwide. The life cycles of the digeneans are complex and in their developmental cycle stages they can parasitize different host species. The Neorickettsia bacteria infect the fluke along the developmental stages of their life cycle and are therefore vertically transmitted. Neorickettsia are unique among the Family Anaplasmataceae because both vertical transmission and horizontal transmission (at least from fluke to vertebrate) have been documented. The typical trematode life cycle involves aquatic snails (e.g. Elimia spp.) as the first intermediate host, an arthropod as the second intermediate host, and a vertebrate as the definitive host (i.e., bats). Neorickettsia risticii was detected by PCR in metacercariae larvae infecting immature and adult aquatic insects (caddisflies, mayflies, damselflies, dragonflies, and stoneflies) collected from a pasture stream in northern California, Pennsylvania, Kentucky, and Ohio. It was demonstrated by Koch’s postulate then that horses develop Potomac Horse Fever (PHF) by ingesting infected cercariae in water or insects infected by metacercariae harboring N. risticii. Because PHF has now been confirmed in many countries, and due to the wider global distribution of the disease, equine neorickettsiosis (EN) is considered a more appropriate designation. Equine neorickettsiosis has been documented in the United States, Canada, Brazil, Uruguay, France, and The Netherlands. Adult flukes may pass their neorickettsial endosymbionts to the definitive vertebrate hosts by an unknown mechanism, and once inside the vertebrate, are capable of invading and multiplying within a variety of cells. At present, there are four distinct diseases attributed to Neorickettsia spp., in which N. risticii, N. sennetsu, and N. helminthoeca are horizontally transmitted to definitive or accidental mammalian hosts of trematodes and subsequently cause disease. In a 16S rRNA sequence analysis of isolates of N. risticii from horses with clinical signs of PHF, a uniquely different organism was identified and it was proposed that this isolate may be a new and distinct species of Neorickettsia. This hypothesis could not be confirmed until recently when this novel species, designated N. findlayensis, was isolated from two horses with clinical signs of EN in Ontario, Canada.
2. Discovery of Novel Rickettsial Species Causing Equine Neorickettsiosis

Equine neorickettsiosis is an acute, seasonal disease of horses characterized by fever, lethargy, anorexia, dehydration, diarrhea, laminitis, and/or abortion, that is commonly known as PHF. Neorickettsia risticii (formerly Ehrlichia risticii) had been recognized as the etiological agent of PHF since being recognized in the mid-1980s. In Canada, PHF has been confirmed in at least five provinces. In the province of Ontario, there is compelling published data of an endemic disease resembling EN that has been present since the late 19th century. Every summer, horses endemic disease resembling EN that has been present in Ontario, there is compelling published data of an endemic disease resembling EN that has been present since the late 19th century. Every summer, horses are presumptively diagnosed and treated for PHF in Ontario, but the etiological agent is not routinely confirmed by the recommended molecular testing in blood and feces. Blood samples (n = 41) from horses with typical clinical signs such fever, lethargy, inappetence, and diarrhea, that resided at various locations in Ontario, Canada, were tested by PCR and cultured from 2015 to 2020 at the Molecular, Cellular, and Environmental Rickettsiology Laboratory, Department of Veterinary Biosciences, College of Veterinary Medicine, The Ohio State University. Sixteen of 41 (39%) samples yielded a positive culture for Neorickettsia spp. organisms, of which 12 culture isolates were analyzed by PCR for four genes, P51, 16S rRNA, Ssa3, and Ssa1, followed by sequencing. Phylogenetic analysis confirmed that 10 of the isolates were strains of N. risticii, whereas the remaining two isolates (Fin17 and Tom16) were a previously uncharacterized Neorickettsia spp. These two Canadian isolates were closely related to Neorickettsia spp. 8. The results were corroborated by whole-genome sequencing of Fin17 and genomic comparison with N. risticii, N. sennetsu, and N. helminthoeca. Further, the phylogenetic analysis of 12/16 of these Ontario isolates demonstrated clustering according to the geographic area of origin.

3. Experimental Infection with Novel Rickettsial Species in Horses

To demonstrate that the isolated organism was in fact the cause of the clinical signs, an experimental study to fulfill Koch’s postulate was undertaken. Two ponies were intravenously inoculated with the uncharacterized Neorickettsia spp. (Fin17-infected P388D1 cells). Pony 1 developed intermittent fever and lethargy, tachycardia, anorexia, and watery diarrhea, on Days 14 to 18 post-inoculation. Pony 2 also developed mild fever on Day 7, and lethargy on Day 11 postinoculation. Seroconversion was detected by Day 6 for both ponies by using indirect fluorescent-antibody assay. Neorickettsia spp. was detected by quantitative PCR (qPCR) in the peripheral blood specimens from both ponies by Day 9 post-inoculation and blood culture was positive on Days 9 and 16 in both ponies. Fecal samples were not tested for the molecular detection of the inoculated bacteria during this experiment. By using PCR and sequencing for 16S rRNA and ssa3V gene, the identity of the inoculated strain (Fin17) was confirmed for the culture isolates from both ponies as identical to each other and to the original Fin17 horse isolate. The experimental inoculation of Fin17 demonstrated that this isolate is capable of infecting horses and causes typical clinical signs of PHF or subclinical infection, and therefore fulfills Koch’s postulates as a novel causative agent of PHF.

4. Summary

Neorickettsia spp. are a small group of Gram-negative, endosymbiotic of digenean flukes with a complex life cycle that involves aquatic and terrestrial environments of vertebrates and invertebrate hosts. The recent discovery of the novel species, N. findlayensis, underscores the importance of cultural isolation and in-depth molecular analysis of the isolated strains. There is currently no commercial molecular test for the diagnosis of N. findlayensis-associated EN. PCR amplification and amplicon size comparison of ssa3 gene can be used to distinguish new Neorickettsia spp. from N. risticii strains. In addition, based on the whole genome sequencing, a new PCR test to detect both species is currently being developed. Concomitant submission of ethylenediaminetetraacetic acid (EDTA) blood and feces is recommended to maximize the diagnosis of EN, nevertheless it has been observed that fecal samples are more likely to yield a positive result. Therefore, in the event that only one sample can be submitted, a fecal sample would be preferred. Further studies will be conducted to investigate the natural history of this new species, the pathogenesis of the disease, and to improve laboratory diagnosis and potentially vaccine development.

Acknowledgments

This research was supported in part by The Ohio State University and Equine Guelph.

Declaration of Ethics

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

Conflict of Interest

The Authors have no conflicts of interest.

References


Nocardioform Placentitis: A Continuing Question

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Nocardioform placentitis (NP) is defined as a focal mucoid placental inflammation in which the bacterial infection (Amycolatopsis spp., and/or Crossiella equi as the most common isolates) is limited to the chorionic surface of the ventral placenta without infection of the fetus. NP was first diagnosed in Central Kentucky in 1986 and continued to result in episodic outbreaks of abortion and preterm birth in mares in 1998, 1999, 2011, 2017, and 2020. To date, the pathogenesis of the disease remains an enigma and attempts to induce an experimental infection in mares have been unsuccessful. While episodic in nature, NP remains a problem to the equine breeding industry, as its diagnosis and treatment remain challenging and economic impact is immense. In some cases, NP can be diagnosed through transabdominal ultrasonography to detect placental separation and the accumulation of hyperechoic exudate. However, since a limited area of the placenta is accurately visualized by transabdominal ultrasonographic scanning, the lack of apparent lesions in the placenta does not exclude the possibility of disease. NP treatment has been empirically applied and has been based on common treatments for ascending placentitis and the results of culture and antimicrobial susceptibility patterns of bacteria isolated from lesions post-foaling. The aim of this paper is to provide a comprehensive review and updates on equine NP.

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1. Introduction

In the United States, approximately 3-5% of Thoroughbred broodmares suffer late-term pregnancy losses due to placentitis, making it the single most common cause of abortions, stillbirths, and perinatal losses.1,2 These outcomes lead to multimillion-dollar losses in the equine breeding industry in addition to their effects on the mares’ well-being and the emotional impacts on the owners.3 Based on the lesion
site, distribution, and pathogenesis, there are 4 recognized forms of equine placentitis: ascending, focal mucoid (nocardioform), diffuse (hematogenous), and multifocal placentitis. Ascending placentitis is the most frequent type of equine placentitis. In this type, the ascending infection (predominately caused by *Streptococcus equi subspecies zooepidemicus*) gains access to the caudal pole of the chorioallantois (cervical star region) through the vagina and consequently breaches the uterine cervical barrier. At the infection site, the bacteria colonize, and the resultant inflammatory response and leukocytic infiltration lead to thickening and separation of the chorioallantois from the endometrium. Multifocal and diffuse placentitis are less common forms and are usually a result of hematogenous spread of microorganisms to the uterus, such as leptospirosis, salmonellosis, histoplasmosis, and candidiasis.

**Etiology**

Nocardioform placentitis is believed to be associated with gram-positive, branching Actinomycetes (Fig. 2) including *Amycolatopsis* spp., *Crossiella equi* along with more recently characterized isolates of *Streptomyces atriruber* and *Streptomyces silaceus*, among others. Characterization of Actinomycetes associated with abortions during the 2011 outbreak of NP in KY revealed that *Amycolatopsis* spp. (49% of cases) was the most common isolate, with *Crossiella equi* (29% of cases) as the next most frequent isolate. It has been suggested that *Crossiella equi* infections may be more likely to result in abortion, whereas infections with other types of Actinomycetes tend to result in live, but premature foals. Although Actinomycetes are a group of common soil microorganisms, attempts in 2011 to culture *Amycolatopsis* spp. and *Crossiella equi* from KY soil samples from the environment of affected mares failed.

**History and Outbreaks**

NP was first diagnosed and characterized in Central Kentucky (KY) in 1986. The term “nocardioform” was historically adopted due to phylogenic relatedness of the causative agents (i.e., *Amycolatopsis* spp., *Crossiella equi*, and *Streptomyces* spp.) to the Nocardia species. Subsequent NP outbreaks occurred in KY and were reported in 1998, 1999, 2011, 2017, and 2020 (Fig. 1). Sporadic cases of NP also have been reported in Florida, Louisiana, South Africa, Italy, Australia, and most recently in New Zealand. Anecdotally, NP cases have also been reported sporadically in California, Indiana, Maryland, New York, North Carolina, Ohio, Pennsylvania, and Texas. Additionally, some of these cases were recorded in Standardbred mares in Ohio and Pennsylvania.

**Fig. 1.** Historical nocardioform placentitis trends from 1991 to 2020 in Central Kentucky. Source UK-VDL 2020.
are involved in mucoid placentitis and/or the difficulty of detecting bacteria following antimicrobial treatment. In support of this notion, the authors’ preliminary data using 16s rRNA and RNA sequencing suggests that focal mucoid placentitis could be associated with other bacteria. The analysis demonstrated that Proteobacteria, Actinobacteria, Firmicutes, and Bacteroidetes were the most abundant phyla in the NP cases, however, it is still not clear whether these bacteria are involved in the pathogenesis of NP or are environmental contaminants.

Possible Predisposing Factors and Occurrence

This form of equine placentitis is likely multifactorial and may involve factors such as host factors and environmental conditions. NP tends to manifest during the last trimester of pregnancy. Abortions typically occur between November and June, with the highest incidence in January and February. Interestingly, the NP epizootic outbreaks in central KY were usually preceded by hot and dry weather in August and September in 2010, 2016, and 2019. For instance, there is a negative association between August and September rainfall and the number of NP
cases submitted to University of Kentucky Veterinary Diagnostic Laboratory (UKVDL) for the subsequent foaling season. Also, there is positive correlation between mean temperatures in August and September and the number of NP cases submitted the next foaling season. The previous observations suggest environmental conditions as a predisposing factor for the disease.

The method of breeding does not appear to be a factor in NP. NP has been reported with live cover, artificial insemination, and embryo transfer. Large farms with higher stocking density are more at-risk.

Lesion (Gross and Histopathological Appearance)
The NP lesions from the 2020 foaling season varied in size (range; 5 X 3 cm – 100 X 50 cm). The distribution of the placental lesion in NP is distinct from those of ascending bacterial placentitis with lesions of NP mainly distributed in the cranial-ventral portion of the placenta near the junction of the uterine horns and body (Fig 3). The lesion is often demarcated from the surrounding normal placenta, and the affected placenta is covered with a thick, mucoid material (Fig. 3). Variation in volume, coloration and consistency of the mucoid material can be seen among different NP cases. It is worth noting that some cases do not have the characteristic mucoid exudate (i.e., avillous chorion with no mucoid exudate), but will have a similar exudate evident microscopically. This distinction might be attributed to disease chronicity or treatment. Of note, cystic adenomatous hyperplasia on the allantoic surface has been reported in association with NP but has also been reported with a variety of placental pathologies. Based on historical findings, it appears that bacterial infection starts at the center of the lesion and then expands outward. This notion
could be explained by the fact that the center of the lesion is most commonly avillous (complete loss of chorionic microvilli), whereas the margin of the lesion has raised, irregular and red areas (Figs. 3 and 4). Therefore, the margin of the lesion is the best sampling site for bacterial culture, PCR, and histopathology. Histologically, the center of the lesion usually demonstrates blunted and atrophied chorionic villi with lymphocytic infiltrates; however, at the margin of the lesion the chorioallantois may demonstrate the infiltration of neutrophils, lymphocytes, and plasma cells with squamous metaplasia, blunting and loss of the chorionic microvilli (Figs. 4 and 5). The surface exudate contains sloughed epithelial cells, leukocytes, and an eosinophilic, amorphous material.

2. Pathogenesis and Pathophysiology

The site and solitary nature of NP lesions does not fit with ascending or hematogenous bacterial infection. The placental lesion in NP is mainly distributed in the cranial-ventral portion of the placenta near the junction of the uterine horns and body. The classic lesion of NP is a thickened, tenacious mucoid material overlying a moderately well demarcated section of the chorionic surface with a central area (chronically infected areas) of tan discoloration exhibiting loss of villi, surrounded by a raised, irregular red-tan chorion (Figs. 3 and 4). The areas correspond respectively to chronic, squamous metaplasia and mild to moderate mononuclear inflammation and more active inflammation with blunting and necrosis of chorionic villi (Fig. 5). As mentioned previously, the pathogenesis of the disease remains poorly understood. Attempts to induce the infection in mares by intrauterine inoculation of *Crossiella equi* at the time of breeding or in pregnant mares via oral, intravenous, and intranasal routes with *Crossiella equi* were unsuccessful. In Australia, experimental trials to induce equine amnionitis and fetal loss (EAFL) by feeding mares with processionary caterpillar resulted in unexpected cases of focal mucoid placentitis. Therefore, the possible implication of sources other than bacteria in NP should not be neglected. NP is associated with three major events: placental inflammation (focal mucoid placentitis), chorionic separation from the endometrium (placental separation), and placental insufficiency. Understanding the molecular mechanisms of these three events holds potential for the development of new diagnostic tools and therapies to forestall NP and its detrimental effects on the pregnancy. Recently, using a transcriptomic approach (i.e., ribonucleic acid (RNA) sequencing), the authors elucidated the key regulators and molecular
mechanisms triggering these events in clinical cases of NP. Results revealed that inflammatory signaling, toll-like receptor signaling, inflammasome activation, chemotaxis, and apoptosis pathways are involved in NP.30 NP is associated with the upregulation of a set of genes encoding the key regulators of the inflammatory cascade.30 These include pattern recognition receptors (PRRs) such as Toll-like receptors (TLRs).30 Of note TLRs are the primary and earliest recognition mechanism for pathogen associated molecular patterns (PAMPs) unique to the microorganisms with subsequent activation of the inflammatory cascade.31,32 Among TLRs, several TLRs (TLR1, TLR2, TLR3, TLR5, TLR7 and TLR8) were significantly upregulated in NP.30 It is noteworthy that TLR1/TLR2 heterodimers are responsible for recognition of gram-positive bacteria, consistent with the Amycolatopsis spp. infection,31 which is the major isolate from NP cases. Taken all together, these findings address the crucial role of TLRs in triggering the inflammatory response associated with NP. Therefore, strategies to block TLRs hold potential for future therapies to mitigate the inflammatory cascade and to forestall NP. This notion is supported by studies showing that TLR antagonists (TLRAs) were highly effective in preventing preterm birth induced by lipopolysaccharides (LPS), heat-killed E. coli or platelet activating factor (PAF) in primates and mice.33,34 The authors’ preliminary data also shows an association between the abundance of metabolically active bacteria and global gene expression patterns in the placenta, suggesting interactions between the host and

![Normal chorioallantois](image1)

![Nocardioform placentitis](image2)

Fig. 5. Microscopic appearance of chorioallantois retrieved from normal postpartum mares (A) and nocardioform placentitis affected mares (B). In images (B1-4), notice the infiltration of neutrophils, lymphocytes, and plasma cells with squamous metaplasia, blunting, and loss of the chorionic microvilli. The surface exudate contains sloughed epithelial cells, leukocytes, and an eosinophilic, amorphous material. Courtesy of Dr. Rebecca E. Ruby.
pathogen. The increased leukocytic infiltration in NP was associated with the upregulation of matrix metalloproteinase (MMP1, MMP3, and MMP8) and apoptosis-related genes, such as caspases (CASP3 and CASP7), which could explain placental separation associated with NP. Preliminary data on the transcriptome of the bacteria in NP cases also demonstrates that bacteria are involved in metabolic pathways and biosynthesis of secondary metabolites in the area of the placenta without a gross lesion, increasing their abundance, perhaps leading to increase leukocytic infiltration and placental separation. Also, NP was associated with downregulation of several placenta-regulatory genes (ABCG2, GCM1, EPAS1, and NR3C1), angiogenesis-related genes (VEGFA, FLT1, KDR, and ANGPT2), and glucose transporter coding genes (GLUT1, GLUT10, and GLUT12), as well as upregulation of hypoxia-related genes (HIF1A and EGLN3), which could potentially be associated with the placental insufficiency accompanying NP. Since NP is associated with angiogenesis dysregulation, the use of therapeutics that improve placental angiogenesis and/or blood flow might be beneficial for NP treatment. The significant pathways associated with placental inflammation, separation and insufficiency are summarized in Fig. 6.

3. Sequel and Pregnancy Outcomes

Multiple outcomes have been associated with NP: late term-abortion, weak yet viable foals, or normal parturition. These different pregnancy outcomes might be dependent on several factors such as size of the lesion, severity of inflammation, and causative bacteria, among others. For instance, increased lesion size is associated with a growth-retarded foal or a dead foal. There is a negative correlation between the lesion size and the foal weight (i.e., the larger the lesion, the smaller the foal and vice versa). NP is also associated with decreased gestational length (10 days less than unaffected mares) and decreased neonatal weight (10 lbs less). Although neonates may be smaller, they do not have abnormal or altered IgG or WBCs. NP lesions may also be seen in the chorioallantois in mares with normal neonates. Interestingly, affected mares have normal postpartum fertility, with an average of 1.5 cycle per pregnancy.
4. Diagnosis

While costs associated with NP can be significant, its diagnosis and treatment remain challenging. Clinically, large NP lesions are usually associated with premature mammary gland development, which might reflect a late stage of the disease. Vulvar discharge is not commonly observed in NP cases unless the mare is about to abort. NP can be diagnosed through transabdominal ultrasonography to detect placenta separation (i.e., separation of the chorion from the endometrium) and the accumulation of hyperechoic exudate (Fig. 7). However, since a limited area of the placenta is accurately visualized by ultrasonography, the lack of apparent lesions in the placenta does not exclude the possibility of disease. Transrectal ultrasound can also be useful in advanced cases where there is sufficient diffuse inflammation leading to an increased combined thickness of the uterus and placenta (CTUP), or the focal lesion is located caudally enough to be identified on transrectal evaluation. There are no clear recommendations for the most appropriate window to screen, but many elect to perform a transrectal and single transabdominal ultrasound evaluation in late gestation to screen for placental and fetal health. The use of more frequent scans is dictated by the history of the mare and the incidence of NP that season. A recent retrospective work has described the serum profile of mares with focal mucoid placentalitis (n=6; two placentas were PCR positive for the *Amycolatopsis* spp, while the other four had no bacteria detected), and found alterations in endocrine, cytokine, and feto-secretory markers in the weekly assessed samples. The focal mucoid placentalitis was associated with an increase in progestins, decrease in estradiol-17B, and increase in the fetosecretory protein Alpha-Fetoprotein (AFP). Additionally, this study showed an increase in pro-inflammatory IL-2 and IFNg, pleiotropic IL-6 and tumor necrosis factor (TNF), and anti-inflammatory IL-5, and IL-10 in the maternal serum. It is important to mention that all of the mares with focal placentalitis within this study produced a viable neonate. While abnormal estrogen and progesterone profiles and changes in serum cytokines have been reported for placentalitis, still there is no sensitive and specific biomarker available to diagnose and predict this condition. This emphasizes the importance and urgent need to develop sensitive and reliable diagnostic blood biomarkers for this disease. Postpartum diagnosis is dependent on the characteristic NP/focal mucoid placentalitis lesion, as mentioned earlier, which could be confirmed by obtaining swabs to culture and isolate nocardioform Actinomycetes on blood agar and Columbia CNA agar with sheep (or horse) blood, and using culture isolates (or swab/placental tissue) to perform PCR for nocardioform Actinomycetes (i.e., *Crossiella equi* and *Amycolatopsis* spp). It is worth noting that the PCR primers used for *Amycolatopsis* spp. are designed to identify Genus *Amycolatopsis*, not specific species. Aborted fetuses had a decreased body weight/smaller than normal, lack of internal adipose tissue, and decreased musculature.

5. Treatment

Given that the pathogenesis of NP is still unknown, and there are no established experimental models to study NP, treatment for nocardioform placentalitis has...
been empirically applied and based on treatments used for ascending placentitis. The treatment protocols vary by practitioner and severity of disease, but tend to include broad spectrum antimicrobials, anti-inflammatory agents, tocolytics and other therapeutics that improve uterine/placental blood flow and help to maintain uterine quiescence as summarized in Table 1. Treatment outcomes might be dependent on several factors (stage of the disease/size of the lesion, causative bacteria, gestational age, among others). Recently, in vitro studies revealed that nocardioform Actinomycetes are susceptible to ceftriaxone, doxycycline, minocycline, linezolid, and trimethoprim/sulfamethoxazole as depicted in Table 2, confirming earlier study results. However, it is unclear whether these antimicrobials are effective in vivo. Further studies to investigate the placental diffusion of these antibacterial treatments are warranted.

### 6. Conclusion

Nocardioform placentitis continues to cause episodic outbreaks of abortion and preterm birth in mares. While NP is an important equine placental pathology and cause of reproductive loss, its route of infection and the pathogenesis is still unknown. Due to the knowledge gap in the pathogenesis of NP, the diagnosis of disease is limited and the current treatment regime is empirical with a debatable success rate. Using high-dimensional biology should improve the understanding of the pathophysiology and the host-pathogen interaction, allowing for the

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Table 1. Common Therapeutic Agents Used to Treat Mares with Placentitis

<table>
<thead>
<tr>
<th>Therapeutic Agent</th>
<th>Suggested Dose</th>
<th>Mode of Action/Proposed Effect</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trimethoprim (TMP)-sulfamethoxazole (SMZ)</td>
<td>15–30 mg/kg, PO, q12h</td>
<td>Antibacterial</td>
<td>40-42</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>6.6 mg/kg, IV, q24h</td>
<td>Antibacterial</td>
<td>41, 43</td>
</tr>
<tr>
<td>Doxycycline</td>
<td>10 mg/kg, PO, q12h</td>
<td>Antibacterial</td>
<td>44</td>
</tr>
<tr>
<td>Enrofloxacin</td>
<td>5 mg/kg, IV, q24h</td>
<td>Antibacterial</td>
<td>45</td>
</tr>
<tr>
<td>Potassium penicillin G</td>
<td>22,000 IU/kg, IV, q6h</td>
<td>Antibacterial</td>
<td>41, 43</td>
</tr>
<tr>
<td>Altenogest</td>
<td>0.088 mg/kg, PO, q24h</td>
<td>Tocolytic (block uterine contractions)</td>
<td>41, 42</td>
</tr>
<tr>
<td>Firocoxib</td>
<td>57 mg (loading dose) then 1 tab q24h</td>
<td>Non-steroidal anti-inflammatory medication (NSAID)</td>
<td>46</td>
</tr>
<tr>
<td>Flunixin meglumine</td>
<td>1.1 mg/kg, PO/IV, q24h, or q12h</td>
<td>Anti-inflammatory</td>
<td>41</td>
</tr>
<tr>
<td>Dexamethasone</td>
<td>40, 35, 25 mg, q24h, IV for 6 d, decreasing dose every 2 d</td>
<td>Anti-inflammatory; stimulate fetal maturation.</td>
<td>41</td>
</tr>
<tr>
<td>Acetylsalicylic acid (ASA, Aspirin)</td>
<td>50 mg/kg, PO, q12h</td>
<td>Improve blood flow to the placenta.</td>
<td>41</td>
</tr>
<tr>
<td>Pentoxifylline</td>
<td>8.5 mg/kg, PO, q12h</td>
<td>Improve blood flow to the placenta; Block endotoxin-induced TNF, IL-6; anti-cytokine activity</td>
<td>39, 40-42</td>
</tr>
<tr>
<td>Estradiol cypionate</td>
<td>10 mg/mare, IM, q3d for 3 treatments</td>
<td>Improve blood flow/angiogenesis in the placenta and improve immunity.</td>
<td>47</td>
</tr>
</tbody>
</table>

### Table 2. In Vitro Anti-Microbial Susceptibility Patterns of Nocardioform*: 2019-2020 Foaling Season

<table>
<thead>
<tr>
<th>Antimicrobials</th>
<th>Amycolatopsis Spp. (n = 91)</th>
<th>Crossiella equi (n = 60)</th>
<th>Unidentified (n = 20)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S (%)</td>
<td>I (%)</td>
<td>R (%)</td>
</tr>
<tr>
<td>Amikacin</td>
<td>90</td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>Amin/Clav. Acid</td>
<td>74.7</td>
<td>16.4</td>
<td>8.8</td>
</tr>
<tr>
<td>Cefepime</td>
<td>26.4</td>
<td>12.1</td>
<td>61.5</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>73.6</td>
<td>16.5</td>
<td>7.7</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>31.9</td>
<td>53.9</td>
<td>14.2</td>
</tr>
<tr>
<td>Clarithromycin</td>
<td>78</td>
<td>7.7</td>
<td>14.3</td>
</tr>
<tr>
<td>Doxycycline</td>
<td>81.3</td>
<td>7.7</td>
<td>11</td>
</tr>
<tr>
<td>Imipenem</td>
<td>0</td>
<td>40.7</td>
<td>59.3</td>
</tr>
<tr>
<td>Linezolid</td>
<td>100</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Minocycline</td>
<td>84.7</td>
<td>3.3</td>
<td>12</td>
</tr>
<tr>
<td>Tobramycin</td>
<td>36.3</td>
<td>12</td>
<td>51.7</td>
</tr>
<tr>
<td>TM/SMZ</td>
<td>80.2</td>
<td>0</td>
<td>19.8</td>
</tr>
</tbody>
</table>

Abbreviation: TMP-SMX, trimethoprim-sulfamethoxazole; S, susceptible; I, intermediate; R, resistant.

*As there are no interpretative criteria for antimicrobial susceptibility of nocardioform actinomycetes for horses, the criteria have been extrapolated from human Nocardia spp. It should be kept in mind that these results are only in vitro test results and may not necessarily be applied to actual clinical placentitis cases in mares. Further studies to investigate the placental diffusion of these antibacterial treatments and their efficacy are warranted.

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EMERGING AND RE-EMERGING INFECTIOUS DISEASES
development of diagnostic tools and therapeutic strategies to prevent losses due to NP.

Acknowledgments
Part of this work was funded by the Koller Emergency Response Fund, the Grayson Jockey Club Research Foundation, and the Albert G. Clay Endowment. The authors would like to thank Dr. Emma Adam and Dr. David Horohov for their help and support. The authors would also like to thank all veterinarians, farm owners and managers, and UKVDL personnel who participated in these studies and surveys.

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

Conflict of Interest
The Authors have no conflicts of interest.

References and Footnotes
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Streptococcus zooepidemicus: Commensal or Pathogen?

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1. Introduction

*Streptococcus equi* subspecies *zooepidemicus* is the bacterium most frequently recovered from the oropharynx of horses.1,2 The prevalence of *S. zooepidemicus* in samples recovered from healthy horses might suggest that this organism may not be a causal agent of disease. However, growing evidence supports an important role for *S. zooepidemicus* in a wide array of diseases of horses and other animals. This review examines the population structure of *S. zooepidemicus*, informed by the analysis of genome sequencing data, to shed new light on the role of this subspecies of bacteria as a causative agent of disease in animals, including humans.

2. The Many Lives of *S. zooepidemicus*

*S. zooepidemicus* is a β-hemolytic Gram-positive Lancefield group C *Streptococcus* that can use lactose and sorbitol but fails to ferment trehalose.3,4 Although *S. zooepidemicus* is frequently recovered from healthy horses,5 its presence is associated with respiratory disease in Thoroughbred racehorses,6,7 uterine infections in mares,8,9 and ulcerative keratitis.10 It is also associated with disease in a wide range of other animal hosts, including cattle,11 sheep,12-15 pigs,16-19 dogs,20,21 and humans.22-26 *Streptococcus equi* subspecies *equi*, a biovar of *S. zooepidemicus* that cannot use lactose or sorbitol,4 is the causative agent of strangles in horses.27 Strangles is one of the most frequently diagnosed disease of horses, which is characterized by fever and the abscessation of lymph nodes in the head and neck.28-31 The often obvious clinical signs and high prevalence of strangles led to this biovar lending its name to the species as a whole following its identification in 1888.27 However, *S. equi* actually clusters within the much broader group of *S. zooepidemicus* strains as just one of a wide range of variants.32 Therefore, these data32 and additional analyses using multilocus sequence typing (MLST)33 and genome sequencing data,34 detailed below, provide evidence that *S. equi* evolved from an ancestral strain of *S. zooepidemicus* and is, in actual fact, a lineage of *S. zooepidemicus*. An enhanced understanding of the genetic diversity of *S. zooepidemicus* strains and the ability of particular lineages to cause specific diseases will shed new light on the evolution of this pathogenic group and direct the development of novel diagnostic tests and vaccines.

3. Identification of Different *S. zooepidemicus* Strain Types

Strains of *S. zooepidemicus* were originally differentiated using sugar fermentation. The *S. equi* biovar fails to ferment lactose and sorbitol, facilitating its
differentiation from the wider population of *S. zooepidemicus*. However, the resolution that biochemical methods provides is extremely limited and can be influenced by a variety of loss-of-function events that might suggest strains are related when in fact the same phenotypic properties arose independently. Therefore, although permitting the identification of *S. equi*, this methodology provided insufficient resolution to determine if certain strain types of *S. zooepidemicus* were more capable of causing specific types of disease in particular host animals. Over time, several other methods have been developed to improve the ability to differentiate strain types. MLST assigns specific allele numbers to each unique 400- to 500-base pair nucleotide sequence of internal fragments of seven housekeeping genes. Each combination of seven allele numbers is then assigned a specific sequence type (ST) number. Much more variation can be detected by MLST, and as the majority of nucleotide variation does not lead to changes in amino acid sequence, different alleles are usually not subjected to functional selective pressure. Furthermore, the nucleotide sequence data that are generated by MLST are fully portable and can easily be compared between different laboratories via electronic databases available on the Internet. These databases are therefore a powerful resource with which to conduct global epidemiological studies. The MLST scheme for *S. zooepidemicus* uses fragments of the seven housekeeping genes *arcC*, *nrdE*, *proS*, *spi*, *tdk*, *tpi*, and *yqiL*. To date, 1,519 different isolates of *S. zooepidemicus* or *S. equi* from 21 different countries have been uploaded onto the online database (Fig. 1). A total of 865 different alleles have been assigned, generating 437 STs comprised of unique allele combinations (*Streptococcus zooepidemicus* [PubMLST, last accessed 27th January 2021]). Therefore, the MLST platform permits the identification of hundreds of different STs within populations of *S. zooepidemicus* in order to identify disease-causing strains.

4. Nonequine Transmission of *S. zooepidemicus* STs

*S. zooepidemicus* is frequently recovered from horses both with and without clinical signs of disease, providing challenges in establishing a causal role in disease. However, *S. zooepidemicus* is less prevalent in other animal species, providing an opportunity to examine the potential of this agent to cause disease. The phylogenetic reconstruction shown in Fig. 2, which was generated using the concatenated DNA sequences of the seven MLST alleles, highlights the diversity of the population of *S. zooepidemicus* as measured by MLST. *S. zooepidemicus* is regularly isolated from cases of disease, including mastitis, wound infections, respiratory disease, and uterine infections in the ruminant population of Spain. MLST analysis identified that the ST-236 group of *S. zooepidemicus* was the dominant strain type recovered from goats and sheep in Spain and that none of the isolates within this ST had previously been recovered from horses. Thirty-two (82%) of the 39 isolates that are ST-236 or a single locus variant (ST-266 and ST-272) were recovered from cases of mastitis, suggesting that this subgroup of *S. zooepidemicus* may have evolved so as to specifically cause disease in sheep and goats rather than in horses. Genome sequencing of the C7 strain of ST-236 identified a novel phosphoenolpyruvate sugar phosphotransferase system, which enables this group of *S. zooepidemicus* to use arbutin. Arbutin is a common component of animal foodstuffs, and the ability to use arbutin may confer a selective advantage to strains infecting animals, the diet of which contains this sugar. Another subgroup of *S. zooepidemicus*, ST-72, was recovered from an outbreak of acute...
nephritis in humans residing in Nova Serrana, Brazil, between 1997 and 1998. Two hundred and fifty-three people were affected, of which seven required dialysis and three patients died. The outbreak was linked to the consumption of unpasteurized dairy products. Interestingly, although two of the five ST-72 isolates listed in the MLST database were recovered from the respiratory tract of horses in the United Kingdom and the United Arab Emirates, another two isolates were recovered from humans. One of these isolates was recovered from the cerebrospinal fluid of a man in Barnsley, UK, during 2006 and the other from a case of nephritis in a dairy farmer in Northallerton, UK, during 1983, which was 14 years before the outbreak of nephritis in Brazil. Therefore, this subgroup of *S. zooepidemicus* may have an increased ability to cross species boundaries and cause rare, but serious infection of humans. A double locus variant of ST-72, ST-10, was responsible for a large outbreak of acute fatal hemorrhagic pneumonia in kenned dogs residing in the United Kingdom between 2000 and 2002, providing further evidence of the ability of this subgroup to infect multiple mammalian hosts. ST-194 is a further subgroup of *S. zooepidemicus* that has been responsible for severe disease in nonequine hosts. This ST was first recovered from pigs in Sichuan Province, China, during 1975, which suffered from painful swelling of the joints, respiratory disease, and diarrhea, leading to the death of over 300,000 pigs in a 2-week period. Cases of *S. zooepidemicus* ST-194 infection in pigs in China has continued, with the genome of a further strain, CY, which was isolated from a pig in Nanjing Province during 1998 and sequenced in 2014. In 2019, thousands of cases of septicemia and death in pigs due to infection with a ST-194 strain of *S. zooepidemicus* occurred in North America, providing evidence that the ST-194 strain had spread beyond...
Asia. Interestingly, the MLST database identifies a human case of septicemia due to an ST-194 strain of *S. zooepidemicus* that occurred in a woman during 2001 after her return to the United Kingdom from abroad. These findings highlight the significant threat already posed by the ST-194 strain of *S. zooepidemicus* to the pig industry in Asia and North America and the potential risk of zoonotic transmission to humans. Given the severity of disease in affected animals, the screening of pigs for *S. zooepidemicus* infection prior to their import into other parts of the world would be a prudent measure.

5. **Respiratory Infections of Horses Associated with *S. zooepidemicus***

Respiratory disease affects a large proportion of young horses around the world, with an incidence of approximately 5 cases per 100 horses per month. The mean duration of clinical signs for each episode is around 8 weeks, and the disease often reoccurs in individual animals, causing considerable disruption to the equine industry. Although several bacterial species, including *S. zooepidemicus*, *Streptococcus pneumoniae*, and *Actinobacillus* spp., and *Mycoplasma* have been associated with respiratory disease in horses, only the prevalence and incidence of *S. zooepidemicus* and *S. pneumoniae* decreased in parallel with respiratory disease and age, consistent with the development of acquired immunity to infection with these pathogens. Recently, cases of respiratory disease in working horses residing in Ethiopia, characterized by coughing, nasal discharge, or altered respiration were significant more likely to test positive for the presence of *S. zooepidemicus* (odds ratio: 12.4, 95% confidence interval: 3.6 to 42.4), with no evidence for the involvement of viral pathogens. Despite this evidence, the investigation of outbreaks of respiratory disease due to *S. zooepidemicus* has continued to be confounded by the diversity of this subspecies and the ability of different strains to establish persistent infection of the tonsils of recovered horses, which resemble the outbreak strain when using traditional typing methodologies. However, MLST analysis permits strains of *S. zooepidemicus* to be identified at much higher levels of resolution, making it possible to link particular STs with outbreaks of disease. *S. zooepidemicus* strain H70 (ST-1), which was recovered from the nasopharynx of a Thoroughbred racehorse in Newmarket, UK, during 2000, is typical of respiratory tract isolates being recovered from horses in the United Kingdom and New Zealand. The genome sequence of this strain was completed, highlighting an array of surface proteins and biochemical pathways that may assist this strain to infect horses. Fifty-five of 71 (77%) strains related to ST-1 were recovered from the respiratory tract of horses, suggesting that this group of strains may be better suited to this niche. In Sweden, a herd of 17 Icelandic horses developed respiratory disease during 2009. Clinical signs included an elevated body temperature, nasal discharge, coughing, and lethargy. MLST was used to identify that a ST-24 strain of *S. zooepidemicus* was recovered from all horses with signs of respiratory disease, and unrelated strains were isolated from healthy horses on the same farm. Interestingly, the ST-24 strain was recovered from one of the affected horses 8 months postresolution of its respiratory disease, providing evidence that this strain was able to persist, and opening up new opportunities for transmission to naive animals. ST-24 is related genetically to ST-79, ST-84, ST-293, ST-339, and ST-418. Fifteen of the 20 (75%) isolates in the ST-24 group that are listed in the MLST database were recovered from the respiratory tract of horses, including from cases of respiratory disease in the United States during 1986 and 1988. Therefore, the ST-24 group appears to be adept at causing respiratory disease in horses. An outbreak of respiratory disease, characterized by purulent nasal discharge and coughing, was identified in New Caledonia-resident horses between October 1997 and July 1998. Attempts at the time to isolate a causal viral agent or to demonstrate seroconversion to equine influenza virus, equine herpesvirus-1, adenovirus, or rhinovirus were unsuccessful. However, *S. zooepidemicus* was recovered from 80% (25 of 31) of affected animals but only 4% (1 of 25) of healthy horses (P < 0.0001, two-sided Fisher’s exact test). An MLST analysis of isolates recovered from affected horses identified a ST-307 strain of *S. zooepidemicus* as the causal agent. ST-307 clusters with ST-3, ST-92, ST-167, ST-249, and ST-369. Seven of the 8 isolates within these related STs were recovered from cases of respiratory infection in horses, providing evidence that this subgroup of *S. zooepidemicus* is proficient at causing respiratory disease in horses. Although MLST has the ability to resolve different strains, it samples only a tiny portion (0.2%) of the core genome of *S. zooepidemicus*, and so the variation that is measured accrues slowly over many years. In some instances, it is important to be able to resolve differences within a ST in order to determine if the variation within that population of *S. zooepidemicus* arose over many years, indicative of an endemic strain, or whether there is little variation within an ST, which would be typical of an outbreak or epidemic strain. An epidemic of respiratory disease affected almost the entire native Icelandic horse population of 77,000 animals in 2010, resulting in a self-imposed ban on the export of horses and significant economic cost to associated industries. The disease was characterized by clinical signs of a dry cough coexisting with mucopurulent nasal discharge and mild conjunctivitis. Rectal temperature remained normal in most horses, and although morbidity rates approached 100%, mortality was infrequent. The incubation period was between 2 and 4 weeks and the duration of clinical signs varied from 2 to 10 weeks. The rate of spread suggested that a viral agent was responsible, but PCR of nasal swabs...
for viral agents of horses and some common respiratory viruses of humans and other animals were negative. Paired blood samples showed a lack of seroconversion to viral agents. Only equine herpesvirus-4 was inconsistently isolated from small numbers of both healthy and clinically affected horses. In the absence of a viral pathogen, it was noted that S. zooepidemicus was isolated from almost all of the nasal swabs taken from coughing horses and from the diseased tissues of occasional fatal cases. Initial MLST analysis identified that four STs accounted for 198 of 257 (77%) isolates recovered from the Icelandic horse population during the epidemic. ST-306 contained 37 isolates obtained from eight horses at the Icelandic Veterinary Institute at Keldur, Reykjavik. Fifty-two ST-248 strains were recovered from 14 horses and 1 dog residing at 8 different farms. Twenty-eight isolates of ST-246 were obtained from 14 horses residing at 10 farms and 1 human isolate. Finally, 81 isolates of ST-209 were recovered from 45 horses residing at 21 premises across Iceland, 1 human, and 1 cat. Mixed populations of 2 different isolates of S. zooepidemicus were recovered from 13 horses, and 3 different isolates were recovered from 2 horses, highlighting the challenges in differentiating endemic from epidemic strains. Based on these MLST data, the epidemic could have been caused by ST-248, ST-246, or ST-209 strains of S. zooepidemicus, and so a whole-genome sequencing approach was used to resolve these STs further. The analysis of genome sequencing data identified that the isolates of ST-248, ST-246, and ST-209 differed from other isolates of their same ST by a maximum of 151, 153, and 25 single nucleotide polymorphisms (SNPs), respectively. Therefore, ST-248 and ST-246 strains had accrued relatively high levels of variation, indicating that these strains were endemic within the Icelandic horse population. The 81 isolates of ST-209 had little variation within their core genome, indicating that they had been transmitted through the Icelandic horse population over a short period of time and were responsible for the epidemic of respiratory disease. The Icelandic-resident horse population is geographically isolated, arising from animals introduced by settlers in the 9th and 10th centuries, with virtually no import of horses for the last 1,000 years. The most likely source of the ST-209 strain was believed to be through the import of contaminated tack. Network analysis identified that the epidemic traced back to yard A, which used a submerged treadmill for the training and rehabilitation of horses from farms located across Iceland. The water used in the treadmill contained no disinfectant and was changed on a once- or twice-weekly basis, providing ideal conditions for the transmission of S. zooepidemicus between the visiting horses. Therefore, it is likely that following entry into Iceland, the rapid epidemic spread of ST-209 was facilitated by exposure of horses from across Iceland to contaminated water in the submerged treadmill. The relatively long incubation period permitted infected horses to return to their original farms before exhibiting clinical signs of disease. On identification of the likely transmission route, the addition of chlorine coupled with regular cleaning and disinfection of water treadmills has been introduced to minimize or eliminate the transmission of S. zooepidemicus or other infectious agents via this route. The epidemic ST-209 strain was also recovered from a cat and the blood of an Icelandic woman who had suffered a miscarriage. A closely related strain of S. zooepidemicus ST-209, Hum3, was recovered from a psoas abscess in a Finnish man during 2011, indicating that ST-209 strains of S. zooepidemicus have the potential to cause zoonotic disease. Bjornsdottir et al. also identified other cases of human infection caused by ST-246 and ST-2 strains of S. zooepidemicus, suggesting that the prevalence of zoonotic transmission of S. zooepidemicus may be underreported.

6. The Future Application of Whole-Genome Sequencing to Track S. zooepidemicus Infection

The analysis reported by Bjornsdottir et al. was the first to report the application of whole-genome sequencing to elucidate the cause of an outbreak on a national scale by the differentiation of an epidemic strain from strains that were endemic in the population. S. equi isolates are closely related to one another and can currently be differentiated into nine different STs by MLST (Streptococcus zooepidemicus | PubMLST, last accessed 27th January 2021), which cluster together as a subgroup of S. zooepidemicus (Fig 2). Therefore, MLST captures insufficient genetic diversity to adequately differentiate the population structure of S. equi and to resolve transmission events. An alternative single locus typing scheme for S. equi measures variation within the 5’ variable region of the SeM gene. This sortase-processed cell surface protein binds to fibrinogen and immunoglobulin to impede the phagocytosis of S. equi by immune cells. However, the utility of SeM typing as an epidemiological tool is limited by homoplasy and the high rate of mutation of SeM. Recently, Mitchell et al. described the application of a novel web-based core genome MLST (cgMLST) bioresource, implemented in Pathogenwatch (https://pathogen.watch/collection/j8c022xfgu8v-globetrotting; last accessed 28th January 2021) to determine the population structure of 670 isolates of S. equi, which were recovered from horses residing in 19 countries (Fig 3). A total of 2,962 variant sites were identified across the 1,286 loci within the core genome, and each pair of S. equi isolates differed from one another by an average of 69 SNPs across the core genome (range, 0 to 181). The analysis identified numerous examples of closely related strains of S. equi in geographically distant nations, highlighting that the lack of pre-export testing, used routinely for many animal diseases, facilitated the international transmission
of *S. equi* (Mitchell et al., in press). The Pathogenwatch resource that was developed for *S. equi* is also available for the analysis of *S. zooepidemicus* genomes. This resource enabled the rapid differentiation of 923 isolates of *S. zooepidemicus* based on 145,408 variable sites across 1,286 loci within the core genome (Fig. 4). The *S. zooepidemicus* population differed by an average of 32,954 SNPs across the core genome (range, 0 to 52,715), providing unprecedented capacity to resolve differences between isolates and track the transmission of pathogenic strains as they are transmitted at national and international levels through multiple animal hosts (https://pathogen.watch/).

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**Fig. 3.** Midpoint rooted phylogenetic reconstruction of the *S. equi* population generated within Pathogenwatch and visualized in Microreact. The dendrogram was constructed from pairwise cgMLST scores using the APE package. The resulting tree was midpoint rooted using the phangorn package. The scale bars relate to branch lengths and indicate the number of core genome SNPs (cgSNPs) that are proposed to have occurred on the branches. Colored circles indicate the country from which the isolates originated as indicated in the key.

**Fig. 4.** Midpoint rooted phylogenetic reconstruction of the *S. zooepidemicus* population generated within Pathogenwatch. The dendrogram was constructed from pairwise cgMLST scores using the APE package. The resulting tree was midpoint rooted using the phangorn package. The scale bars relate to branch lengths and indicate the number of cgSNPs that are proposed to have occurred on the branches. Colored circles indicate the location of STs described in the text.
7. Summary

The diversity of *S. zooepidemicus* cannot be captured by traditional phenotypic strain typing methods, which were only capable of differentiating *S. equi* subspecies *zooepidemicus* from *S. equi* subspecies *equi*. The failure of these early typing schemes resulted in an assumption that all strains of *S. zooepidemicus* were commensal organisms despite the fact that certain strains can be highly virulent, leading to severe disease and the death of some animals, including humans. The availability of the MLST and Pathogenwatch resources for the identification and tracking of pathogenic strains herald a new era for the study of this neglected pathogen. With these tools, it is now possible to identify and track outbreak and epidemic strains as they are transmitted through and between populations of animals around the world. The finding that some strains such as ST-194, ST-236, and ST-72 are adapted to cause disease in pigs, ruminants, and humans, respectively, and that other strains including ST-1, ST-24, ST-209, and ST-307 may be more capable of causing respiratory disease in horses provides an opportunity to identify genes that influence host and tissue specificity and that are required for *S. zooepidemicus* to cause disease. This knowledge will have enormous potential for the rational development of novel vaccines and therapeutics with which to prevent and treat disease.

Acknowledgments

Dr. Hayley Wilson is funded by a grant from the Petplan Charitable Trust (S19-741-780).

Declaration of Ethics

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

Conflict of Interest

Dr. Andrew Waller is employed as Chief Scientific Officer for Intervacc AB, Stockholm, Sweden. Dr. Hayley Wilson has no conflict of interest.

References

1. Introduction

Equine herpesvirus-1 (EHV-1) belongs to the family of herpesviruses and the subfamily of Alphaherpesvirinae. EHV-1 is ubiquitous in horses worldwide, and most horses are infected with EHV-1 early in life with overall low morbidity. Following this primary infection, a life-long latency is established, and it is currently estimated that ~80% of horses are latently infected with EHV-1. Combined with frequent reactivation, this is the dominant feature ensuring the virus’ survival in the horse population. In addition, viral survival depends on a number of immune evasive and suppressive mechanisms that are initiated by the virus and prevent the host’s immune system from establishing long-term protective immunity.

2. Clinical Disease Manifestations

Clinically, EHV-1 is the cause of viral respiratory disease, late-term abortions, neonatal foal death, equine herpesvirus myeloencephalopathy (EHM), and chorioretinopathy. EHV-1 has also been shown to infect the male reproductive tract, residing in the testicular vasculature and seminal fluid following infection. However, the clinical relevance of these findings has yet to be determined. Differences in the pathogenic potential of viral strains can influence clinical outcome as do several additional host and environmental factors.

Respiratory Disease

Respiratory disease caused by EHV-1 can be mild or subclinical in older or previously infected horses. In contrast, the respiratory disease observed in young immunologically naïve horses can be severe, lasting for 2 to 3 weeks. This form is characterized by a biphasic fever, lethargy, anorexia, coughing, and nasal and ocular discharge that is initially serous and then becomes mucopurulent.

Abortions and Neonatal Foal Death

EHV-1 is also the cause of late-term abortions in the third trimester of pregnancy, in addition to the premature delivery of foals that die soon after birth. Mares infected with EHV-1 can appear healthy and abort 2 weeks to several months after infection or reactivation of the virus. Sporadic abortions in individual mares are most common, but EHV-1 outbreaks with high attack rates (so-called abortion storms) have been reported and depend on herd management, immune status, and viral factors.

EHV-1 Myeloencephalopathy

In addition to respiratory disease and reproductive consequences, EHV-1 also causes a neurological disease apparent in up to ~10% of infected horses in
outbreak situations. Onset of EHM typically occurs between 9 to 13 days after primary infection following a secondary fever response that is associated with a cell-associated viremia. Clinical signs range from mild, temporary ataxia to paralysis that can lead to recumbency and urinary incontinence, bladder distension/tony, and urine dribbling, often resulting in euthanasia. The caudal spinal cord is often affected more severely, resulting in weakness of hind limbs, ascending hind limb paralysis, bladder dysfunction, and sensory deficits in the perineal area.

EHV-1 Chorioretinopathy
EHV-1 infection can lead to chorioretinopathy, causing permanent “shotgun” lesions of the retina in a substantial proportion of infected horses (Fig. 1). Ocular lesions primarily affect the choroidal vasculature and appear between 4 weeks and 3 months after primary infection. Clinically, lesions do not have a significant effect most of the time, but histopathological changes mirror those observed in the central nervous system (CNS) and uterus.

3. Pathogenesis of EHV-1 and EHM
Pathogenesis of Infection
Initial infection with EHV-1 commonly occurs within the first few months of life via respiratory secretions, fomites, or exposure to placental or fetal materials containing the virus. After infection, a life-long latency is established in the trigeminal ganglion and lymphoid tissues and reactivation can occur during periods of stress and lead to renewed shedding, clinical disease, and infection of other horses. Primary infection with EHV-1 occurs via the respiratory tract and results in replication, shedding, and spread of the virus via the basement membrane to the underlying tissues and local lymph nodes of the head. After further replication and infection of leukocytes, a cell-associated viremia is established between days 4 and 10 postinfection.

This cell-associated viremia is central in the pathogenesis of EHV-1 because peripheral blood mononuclear cells are a robust immune and inflammatory cell population in the vasculature, as well as carriers of EHV-1, and transport the virus to sites of secondary infection including the CNS. In the vasculature of the CNS, contact between infected leukocytes and the vascular endothelium leads to endothelial cell infection, inflammation, thrombosis, tissue necrosis, and ultimately EHM directly after viremia on day 9 to 13 postinfection.

Viral and Host Factors
A number of recent studies have examined viral and host factors that contribute to the establishment of viremia, virus and host interaction during viremia, and transfer of virus to vascular endothelial cells of the spinal cord. For viral factors, the identification of a single nucleotide polymorphism in the viral polymerase gene that results in a coding change (D752 vs. N752), is the most studied and has been shown to be strongly associated with an increased duration and magnitude of viremia and neuropathogenicity. Other viral proteins that have thus far been identified to be directly involved in viral spread and cell-to-cell transfer in vitro include ORF2, ORF17, gB, gD, gp2, and UL3. For host factors, increased age, breed (Standardbred, Warmblood, Thoroughbred, Quarter Horse, Paint, Appaloosa, Spanish horses, Fjord, Draft, and Lipizzaner), female sex, increased magnitude and duration of viremia, and pregnancy or nursing have been identified with an increased incidence of clinical EHM. In addition, more recent in vitro studies have shown that cellular mechanisms that contribute to EHM pathogenesis include the induction of interferons, chemokine responses, activation of the mitogen-activated protein kinase (MAPK) pathway, regulation of adhesion molecules, and cell-to-cell contact. In addition, a dysregulation of hemostasis after EHV-1 infection has been shown in vitro and in vivo and is thought to play an important role in the neuropathogenesis of EHV-1.

4. Control of EHV-1 and EHM
Vaccination and Immunity
Currently, there are a number of inactivated and modified live vaccines for EHV-1 commercially available. Vaccination regimens are commonly implemented in large breeding or stud operations and in the racing industry to reduce viral spread. However, although current vaccines can limit viral nasal shedding and clinical signs of respiratory disease, the reduction of viremia is more limited, as is prevention of abortion. Currently only two vaccines are licensed for providing some protection from abortion, and none of the current vaccines appear to reduce the incidence of EHM. AAEP guidelines
recommend vaccination after the initial series at 6-month intervals for horses younger than 5 years of age, horses on breeding farms or exposed to pregnant mares, horses on premises with frequent movement, and performance or show horses (https://aaep.org/guidelines/vaccination-guidelines/risk-based-vaccination-guidelines/equine-herpesvirus-rhinopneumonitis). However, in the face of an outbreak, vaccination of sick or already exposed animals is not recommended. Vaccination of healthy horses in neighboring premises may aid in the limit of viral spread but should not be performed with the expectation of preventing EHM. Furthermore, in light of some reports suggesting an increased incidence of EHM as a result of frequent vaccination, initiation of EHV-1 vaccination should be carefully considered.24,32 Some of the problems related to developing better vaccines for herpesviruses are the establishment of latency and the fact that herpesviruses use many immune evasive mechanisms to subvert induction of protective immunity. In addition, EHV-1 affects multiple sites of the body, and a better understanding is needed to define what protection entails. This includes whether the goal is to protect from respiratory disease, abortions, and EHM or whether also trying to prevent viral nasal shedding, viremia, and possibly establishment or reactivation from latency. In order to achieve any of these goals, it is vital to understand what is required for protection from these different aspects of EHV-1 infection. After natural infection, a short period of immunity protects against reinfection, and horses are likely protected from all clinical disease manifestations as well as shedding and viremia.33 This immunity includes a combination of virus-neutralizing antibodies, which play a role in reducing nasal viral shedding;33 and cytotoxic T-lymphocytes (CTLs), which are most critical for protection from secondary clinical disease and viremia.19,34 as well as multiple components of mucosal and systemic innate immunity.35,36 However, the only identified reliable correlate of immunity for protection from EHM is precursor frequencies of CTLs (pCTL).19,34 Unfortunately, good laboratory assay systems for regularly evaluating pCTL are lacking. Some studies have also identified high preinfection EHV-1-specific IgG and IgG3/7 (IgGb) titers in serum as well as a rapid induction of IgG3/7 in the absence of interferons and chemokines in nasal secretions after infection as an indicator of protection from respiratory disease, shedding, and viremia,37 but these studies did not include horses that showed clinical EHM or abortions. Consequently, predictions may not apply the same way to secondary EHV-1 manifestations. An earlier study found that protection from EHM was associated with increased IgG3/7 (IgGb) and decreased IgG3/5 (IgGT) levels and lower IgG3/5 to IgG3/7 or IgGl(IgGa) ratios in serum prechallenge.38 A third study that used the neuropathic strain Ab4 and induced clinical EHM in 3 out of 8 yearling horses identified decreased induction of interferon (IFN) alpha and increased induction of interleukin (IL)-10 in nasal secretions collected from horses that went on to develop clinical EHM.7 Together, these results suggest that a shift from a T-helper 1 type cellular immunity to a T-helper 2 type immunity may correlate with an increased risk for developing EHM. These data are supported by a study evaluating host risk factors correlated with EHM by using the “aged mare model.”39 However, studies that include horses of all ages, sexes, and breeds that are affected by clinical EHM would be needed to further confirm host risk factors for EHM, correlates for protection from EHM, and ultimately development of more efficacious vaccines.

Management and Biosecurity
In the absence of better vaccines, current measures to protect horses from EHV-1 and EHM outbreaks should rely on the implementation of effective management practices aimed at reducing the likelihood of introducing and disseminating EHV-1 infection.10 On a day-to-day basis, this means quarantine for new incoming horses or horses returning from an on-site event. At a show or event, this means avoiding sharing food/water buckets or other equipment that could be contaminated, washing hands, limiting stress as much as possible, and generally keeping horses away from others that are not from the same cohort. Horses presenting with fevers and nonspecific symptoms that may or may not include neurological signs should be isolated until a diagnosis is secured. If an acute EHV-1 infection and shedding have been diagnosed on the premises, stringent biosecurity measures should be taken, including isolation of potentially infected horses for 21 to 28 days after the last new infection (https://aaep.org/sites/default/files/Documents/EHV1_4_Final.pdf).10 In an outbreak situation, all animals on the premises should be monitored for clinical signs which includes taking temperatures twice daily. Sick or exposed animals with a confirmed diagnosis should be segregated using distance, airspace, or a separate building and supported by separate personnel and equipment. Foot traffic should be kept out of the primary biosecurity perimeter, and hygiene measures should be implemented (i.e., foot baths, hand washing, and protective clothing). Other horses on the premises that have not been diagnosed and appear clinically healthy should be monitored daily for fevers, respiratory signs, submandibular lymph node enlargement/sensitivity, signs of limb/ventral edema, or neurological signs and tested for viral nasal shedding and viremia if any clinical signs suggestive of EHV-1 are detected. Quarantined healthy horses should only be lightly exercised separately from nonquarantined horses, preferably outdoors. Furthermore, exposed horses should not be moved until it is determined they are not going to develop disease or pose a risk to unexposed horses. Vaccination of sick or already exposed animals on the premises is not recommended. For diagnosis, a combination of nasal swabs and whole blood collected in ethylenediaminetetraacetic acid (EDTA) tubes is recommended to...
evaluate nasal shedding and viremia associated with EHV-1 infection. Testing of asymptomatic horses is done to verify the absence of shedding at the end of the quarantine period. 

Viruses isolation is considered the “gold standard” test for making a laboratory diagnosis of EHV-1 infection and should be attempted, especially during epidemics of EHM, concurrent with use of rapid diagnostic tests such as quantitative polymerase chain reaction (qPCR), to achieve retrospective biological and molecular characterization of the viral isolate. Serology that demonstrates a 4-fold or greater increase in serum antibody titer, using serum neutralization or complement fixation tests, on acute and convalescent samples collected 7 to 21 days apart can also provide presumptive evidence of infection.

5. Summary

In conclusion, EHV-1 is ubiquitous in horses worldwide, and >80% of horses are estimated to be latently infected with the virus. EHM is a relatively rare but devastating sequela of EHV-1 infection that can occur in up to ~10% of infected horses in outbreak situations. The mechanism underlying CNS endothelial infection is unknown, as are the risk factors that determine its occurrence. Although viral factors are certain to be important, host factors including age, breed, sex, pregnancy, and magnitude and duration of viremia as well as environmental factors are also critical. Immunity after infection or vaccination offers limited protection in particular regarding EHM. This lack of an induction of protective immunity to EHV-1 is likely due to immunomodulatory properties of the virus and latency establishment. Thus, early recognition and diagnosis of suspected cases and monitoring of high-risk horses, as well as stringent biosecurity measures, currently represent the most reliable means of preventing outbreaks of EHM.

Acknowledgments

Declaration of Ethics

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

Conflict of Interest

The Author has no conflicts of interest.

References


Trouble with Gamma-Herpesviruses

Lutz S. Goehring, DVM, MS, PhD, DACVIM, DECEIM

Nasal swabs/conjunctival swabs PCR-positive for equid herpesvirus (EHV) -2 or -5 are poor indicators of disease. Testing for EHV-2/-5 in multiplex PCR panel assays is worth rethinking. PCR EHV-5 detection on peripheral blood and/or bronchoalveolar lavage fluid (BALF) from horses that presented for a respiratory disease work-up should prompt chest radiographs, especially when BAL cytology does not support the level of clinical disease/exercise intolerance. Conditions or diseases associated with EHV-5 are unlikely to benefit from (systemic or local) valacyclovir (or similar) therapy. Author’s address: Wright Markey Chair and Professor of Equine Infectious Diseases MH Gluck Equine Research Center, University of Kentucky, 1400 Nicholasville Road, Lexington, KY 40546-0099; e-mail: l.goehring@uky.edu. © 2021 AAEP.

1. Introduction

Herpesviruses are all enveloped (double-strain) DNA viruses and are divided in 3 sub-classes: alpha, beta, and gamma. Currently, there are no known beta-herpesviruses known in equids; however, several alpha-herpesviruses are known to cause disease in horses (EHV-1, -3, -4). Equid herpesvirus-2 (EHV-2) and -5 (EHV-5) are two of five currently known gamma-herpesviruses of equids. EHV-5 and EHV-2 are frequently detected in combination and seem to be more strongly associated with horses, while 3 gamma-herpesviruses, (asinine; AHV)-2, AHV-4 and -5 are more commonly found in donkeys. However, crossover and co-existence in horses or donkeys have been observed. For decades, both gamma-herpesviruses played an unclear role in mild to, on occasions, moderate disease in horses of the respiratory tract or were associated with keratoconjunctival disease. In 2007, a novel and seriously debilitating disease was described in five horses that seemed strongly associated with EHV-5. Due to location and macroscopic appearance, the disease was named equine multi-nodular pulmonary fibrosis (EMPF). Since then, EMPF has been diagnosed as an incidental but severely debilitating disease on all continents. EHV-5 was first described in horses in Australia. Both EHV-2 and -5, when in their latent stage of chronic-persistent infection, have been found in peripheral blood mononuclear cells (PBMC), mainly in T- and B-lymphocytes. These viruses differ in their ease of recovery using viral culture techniques. While both require co-culture techniques to be isolated from PBMC, it has been easy to recover EHV-2 and notoriously difficult to recover EHV-5. With the introduction of (quantitative) PCR, the extensive prevalence of EHV-5 (and EHV-2) was discovered. Both viruses have a world-wide distribution and can even be found in isolated or remote horse (or donkey) populations. As a likely result of a parliament decision from the 1880s to
not allow the further import of mammals onto the island, the Icelandic horse population is, for example, negative for EHV-1, Strep equi spp. equi, and equine influenza; yet both gamma-herpesviruses EHV-2 and -5 (and alpha EHV-4) have been detected in Icelandic horses. This shows the longstanding pathogen and host interaction for centuries probably, which requires significant adaptation and only mild pathology during replicative phases of the pathogen, if at all.

2. Clinical Presentation

Since their detection, EHV-2 and -5 have been described as fairly benign causes of respiratory tract disease and possibly keratoconjunctivitis (KCC) in foals and young horses upon first-time exposure to these pathogens. Upon infection, there can be a fever, serous to mucoid nasal discharge, conjunctivitis, and lymphadenopathy of the mandibular/retropharyngeal lymph nodes.2 There are few single case reports published on EHV-2 and EHV-5 associated with dermatitis, lymphoma, or esophageal disease.3 In a larger number of studies, KCC has been debated as a result of EHV-2 and/or -5 infection. One large study in approx. 250 (single breed) horses, examined on four occasions at 6-month intervals, did not show an association with ophthalmic disease and equine gammaherpesviruses. Forty-five to 60% of horses with KCC were at least once positive for either EHV-2 and/or -5 on a conjunctival swab, while 13 (30%) of clinically normal horses were also positive.4 EHV-5 has been demonstrated in endometrial lavages in about 15% of a total of 60 mares with reproductive disorders,5 while less than 3% or 2%, respectively, of abortion/stillbirth/neonatal death/placentitis cases were PCR positive with EHV-2 or -5.6 EHV-5 and EHV-2 have also been identified in gastric mucosa by novel in situ hybridization technique and PCR; however, EHV-5 was not associated with ulcerative lesions.7 Several research groups have focused on the question whether EHV-2 or -5 infections are involved in inflammatory airway disease (IAD) in (young) performance horses. Houtsma and collaborators8 looked at two groups, an IAD group vs. a healthy control group at a single time point. Results suggested a positive relationship between EHV-2 presence in nasal swab and IAD. Interestingly, EHV-5 was only detected in (few) BAL samples, however, not in nasal swabs of IAD horses. The group concluded an association of gamma-herpesviruses presence and IAD in their study. Doubli-Bounoua and collaborators9 conducted their study on 50+ Standardbreds, which were followed for over two years with once-a-month collection of a nasal swab and tracheal aspirate (TA) collected via endoscopic examination. EHV-2 and EHV-5 were the most frequently identified respiratory tract viruses, often in combination, in this study. These authors found a correlation between EHV-2, coughing, and tracheal mucus volume, while EHV-5 positive samples seemed evenly distributed between affected and unaffected horses. Similar conclusions came from Sweden, where a study focused on EHV-5 presence in the respiratory tract of about 60 elite Standardbred horses and their respective performance levels.10 Horses were followed for one year with monthly sampling (13 sampling episodes), where TA and nasal swabs were collected each time. Performance status at time of sampling was determined using a standardized exercise test. At least one sample (NS or TA) per sampled horse was positive once for EHV-5 using qPCR during the observational period. Several horses had multiple samples positive for EHV-5 with highly variable quantities of viral copy numbers. However, regardless of the viral quantity, there was no association between EHV-5 presence and change in ability to perform. Viral strains of the study (and two archived strains collected from EMPF horses) were sequenced for viral gB, which allowed allocation of the Swedish strains into one of four genotypes (I – IV). Most strains were either in group I or III. As these horses were sampled multiple times at monthly intervals, it is worth mentioning that genotypes differed between monthly samplings, and the two strains from archived EMPF samples were assigned to EHV-5 genotype III.11 In 2007, EMPF was described as a novel disease of the lower respiratory tract of horses. Furthermore, EMPF appears to be the most severe clinical presentation to date consistently associated with EHV-5. Horses at a more advanced stage of disease present with tachypnea, exercise intolerance, and an undulating fever. Cytology of tracheal aspirate or BALF can be surprisingly modest regarding its cellularity. There are typically nodular opacities visible on chest radiographs, while peripheral areas of lung consolidation can be also visualized by ultrasound imaging. Lung biopsies show interstitial fibrosis with marked influx of inflammatory cells, mainly lymphocytes. In a first case series publication describing a little more than 20 cases, necropsy results revealed distinct nodular fibrosis demarcated from more normal (aerated) lung tissue, with a predominantly lymphocytic interstitial cell infiltrate.12 Remaining but deformed alveolar spaces were filled with a moderate exudate of mixed neutrophilic granulocytes and macrophages, while the remaining alveolar-like architecture is lined with cuboidal epithelial cells. An increased number of myofibroblasts has been noticed in the affected areas. Occasionally, macrophages contained viral inclusion bodies (IB), which, on electron microscopy (EM) investigation, showed viral particles. An EHV-5 specific in situ hybridization was positive for the IB and for sections of (remaining) alveolar spaces for macrophages there. PCR analysis specific for consensus herpesvirus and for equid herpesviruses -1, -2, -4, -5 was positive for EHV-5 in all 20+ cases and for EHV-2 in about one third of cases. More so, clinical disease (and macro/microscopic disease) has been successfully evoked via instillation of cultured EHV-5 that
3. Diagnostics

It appears that neither nasal nor conjunctival swabs positive for EHV-5 and/or EHV-2 are likely predictive for disease in the upper respiratory tract or for KCC. An EHV-5 positive BALF sample might be indicative for EMPF and warrants further investigation. Pusterla and collaborators compared four groups of horses with EMPF, other interstitial (non-EMPf) pneumonia, IAD, and a control group without signs of respiratory disease. They collected peripheral blood, nasal swabs, and BALF from all horses. Interestingly, almost all horses with EMPF were EHV-5 positive in peripheral blood samples, NS, and BALF. Animals of any of the other groups were rarely positive on peripheral blood samples, and only one horse out of 32 of the IAD-group was EHV-5 positive on a BALF sample. However, there was a relatively large proportion of EHV-5 positive (between 45-70%) NS samples in any of the non-EMPf groups. The authors concluded that BALF could be a valuable diagnostic sample in the diagnosis of EMPF.

4. Treatment

Current therapy focuses on anti-inflammatory/ immunosuppressive therapy with dexamethasone combined with additional inflammation-modulatory therapy with doxycycline. There are no long-term studies on EMPF therapy efficacy and clinical outcome. Virustatic drugs such as valacyclovir or ganciclovir have been implemented as therapeutics during herpesvirus-induced disease in the horse. A study on a small group of EMPF horses treated with an established valacyclovir dosing regimen over 10 days has failed to lower EHV-5 load in BALF prior to and after (10 day) treatment. An explanation for treatment failure using virustatics could be the virus' rapid cell entry into B- and T-lymphocytes, which it uses as a vehicle to be transported throughout the body; however, this cell entry also switches the virus to its latent or dormant form. As valacyclovir or ganciclovir are drugs that interfere with active (lytic) viral replication, a latent stage of virus will be left untouched. Still, this does not explain EMPF pathology, and it is speculated that EHV-5 is transferred to pulmonary epithelial cells, where virus replication could be temporarily more robust, and into myofibroblasts that are activated by the virus to produce collagen. It seems quite likely that without transcriptome and proteome analysis, the question of the role of EHV-5 as the innocent bystander, the accomplice, or the perpetrator cannot be answered any time soon. However, only with a better understanding of this virus and its pathogenesis is there a chance for more effective EMPF or other disease entities therapy.

5. Conclusion

In summary, there seems to be many body systems where EHV-2/5 can be found under physiological or pathological circumstances. There seems to be a stronger association of EHV-5 prevalence and (upper) respiratory tract; however, a causal relationship of airway disease or impairment of respiratory tract function has yet to be elucidated. At this stage, the role of these viruses is unclear, whether they are innocent bystanders due to their latency location and became involved in immune-mediated disease, or they are partners in crime, potentially activating immune cells. Availability of transcripational data, RNA-data that elucidates gene-activity, would be valuable information on the pathogenesis of equine gammaherpesviruses.

Acknowledgments

Declaration of Ethics

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Conflict of Interest

The Author has no conflicts of interest.

References


Equine Rhinitis Viruses: The Upcoming Respiratory Pathogens

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1. Introduction and Epidemiology

Equine rhinitis viruses (ERVs) are an important cause of acute respiratory disease in horses across the globe, with cases previously reported in Australia, North America, Europe and Asia.1–5 Equine rhinitis A and equine rhinitis B viruses (ERAV and ERBV, respectively), formerly called equine rhinoviruses-1 and 2/3, were originally discovered in the 1960’s (ERAV) and 1970’s (ERBV) and are RNA viruses of the picornavirus family.6,7 ERAV is of the genus aphthovirus and is closely related to foot-and-mouth disease virus, whereas ERBV is the sole member of the genus erbovirus.8–10 Only one serotype of ERAV has so far been identified; however, three distinct serotypes of ERBV have been isolated: ERVB1.1436/71, ERBV2.313/75, and ERBV3.11,12 Initial infection with ERVs results in local viral reproduction in the nasopharynx. In ERAV, this is followed by a viremia lasting 4–5 days.13 Transmission between horses occurs primarily through close contact and inhalation of respiratory droplets; however, indirect transmission through contaminated equipment or waste from infected horses may also occur. As with other viral respiratory pathogens, shedding occurs from the nasopharynx; however, shedding through urine and feces may also play a significant role in allowing ERVs spread through a facility.13–15 This shedding may persist in some horses long after clinical resolution. A study by Lynch et al, 2013 found high levels of ERAV shedding in urine persisted for at least 37 days post-infection (the length of the study).15 Interestingly, a case study by Johnson et al, 2012 also found ERAV in a semen sample from a healthy stallion, although the significance of this finding for viral transmission is unknown.16 Since their discovery, the contribution of ERVs to the burden of equine respiratory disease has been unclear. Several studies investigating the seroprevalence of ERVs have found that a high proportion of mature performance horses had serum neutralizing antibodies to ERAV (~37–75%) and ERBV (~50–80%).10,17–19 Seroprevalence in some populations was even higher; for example, a study of 200 clinically healthy horses stabled across Austria found seroprevalences of 90% and 83% to ERAV and ERBV, respectively. Many foals acquire passive immunity to ERVs from their dams at birth, but immunity wanes significantly by 5–6 months of age and, in the case of ERAV, may disappear completely at 10–12 months.18 Many horses seroconvert to ERAV as yearlings and 2-year-old’s, often coinciding with introduction into training facilities.2,20,21 ERAV seroreversion is not typically observed after initial infection suggesting immunity is long-lasting.18 In contrast, seroconversion to ERBV can happen at an earlier age (<6 months) than ERAV and, as there may be overlap in time between the presence of maternal antibodies and actively acquired immunity due to exposure, interpretation of changes in titer may be difficult. Unlike ERAV, ERBV seroreversion is commonly observed and
repeated episodes of reinfection or recursion may occur throughout a horse’s life.\textsuperscript{18} Seroprevalence studies suggest ERV infections are common throughout the world. However, their importance as a cause of acute respiratory disease in horses has often been overlooked due to several factors. While many performance horses are seropositive for ERVs, these viruses are rarely isolated from clinical cases of acute respiratory disease.\textsuperscript{22} This discrepancy may result from the well-documented difficulties in isolating ERVs using standard virus isolation techniques.\textsuperscript{2,20,23,24} The reason for this difficulty is unclear. ERVs may occur undiagnosed, or be attributed to other respiratory pathogens. To further confuse the issue, ERVs have been recovered in clinically healthy horses and are frequently found in combination with other viral or bacterial pathogens.\textsuperscript{2,5,23} These findings suggest the principal role of ERVs may be to increase the severity and duration of other respiratory diseases through co-infection, rather than as stand-alone pathogens. However, previous studies have documented outbreaks of acute respiratory disease caused by ERVs without coinfection with other pathogens.\textsuperscript{21,22,25,26} Occurrence of clinical disease often coincided with entry into training yards.\textsuperscript{21,22,26,27} These findings are not surprising as horses are often transported to training facilities and/or yearling sales as yearlings or 2-year-olds and encounter an increased number of other horses and handlers. Clinical cases varied widely in severity, with some horses exhibiting mild respiratory signs lasting 1–3 days and others experiencing more severe signs lasting several weeks. Probability of severe disease was found to be highest in yearlings and 2-year-olds.\textsuperscript{21,28} Age, number of horse-to-horse contacts, and the stress of transportation and/or early training have all been suggested to increase susceptibility to ERVs and other respiratory pathogens.\textsuperscript{21,27–30} Therefore, while ERVs may not represent a common source of morbidity in mature horses, evidence suggests these viruses significantly contribute to the burden of respiratory disease in young animals, particularly those in performance disciplines.

2. Clinical Presentation

Clinical disease is characterized by a febrile period of 1–3 days, cough, anorexia, serous nasal discharge, which progresses to mucopurulent, and enlargement of the lymph nodes of the head and neck. Distal limb edema is also occasionally observed. These infections are usually transient; however there is wide variation in the duration and severity of clinical signs with a small proportion of horses experiencing prolonged illness.\textsuperscript{28} An experimental study of ponies inoculated with ERAV found that in addition to upper airway signs, clinical cases also developed adventitial lung sounds and mucopurulent discharge in the lower airways on endoscopic examination.\textsuperscript{25} These findings suggest ERAV infection is not confined to the upper respiratory tract. In addition to clinical illness, ERAV infections are a source of missed training days and failure to race.\textsuperscript{27,28} In an outbreak of ERAV in 51 yearlings stabled at a Standardbred training facility, clinical signs persisted for 1–40 days, and 6 horses were removed from training for 8–12 weeks due to the severity of their condition.\textsuperscript{26} Subclinical ERV infection and seroconversion has commonly been observed in stables experiencing outbreaks.\textsuperscript{2,21}

3. Diagnosis

Laboratory diagnosis of ERAV and ERBV can be made by measuring antibody titer in paired acute and convalescent sera. Serum samples should be taken at the onset of clinical illness and 2–3 weeks post-infection. Antibody titer is commonly measured using standard viral neutralization assays; however, enzyme-linked immunosorbent assays (ELISAs) have recently been introduced. As there is evidence of persistent shedding of ERVs after clinical resolution, use of paired titers can distinguish between acute and chronic infections by detecting the timing of seroconversion. Due to the high seroprevalence of the ERVs in mature horses and the high titers observed in horses previously infected with ERAV, increases in antibody titers may not occur with future exposures.\textsuperscript{25} Therefore, caution should be used when interpreting serology data from mature horses. Several studies have reported difficulties in isolating ERVs from nasopharyngeal swabs using standard virus isolation techniques.\textsuperscript{2,20,23,24,26} The reason for this difficulty is unclear; however, there is some evidence that choice of rapidly dividing cell cultures and addition of MgCl\textsubscript{2} enhances the growth of ERBV serotypes, thereby improving diagnostic sensitivity.\textsuperscript{24} Given the poor diagnostic sensitivity of virus isolation, and the documented isolation of ERVs from clinically healthy horses, use of virus isolation alone as a diagnostic tool is not recommended. However, it may be a useful adjunct to other tests. In recent years, reverse transcriptase polymerase chain reaction (RT-PCR) has been used to detect ERVs from nasopharyngeal or urine swabs with improved sensitivity over virus isolation, and 100% specificity.\textsuperscript{23,24,31} Another advantage of RT-PCR is its shorter turnaround time compared to virus isolation or serology, allowing for more rapid diagnosis. Given the difficulty of isolating ERVs and the occurrence of persistent viral shedding, it is recommended that practitioners use serology alone or in combination with RT-PCR as a means of diagnosis.

4. Treatment

As with other viral respiratory diseases, treatment is largely supportive. Anti-inflammatories can be administered to reduce inflammation and discomfort. Antibiotics are not indicated unless
there is evidence of secondary bacterial infection. Horses should be rested for at least two weeks, although a previous study investigating plasma fibrinogen in ERAV cases found that levels remained increased 4-6 weeks postinfection, even in uncomplicated cases.26 Therefore, the duration of systemic recovery may necessitate longer periods of rest before horses return to their full training regime, to prevent potential complications. Early return to training may prolong duration of clinical signs and increase the risk of complications such as bronchopneumonia.26

5. Prevention

There are currently no approved vaccines against ERBV; however, a vaccine against ERAV has been developed by Boehringer Ingelheim Animal Health and been granted a license for conditional use by the USDA where there is demonstrated need. Even so, to date no studies have been published demonstrating the efficacy or safety of this vaccine and therefore, its usefulness in preventing ERAV is unknown. Implementation of effective farm level biosecurity and disinfection protocols is the best approach to prevent the introduction and spread of ERVs in stables. These protocols should include quarantining or cohorting new or returning horses by date of entry or seller, before allowing them to mix with other animals in the facility. In the event of an outbreak, sick horses should be stabilized in isolation stalls away from healthy horses where possible. In addition, disinfection of common surfaces and equipment, staff hygiene, and proper waste management will reduce the chance of transmission between animals. Although there are no published studies on the persistence of ERVs in the environment, other picornaviruses such as foot and mouth virus can survive on surfaces for at least 14 days.32 Therefore, disinfection protocols should include cleaning of surfaces with agents effective against non-enveloped viruses such as chlorine or iodine compounds.33 As stress has been proposed to increase the incidence and chronicity of clinical ERAV infections in yearlings and 2-year-olds, a period of acclimation after sale or relocation is recommended before commencement of training.1,28

Acknowledgments

The Author would like to thank Dr. Matthew Wittenrich for his help with editing this paper.

Declaration of Ethics

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

Conflict of Interest

The Author has no conflicts of interest.

References


Comparison of Equine Synovial Sepsis Rate Following Intrasynovial Injection in Ambulatory vs. Hospital Settings

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Frequency of synovial sepsis was not different when comparing intrasynovial injections performed in the field versus in-hospital and with or without antibiotics. Authors’ address: Colorado State University, 300 West Drake Road, Fort Collins, CO 80523; e-mail: lynn.pezzanite@colostate.edu. *Corresponding and presenting author. © 2021 AAEP.

1. Introduction
Frequency of synovial sepsis in horses following intrasynovial injection has been reported, but not compared with respect to environment in which the injection was performed. The objective of this study was to describe occurrence of synovial sepsis following intrasynovial injections performed in ambulatory versus hospital settings.

2. Materials and Methods
Records from Colorado State University were evaluated (2014-2018) and horses receiving intrasynovial injections identified. Patients presenting for septic synovial structures were excluded. Patient signalment, primary supervising service, medications injected, location (field/hospital), whether synovial sepsis resulted, and at what time sepsis was recognized were recorded. Logistic regression was used to estimate the contributions of covariates to the occurrence of synovial sepsis following injection.

3. Results
During the study period, 3866 intrasynovial injections were performed in 1112 horses during 1623 sessions (643/1623 sessions in the field). Performing injections in the field vs. hospital \( p = 0.2 \) or without antibiotics \( p = 0.7 \) did not alter risk of synovial sepsis. Most frequently used medications were hyaluronate (846/1623, 52.1%), triamcinolone acetonide (780/1623, 48.1%), and amikacin sulfate (684/1623, 42.1%). Four horses developed synovial sepsis (0.2% sessions, 0.1% synovial structures); 3/4 injected in the field, 2/4 received antibiotics concurrently. Frequency of septic synovitis was 10.4 cases per 10,000 injections, or 1 in 967 injections.

4. Discussion
These data may help to inform practitioners and clients regarding the relative potential risk of complications following intrasynovial medication in different environmental settings.

Research Abstract—for more information, contact the corresponding author

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Declaration of Ethics

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Conflict of Interest

The Authors have no conflicts of interest.
Effects of Racing on Systemic Cytokine mRNA Expression in 2-Year-Old Thoroughbreds

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This is the first study evaluating the effects of racing on cytokine profiles in 2-year-old Thoroughbred racehorses. This study confirms a major role of the immune system on the adaptation of young equine athletes to strenuous exercise. Authors’ addresses: Department of Veterinary Clinical Sciences, Washington State University, Pullman, WA 99164 (Sanz, Bayly); and Maxwell H. Gluck Equine Research Center, University of Kentucky, Lexington, KY 40546 (Page, Horohov); e-mail: macarena@wsu.edu. *Corresponding and presenting author. © 2021 AAEP.

1. Introduction
Two-year-old Thoroughbreds are more susceptible to developing respiratory disease, but the effect of racing on their immune system is not well described. Systemic mRNA cytokine expression before and after racing was evaluated.

2. Materials and Methods
Two-year-old Thoroughbreds (n = 98) were included with owner consent. Blood was collected before (up to 24 hours) and after (within 2 hours) racing for mRNA expression of 12 cytokines with roles in inflammation and immune system function. No furosemide was administered preracing. A paired t-test/Mann–Whitney test was used to compare before and after racing samples. Relationships between cytokines were assessed with Pearson correlation. Significance was set at p < .05.

3. Results
After racing, CCAAT enhancer binding protein beta (CEBPB), hypoxia-inducible factor (HIF)-1α, interleukin (IL)-8, IL-10, and IL-17 increased, whereas insulin-like growth factor-1 and vascular endothelial growth factor, decreased (p < 0.001). Interferon-γ, IL-1β, IL-4, IL-6, and tumor necrosis factor-α did not change with strenuous exercise. Strong correlations (p < .001) were found between CEBPB and HIF-1A (0.81), HIF-1A and IL-10 (0.64) and IL-1β and vascular endothelial growth factor A (0.61).

4. Discussion
Racing induced significant changes in cytokine expression. Like that seen in older racehorses, pro-inflammatory and anti-inflammatory cytokines were increased. Cytokines involved with muscle repair and growth also changed. These
cytokines have not been evaluated in young racehorses and the findings may represent a new conduit for the investigation of fitness, and the impact of training programs on equine health.

Acknowledgments

Declaration of Ethics
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Conflict of Interest
The Authors have no conflicts of interest.
Exercise Affects Proximal Sesamoid Bone Pathology in Thoroughbred Racehorses

Sarah K. Shaffer, BS*; Susan M. Stover, PhD, DVM, DACVS; and David P. Fyhrie, PhD

Proximal sesamoid bone fracture is a common fatal injury among Thoroughbred racehorses. A focal region of bone loss and high microcrack density occur before proximal sesamoid bone fracture. Bone loss and microdamage are related to horse training history. Authors’ addresses: Department of Mechanical Engineering (Shaffer), Department of Radiological and Surgical Sciences, School of Veterinary Medicine (Stover), Department of Biomedical Engineering (Fyhrie), Department of Orthopedics, School of Medicine (Fyhrie), University of California-Davis, One Shields Avenue, Davis, CA 95616; e-mail: skshaffer@ucdavis.edu. *Corresponding and presenting author. © 2021 AAEP.

1. Introduction
A radiolucent subchondral bone lesion (SBL) often precedes proximal sesamoid bone (PSB) mid-body fracture. The relationships between horse exercise history, focal bone loss, and microcracks were examined for racehorses that did (case) or did not (control) suffer biaxial PSB fracture.

2. Materials and Methods
Bone volume fraction and microcrack density (Cr.Dn) were quantified using microcomputed tomography and undecalciﬁed histology, and compared among 10 fractured medial-PSBs from case horses (FX-PSB), 10 intact medial-PSBs from the opposite forelimb of case horses (CLI-PSB), and 10 intact medial-PSBs from control horses without PSB fracture (CTRL-PSB) using ANOVA. Relationships with lifetime gallop-speed exercise history variables were assessed using regression ($\alpha \leq 0.05$).

3. Results
SBLs were more prevalent in FX-PSBs (9) and CLI-PSBs (7) than CTRL-PSBs (0). Bone volume fraction was 8% and 6% lower in FX-PSBs than in CTRL-PSBs and CLI-PSBs, respectively. Cr.Dn was 187% higher in CLI-PSBs than in CTRL-PSBs and 260% higher in FX-PSBs than in CTRL-PSBs. Higher exercise frequency was associated with more bone loss and microcrack formation, especially in the most recent 10 months.

4. Discussion
Bilateral, osteopenic, microdamaged SBLs were observed in most case horses. The relationship between SBL tissue properties and horse exercise is consistent with damaged-induced bone remodeling. Horse training history is associated with fracture risk.

Research Abstract—for more information, contact the corresponding author

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Acknowledgments
This project was supported by the Grayson Jockey Club Research Foundation, Inc., the Maury Hull Fellowship (University of California Davis), the Louis R. Rowan Fellowship (California Thoroughbred Foundation), and the University of California Davis Center for Equine Health with funds provided by the State of California satellite wagering fund and contributions by private donors.

Declaration of Ethics
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Conflict of Interest
The Authors have no conflicts of interest.
Chiropractic Treatment of Lameness and Concurrent Axial Skeleton Pain and Dysfunction in Horses

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Chiropractic care had no significant effect on limb lameness; however, significant reductions in axial skeleton pain, stiffness, and muscle hypertonicity did occur. Authors’ address: Gail Holmes Equine Orthopaedic Research Center, Department of Clinical Sciences, College of Veterinary Medicine and Biomedical Sciences, Colorado State University, 300 West Drake Road, Fort Collins, CO, 80523; e-mail: kevin.haussler@colostate.edu. © 2021 AAEP.

1. Introduction
Chiropractic is a common treatment modality used to treat back pain and stiffness. Clinically important interactions occur between axial and appendicular regions with regards to poor performance and lameness. The objective of this study was to evaluate the effects of chiropractic treatment on limb lameness and concurrent axial skeleton pain and dysfunction.

2. Materials and Methods
Twenty collegiate polo horses with Grade 1-3/5 lameness (AAEP scale) within at least one limb were randomly assigned to treatment and control groups. Subjective and objective lameness examinations and scores for spinal stiffness, muscle hypertonicity, and mechanical nociceptive thresholds were performed on Days 0, 14, and 28 by a blinded examiner. Chiropractic treatment was applied on Days 0, 7, 14, and 21. Data was analyzed by a mixed model fit separately for each response variable ($p < 0.05$).

3. Results
No changes were observed in measures of lameness or mechanical nociceptive thresholds. Measures of muscle hypertonicity and spinal stiffness decreased significantly across sites within the axial skeleton. Spinal pain scores significantly decreased in the number of affected cervical vertebral levels and total severity.

4. Discussion
Chiropractic care had no effect on limb lameness. The reductions in spinal pain, stiffness, and muscle hypertonicity as they relate to...
Compensatory limb lameness mechanisms are clinically relevant. Limitations include the large variability in outcome parameters within horses and the lack of standardization of chiropractic treatment between horses.

Acknowledgments

Research funding was provided by the Colorado Racing Commission Funds, College of Veterinary Medicine and Biomedical Sciences, Colorado State University.

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Conflict of Interest
The Authors have no conflicts of interest.
Pharmacokinetics, Pharmacodynamic Efficacy, and Safety of Acetaminophen in Adult Horses with Naturally Occurring Chronic Lameness

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Acetaminophen is safe dosed at 30 mg/kg, PO, q12h for 21 days but not suitable as a monotherapy for analgesia. Authors’ addresses: Department of Biomedical Sciences and Pathobiology (Mercer, Davis, Cecere); Department of Large Animal Clinical Sciences (McKenzie, Byron, Trager-Burns, Wilson); Marion duPont Scott Equine Medical Center (Kelleher); Department of Population Health Sciences (Werre), Virginia Maryland College of Veterinary Medicine, Blacksburg, VA 24060; e-mail: mmercer1@vt.edu. *Corresponding and presenting author. © 2021 AAEP.

1. Introduction
Prolonged use of traditional NSAIDs is associated with adverse effects in horses. Acetaminophen has been combined with NSAIDs for treatment of orthopedic pain in horses. This study aimed to determine the pharmacokinetics, safety, and efficacy of acetaminophen in naturally occurring lameness.

2. Materials and Methods
Twelve horses with chronic lamenesses were administered acetaminophen (30 mg/kg, PO, q12h) for 21 days. Pharmacokinetic analysis was performed on days 7 and 21 and analyzed via liquid chromatography-tandem mass spectometry (LC-MS/MS). Clinicopathologic analysis, gastroscopy, and liver biopsy were performed on days 0 and 22. Objective lameness evaluation was performed over 12 hours without drug (control) and on day 21 of treatment.

3. Results
Maximum plasma acetaminophen concentrations were 22.70 ± 10.25 ug/mL occurring at 0.44 ± 0.22
hours on day 7 and 18.37 ± 6.91 ug/mL at 0.71 ± 0.26 hours on day 21. Squamous mucosa ulcer scores improved from day 0 (0.73 ± 0.11) to day 22 (0.28 ± 0.12; p = 0.03). There were no clinically significant differences in liver biopsy scores or clinicopathologic parameters. There was a statistically significant improvement in the vector sum total head height compared to time 0 at 1 hour post treatment (11 ± 45%) compared to baseline control (−25 ± 60%; p = 0.003).

4. Discussion
Absorption of acetaminophen was variable and dependent on the rate of gastric emptying similar to previous reports. Acetaminophen is safe following 21 days of treatment in adult horses but may not be effective alone for management of chronic naturally occurring lameness.

Acknowledgments

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Conflict of Interest
The Authors have no conflicts of interest.
Characterizing the Cytokine Environment in Acute Tendon Injury to Enhance Stem Cell Therapy

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A novel ultrafiltrate probe implanted within surgically created core lesions of the superficial digital flexor tendon allowed for cytokine characterization in acute tendon injury over 21 days. Inflammatory mediators peaked within 48 hours. Authors’ addresses: Department of Clinical Sciences, College of Veterinary Medicine (Koch, Berglund, Gilbertie, Ellis, Schnabel); Comparative Medicine Institute (Koch, Berglund, Gilbertie, Schnabel); Department of Molecular Biomedical Sciences, College of Veterinary Medicine (Messenger), North Carolina State University, Raleigh, NC 27606; e-mail: dwkoch@ncsu.edu. *Corresponding and presenting author. © 2021 AAEP.

1. Introduction
Tendon injury in the horse commonly affects the superficial digital flexor tendon (SDFT). Although mesenchymal stem cell (MSC) therapy has improved clinical and experimental SDFT lesions, little is known about how MSCs exert their influence in this environment. Further, the cytokine profile in acute tendon injury has been poorly characterized. In an equine model of acute tendon injury, the objective was to track the temporal change in inflammatory cytokines. The hypothesis was that these inflammatory cytokines change over time.

2. Materials and Methods
Horses (n = 7) underwent surgical induction of bilateral forelimb SDFT lesions with (n = 6) or without (n = 1) bilateral ultrafiltrate probe placement. Tendon ultrafiltrate was collected from the probes immediately postoperative and then every 12 hours for 21 days.

3. Results
Pro-inflammatory cytokines interleukin-1 beta, -6, and -8 along with fibroblast growth factor-2 were noted to peak by 48 hours. Percutaneous ultrasound-guided aspiration of control horse lesions was inconsistent and with analytes below the quantifiable level.

4. Discussion
The data support the hypothesis that a temporal change in inflammatory cytokines occurs following...
acute tendon injury with peak inflammation occurring by 48 hours. Future in vitro work will examine the MSC secretome following stimulation with a cytokine profile that represents peak versus post-inflammatory tendon injury to determine if the efficacy of stem cell therapy in tendon injury can be enhanced.

**Acknowledgments**

**Funding Sources**

Grayson-Jockey Club Research Foundation, NIH 5T32OD011130-13 (DWK), and NIH 5K01OD027037-02 (AKB).

**Declaration of Ethics**

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**Conflict of Interest**

The Authors have no conflicts of interest.
Histologic and Biomechanical Evaluation of Biopsy Samples of the Equine Digital Cushion from Forelimbs

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Biopsies of the equine digital cushion can be successfully obtained and used for mechanical and histological analysis. The digital cushion has varying properties depending on location of the sampling. Authors’ addresses: Aspen Meadow Veterinary Specialists, 104 S Main Street, Longmont, CO 80501 (Damone); Equine Field Service (Bass), Translational Medicine Institute (Gadomski), Microbiology, Immunology, and Pathology (Magunda), Colorado School of Public Health (Rao), Colorado State University, College of Veterinary Medicine, Fort Collins, CO 80523; University of Georgia, Department of Large Animal Medicine, College of Veterinary Medicine, Athens, GA 30602 (Moorman); e-mail: jamesdamonedvm@gmail.com. *Corresponding and presenting author. © 2021 AAEP.

1. Introduction
The equine digital cushion (DC) remains poorly understood in form and function. The first hypothesis was that adequate samples could be obtained from the DC for testing. The second hypothesis was that there would be differences between lame and nonlame limbs.

2. Materials and Methods
Forefeet from 16 horses with induced lameness were radiographed prior to humane euthanasia. Radiographs were used to guide sampling of the DC postmortem. Two samples, palmar and dorsal, on midline were acquired via punch biopsy. Compressive loading was performed followed by histological examination of collagen, adipose, ground substance, elastic fibers, and number of blood vessels. Mechanical and histologic variables were compared between lame and nonlame limbs, as well as between dorsal and palmar sites, with \( P < 0.05 \).

3. Results
There was no significant difference between lame and nonlame forelimbs in elastic modulus or histological variables. Significant differences in elastic modulus and percentage ground substance were found between dorsal and palmar samples.

4. Discussion
Satisfactory samples for mechanical and histological analysis were obtained. No significant differences in
mechanical or histological properties between lame and nonlame limbs were found, which may be from limitations of the lameness model. Differences between dorsal and palmar samples in elastic modulus and amount of ground substance may reflect different roles of the DC based on its location.

Acknowledgments

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Conflict of Interest
The Authors have no conflicts of interest.
How to Incorporate a Modified Hoof Cast into Equine Veterinary Practice

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1. Introduction
Maintaining a healthy equine foot as well as successfully treating lameness attributed to the foot requires appropriate farriery, which is of paramount importance in equine veterinary practice. The equine veterinarian is responsible for the overall health of the horse, including the foot; however, the foot is the one area of the horse that generally requires input from another professional—the farrier. Historically, the use of hoof casts is not new to veterinary medicine as they are used to treat distal phalanx fractures, laminitis, hoof capsule avulsions, and countless other problems affecting the equine foot. Recently, hoof casts have gained popularity in farriery to maintain horses in the barefoot state, to treat hoof wall defects and white line disease, and to manage compromised feet where shoes are not an option. However, there are innumerable occasions when a hoof cast could be beneficial in routine equine veterinary practice. This paper describes the application of a modified hoof cast that is relatively simple for any equine clinician to apply. The cast application described here is basically limited to the outer hoof wall, which is different from the traditional foot cast used in equine practice.

2. Hoof Casts
Hoof casts are used in veterinary practice to treat certain foot problems; however, the first author was never an advocate for using them in farriery. The main concern was the restrictive nature of the cast that impeded the physiologic function of the foot, especially the heel structures, and the inconsistent methods of application. Recently, a revolutionary new technique for cast application has been introduced—revolutionary in the sense that the cast adheres to and is limited to the outer hoof wall. This allows the section of the cast across the heels to be removed, thus eliminating the constrictive nature of the cast. The technique is geared toward providing strength, protection, and stability to the hoof capsule yet does not interfere with or compromise physiologic foot function. There are many reasons why this cast application should become a routine treatment for foot issues in equine veterinary practice. The use of a foot cast presents a viable option to equine veterinarians that can be used on its own merits when necessary or combined with traditional farriery.

3. Indications
Injuries to the equine hoof capsule and lameness related to the foot are frequently encountered in equine practice. Injuries to the hoof capsule, especially in the acute stage, can often be treated with a hoof cast. Damage to the hoof capsule such as hoof wall loss, toe or quarter cracks, white line disease resections, avulsions, etc. are often acutely painful

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and can be stabilized using a hoof cast. Obviously, the appropriate drain will need to be incorporated into the cast when necessary to prevent infection. The first author has been successful in rehabilitating many hoof capsule distortions in the barefoot state.\textsuperscript{1,2} Reluctance to remove shoes and leave the horse barefoot has always been for fear of damage to the hoof capsule and resultant discomfort for the horse. Here, the cast affords protection, which promotes comfort while allowing the physiological benefits of being barefoot. Another use is when a horse is trimmed too short or sustains foot bruising; a cast can be applied that protects the compromised structures of the hoof capsule. Scenarios where a cast is beneficial in equine practice include the following:

- Rehabilitating the foot in the barefoot state
- Transitioning the horse from being shod to barefoot
- Permitting light exercise when the horse is being maintained in the barefoot state
- Stabilizing/increasing thickness of a thin or compromised hoof wall
- Stabilizing hoof wall defects (toe, quarter, and heel cracks)
- White line disease
- As an alternative to glue-on shoes

4. Application of the Cast

The cast application described here will be different than the conventional application. Trimming the foot prior to cast application is always a crucial step, and the basic guidelines for trimming will apply whether the foot is being rehabilitated for a hoof capsule distortion, being transitioned to remaining barefoot, or reinforcing the hoof wall prior to applying shoes.\textsuperscript{2,3,4} Briefly, the solar surface of the foot to be cast is brushed briskly with a wire brush, and no horn is removed with a hoof knife. A line is drawn across the widest part of the foot, and the heels of the hoof capsule are trimmed to the base of the frog or to the same plane as the frog. If the frog is receded below the bearing surface of the hoof wall, every attempt should be made to trim the heels to the same plane as the frog. If the frog is prolapsed below the ground.
surface of the foot, a few days of weight bearing on the frog will put it on the same plane as the frog. Toe length is decreased using the nippers or rasp in a vertical direction, thus preserving the horn on the solar surface and creating approximate proportions on either side of the line drawn across the middle of the foot (Fig 1A). Finally, the edges of bearing border of the hoof capsule are rounded using the rasp at an angle. To apply the cast, the outer surface of hoof wall at the heels and frog sulci are cleaned with a rasp, and the remainder of the outer surface of the hoof wall is sanded using a medium grit sanding block (Fig. 1B and C). A thick focal layer of an acrylic adhesive compound is applied to the outer surface of the hoof wall at the heels, which will act as the anchor point to stabilize the cast. A thin layer of acrylic is randomly spread on the remainder of the outer hoof wall stopping below the coronet. Starting at the heels, a roll of either 2- or 3-inch casting tape, depending on the size of foot and thickness of the cast desired, is wrapped around the perimeter of the foot (Fig. 2A–C). The cast is applied without being immersed in water first as this allows the acrylic to permeate between the fibers of the cast, thus forming a bond with the outer hoof wall. The cast will extend to just below the coronet and at least .5 inch or more below the hoof wall to create a fold onto the ground surface of the foot. The cast can be further thickened by creating some folds at the heels and/or the quarters by layering the cast back and forth and then enveloping the folds as one continues to wrap around the perimeter of the foot. With the cast in place, a sponge soaked in water is used to thoroughly wet the cast, causing it to cure. This step is unique to this application as the acrylic incorporated in the first few layers of the cast that adheres to the outer hoof wall is now beginning to cure, and the water added to the remainder of the cast causes the resin to begin the curing process. Interestingly, neither the reaction of the acrylic nor the cast resin interferes with the other. Finally, the cast is covered with plastic compression wrap, and the foot is placed on the ground to flatten the folds of the cast on the solar surface of the foot. (Fig. 3 A–D). The unique part of this application is the removal of the section of cast that bridges the bulbs of the heels. Once the cast has cured, using the edge of the rasp, the cast is scored on either side of the frog from the frog sulci at the base of the frog to the edge of the cast at the hairline of the heel bulb. The score lines are now cut through using a thin hoof knife or some form of saw blade or gigli wire. The first author uses a hacksaw blade inserted in a handle, which can be used with one hand and cuts easily through the cured cast. The section of cast that covers the base of the frog is removed, and the edges of the cast are smoothed with a rasp or sanding block and blended with the hoof wall at the heels (Figs. 4A and B; 5C). Finally, the foot is placed on a hoof stand, and any cast material above or at the coronary is removed by light rasping or again using the sanding block to blend the cast material with the dorsal hoof wall as the acrylic does not extend to the coronet and the cast is not adhered to this area (Fig. 5). It should be noted that when the application of this modified cast is complete, the cast is limited to the outer hoof wall and the physiological function of the
deformable structures in the palmar foot are not disturbed (Fig. 5A–C).

The hoof cast is worn up to 60 days on average in general practice and is trimmed the same as the outer hoof wall during regular shoeing intervals. If the horse is kept in a continuous wet environment, the cast is waterproof, but the moisture will affect the adhesive properties of the cast, and it will begin to loosen and peel off. The cast is simply removed by rasping it off the outer hoof wall. The authors have seen no contraindications for using this casting technique; however, casts should not be used to cover areas of infection, sensitive tissue, or full-thickness defects without taking the appropriate precautions such as a protective barrier or a drain.

5. Discussion

It should be noted that the cast described in this paper differs from other casting tape presently being used. The fibers in the weave of this cast are not packed as tight, which allows the acrylic adhesive to permeate the cast and makes the cast lighter. Furthermore, 25% more resin has been added to the cast to expedite the curing process. The emphasis here should not be placed on the casting tape but rather on the unique application of the cast. It is readily apparent that the application of a hoof cast as described above adheres to the outer surface of the hoof, affords protection, and provides strength and stability to the hoof capsule while allowing the full physiological function of the foot. The increase bulk added to the hoof wall allows a shoe to be attached in cases where nailing was difficult. Furthermore, the cast forms an interface at the heels that adds protection and decreases wear that arises from the heel moving against the shoe or the ground during weight bearing. There are innumerable foot problems that can be treated with this type of cast methodology in equine veterinary practice. The ability by veterinarians and farriers to confidently apply this casting technique is easily acquired after doing a few cases. It can also be used as a topic to enhance communication between the veterinarian and farrier. The structures of the equine foot have the unique ability to adapt, strengthen, change shape, and improve the integrity of the hoof capsule structures if given a period without shoes. This is important in lameness cases that are localized to the foot as most of these cases will be associated with some form of hoof capsule distortion. Horses wanting to be left barefoot after being shod for any length of time require a transition period to allow the feet to strengthen and adapt after having the interface (shoe) removed. In both instances, applying a cast affords stability and protection to the hoof capsule without interfering with any of the physiological functions of improving the structures. The second author has found a marked benefit from using a hoof cast on feet, especially horses with thin hoof walls that previously had glue-on shoes applied. Here, the shoe is nailed into the cast material rather than the hoof wall, thus allowing numerous shoe applications without further compromising the hoof wall integrity. This technique is quite helpful in thin-walled horses as it prevents a close or misplaced nail. Damage to the hoof capsule including hoof wall loss, hoof wall defects such as toe and quarter crack, white line disease, and the necessity to perform hoof wall resection are frequently encountered in equine practice. Detached or diseased hoof wall can be removed, the area debrided when necessary, impression material added to act as an interface and restore shape to the capsule, and then placed in a cast (Fig. 6). By determining the cause and when combined with the appropriate farriery, both a toe crack and quarter crack can be
stabilized using this type of hoof cast. A cast is used on the entire hoof capsule with a toe crack, and the cast is sectioned from the toe quarter to the heel on the affected side of a quarter crack. This type of repair is easy and strong, adheres well, and is non-invasive. In fact, the authors feel that using a full or partial hoof wall cast for stability may become the treatment of choice for repairing hoof wall defects.

6. Conclusion
This method of cast application has huge implications for both veterinarians and farriers, whether the horse is shod or barefoot. The reluctance of many veterinarians, farriers, and horse owners to remove the shoes to rehabilitate the hoof capsule or transition to being barefoot is due to the initial discomfort the horse may experience and the damage to the hoof capsule without the protection of a shoe. Here is a hoof cast application that will bridge that gap while allowing the structures of the hoof to adapt, strengthen, change foot conformation, and restore itself. The potential uses for this hoof cast application are unlimited and only now being imagined.

Acknowledgments

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

Conflict of Interest
Dr. Steve O’Grady has no financial interest in any product described in this manuscript. Derek Poupard is the owner of Hoofcast LLC and is currently involved in the commercialization and distribution of the casting tape described in this manuscript.

References and Footnotes

*a3M® sanding sponge - 3M Center St. Paul, MN 55144-1000.*
*bEquilox® adhesive system - Equilox International, Inc., 110 NE 2nd Street Pine Island, MN 55963.*
*cHoofcast® - Farrier Products Distribution, 361 Haven Hill Rd., Shelbyville, KY 40065.*
*eHacksaw blade handle – Amazon.com, inc.*
*fDeLite® impression material - Farrier Products Distribution, 361 Haven Hill Rd., Shelbyville, KY 40065.*
Effect of Arena Surface Composition on Shear Ground Reaction Forces

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Shear mechanical behavior is more dependent on surface compositional properties than surface type categories (dirt/synthetic). Authors’ addresses: JD Wheat Veterinary Orthopedic Research Laboratory (Rohlf, Garcia-Nolen, Stover), Biomedical Engineering Graduate Group (Rohlf, Fyhrie, Stover), Department of Surgical and Radiological Sciences (Rohlf, Garcia-Nolen, le Jeune, Stover), University of California, Davis, CA 95616; Racing Surfaces Testing Laboratory, University of Kentucky, Lexington, KY 40502 (Peterson); e-mail: cmrohlf@ucdavis.edu. *Corresponding and presenting author. © 2021 AAEP.

1. Introduction
Shear forces at the surface-hoof interface affect hoof slide and surface grip, influence forces transferred to the limb, and affect injury risk. The influence of surface composition and management on shear ground reaction forces were quantified.

2. Materials and Methods
Shear force was measured at 4 sites on 12 arena surfaces (5 dirt; 7 synthetic) with 5 increasing normal loads. Surface composition, surface temperature, cushion depth, and moisture content were also measured. Coefficient of friction and adhesion were calculated from shear data. The effects of surface on shear properties were assessed using ANOVA (p < 0.05). Surface composition was correlated with shear properties.

3. Results
Adhesion and coefficient of friction were not different between dirt and synthetic surface categories. Correlations between adhesion and coefficient of friction with surface fiber content were observed. These trends predict that more fiber will decrease soil adhesion (r = −0.75; p < 0.01) and increase the coefficient of friction (r = 0.81; p < 0.01).

4. Discussion
Although surface type (dirt, synthetic) was expected to significantly affect shear surface properties, no significant relationships were found. However, when looking at surface type as a gradient with the percentage of fiber content, significant relationships between compositional and shear

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properties were observed. This suggests that arena owners can influence shear mechanical behavior of a surface by adjusting fiber content; however, future studies are necessary to directly determine the relationship of shear surface properties and equine musculoskeletal health.

Acknowledgments

Special thanks to Kaleb Dempsey from the Racing Surfaces Testing Laboratory for determining soil compositional properties.

Funding Sources

Supported in part by the Center for Equine Health with funds provided by the State of California satellite wagering fund and contributions by private donors.

Declaration of Ethics

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

Conflict of Interest

The Authors have no conflicts of interest.
Current Joint Therapy Usage in Equine Practice: Changes in the Last 10 Years

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Differences in the use of various joint therapies were observed over time. While some observations are in agreement with the scientific evidence, others are not fully concordant with the current literature. Authors’ addresses: Department of Large Animal Clinical Sciences, College of Veterinary Medicine and Biomedical Sciences, Texas A&M University, College Station, TX 77843 (Zanotto); Department of Clinical Sciences, College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Fort Collins, CO 80523 (Frisbie); e-mail: David.frisbie@colostate.edu.

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1. Introduction
There has been substantial growth in the understanding of osteoarthritis and in the development of novel therapies over the past few years. The aim of the present study was to compare the use of various joint therapies over the past 10 years.

2. Materials and Methods
An electronic survey was administered to American Association of Equine Practitioners members. Questions from a similar survey administered 10 years ago were repeated, and new questions were added. The responses were tabulated, analyzed, and compared to those of the previous survey.

3. Results
Methylprednisolone acetate was significantly less commonly used for high-motion joints than previously reported (odds ratio = 2.39, p < 0.001). The likelihood of respondents having used autologous conditioned serum was almost 3 times higher now (odds ratio = 2.97, p < 0.001). There is an increased use of concomitant antibiotic therapy with intra-articular medication.

4. Discussion
Triamcinolone acetonide remains the most commonly used therapy to treat high-motion joints. The use of methylprednisolone acetate to treat high-motion joints has significantly decreased, which is likely associated with its reported harmful effect on cartilage. The use of biological therapies in joints has become more popular, which could be associated with recent supportive scientific evidence and increased availability of these products on the market. The increased use of antibiotics with intra-articular therapy is not in agreement with the current literature's recommendations.

Research Abstract—for more information, contact the corresponding author

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Acknowledgments

The Authors would like to thank the AAEP Performance Horse Committee and Mr. David Foley for all the assistance with the survey.

Declaration of Ethics

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

Conflict of Interest

The Authors have no conflicts of interest.
Evaluation of Autologous Protein Solution Injection for Treatment of Tendonitis in an Equine Model

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Intralesional injection of autologous protein solution (APS) may result in superior and faster healing of tendonitis. Authors’ addresses: Department of Clinical Studies, New Bolton Center, School of Veterinary Medicine, University of Pennsylvania, Kennett Square, PA 19348 (Gaesser, Underwood, Linardi, Even, Reef, Ortved, Engiles); Department of Pathobiology, New Bolton Center, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA 19104 (Engiles). McKay Orthopaedic Research Laboratory, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA 19104 (Shetya, Mauck). Owl Manor Veterinary, Inc., 720 E Winona Avenue, Warsaw, IN 46580 (King); e-mail: agaesser@vet.upenn.edu. *Corresponding and presenting author. © 2021 AAEP.

1. Introduction
The use of autologous protein solution (APS) in tendon and ligament injuries hasn’t been studied in vivo in the horse. The objective of this study was to evaluate the effect of APS on tendon healing in an equine superficial digital flexor (SDF) tendonitis model. The authors hypothesized that intralesional injection of APS would result in superior structural and biomechanical healing.

2. Materials and Methods
SDF tendonitis was induced in both forelimbs of eight horses using collagenase injection. One forelimb received an intralesional injection of APS, while the other was injected with saline. Ultrasonographic examinations were performed at 2 week intervals following treatment. At 12 weeks, horses were euthanized and SDF samples harvested. Histologic evaluation, biomechanical testing, gene expression analysis and total glycosaminoglycan (GAG), and total DNA quantification were performed.
3. Results
Collagen type III (COL3A1) expression was significantly higher (p = 0.028) in saline-treated tendon than in normal tendon. Mean total DNA content was significantly higher (p = 0.024) in saline-treated tendons than normal tendons, whereas total DNA content was not significantly different between APS treated tendon and normal tendon. No other statistically significant results were reported.

4. Discussion
Reduced expression of COL3A1 in APS-treated tendon may indicate superior healing. Increased total DNA content in saline-treated tendons may indicate ongoing healing processes, versus APS-treated tendons which may be in the later stages of healing. Limitations include a short study period and inconsistency in size and severity of induced lesions.

Acknowledgments

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

Conflict of Interest
This study was funded by Owl Manor, the manufacturer or Pro-Stride® APS. William J. King is the Director of Research and Development at Owl Manor. The remainder of the authors have no conflicts of interest.
Leverage the Talent of Your Team

Amanda L. Donnelly, DVM, MBA

This paper outlines how to identify factors that may contribute to inefficiency, what systems need to be improved, and how to increase employee productivity. Author's address: ALD Veterinary Consulting, LLC, 712 Meadowcroft Lane, Nolensville, TN 37135; e-mail: adonnelly@aldvet.com. © 2021 AAEP.

1. Introduction

Team development is unquestionably one of the greatest challenges for most veterinary practice leaders. This includes how to effectively onboard employees and build an efficient, productive team. Another frustration occurs if practice leaders promote seemingly talented employees to supervisory roles only to find they don’t excel in their new job roles. One of the keys to addressing these challenges is to determine whether the practice has a systems problem vs. a people problem or both. For the practice to run smoothly, the business must have systems in place that enhance efficiency and productivity. Likewise, the business needs to have well-trained, engaged team members who desire to contribute to the success of the organization.

2. Practice Assessment

Before deciding on solutions to implement to increase team development and efficiency, it’s essential to first assess possible underlying causes of any problems. Practice leaders can begin by looking for signs of inefficiency in daily operations. One way to do this is to think of daily challenges or slowdowns in workflow that occur on a regular basis. For example, are there problems with scheduling? Are team members consistently trying to locate missing paperwork, charts, or equipment? Do managers or doctors find they are constantly interrupted by team members asking for direction? It’s also helpful to track and evaluate any inefficiencies to determine the significance of the problem. For example, if scheduling truly is a problem, this may be reflected in a decline in the number of farm calls each month. Likewise, to track the inefficiency associated with employee interruptions, doctors can track whether this slows down their productivity on a regular basis or whether it only occurs for a short time with new hires. Once the signs of inefficiency are identified, consider factors that may be causing the problem. Staff shortages, for example, are definitely a major problem for many practices. But other causes for inefficiencies include unclear or inconsistent protocols, problems with scheduling, and disorganized team members. Likewise, poorly defined job roles and a lack of training can cause slowdowns in workflow. When thinking about the causes of inefficiency, look at whether one or more team members are involved. If multiple employees demonstrate a lack of accountability or inefficiency, the problem is most likely a systems problem. On the other hand, if one or two team members keep
making the same mistake or demonstrate inferior job performance, this indicates a people problem. When assessing efficiency, it’s also wise to evaluate key performance indicators (KPIs) to see how the practice is performing. Look at the revenues and doctor transactions, for example, to see if they are consistent or higher than previous quarters or years. The KPIs can indicate if there is a problem with business performance, how much growth has occurred, and whether the perception of inefficiency is related to the stress and possibly burnout of team members. If the number of transactions hasn’t increased but the team is unhappy and complaining about inefficiencies, then the problem may be staff shortages or decreased employee well-being. On the other hand, if transactions have increased significantly, then inefficiencies might be related to a lack of training for new hires and lack of clarity on protocols.

3. Identifying Processes to Improve Operational Efficiency

The practice assessment is likely to uncover one or more of the following common systems problems. Taking action to improve these processes will result in a business that runs more smoothly even when the practice manager and owner aren’t present.

Define Clear Protocols

When contemplating the practice’s operational protocols, think about whether any protocols are missing or lack clarity as well as whether they are documented in writing. Often protocols are well known by experienced team members but may not be clear to those with less experience or new hires. When protocols are written, it makes training much easier and helps to increase employee accountability. To develop written protocols, ideally practice leaders should include the entire team. Team members who are involved in providing services or completing job duties are the ones best suited to determine if protocols are missing and whether they are clear. The team can create a list of protocols that need to be drafted or updated. Putting protocols in writing can be a daunting task, so it makes sense to prioritize the list of necessary protocols. Focus first on those that are most important for patient care and daily operations. For example, a protocol for how to complete a routine procedure or frequent client communications would take precedence over one for how to complete a job task that is only done occasionally. To make progress, practices should set a goal to complete the protocols according to a specified timeline. Many protocols can be drafted using bullet points and are no longer than one page. In this case, it may be possible to draft at least one protocol every one to two weeks.

Implement Standard Operating Procedures

Often practices have team members that complete work in many different ways. Likewise, it’s not uncommon to have different doctors with different standards or ways of doing procedures. Within reason, practices should determine the most appropriate, efficient way to complete routine procedures, treatments and surgeries. Not only does this help increase efficiency, it helps eliminate confusion and makes training more effective when onboarding employees.

Organize Workflow

Practices should establish specific organization procedures for all areas of the business as this can help employees be more productive and save time. For example, making sure charts, check-in sheets, lab results, or travel sheets have a designated home helps with efficiency, so people don’t waste time trying to find paperwork. Likewise, stocking rooms and trucks with all the necessary supplies and equipment means team members don’t have to waste time trying to find something. Remember that implementing multiple action steps to organize workflow can add up to significant improvements in operational efficiency.

Clearly Define Job Roles

Completing certain job tasks and fulfilling job duties often falls to multiple team members in the same department. Unfortunately, if job roles and expectations aren’t well-defined, employees may claim “I didn’t know it was my job” or say “I thought Bob was going to do it.” And even when team members are committed to doing an excellent job, there may be confusion about job responsibilities and job expectations if they aren’t clearly defined.

Leveraging the Use of Technology

It’s always wise to determine if technology solutions can help increase operational efficiency. This may include having clients fill out new client information forms or the patient’s history online prior to coming in which saves time at the beginning of appointments. Leveraging the use of technology includes having prepared treatment plans for all common medical procedures, treatments, and surgeries so team members don’t have to generate a new treatment plan for every client. Managers can link service codes in the practice management software for those services that are typically done together when creating treatment plans. It also helps save time to reduce the number of computer codes for services, so employees don’t have to stop and ask a co-worker or the doctor which code to use.

4. Improving Team Productivity

Even practices with effective systems can experience challenges with team development. Aside from having insufficient staff, the following people
problems can cause inefficiency and a lack of team productivity:

- Lack of trained and/or skilled team members
- Lack of accountability
- Employee conflict, drama, and problem behaviors
- Lack of employee empowerment
- Lack of motivation and self-leadership

The first step to creating a productive team is to make sure the business sets employees up for success with excellent orientation and onboarding systems.

Improving Orientation and Onboarding

Employee orientation includes all the activities involved with helping integrate new hires into the organization. Unfortunately, orientation is often ineffective in many practices because it’s essentially non-existent or too short. Simply telling employees how to clock in and giving them the employee manual and details about their schedule is not orientation. A half-day of direction is also not sufficient orientation. Orientation shouldn’t be rushed as this critical time period can make or break whether a new hire turns out to be an excellent addition to the team. To implement an orientation process that leads to enhanced retention, consider that the entire 90-day introductory period for a new employee is their orientation period. This doesn’t mean they won’t undergo training during this time, but this paradigm helps to ensure practice leaders recognize the need to pay attention to employees during their first few months. Using a detailed checklist is a good way to outline all the activities that should occur during orientation. It can include items such as completing relevant new hire paperwork, learning about employee benefits, reviewing job duties and expectations, and learning practice protocols. The checklist should also include a schedule for training sessions and when supervisors or managers will complete reviews of job performance. Another often overlooked part of orientation is to include a meeting for new hires to learn about the practice history, mission, vision, core values, and culture. This helps new team members gain an understanding of what it means to work at the practice and how they fit in. Employee onboarding is the process for new employees to gain the requisite knowledge, skills, and proficiencies to be able to do their job competently and contribute to the success of the business. The length of time for onboarding is variable, but most businesses consider the first 6-12 months to be the onboarding period. As with orientation, managers can improve onboarding by using a checklist to track the progress of learning for new hires. Effective onboarding includes the need to develop and implement comprehensive training programs. Given the scope of this project, it’s best to enlist the participation of multiple team members who can be part of a training team for the business.

Employee Training and Development

Essentially, employee development is all the efforts to assist in the professional growth and learning for team members. To determine whether the practice has an effective employee developmental program in place, consider the answers to the following questions:

- Does the business have a formal training program for new hires?
- Is there a plan to provide ongoing training to all employees?
- Does the practice utilize multiple resources to provide training for employees?
- Do training programs have supporting tools and materials?
- Do employees meet regularly with managers to discuss their training needs?
- Are employees encouraged to develop new skills?
- Does the practice have a training budget?
- Are employees empowered to utilize their skills?
- Does every employee have a written developmental plan reviewed with managers quarterly or bi-annually?

Each employee should have a written development plan that outlines their goals and action steps to achieve the goals. The action steps need to be specific and include a timeline. Bear in mind that it is far better to establish a reasonable number of goals each year rather than try to do too much given the busy work schedules of most employees. Managers should meet with employees at least quarterly to discuss their progress.

Developing Managers

All too often, employees are promoted into supervisory or management positions, but they don’t excel in their new roles. In part this may be because the team member is not suited for the leadership position. However, the problem is often related to a lack of training and leadership coaching that is necessary for the employee to understand how to be successful completing their new job duties. Before promoting employees, the practice manager or owner should meet with them to discuss the job expectations and duties that go along with the new position. For example, will the new position require working different hours or being available after-hours? Perhaps the new position will require attendance at leadership meetings or continuing education seminars. Often job duties for middle managers include being involved in supervising their co-workers and the employee review process. It’s important to make sure new managers understand these new responsibilities. A
development plan should be put in place for managers who are promoted so they can learn and grow. The plan needs to outline specific action steps for the team member to gain knowledge and skills they need to be a successful manager. This may include taking advantage of resources such as books, articles, webinars, and professional meetings. New managers also need to receive ongoing guidance and feedback as they assume their new job role. Most people need coaching in areas such as communication skills, being a role model, and giving co-workers feedback. The practice needs to identify who will serve as a mentor or coach for the manager. This could be the practice owner, an associate veterinarian, the practice manager, another manager, or an outside consultant.

Enhancing Employee Empowerment and Problem-Solving

One of the biggest drains on practice efficiency occurs when team members have to seek out supervisors for assistance or approval before taking action. Consider the following examples of how efficiency and teamwork suffer because employees aren’t trained or empowered to be problem-solvers.

- Client service representatives who must check with the practice manager before making a minor adjustment to an invoice.
- Team members who must check with the practice manager about payment options for the client.
- An owner or trainer who waits while staff check to seek approval for them to drop off a patient outside the normally scheduled times.
- Technicians wait to proceed with procedures to see in what order the doctor wants them done.
- The inventory manager can’t complete the drug order because they have to check with the doctor about a substitution; this slows the order and necessitates more phone calls.

If any of these scenarios sound familiar, then practice leaders should take advantage of the opportunity to increase employee empowerment and the problem-solving capabilities of their team. Not only does increasing employee empowerment increase efficiency, it is a well proven strategy to increase engagement. When team members are highly engaged, they have greater job satisfaction and contribute more to the success of the business. To empower employees, practice leaders should begin by making sure job expectations are clear and team members know their boundaries related to what action they can take. Let’s look at the example of a disgruntled client who isn’t happy with their bill. Perhaps they feel they weren’t properly informed about the cost of a service. A fully empowered team member is one who knows to provide the highest possible service and what actions they can take to enhance client satisfaction. This includes knowing the boundaries for how much (if any) they can adjust a bill and whether there are other actions they can take to help the client. Another way to increase empowerment is to encourage employees to offer ideas and possible solutions for how they think a problem should be handled rather than just asking how they should proceed. In time, employees will learn to present possible solutions to problems and feel comfortable making decisions on their own about minor variations in daily operations. Excellent questions to ask team members to encourage problem-solving include the following:

- “What have you done so far to solve this problem?”
- “How do you think we can resolve this issue?”
- “What ideas do you have to address this situation?”

5. Conclusion

With proper training and coaching, employees can be more efficient and effective in their jobs. Team members that can quickly respond to a variety of situations and client demands are invaluable since every time an employee has to seek out a manager or veterinarian to make a decision, efficiency suffers. With increases in efficiency, practice productivity increases as well. Efficient, well-trained healthcare teams are capable of seeing more patients, performing more procedures, and scheduling more surgeries.

Acknowledgments

Declaration of Ethics

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

Conflict of Interest

The Author has no conflicts of interest.
Integration of Telemedicine/Virtual Care into Equine Veterinary Practice—The Why and How

Eleanor Green, DVM, DACVIM, DABVP*; and Richard Markell, DVM, MRCVS, MBA*

Telemedicine is an essential component along the continuum of patient care with many benefits to the patient, the veterinarian, clients, and staff. Strategic thought along with best-practice guidelines for including virtual care/telehealth in daily practice will elevate access to healthcare, improve patient outcomes, and evolve fundamental care for patients. Virtual care can be an integral part of the standard of care in veterinary medicine. Authors’ addresses: Animal Policy Group, 13802 N Scottsdale Road, Suite 151-25, Scottsdale, AZ 85254 (Green); IlluminX Consulting, Inc., 906 3rd Street, Encinitas, CA 92024 (Markell); e-mails: dreleanorgreen@gmail.com, illuminxconsult@gmail.com. *Co-corresponding and presenting authors. © 2021 AAEP.

1. Introduction

A look at the telemedicine trends in human healthcare can provide valuable insights for veterinary medicine, which often follows trends that are relevant and applicable within veterinary healthcare. Additionally, human patients are the animal owners that have come to expect a level of veterinary healthcare that rivals human healthcare. Their adoption rate of telemedicine in their own care will surely lead to similar expectations for their own animals to receive veterinary telemedicine. Telemedicine/telehealth/virtual care (referred to in this paper as telemedicine) is the natural evolution of healthcare in the digital world and has been ignited to the point that it has become fundamental in how human healthcare professionals deliver care to patients, from urban centers to rural communities. Telemedicine is the practice of medicine using technology to deliver care remotely such that treating and monitoring patients no longer has to begin and end in a hospital or clinic. Telemedicine is well positioned to become a standard service in both human and veterinary healthcare. The American Telemedicine Association was established 38 years ago, in 1983, with a goal to promote access to care via telecommunications technology.1 Since then, the upward trajectory of adoption of telemedicine has been slow but steady until 2020, when COVID became a substantial catalyst that spurred exponential growth in the use of telemedicine by both healthcare providers and patients. By April 2020, nearly all primary care physicians (97%) were using telemedicine to treat patients.2 Telemedicine use by rural health centers increased during the pandemic, peaking at 54% in the last week of April 2020.3 Medicare beneficiaries using telemedicine exploded from just 13,000 per week before the pandemic to 1.7 million per...
week. Stanford Healthcare, one of the notable leaders in telemedicine, reports that 30% to 40% of visits remain remote despite the resumption of in-person visits. They cite reliability, efficiency, and convenience in allowing patients to receive routine care they need virtually anytime, anywhere. More than 75% of physicians surveyed said telemedicine provided better care for patients. Studies have shown that in most cases, telehealth was equivalent to in-person care, and in some areas, it was better. Most, if not all, veterinary clients are also human patients; thus, the opinion of these end users matters in guiding both human and veterinary healthcare. At least 28 studies have shown that > 80% of patients indicated satisfaction with telemedicine, including general practice, gynecology, psychiatry, neurology, prenatal, oncology, diabetes, and rheumatology. Levels of patient satisfaction for telemedicine are among the highest of all healthcare, insurance, and financial service industries. Patient responses indicated that 85.5% said telemedicine made it easier to get the care they needed, and 77% said they were completely satisfied with virtual care. Of patients surveyed, 75% said they expect virtual care to be a standard part of their care, with 50% saying they would switch providers to have virtual care visits on a regular basis. Their three top drivers were convenience, speed of access, and safety of being seen at home. In contrast to what was predicted, 45,000 patients said video visits resulted in greater satisfaction than in-person visits. Some of the major factors that enhance telemedicine are the rising development and adoption of technologies, such as the Internet of Things and artificial intelligence, by human patients and animal owners. These data have substantial consequences to veterinarians because they suggest that veterinary clients are expecting telemedicine as an option in their own care, with the implication being that they will demand the same in the care of their animals. Ignoring these trends, along with client opinions and preferences, could be disadvantageous to veterinary practices and their potential for the highest level of success.

2. Definitions

Virtual care is an umbrella term that encompasses all forms of telehealth. Telehealth is a next-level overarching term that includes use of technology to remotely deliver care, information, and education. Telemedicine refers to the provision of patient care by remote application of technologies, including the transmission of information via electronic communications, such as video, text, instant messaging, telephone, etc. Synchronous telemedicine is essentially real-time virtual visits, using video, telephone, or live chat. Asynchronous telemedicine refers to the “store and forward” technique in which clients collect and provide medical history and other supporting information and send it to the veterinarian for later evaluation, and/or veterinarians collect medical history and other supporting information, such as images and pathology reports, and send them to a veterinary specialist for diagnostic and treatment expertise. Teletriage provides timely guidance, not a diagnosis, with the opportunity to obtain the patient’s medical history, learn about the current problems, and determine whether a virtual visit is sufficient to address the problem or whether an in-person visit is indicated, including how urgent that visit should be. Telemonitoring allows postsurgical and postdischarge monitoring of patients remotely. The capabilities of telemonitoring will be expanded substantially in concert with further developments of technologies, like mobile monitoring devices, wearables, insideables, etc. Digital technologies will be able to allow monitoring of vital signs, electrocardiograms (ECGs), glucose, and other relevant parameters. With teleconsulting, it is possible to assemble specialists from every specialty along with other consultants virtually in “one room,” as needed by the patient. Telecardiology has been in use for decades by means of the transmission of ECGs over telephone lines and interpretation by cardiologists or internists. Likewise, teleradiology continues to allow practices everywhere to have access to board-certified radiologists, elevating the quality of patient care. Teleultrasound services have now been introduced into equine practice with increasing adoption. Teledermatology is uniquely suited for telemedicine as it is possible in some cases to provide accurate diagnoses and treatment recommendations. Results from telepathology, telemicroscopy, and telecytology have been shown to be in agreement with conventional methods, even exceeding them in some cases. Teleophthalmology is becoming more achievable with development of technologies, including smartphone technology and an indirect ophthalmology lens, as is teleradiology. Veterinarian-to-veterinarian teleconsulting affords mutual support among colleagues.

3. Telemedicine in Veterinary Healthcare

Veterinary telemedicine has been following in the wake of human healthcare. The global veterinary telehealth market size was valued at $92 million in 2020 and is expected to expand at a compound annual growth rate of 19.5% from 2021 to 2028, with a predicted revenue forecast for veterinary telehealth in 2028 of $417.1 million. Whether recognized or not, telemedicine is already an important pillar of nearly all equine practices, from large referral centers to rural practices. Telemedicine is working. While the in-person physical examination will never be replaced, and should not be, telemedicine is an essential component of the continuum of the highest-quality, comprehensive care for equine patients; in fact, telemedicine affords additional “touch points” with both clientele and patients. Telemedicine can offer insights that augment a single in-person visit. Responsible virtual
examination, diagnosis, and consultation, when appropriate, can be legally and effectively incorporated into practice. Another benefit of telemedicine is that it can be useful as an alternative or temporary substitute for in-person visits that need to be cancelled. Telemedicine is being used in equine medicine for an abundance of problems, such as basic triage, dermatology cases, lameness, minor lacerations, colic, basic ophthalmology, progress checks, postoperative follow-up, and long-term care. Telemedicine with video allows the veterinarian to visit the premises virtually with the potential to enhance the examination by observing the horse in its own environment, other animals, grain, hay, feed storage, facilities, level of management, and more. Telemedicine allows access by practitioners synchronously or asynchronously, as the case indicates. Using equine lameness as an example, much valuable information can be obtained virtually. The complete medical history can be recorded and added to the existing medical record. Still images and videos can be transmitted to the veterinarian by the owner, the trainer, or potentially a veterinary technician. With real-time video, such as Zoom, the veterinarian can guide the examination directly, following the American Association of Equine Practitioners Guidelines for Evaluating the Lame Horse, as far down the list as possible. The horse can be examined at rest, observing posture, stance, expressions of pain, weight bearing, swelling, wounds, visible foreign bodies, etc. In motion, video can capture the horse as it walks and trots toward and away from the camera, laterally to the camera, longeing both directions, and under saddle performing its routine exercise tasks. Depending upon the expertise and reliability of the owner or trainer, watching the horse’s response to focal manual palpation, hoof testers, and flexion tests is possible. While more definitive diagnostic tests, like nerve blocks and imaging, cannot be performed virtually, sound judgments can be made about whether the horse must have an in-person examination, and especially how urgent that examination should be. The severity of the lameness and how many limbs are involved can be assessed. In some cases, treatment can be recommended with a well-calculated follow-up plan for treatment and further assessment. One example might be a horse with sudden onset of unilateral lameness soon after shoeing that is positive to hoof testers over one nail and shows no other abnormalities. In such a case, the farrier might be called to check the shoe, in which case the veterinarian and farrier can communicate and the follow-up plan determined accordingly. Other horses may be obvious as urgent cases needing immediate attention, whether on the farm or in the hospital. One instance would be a horse displaying signs of a classic grade-IV laminitis. Another would be a non-weight bearing lameness consistent with a severe injury, like fracture. Ruptured tendons could be suspected because of local swelling and potentially hyper-extension of joints supported by affected flexor tendons. The amount of swelling and pain associated with cellulitis are observable by video, especially the excessive pain response to touch. Obvious muscle atrophy will raise suspicion of a neurologic condition. In each telemedicine case, the veterinarian gleaned important information that guides next steps, including emergency in-person visits. In equine practice, rural settings are common, validating a look at the benefits of telemedicine in human healthcare in rural communities, where telemedicine has been shown to be invaluable. The results are improved patient care, patients seeing more specialists with shorter wait times, reduced costs and time required, increased physician utilization, and 100% positive feedback from patients. All these benefits apply to equine practice. Justifiably, equine telemedicine has been referred to as the “new farm call.” Additionally, telemedicine can help address the challenges associated with lack of access to adequate veterinary care in rural communities, especially considering minimal availability of veterinarians concentrating on equine medicine. Some practices have taken advantage of a model in which a central hospital is supported by outposts in surrounding communities that are visited on a regular basis. Telemedicine allows these distant communities to be served daily with more consistency than without telemedicine. Even when mixed-animal veterinarians are located in rural communities, through telemedicine, they have constant access to the best equine-only veterinarians and specialists, as needed. Challenges of access to care pertain to more than just rural communities. There are numerous circumstances in which horses cannot be transported to a hospital or clinic, as there are for veterinarians not able to make the trip to the horse. Veterinary technicians can extend the reach of the veterinarian through telemedicine, depending upon the varying regulations—for example, the definitions of direct supervision. Hopefully, veterinary technicians will be able to be better leveraged in telemedicine services in the future. Monetization of telemedicine can benefit the financial health of a practice and requires a plan for protocols, just as with in-person visits, that are individualized for the practice and/or for its veterinarians. The first step is establishing a defined price structure that includes specific fees for telemedicine examinations. Some recommend setting the telemedicine examination fee equal or comparable to an in-person examination, the latter of which can be waived if the telemedicine examination results in a clinic visit for that problem. Others reduce the fee for a telemedicine examination because the reduced fee can be justified when the telemedicine examination is more time efficient than in person; however, telemedicine can add a layer of time commitment for communications, billing, etc. One must also take into account client convenience and minimizing stress for the patient. The practice will have to determine how it wants to collect payment—for example, credit card, existing online payment programs, credit, etc.—in the most secure manner. An essential component
of the introduction of fees for telemedicine is a robust customer awareness campaign. Clients must be informed of the transition not only to providing telemedicine services in the practice but also to requiring payment for these telemedicine services. Proactive communication and transparency are key. At the time of scheduling a telemedicine visit, clients can be gently reminded of the fees while they are assured that telemedicine meets standards of care and the results are part of the horse’s permanent medical record. Marketing materials can highlight benefits of telemedicine, including its fulfillment of standards of care. When properly explained, most clients will accept these fees, especially when they understand the benefits. The capabilities of virtual examination will expand in concert with the conception and refinement of new technologies. Digital radiology has opened the door widely for teleradiology, with consequent creation of new job opport-unitics. Teleradiologists can work from any location with vast work schedule options. Digital ultrasound offers comparable options for teleultrasound, including remote teleguidancea (remote ultrasound teleguidance). This ultra- portable ultrasound enables real-time remote viewing and image optimization. This can be used for remote consultation as well as education and coaching regardless of the operator’s global location. Telecardiology services have been in place for many years for veterinarians. Today, personal smartphone ECG monitoring devices are used in human healthcare that allow a medical grade ECG anytime, anywhere, and at an affordable price (AliveCor’s KardiaMobile). Eko Duo is a combination ECG and digital stethoscope that is powered by artificial intelligence; Eko has also developed a similar digital stethoscope for veterinarians. Smartphone-based portable ECG devices are currently under development for horses. PonyUp Technologies provides monitoring devices for horses, such as VetCheq, which remotely and noninvasively measures pulse, respiration, and central venous pressure with Bluetooth technology. For teleneurology, an ambulatory electroencephalogram system is in progress for horses with promising results.16 Tytocare allows a full examination at home with physicians able to hear heart and lung sounds, conduct ear exams, see the throat, and receive examination results by secure e-mail. In-home diagnostic kits are flourishing in human healthcare to test for COVID-19, human papilloma virus, HIV, trichomonas, cholesterol, thyroid hormones, allergies, food sensitivities, colon cancer, heavy metals, and more. Zoetis offers a stall-side test for equine inflammation and infection, and TargetVet has stall-side equine progesterone and IgG tests. VetGuardian has developed the first zero-touch monitoring device for veterinarians that measures vital signs accessible by a smartphone. Imagine the benefit to horses, clients, and the veterinary healthcare team when these technologies can be integrated. Conversations within the profession about telemedicine almost always include opinions about the veterinary client-patient relationship (VCPR). In human healthcare, regulations regarding telemedicine continue to evolve, as they are in veterinary healthcare. Today, all states, except one, allow virtual creation of the physician-patient relationship. While the regulatory trajectory for veterinary telemedicine lags behind human telemedicine, there are recent notable changes in veterinary regulations. Michigan and New Jersey just passed legislation to allow a telemedicine VCPR to be established virtually under the judgment of the veterinarian. The American Association of Veterinary State Boards (AAVSB) established guidelines for telemedicine as a model for state boards. Their language reads, “The veterinarian must employ sound professional judgment to determine whether using telehealth is suitable each time veterinary services are provided and only furnish medical advice or treatment via telemedicine when it is medically appropriate. A veterinarian using telemedicine must take appropriate steps to establish the VCPR, obtain informed consent from the client, and conduct all necessary patient evaluations consistent with currently acceptable standards of care. Some patient presentations are appropriate for the utilization of telemedicine as a component of, or in lieu of, hands-on medical care, while others are not.” In other words, the veterinarian’s judgment should be the determining factor. This language supports the notion that veterinarians are intelligent, compassionate, and devoted to their patients. As such, they should be trusted to determine when a VCPR can be formed remotely and when it cannot; most certainly, remote establishment of a VCPR is not always appropriate. Those who oppose the remote establishment of the VCPR offer concerns surrounding the differences between human and veterinary healthcare, including diverse species, unique health risks for owners, dissimilar payment models, and disparate regulations regarding pharmaceuticals and products. As veterinarians use the VCPR, whether remote or in person, they must be especially mindful of the Animal Medicinal Drug Use Clarification Act (AMDUCA), which provides veterinarians acting within the VCPR greater prescribing and dispensing options so animals can receive the medications they need when they need them. It is critical that any relaxed regulations about the VCPR must not create concerns that could jeopardize AMDUCA. One point of consideration is that the AMDUCA requirements for an established VCPR were established long before virtual creation of a VCPR was being contemplated. Conversations with appropriate federal agencies will be necessary to clarify. COVID-19 provided the ultimate experiment for virtual establishment of the VCPR as it accelerated telemedicine in both human and veterinary medicine. State medical regulatory agencies reset many of the barriers to entry for telemedicine, and the experiment has proven beneficial for all. The Veterinary Virtual Care Association (VVCA)
contacted the veterinary medical state boards in those states in which the telemedicine regulations were relaxed to determine if any adverse events were attributable to telemedicine. To date, documented complaints related to telemedicine are essentially nonexistent. The VVCA has also remained in close contact with those in Ontario, where the creation of the VCPR through telemedicine was legalized in 2018. The Ontario VMA has been conducting surveys since COVID-19 forced them to restrict in-person visits. Within 2 months, 90% of veterinarians were using telemedicine. When restrictions were relaxed, > 80% of veterinarians continued to use telemedicine. Their veterinary medical governing board has received zero complaints related to telemedicine. As such, concerns related to the relaxation of the VCPR (and other regulations) seem unfounded, as do concerns about long-term acceptance of virtual creation of the VCPR. When considering and implementing telemedicine into a practice, valid concerns must be considered. The VVCA recently completed a survey asking the top reasons why practitioners were hesitant to implement virtual care in their practices. Regulatory/legal concerns ranked number 1. Other concerns were clients, technology, and staff. All of these can be addressed effectively. Additionally, there are good indicators that clients are willing to pay for virtual services. The value of hands-on examination must never be ignored. Fear of misdiagnosis and liability are trepidations for some, yet others feel they are similar to in-person visits. Communicating via technology is different from face-to-face communications, necessitating preparation and perhaps additional training to be most effective in exchanging information effectively with the appropriate level of compassion and personal touch, which can be achieved. Monetization of telemedicine can be imposing but is accomplishable and generates profits. While technologies are considered assets, they also have limitations. Internet access can be inconsistent, especially in areas with the greatest need. Some veterinarians are apprehensive about the time commitment of implementing telemedicine into a practice and have related questions about transition requirements, alteration of workflow, re-quired staff, client acceptance, and ultimate cost-benefit ratio. Some have become secure with their current practice model and are reluctant to change. Because younger generations are more comfortable with technologies and have high expectations for their use, multiple generations in the practice can impose issues related to differing expectations for modernization, technology use, telemedicine, etc. State regulations can be restrictive and most certainly lack consistency nationwide. The VVCA was created in 2020 to provide valuable resources to veterinarians while ensuring that virtual care becomes a part of the standard of care by advocating best practices for veterinary healthcare providers. These best practices are under constant revision and can be found on the website at https://vvca.org. Some of

the website features contain a quick-start guide, guidelines for telehealth, best practices for telemedicine, best practices for legal and ethical issues, a client consent form, workflows and decision trees, a front-desk phone guide, blogs, videos, and current newsworthy items. Comparative policies of the American Veterinary Medical Association, American Association of Veterinary State Boards, and Veterinary Innovation Council feature organizational views. A very useful tool is the constantly updated veterinary telemedicine regulatory map showing current regulations for each state. Increasing access to veterinary healthcare is a core responsibility for all in the profession. By effectively leveraging simple technology, practices can significantly improve patient outcomes (earlier diagnosis and treatment), owner compliance, client satisfaction, and loyalty. Telemedicine can increase the time efficiency and productivity for the practitioner. An additional revenue stream is captured, while reduced cost to clients in some cases and improved access to care and improved outcomes and satisfaction for all stakeholders can be realized. Telemedicine is not meant to replace the in-person patient exam, and in many cases it cannot. It may, however, allow a triage for those cases to determine and schedule an in-person veterinarian visit, when indicated; in fact, it has been shown that telemedicine stimulates substantially more hospital and in-clinic visits. Veterinarians historically have given away many of their services while powerlessly watching a number of revenue streams be stripped away. Telemedicine offers a viable path to monetize their valuable services, recapture some lost revenues, and do so by leveraging their medical training and that of their professional staff. In the end, veterinarians have the option to choose telemedicine, and if they do not want to offer telemedicine services, they should not; however, new associates may seek practices that use telemedicine. If animal owners do not like telemedicine, they will not use it; however, if they do like it, they will find a practice that offers it. An essential point is that telemedicine will not turn good veterinarians into bad veterinarians. It has been said that technologies will not replace doctors/veterinarians, except those who do not use them. Telemedicine is here to stay; can be leveraged to benefit the patient, client, veterinarian, staff, and practice; and can contribute to veterinarians being better caregivers to the animals to which they have dedicated their lives.

4. Recommendations

1. To get started, seek resources such as TeleMed Best Practices, Legal and Ethical Issues; sample client consent forms, front-desk phone guides, and prescribing medications; as well as sample telemedicine flow charts that can be found on the VVCA
3. Determine the most appropriate inclusion of staff in offering telemedicine services in your practice, e.g., veterinarians, veterinary technicians, front-office staff, billing office, practice managers, etc. Engage them all in crafting a workable plan that is most likely to be well received and successfully adopted.

4. Provide training as necessary for the practice as a whole and for individuals involved in different aspects of your telemedicine services. One example is communications training to ensure that communications via technologies convey the practice culture desired. Another is the individual roles and responsibilities with seamless incorporation of each 24/7.

5. Plan on the structure inside your practice for managing calls, veterinarian’s response, scheduling, billing, medical record entry, etc. as well as an evaluation of charges/value for telehealth—time base (10 minutes vs. 60 minutes), by the job (prepurchase radiograph evaluation), etc.

6. Establish and maintain policies and procedures for medical records generated through telemedicine. This includes the secure transmission of records. Create an integration pathway to link your existing medical record program to your telehealth records or explore add-on programs available to facilitate and streamline that process. Ensure confidentiality of telemedicine records.

7. Implement practices to maximize cybersecurity and minimize cybercrime regarding medical records and data management. This applies to all practice information, not just telemedicine.

8. If a platform vendor is preferred, ask many questions before signing on, such as their level of confidentiality, the extent of their insurance coverage, how they manage and use their data, etc.

9. Ensure that connectivity, equipment, and processes are working and then double-check them frequently. Obtain client and staff feedback to continually improve processes.

10. Inform your malpractice insurance provider of the inclusion of telemedicine practices in your practice and obtain confirmation that they are covered.

11. Evaluate how much and what type of telemedicine you are currently providing, e.g., triage, rechecks, telemonitoring, remote diagnosis, etc. Determine the value to the practice of each. Also, evaluate what circumstances have been gratis, what circumstances should be/should have been billed, and what the value of those services is. What questions can be answered by staff or should be answered by a veterinarian? What is “good will for a great client” as these and many others are certainly individual for different clients and different practices? Many of us have a history of undervaluing the worth of our time and expertise as well as underestimating the willingness to pay of many of our clients. There are circumstances in which friends seek advice from colleagues as they do not want to bother their own veterinarian/friend, knowing they would not be billed by them; they want to pay a veterinarian so they could ask more questions.

12. Define the possibilities and limitations of remote diagnosis in your practice.

13. Obtain written client consent before the telemedicine visit. The VVCA offers a list of concepts to be included in this form.

14. Educate clients about the option of telehealth for their horses and its many benefits. Obtain client feedback as many will have already experienced telemedicine for their own healthcare. Early engagement of clients will set up the expectation for charges, as well as the gentle nudge for “the value of a veterinarian’s time.”

15. Add telehealth promotion and education to your existing market plan to increase awareness that your practice offers this service in the continuum of care practice paradigm.

Acknowledgments

Declaration of Ethics

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

Conflict of Interest

Dr. Green serves on the board of Vet Guardian. Dr. Markell is a consultant for Butterfly Network.

References and Footnote


*Butterfly Networks iQ Vet + (https://www.butterflynetwork.com/vet/teleguidance-vet).*
Frontiers in Athletic Rehabilitation: What Is Translatable to the Horse?

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1. Introduction

The objective of this outline is to discuss emerging rehabilitative approaches used in elite human athletes, provide an overview of the research supporting their translational use, and discuss how their incorporation may apply to the equine athlete. This review follows in two sections: (A) nonoperative strategies (for use in athletes in some level of active competition or training) and (B) pre- and postoperative strategies (prehabilitation and rehabilitation for the surgical patient transitioning out of and then back into athletic workload). Appropriate incorporation of any modality or therapeutic exercise into a successful rehabilitation program relies on an accurate diagnosis. Unfortunately, universal recommendations regarding the timing, frequency, and specific indications of many of the below-described modalities are still lacking. As further research is able to define specific parameters, significant advancements within the rehabilitation field can be expected.

2. Translational Nonoperative Strategies

Objectively Assessing Athletic Workload

Accurately determining the balance between appropriate training rigors and recovery to strategize the timing of peak athletic performance while mitigating risk of injury is an active area of research in human athletics.1,2 This careful equilibrium not only affects in-season management, but also subsequent contract negotiation and television rights in professional human athletes. Specifically, the distinction between “overtraining” and “overreaching” has been investigated in relation to acute fatigue and markers of decreased performance.1 By definition, “overtraining” encompasses an accumulation of training resulting in long-term decreases in performance capacity which may take several weeks or months to restore.1 In contrast, “overreaching” refers to an accumulation of training resulting in short-term decreases in performance, which may take several days to several weeks to resolve.1 Although the interplay between the two scenarios remains unclear, it is thought that overreaching precedes overtraining in a continuum of
Blood flow restriction (BFR) therapy applied in A, a human patient during step exercise; B, human patient during side lunges; C, a human patient during leg presses; D, an equine forelimb in preparation for walking exercise. Blood flow restriction training is being utilized by human physical therapists to prescribe controlled exercise following orthopedic injury to improve muscular strength in absence of damaging loads. Its equine applications are of current research interest. Human BFR photos courtesy of Dr. Brian Noehren. Equine BFR photo courtesy of Dr. Sherry Johnson.

Symptoms. Currently implicated physiologic biomarkers associated with altered (positive and negative) exercise performance include muscle oxidative capacity, hormonal and immune measures such as neutrophils, glutamine, urea, and testosterone/cortisol ratios.3 Wearable fitness tracking technology and sophisticated motion analysis software systems have also been developed to aid in early recognition of physiologic fatigue indicators (heart rate, gait parameters, for example) to further guide judicious exercise prescription. The use of Global Positioning System (GPS) technology in team sports to assess player position, velocity, and movement patterns has become integrated at the professional level with authors noting an improved ability to regulate training loads for individual athletes at crucial times of the training season.4 Incorporation of such technology has even helped improve detection of signs of injury, fatigue and overtraining as injury-prediction models have continued to emerge.4,5 On-body technology, however, is still considered new and evolving with simultaneous incorporation of live motion analysis described as the future iteration.4

Similarly, objective tracking performance devices to assess physiologic workload parameters have been recently developed for use in the equine athlete.6,7 A recent investigation evaluated a three-axis accelerometer at three anatomic locations to determine accuracy in detecting step count and threshold acceleration values for the walk, trot, and canter.6 Although equine-specific metrics to aide in interpretation of real-time data acquisition are still largely lacking, technology incorporation of such sensor use into mainstream clinical practice is expected to broaden preventative efforts associated with training fatigue.7 Interdisciplinary integration into equine competition and training strategies would be facilitated by defining inherent individual variability associated with an equine athlete falling outside of “its own optimal window,”7 thus solidifying individualistic approaches to maximize data interpretation that have been incorporated with human use. The utilization of validated sensor systems to record accurate data, followed by determining the clinical significance of select measured output in horses will be the key to successful application.

Blood Flow Restriction Training

Low load exercise training with blood flow restriction (BFR) has become increasingly used by human physical therapists to prescribe controlled exercise following orthopedic injury.8 Through application of a specialized tourniquet to temporarily reduce blood flow to an exercising limb (Fig. 1), patients are able to increase strength and muscular hypertrophy using only light weights or low-intensity exercise (cycling or walking).9 In essence, BFR is being used to increase strength via low intensity training to a level typically only achieved with mid to high intensity training. Patients with a variety of physical limitations are able to safely perform BFR to improve muscle strength while protecting fragile, healing tissue from excessive loading that occurs during traditional exercise programs, thus improving physical function.10,11 Blood flow restriction training is being safely used as a progressive clinical rehabilitation tool in the process of return to heavy-load exercise for a variety of human conditions including knee osteoarthritis, soft tissue injuries and geriatric sarcopenia.10,12 The most consistently documented therapeutic benefits of BFR within the human literature are muscular hypertrophy and subsequent increases in strength secondary to elevated levels of growth hormone (GH).13,14 The metabolic accumulation of GH has been demonstrated to reach supraphysiologic levels (290 times that of baseline) during human BFR training and is thought to be the main activator of resultant muscular hypertrophy.13,14 Also noteworthy is that the use of patient-specific BFR occlusion pressures have become the standard of care in human practice due to improved comfort and minimized safety risks.15

Although numerous studies have been performed validating human BFR use with compelling results, investigations to assess equine-specific application are considerably sparse.16,17 An initial pilot study
evaluated cuff application to a single equine forelimb \((n = 6)\) using an occlusion pressure of 200 mm Hg applied only at rest.\(^\text{16}\) Following three sets of occlusions for five minutes, muscle thickness was assessed ultrasonographically in addition to peak flow velocity and flow velocity integral of the radial artery. Authors noted that extensor and flexor muscle thickness was increased in the occluded limb, but not the control limb. Authors concluded that acute vascular occlusion may be safe and tolerable in horses.\(^\text{16}\) Subsequently, the same research group confirmed that horses would tolerate BFR at a walk pace.\(^\text{17}\) Six unfit Standardbred mares performed BFR of both forelimbs at a walk pace with 200–230 mm Hg cuff pressure once per day, 6 days per week for a total of 2 weeks. Skeletal muscle thickness and tendon thickness were assessed ultrasonographically and serum growth hormone (GH) concentrations were determined 5, 15, and 60 min following BFR exercise sessions. Authors confirmed that similar to humans, BFR use in horses resulted in elevations in GH and muscular hypertrophy. In contrast to the use of blanket occlusion methodology in the aforementioned equine investigations, a recent study describes short-term beneficial effects of BFR on equine superficial digital flexor (SDF) muscle oxidative capacity over a 10-day study period using BFR pressures based on daily readings.\(^\text{18}\) Specifically, SDF muscle oxidative capacity increases were consistent with acute metabolic adaptations of increased mitochondrial density and an improved ability to oxidize fuels.\(^\text{18}\) Other commonly used rehabilitation modalities such as underwater treadmill and conventional treadmill exercise, however, have failed to affect SDF muscle metabolic responses over four- and eight-week time periods,\(^\text{19,20}\) suggesting that neither modality alone increases muscle oxidative capacity to the same extent as other forms of resistance training.\(^\text{21}\)

Equine athletes competing with chronic injuries, recovering from orthopedic procedures and geriatric horses all represent patients with physiologic limitations to consider when prescribing exercise and strengthening programs. Blood flow restriction training may represent a strategy to optimize equine neuromuscular adaptations during extended recovery periods, but BFR cuff placement and type of exercise used thereafter is a significant application difference between humans and horses (Fig. 1). Despite these differences, however, BFR may represent a novel, medication-free bio-solution to consider in the process of return to heavy-load exercise. Its titrated use under trained professionals may augment historic exercise prescription currently limited by the presence of fragile, healing tissue and the inability to predict spontaneous overexertion in the horse. Ongoing investigations are expected that may further refine recommended use and safe practices for use in horses.

Contrast Therapy

Contrast therapy is defined as the application of alternating cycles of both cold and heat in a repeated manner with the goal of increasing blood circulation through cyclic vasoconstriction and vasodilation.\(^\text{22,23}\) Optimal skin tissue temperatures in humans have been documented to be 10–15°C for cryotherapy and 38–43°C for heat application.\(^\text{23,24}\) A significant body of work exists within human literature investigating the effects of contrast water therapy on postexercise recovery outcomes, with conflicting results.\(^\text{25,26}\) Recently, when the effectiveness of various methods of 10-minute thermal therapy (cold water immersion at 10°C, thermonutral water immersion at 24°C and contrast water therapy alternating at 10° and 38°C) were compared using physical and mental performance measures and physiological responses, the self-perceived feeling of relaxation after 6-min recovery was significantly better after cold water immersion and contrast water therapy, leading authors to conclude it may play a positive role in athletes’ performance and overall well-being.\(^\text{2}\) In contrast, previous work has not documented improvements in strength and sprint performance after contrast water therapy compared to passive recovery alone.\(^\text{25,26}\) Despite conflicting results, contrast therapy is being increasingly utilized by professional athletes to augment recovery strategies. Contrast therapy has technically always been available for use in the equine athlete, but historic focus has largely been on the effects of cryotherapy and superficial heating modalities used independently,\(^\text{27–30}\) and its utility in equine rehabilitation remains largely unknown.\(^\text{31}\) Recently, tissue temperatures at different tissue depths relative to the digital flexor tendons as assessed by thermistor placement during serial heating and cooling cycles using a human contrast therapy device\(^\text{32}\) was found to consistently induce therapeutic cooling (<15°C) and heating (>40°C) of tissues to the depth of the deep digital flexor tendon (DDFT).\(^\text{32}\) Interestingly, target tissues at the skin were reached within 9.5 min when heat was applied first but required up to 12.5 min when cold was initially applied.\(^\text{32}\) Also noteworthy is that in the majority of limbs, tissues deep to the DDFT did not reach target tissue temperatures during the 15-min heating or cooling cycles,\(^\text{22}\) obviating the limitation to significantly affect the proximal suspensory ligament’s temperate. Authors hypothesized that a countercurrent temperature mechanism played a role in the inability to heat or cool tissues deeper than the DDFT compared to more superficial tissue.\(^\text{22}\) Application of cold or hot thermal therapy through ice packs, water immersion, heat packs, heated blankets, etc. has certainly always been physically available to the equine clinician but having a commercially available dry-interface system that can reliably and consistently induce therapeutic tissue changes to a known tissue depth is certainly of value, with the next logical investigative step being to evaluate contrast therapy’s clinical efficacy in injured tissues. Continued efforts to expand knowledge around contrast therapy’s efficacious use can be expected.
Advances in Compression Technology

Recovery acceleration through the use of various rehabilitative strategies including stretching, massage, compression garments and combinations of the aforementioned are being increasingly used in human athletes to minimize days out of competition and speed active recovery.\textsuperscript{31} Accelerating lymph circulation has been demonstrated to beneficially remove metabolic products, improve body fluid dynamics, cause changes in microcirculation and reduce venous blood return.\textsuperscript{32–34} Various methods of improving human lymphatic flow include the use of mechanical stimulation, deep oscillation, and physical stimulation\textsuperscript{35} but reported methodology is so varied that universal clinical practice guidelines for use in sports medicine and rehabilitation are lacking.\textsuperscript{36} Nonetheless, integration of manual lymphatic drainage techniques alongside conventional rehabilitation therapies has become a mainstay of athletic physical therapy programs. Similarly, lymphatic compression technology available for use in the equine athlete includes both full and distal limb systems (Fig. 2). Although the use of compression wrapping has been in use and well tolerated by horses for decades, recent noteworthy advances include the incorporation of full limb, human-grade pneumatic compression technology\textsuperscript{b} with automatic cycling that works to dynamically push lymphatic fluid retrograde in between cycles. This product shares the same manufacturer and pump system as the FDA approved human device\textsuperscript{c} and is currently being investigated for the treatment of lymphangitis as well as for athletic recovery.\textsuperscript{4} Distal limb compression wraps using medical-grade bandages custom-fit based on anatomic measurements\textsuperscript{c} also offer the clinician an avenue through which distal to proximal pressure gradients can be maintained based on the contour of an individual horse's limb. Such systems when combined with contrast therapy, appropriate exercise, retrograde massage and titrated, supervised application have resulted in significant clinical improvements of chronic distal limb edema/lymphedema based on serial, circumferential limb measurements.\textsuperscript{7} With continued investigation of specific application parameters and correlation to performance outcome measures, further recommendations can be expected.\textsuperscript{37}

3. Translational Pre- and Postoperative Strategies

Preoperative Management to Maximize Postoperative Results (Prehabilitation)

There is considerable interest in determining if the use of prehabilitation (receiving physical therapy before a human patient undergoes surgery) results in better outcomes. By performing rehabilitation before surgery, the goal is to help the patient be more prepared for the period of postoperative care that is characterized by limited physical activity, immobility, and reduced function.\textsuperscript{38} The majority of the research on the effectiveness of prehabilitation in humans has centered around anterior cruciate ligament injuries (ACL) of the knee. Because of the acute nature of the injury, there is debate as to whether it is better to quickly reconstruct the ACL or wait for a period of time for the resolution of swelling, limited knee motion, pain, and muscle strength inhibition. Programs to prepare the human athlete for surgery through a physical therapy program involve several components. First is aggressive management of swelling or edema around the knee. This component of care is considered critical as swelling results in muscle inhibition, limits range of motion, and contributes to feelings of instability. However, the focus cannot entirely be on reducing swelling as the clinician must also attempt to maximize recovery by engaging the patient in exercise that does not exacerbate swelling or significant pain. Careful consideration of balancing multiple priorities i.e., regaining strength and range of motion often means having a patient use a crutch or limit too much other physical activity. Another aspect of care that is critical to address before surgery is the incorporation of exercises that focus on neuromuscular re-education. This can take the form of using electric stimulation to help the athlete learn to recruit and use the muscle again. In addition, the athlete should begin to perform exercises that excite the neuromuscular system. For ACL reconstruction rehabilitation, electric stimulation is performed over the quadriceps muscle with the intensity as high as tolerable for the athlete set to a Biphasic or Russian stimulation. The pulse duration is usually on order of 250–300 plus microseconds, with a pulse frequency of 50 Hz with the subject performing a 10-s hold during the stimulation followed by 50 s of rest. The typical duration of this treatment is 10 min. The 10-s hold followed by 50 s of rest is done to minimize fatigue. In addition, dynamic neuromuscular

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\caption{Two emerging compression technologies consisting of A, Full-limb pneumatic dynamic compression device to encourage retrograde movement of lymphatic fluid. Photo courtesy of EQ Press, Vetletics Morrisville, NC; B, EquiCrown® Canada “FIT” compression sleeves. Photo courtesy of EquiCrown Canada.}
\end{figure}
control can be facilitated by having the patient work on balance training while standing on an increasingly unstable surface and/or perturbation training whereby the athlete is on an unstable surface with a physical therapist manipulating the direction and magnitude of perturbation. Lastly, during this period of time the patient typically engages in open chain strengthening of the quadriceps muscle to offset any loss of strength due to initial inhibition. Patients will train for three to four sets with a weight that will result in fatigue (i.e., failure to complete an additional repetition by the end of the third set). Typical length of these programs is anywhere from 4 weeks or in some cases 10 sessions. Each session takes the physical therapist about 60–90 min to complete with the patient. Several clinical trials and systematic reviews have been done to investigate the effect of a prehabilitation program.38–41 Seminal work by Failla et al. showed that a prehabilitation program that included perturbation training resulted in a greater percentage of athletes returning to sport and having good patient reported outcomes.38 In addition, Shaarani et al. incorporated a 6-wk prehabilitation program and found increased single leg hop distance, better patient reported outcomes, and a trend towards a higher percentage of Type 2 muscle fiber expression at a 12-week postoperative follow up as compared to subjects without prehabilitation.39 Although results of these studies are encouraging, recent results of two systematic reviews indicate more work is needed to establish the effectiveness of such programs.40,41 There remains a need for large well controlled trials to evaluate the potential benefit of such programs. However, the evidence to date is encouraging that some form of prehabilitation will better prepare an athlete for their postoperative recovery. In the equine athlete, there are many important factors for prehabilitation that clinicians have started to consider. Of critical importance, and of relevance to the psychosocial discussion below, is the importance of finding the optimal environment for the horse to thrive and recover in. Prior to surgery, the horse should ideally be acclimated to prolonged periods of stall rest as well as any bandages, garments, equipment, and exercises that will be used postoperatively. For very fit horses accustomed to working and/or turnout, stall rest can be mentally and physically challenging to them and dangerous to both them and their handlers. In some cases, moving the horse from an active show or competition barn to a quiet rehabilitation barn with all horses in stalls and on similar schedules is enough to calm an anxious horse and prevent the development of stall vices or dangerous behavior. In other cases, however, the use of long-term sedatives such as reserpine42 or trazodone43 are needed to facilitate calm stall rest and controlled exercise. Once the proper environment has been achieved, there are many ways that prehabilitation for the equine athlete can be pursued, similar to what is being done for human athletes. In the authors’ opinion, all horses can benefit from mobility, strength building, and core stability exercises both during the prehabilitation period and the rehabilitation period. Such exercises are well described,44,45 simple to perform, and have been proven to have an effect, particularly on increasing the cross-sectional area of the multifidus muscle.46 In addition, both active and passive range of motion exercises can be used preoperatively for certain injuries, to prepare the horse for rehabilitation, and to try to limit fibrosis preoperatively. Such exercises may be particularly useful for surgeries of the tendon sheath and fetlock joint where adhesions and fibrosis commonly occur. The use of electrical stimulation in equine physical therapy for muscle activation and training is being explored and certainly warrants further investigation. A recent study examined the use of a commercially available electrical stimulation unit FES 3106 applied using a back treatment pad over the sacral region to generate movement in the lumbosacral region of standing horses without known pathology.37 The authors found that although the movement caused by the electrical stimulation was smaller than that caused by manual pelvic inclination, its effects were documented in a wide area including the muscles of the hind limb. In addition, the authors state that electrical stimulation could easily be applied over a longer period and in a higher frequency than is possible for manual pelvic inclination, providing justification for future studies into its use for stabilizing the sacroiliac joint and potentially for training of the quadriceps muscles. Also similar to neuromuscular reeducation in human athletes is the use of proprioceptive retraining techniques in horses. Examples of such techniques include kinesiotaping,48,49 tactile stimulators,50,51 and more recently equine balance pads (Fig. 3).52

Fig. 3. Balance pad used in horses referred for professional rehabilitation. A, Bilateral forelimb application while standing on medium density (purple) Sure Foot balance pads; B, Bilateral hindlimb application while standing on firm density (green) Sure Foot balance pads. Photos courtesy of Equine Sports Medicine & Rehabilitation, Whitesboro, TX 76273.
The Influence of Psychosocial Factors on Physical Recovery During Postoperative Care

The role of psychosocial factors is receiving increased attention for the influence they have on the physical and patient reported outcomes of human athletes after injury. Several constructs have been considered, which include self-efficacy, fear of re-injury, and psychological readiness to return to sport. Prior work has found that a more positive assessment of self-efficacy, a lower fear of re-injury, and feelings of greater psychological readiness to return to sport result in higher likelihood of returning to preinjury levels as well as reduce the risk of second injury. In addition, there is evidence to suggest that lower psychological readiness to return to sport after an ACL reconstruction resulted in the human athlete having greater deficits in their running gait in the injured knee as compared to the uninjured knee.57 In addition, work from Beischer et al. shows that patients with higher knee self-efficacy at 4 months after surgery had increased odds for symmetrical muscle function at 1 year after surgery.58 Clearly, psychosocial aspects of recovery are important adjuncts that must be considered during the recovery from surgery. How to best help and treat athletes who are not coping well with their recovery is also an area of active investigation. For athletes with significant postoperative challenges in coping with the response to injury referral to a sports psychologist or other mental health professional is warranted. Many athletes though do not have access to psychologists or may have an important but modest or moderate negative change in domains such as self-efficacy and fear of re-injury and not be willing to seek care. Within those cases positive supportive discussion, goal setting and engaging the athlete in the rehabilitation process are all potential adjunct therapies to consider. For example, engaging the athlete for their input on what is done in the rehabilitation program on a given day may help them feel that they have control and an active voice in their recovery process. Also, careful consideration of word choice and non-verbal behavior when interacting with human athletes can communicate a lot of how the practitioner feels about their recovery that they then internalize. In regard to the equine athlete, psychosocial factors may be relevant to both the horse themselves as well as their caretakers, trainers, and owners. Horses can certainly become “sour” or resistant to training methods, specific movements, or even pieces of tack that they associate with pain from an injury. Breaking the pain cycle of an injury is imperative to overcome the negative associations that a horse has developed. In addition, some horses seem to require a temporary, or in more severe cases, permanent change in training methods or tack for them to return from an injury and advance in their career. In the authors’ opinion, pain from the axial skeleton and especially the back, can be particularly difficult for equine athletes to overcome in this regard. Horse caretakers and riders also commonly have their own fears and other psychosocial concerns. Helping caretakers and riders by using the same processes done in human athletes such that the equine athlete is perceiving that those around them are showing positive response about how they are doing is a very interesting area to explore. Although rider fears may be of themselves getting injured or for their career, they often also have fears or concerns that the horse might still be in pain or discomfort, even if not overtly lame. The Ridden Horse Pain Ethogram (RHpE) as developed and evaluated by Dyson et al. may be very useful for such riders and for veterinarians as well.59-63 In the most recent of the RHpE comprised of 24 behaviors, a RHpE score of greater than or equal to 8, meaning eight or more of the behaviors were displayed, was found to be a good indicator of musculoskeletal pain.59

Exercise Specificity

The balance between exercises that simulate or recreate the event the human athlete has to engage in versus targeting the muscle or tissue specifically injured is an area that continues to be debated within the physical therapy community. There is clearly a need for recreating situations that the athlete may experience and training the individual neuromuscular system to respond appropriately. A series of studies by Hewett and colleagues showed, for example, that by focusing on neuromuscular training one could alter movement patterns and reduce risk for subsequent injury.64-66 Further, work by Noehren et al. shows the benefits of programs such as gait retraining which emphasize the benefits of directly addressing the faulty movement mechanics using real-time visual feedback to reduce pain in patients with patellofemoral pain.67 Although these individual studies are supportive of programs that focus on improving movement mechanics the studies were not designed to evaluate a comprehensive program. Clinical experience would dictate that without sufficient underlying muscle strength and control, engaging in such gait retraining programs would be detrimental to recovery. It is established, for example, that following an orthopedic injury such as an ACL tear that regaining quadriceps muscle strength predicts normalization of gait, function, self-reported outcomes, return to play and risk of developing post traumatic osteoarthritis.68-74 Consequently, it is critical that as recovery progresses, loads increase to stimulate adequate muscle hypertrophy. During the early phases of healing using adjuncts such as blood flow restriction training as previously discussed may be an excellent way to slow or reverse muscle atrophy during times when other tissues such as ligaments need to be protected to fully heal. In addition, one can have the athlete use a lighter weight and exercise to the point of muscle fatigue to help stimulate the muscle. Only by carefully considering the underlying impairments and how that contributes to altered movement mechanics can one help the human athlete return to their preinjury levels of function. In addition, careful
attention to movement mechanics as the athlete progresses in rehabilitation is critical to reduce risk of reinjury and long-term complications.

4. Conclusion
Novel physiotherapeutic strategies to augment healing in the equine athlete are evolving, with many modalities applicable to both humans and horses. Veterinarians should refer to specific show jurisdiction guidelines for recommended use in the actively competing equine athlete. Continued collaboration to develop, evaluate, and implement effective translational approaches will redefine historic paradigms in both human and equine practice.

Acknowledgments

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

Conflict of Interest
Dr. Sherry A. Johnson is a co-founder and partner of Equine Core, Inc (Fort Collins, CO), an entity that is developing equine-specific blood flow restriction devices. Dr. Lauren V. Schnabel is a co-founder of Vetletics Inc. (Morrisville, NC).

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How to Perform a Thorough Oral Examination

Leah Limone, DVM, DAVDC-Equine

1. Introduction

A complete visual oral examination should be the basis of every dental visit and is a high standard of care. A comprehensive visual examination of the head and oral cavity is necessary to arrive at a diagnosis, perform appropriate treatments, and plan for management of challenging dental problems. The welfare of the horse depends on skilled and knowledgeable veterinarians that can characterize normal and abnormal findings both systemically and relative to the oral cavity. Clients that observe a comprehensive clinical dental examination of their horse will then compare it to their own dental care in a dentist’s office and are able to recognize the difference between veterinary dentistry and improper non-veterinary dental care. When horse owners recognize that dental treatments are based on medical expertise and accurate diagnoses, they begin to request exams for their horses instead of simply a “float.” A methodical oral and dental examination is required to diagnose diseases affecting the oral cavity. This inspection can be applied with the same degree of objectivity as would be the case during a lameness evaluation or any other body system examination. As with any examination, conclusions allow the clinician to diagnose early disease as well as identify and manage chronic problems, thus improving quality of life and benefitting the long-term general health of the horse.

2. Materials and Methods

Equipment, Location, and Sedation Considerations for a Dental Examination

Basic dental examination equipment is readily available by numerous veterinary suppliers. Recent instrumentation advances allow more efficiency and ease, and modern equine dentistry does not need to be cumbersome and labor intensive. The following basic equipment and instrumentation list is required for an oral examination:

- Sedation
- Headstand, head support, or dental halter
- Large volume dosing syringe or other method to thoroughly rinse ingesta from the oral cavity
- Full mouth speculum
- Buccal and/or lingual retractor
- Bright light source: headlight or magnetic speculum light
- Dental mirror – available in various sizes
- Extended handle periodontal probe with marked measurements
- Extended handle occlusal surface explorer
- Right angled alligator forceps and dental picks to remove feed from gingival pockets

NOTES
HOW-TO SESSION: ADVANCING DENTISTRY FOR FIELD USES

- Short handle double-ended periodontal probe and explorer for incisor evaluation
- Forceps – incisor and molar cap extractors to test for tooth mobility
- 4 x 4 gauze
- Disposable nitrile or latex gloves
- Dental chart

Additional equipment that may facilitate examination and recordkeeping, and ancillary diagnostics include the following:

- Trained technical assistant
- Oral endoscope with light source
- Digital intraoral camera
- Digital radiography system
- Stationary stocks for restraint

Appropriate sedation of the patient is mandatory to complete a comprehensive visual oral examination. An oral examination is an invasive procedure to an unsedated horse. Although compliant horses may tolerate a brief examination of their mouth when held open, and even may tolerate the speculum, it should be noted that an unsedated horse wearing a speculum is capable of inflicting serious injury to itself and handlers. Following cardiac auscultation and physical examination, sedation is administered to allow patient relaxation and compliance. Muscles of mastication should be relaxed to allow speculum placement, and the tongue should be relaxed to allow complete visualization without excessive tongue motion. The author’s preference is an alpha-2 agonist/opiate combination (0.012 mg/kg detomidine combined with 0.01–0.02 mg/kg butorphanol IV). Ergonomics of the clinician and the patient should be considered, and the use of an appropriate head support should be used. An adjustable head stand, sling, or dental halter helps to stabilize the sedated patient, and allows the veterinarian to work in a comfortable position. A quiet indoor environment is best to avoid external distractions to the patient and working indoors with moderate ambient light allows better illumination of the oral cavity with a headlight or speculum light. Consideration should be given to flooring, which should be level and a non-slip surface. Stocks are essential in a clinical setting for treatments including extractions, and while they are not typically required for a basic examination, they can certainly facilitate the process.

Dental History Taking

History taking helps complete the clinical picture in any medical examination and a thorough oral exam is no different. A presenting complaint of a specific dental problem should not preclude the veterinarian from obtaining a complete dental history and performing a thorough dental and physical examination. The general history includes information such as the patient’s signalment, athletic use, medical history, dietary history, husbandry and environment, vices, and recent changes noted. Overall medical history such as prior response to sedation, musculoskeletal and neurologic status, cardiac abnormalities, respiratory disease, underlying renal or hepatic disease, and endocrine status all have an impact on how the horse is evaluated. Oral/dental specific history includes prior dental treatments, feeding habits and any perceived difficulty masticating, fecal consistency, recently noted physical abnormalities such as nasal discharge, maldor, or abnormal swellings, and biting history and any perceived discomfort and progression of such.

Observation and General Physical Examination

Observation of the animal in its normal surroundings can provide important information. Objective data about body condition can be recorded with photographs, weight tape or scale, and can be valuable in management of patient nutrition. Abnormalities with the horse’s posture and ambulation should be observed and taken into consideration for a sedated examination. Dropped grain or partially chewed boluses of hay (quids) can be observed in the stable environment and feces should be examined for volume and consistency, as long forage stems or whole grain visible in feces indicate poor mastication. Observation of the mastication process while the horse eats several different types of food can be time consuming, but it can lend important information to the examination as well as address client concerns if they have noticed more difficulty with a particular feed or hay. It can be particularly useful to observe the horse eating to evaluate for mastication difficulties, as prehension problems look very different from dysphagia. It is important to remember that the horse as a whole should be taken into consideration when being evaluated for dental disease. Oral health is impacted by systemic health and systemic health plays a role in oral health, and the two must be considered collectively in order to make an effective treatment or management plan.

Comprehensive Oral and Dental Examination

Routine comprehensive examinations, beginning with an oral examination at birth for congenital abnormalities, should be performed at an interval that allows for detection and treatment of problems while they are still minor. For most horses, this will be every 6–12 months depending upon their eruption and development of dental problems as they age. The appropriate schedule is variable between individual horses and will be affected by variables such as their own anatomy, use of the horse, stage of development and oral conditions present or developing, and previous treatments performed.

For complete evaluation of all oral/dental structures the comprehensive dental examination needs to be
systematic and organized (Table 1). Extraoral examination is completed first, followed by examination of occlusion, soft tissues, periodontal status, and endodontic status for both the rostral oral cavity (incisors and canines) and then the caudal oral cavity (including premolars and molars). Exam specifics will be outlined in this manner as this is how the author performs a dental exam. The reader should develop their own methodical approach to completing the oral exam to include the components outlined here. Performing a methodical examination will reduce the chances of failing to identify abnormalities so that the veterinarian can ensure that all aspects of oral health are assessed and then apply those findings to reach a diagnosis and treatment or management plan.

**Extraoral Examination**

The dental examination begins with an external visual inspection and palpation of the structures of the head for evaluation of the following:

- **Skull symmetry, conformation, shape**: The ears, eyes, facial crests, musculature, nasal bones, and muzzle should be symmetrical. Breed differences and head conformation may predispose to certain dental concerns, and awareness will aid the practitioner in approaching the dental case.
- **Bony enlargements, thickening, or depressions involving the maxillary region and the mandible**: Gross abnormalities such as these with heat, swelling, or associated with a draining tract may indicate apical infection if unilateral in nature.
- **Symmetry of muscles of mastication**: Temporalis, masseter, and pterygoid muscles should be bilaterally symmetrical without atrophy or hypertrophy.
- **Temporomandibular joints (TMJ)**: These should be bilaterally symmetrical, free of effusion, and nonpainful on palpation. There will be individual variation, but the practitioner should acquire a sense of normal TMJs to be able to recognize abnormal bony enlargement of the TMJ.
- **Parotid salivary glands and intermandibular lymph nodes**: All lymph nodes and salivary glands should be palpated for enlargement and symmetry.
- **Nasal airflow, odor, or discharge**: Airflow should be symmetrical from each nostril, and odor from each side should be assessed. Nasal discharge should be assessed for character, consistency, volume, and odor. Dental sinusitis typically presents as a fetid unilateral mucopurulent discharge. If a noxious odor or discharge from one side is present, the practitioner should plan to evaluate the maxillary dentition meticulously visually on that side for indications of disease.
- **Ocular structures**: Orbital structure, eyes and eyelids should be symmetrical, and their canines should be palpated for enlargement and symmetry.
- **Discharging tracts or nonhealing wounds**: Any wound or draining tract over maxillary structures or mandibular rami should be assessed as these typically will have a dental or traumatic origin.
- **Muzzle and lips**: These should be evaluated for swelling, wounds, or masses. The commissures of the lips are common places to find scars or ulcers from bit injuries, as well as

<table>
<thead>
<tr>
<th>Table 1. A Step-by-Step Comprehensive Oral/Dental Examination</th>
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<tbody>
<tr>
<td><strong>General physical exam and history taking</strong></td>
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<tr>
<td><strong>Equipment setup and sedation administration</strong></td>
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<tr>
<td>– Position horse in safe work area with head support</td>
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<tr>
<td>– Rinse ingesta from the oral cavity</td>
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<tr>
<td>– Operator preparation: headlight, exam gloves, prepare exam instrumentation, ergonomic considerations</td>
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<tr>
<td><strong>External exam</strong></td>
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<tr>
<td>– Facial/skull/muscular/TMJ symmetry</td>
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<td>– Nasal odor/discharge</td>
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<td>– Wounds</td>
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<tr>
<td>– Neurologic abnormalities</td>
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<tr>
<td><strong>Examine incisors and canines</strong></td>
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<tr>
<td>– Occlusion</td>
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<tr>
<td>– Soft tissue examination</td>
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<tr>
<td>– Periodontal status – use periodontal probe</td>
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<tr>
<td>– Endodontic status – use dental explorer</td>
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<tr>
<td><strong>Place speculum – Examine caudal oral cavity</strong></td>
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<tr>
<td>– Occlusion</td>
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<td>– Soft tissue examination</td>
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<tr>
<td>– Examine each arcade – 100, 200, 300, 400</td>
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<tr>
<td>– Count teeth, use mirror or endoscope to examine occlusal surface and all sides/gingival margin of each tooth</td>
</tr>
<tr>
<td>– Periodontal status – mirror, periodontal probe, dental picks</td>
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<tr>
<td>– Endodontic status – mirror and dental explorer</td>
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<tr>
<td><strong>Complete dental chart, take photos or videos</strong></td>
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<tr>
<td><strong>Treatment/management plan – or additional diagnostic plan (radiographs)</strong></td>
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<tr>
<td><strong>Make follow-up recommendations</strong></td>
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melanomas in gray horses. Muzzle deviation to one side can indicate neurologic abnormalities secondary to temporomandibular osteopathy (THO) that should be assessed prior to any significant dental procedures.4–10

**Intraoral Examination**

The intraoral examination includes evaluation of occlusion, oral soft tissues, periodontal, and endodontic status of all dentition. It is helpful to separate the intraoral examination into two parts: the rostral oral cavity including incisors and canine teeth, and the caudal oral cavity including premolars, molars, bars, tongue, and palate. The oral cavity should be rinsed thoroughly to remove all feed material so that visualization of dental structures is not obscured, especially the dental occlusal surfaces and gingival margins. Complete endodontic, periodontic and infundibular assessment requires the use of occlusal and periodontal depth probes to objectively quantify the degree and type of pathology present, this is significantly aided with appropriate lighting and sedation. Most of the rostral examination should be performed prior to speculum placement. The incisors and canine teeth should be evaluated for the following:

- **Occlusion:** Orthodontic status of incisors should be assessed with the horse’s head in a natural position, not manually elevated by the examiner or assistant as this can give the false impression of maxillary prognathism (overjet).11 Rostrocaudal movement of the mandible can be evaluated by observing the relationship between the upper and lower incisor when the chin is raised and lowered.4 When evaluating occlusion, the examiner should consider whether occlusion or malocclusion is due to a dental or skeletal abnormality or asymmetry. Incisor diagonal malocclusion has been reported to be due to skeletal asymmetry and not a primary dental malocclusion.12

Number and alignment of incisors and canines should be assessed. Presence of diagonal or dorsal/ventral curvature incisor malocclusion should be recorded. The dental age should be estimated and compared with the known age of the horse. The lateral excursion to molar contact test (LMC) may be used to evaluate for masticatory symmetry and suspected oral or dental pain.4,13–18 Normal lateral excursion produces a relatively even, subtle to moderate vibration and sound. Deviations from this can be an indication of abnormal dental contact due to cheek teeth overgrowths. It must be kept in mind that this maneuver does not replicate the chewing motion of the horse. If the horse resists this part of the dental examination, sedation may be indicated to help the horse relax and allow a more thorough visual examination.4

- **Soft tissues:** Any masses, wounds, ulcers, or erosions to labial gingiva and mucosa should be noted. Attached and free gingiva should be evaluated for indicators of inflammation and subgingival disease, fistulae, recession, or hyperplasia.
- **Periodontal status:** A double ended periodontal probe and occlusal explorer used for small animal or human dentistry is useful for incisor and canine examinations. The periodontal probe with depth markers is useful to assess degree of attachment loss, and teeth should be palpated and manipulated individually for mobility scoring. Pain response such as chattering or resistance to probing should be recorded, heavily sedated horses will still show significant pain responses to light probing, particularly with equine odontoclastic tooth resorption and hypercementosis (EOTRH) affected incisors and canines.19 Accumulated calculus should be scaled to accurately assess the gingival margin.
- **Endodontic status:** Canines and incisors should be assessed for eruption status, traumatic damage, resorptive lesions, and hypercementosis. Occlusal surfaces of all incisors and canines should be examined for pulp exposure and defects.

Examination of the caudal oral cavity will include visual evaluation of the premolar and molar dentition and gingiva, buccal and lingual mucosa, bars, tongue, hard and soft palate. When the patient is appropriately sedated, a speculum may be applied, and the head rested on a headstand. The veterinarian equipped with a bright headlight or speculum light can visualize structures deep within the oral cavity but does need to use a dental mirror, intraoral camera, or a dental endoscope to visually examine oral and dental structures.2 If the horse is inadequately sedated, effective examination with a mirror is almost impossible. The veterinarian should practice using a mirror or endoscope with every dental examination. Although it may be frustrating at first, skill is acquired with consistent practice. Occlusal and periodontal lesions will no longer be missed, and it will elevate the quality and breadth of the examination. To use a mirror (or endoscope, the same general procedure may be performed with either one) to systematically evaluate all surfaces of the teeth and oral soft tissues, start with the mirror placed between the right maxillary and mandibular arches so that the occlusal surface of 106 is reflected in the mirror. The mirror is then advanced caudally to visualize the occlusal surface of each tooth in the
100 arcade. When 111 is reached, the mirror is then tipped to visualize the palatal aspect of 111, and then withdrawn rostrally to examine the palatal mucosa and interdental spaces. When 106 is reached, the mirror is again advanced to distal 111 and withdrawn rostrally but tipped to visualize the buccal mucosa and vestibular interdental spaces. The same process is then repeated for the 200, 300, and 400 arcades to evaluate all aspects of each tooth in addition to surrounding soft tissues. Examination of all structures should be completed in the same systematic manner as described below.

- Occlusion: Orthodontic assessment of premolars and molars is performed, including counting teeth in each quadrant and recognition of general wear abnormalities and focal malocclusions. Note the position of the clinical crown of each tooth and its orientation and relationship to adjacent and opposing teeth. Any defects or asymmetry in the occlusal crown surface in one row is usually reflected in a wear abnormality or defect in the opposite row. Assess each quadrant of premolar and molar teeth as a functional unit.6

- Soft tissues: Examine all oral cavity soft tissues including all surfaces of the tongue as far caudally as vallate papilla, lingual folds, sublingual mucosa, the hard and soft palates, the gingiva, mucogingival junction, and alveolar and vestibular mucosa. Any masses, wounds, ulcers, or erosions should be noted and their association with dental structures or trauma noted. Attached and free gingiva should be evaluated for indicators of inflammation and subgingival disease, fistulae, recession or hyperplasia.

Visualization and palpation of the interdental space (bars) should be performed for unerupted (“blind”) or vestigial wolf teeth or canine teeth, subgingival remnants, periosteal exostoses (“bone spurs”), or bitten trauma.21,22

- Periodontal status: A long-handled thin periodontal probe is necessary for premolar and molar periodontal assessment. Examination may reveal halitosis, bleeding at the gingival margins of the teeth, pocketing and necrosis of tissues around the teeth, and loose teeth.7 If there is impacted feed or supragingival calculus in between or around the teeth, remove it entirely with long-handled oral picks or forceps to enable accurate probing and gingival assessment. The periodontal probe with graduated markings can be used to measure gingival pocket depth and assess degree of periodontal attachment, which will range from 0.5 to 5 mm for normal teeth.23 It has been shown that gingival pocket depth measurements at the corners of the teeth significantly increases with periodontal disease.24,25 Diastemata width and depth, periodontal pocketing, and interproximal space anatomy should be recorded. Teeth should be palpated and manipulated individually for mobility scoring and any pain response noted.

- Endodontic status: A detailed examination is performed to evaluate the physical structure of each tooth. Defects over the pulp horns on the occlusal surfaces of endodontically infected teeth can be detected by carefully probing the secondary dentin of the occlusal surfaces of suspect teeth.26,27 A long-handled occlusal explorer is used to assess occlusal surface for abnormal secondary dentin or presence of tertiary dentin, open pulp horns, fissures/defects in dentin, enamel or cementum, crown fractures, infundibular cemental hypoplasia, or caries. Peripheral cementum is also examined for dysplasia, hypercementosis, and peripheral caries. Infundibular status is assessed using the Modified Honma system of grading of occlusal caries.28

**Dental Recordkeeping and Documentation**

Completion of a detailed dental chart and images (intraoral photography, video, or image capture from endoscopy) documenting examination findings should be included to complete the medical record. Accurate records are critical for the veterinarian to be able to thoroughly follow a case, communicate with other veterinarians, and provide a medical/legal document if necessary. In dental charting, the dental formula and anatomical locations in the mouth must be standardized to make documentation consistent. Use of standard abbreviations29 for dental terms to describe anatomical boundaries, abnormalities, diagnostics, and therapeutic procedures make communication possible between equine practitioners and other colleagues in both the veterinary and human dental professions.7 Important information recorded in the dental chart includes the following:

- Horse identification and signalment
- Owner information and date and time of examination
- General physical exam abnormalities, vital signs, and body condition score
- Presenting complaint and case history
- Record of sedation and medication administration
- Objective findings for extraoral and intraoral exam components including measurements taken, sketches of visible abnormalities such as fractures or focal malocclusions
Fig. 1. Sample dental chart.
The detailed dental chart should help the veterinarian progress through the exam methodically. The completed record should integrate and interpret examination findings, taking into consideration the history and presenting complaint. This record should assist the practitioner in coming to a diagnosis and a subsequent management or treatment plan. Recording images, videos, and radiographs digitally allows these images to be incorporated in the computerized dental record. Integration of the history and examination findings with the radiologic findings and other diagnostic procedures greatly improves the chances of an accurate diagnosis being formed. A sample dental chart used by the author is included (Fig. 1).

3. Conclusions

The thorough oral examination is the mainstay of the routine dental visit. The veterinarian must have a thorough working knowledge of gross and ultrastructural anatomy of the dentition, as well as an understanding of the etiology and progression of dental pathologies to understand the findings and develop a management plan. Developing a consistent and efficient examination procedure will allow the veterinarian to reliably diagnose pathologies in the early stages of development. Improving the quality of the oral examination will increase the quality of dental care provided to patients. It is every clinician’s responsibility to obtain the evidence and use evidence-based decision making to improve the way in which oral care is administered.

Acknowledgments

Declaration of Ethics

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

Conflict of Interest

The Author has no conflicts of interest.

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How to Obtain Diagnostic Dental Radiographs

Leah Limone, DVM, DAVDC-Equine

1. Introduction
Digital radiography has significantly improved the ability to diagnose dental disease in the horse. Direct digital radiography units allow diagnostic images to be readily obtained. However, practitioners may find difficulty in obtaining radiographs of the horse’s skull that are of diagnostic quality. Familiarity with standard radiographic techniques is critical to obtaining quality images and then correctly interpreting dental radiographs. Standard radiographic projections have been described in depth in multiple publications, and these are recommended for the reader to explore additional sources of information.1–7 The recommendations offered in this manuscript can help the practitioner improve their skills in dental radiography, which will then improve recognition of radiographic signs of dental and paradental pathology.

2. Materials and Methods
Radiography complements but does not replace a thorough visual oral examination. A detailed physical examination, appropriate sedation, and complete oral and dental examinations will allow the veterinarian to identify abnormalities that will indicate radiographs as the next diagnostic step. It is the exam that identifies potential problems within an area in the mouth so that a complete imaging study can be performed and the practitioner can then focus on identifying subtle radiographic changes with the tooth or teeth in question that were identified during the detailed examination. In dental referral practice, the author reviews and consults on referral radiographs on a daily basis. Some of the most common problems encountered when taking dental radiographs are consistent with those previously reported.8

- Motion artifact.
- Exposure irregularities and image processing errors.
- Positioning errors and superimposition of individual teeth and arcades.
- Obtaining an incomplete series of radiographs of the affected arcade.
- Failing to image the opposite “normal” side.
- Lack of radiographic marker or incorrect orientation of radiographs.
- Lack of a complete oral exam resulting in inability to correlate radiographic findings to examination findings.

Profound sedation is required to help reduce motion artifact in dental radiography. The author’s preferred sedation protocol is intravenous administration of detomidinea (0.01–0.02 mg/kg) used in combination with butorphanolb (0.005–0.01 mg/kg). Appropriate sedation lowers the horse’s head, which can then be rested on a low support, such as a stool or

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headstand. Stabilizing the radiographic sensor plate on the same support object greatly diminishes motion artifact. When imaging incisor and canine teeth via an intraoral technique, it is especially important (even if a speculum with radiolucent bite plates is used) that sedation is adequate so that the horse is not stimulated to chew when the plate is felt in the mouth. Differences in tissue density may require more than one radiographic exposure; however, most digital system algorithms can be adjusted to compensate for variations in tissue thickness. The use of a large (12 in x 14 in to 14 in x 17 in) sensor plate or cassette allows imaging of entire arcades, which helps to maintain spatial relationships when evaluating the radiograph. Appropriate positioning of the sensor plate and x-ray generator are necessary to obtain diagnostic images. Knowledge of skull anatomy and topographic landmarks is necessary to guide correct positioning of the patient’s head, sensor plate, and x-ray generator. With closed-mouth radiographic projections, erupted crown can be obscured by superimposition of the crowns of the opposite arcades. Superimposition is addressed by obtaining open-mouth views via use of a speculum or other method to open the mouth. Standard full-mouth speculums may have too much metal laterally that will interfere with imaging. Aluminum speculums are more radiolucent but still produce opacity on the image. Other options to keep the mouth open include a 4 in x 4 in block of wood, a PVC pipe, or a large rubber dog chew toy. Open-mouth lateral oblique views allow for optimal image quality as the superimposition of contralateral cheek tooth rows is reduced or eliminated. The practitioner should become well versed at obtaining standard views for maxillary and mandibular cheek teeth, incisors, canines, and sinuses. Taking a complete series of radiographs is important to allow evaluation of each tooth and the arcade as a functional unit. Limiting radiographs to one view of the arcade in question generally does not provide a diagnosis. A minimum series of 6 standard views for the skull and cheek teeth (dorsoventral, latero-lateral, open-mouth right and left lateral dorsal 30° ventral oblique, and open-mouth right and left lateral ventral 50° dorsal oblique; Figs. 1B, 1C, 2B, 2C, 4B) is
recommended. Intraoral imaging of incisors and canines is best performed using a radiolucent tunnel or speculum with radiolucent bite plates to protect the sensor and avoid obscuring the area of interest. A series of 6 views (bisecting angle technique, dorsoventral and 15° oblique angle to the left and right of maxillary incisors and ventrodorsal and 15° oblique angle to the left and right of mandibular incisors; see Fig. 3B–D) is warranted to assess all rostral dentition. Additional intraoral obliques or extraoral imaging of canines may also be necessary. The dorsoventral or ventrodorsal angulation outlined in the recommended standard lateral oblique views also may need to be adjusted depending on the age of the horse and length of reserve crown. Slight repositioning in a caudo-rostral or rostro-caudal direction may also be required to eliminate obliquity and overlapping of adjacent cheek teeth. Practitioners are encouraged to image both left and right views for comparison of the “normal” side to the side of interest. Bilateral images are necessary for reliable radiographic interpretation; because of the changing angles and anatomy of dentition with age, the horse serves as its own control subject. Subtle radiographic changes should be compared to the normal side to aid in radiographic diagnosis and help decision-making if referral for more advanced diagnostics, such as computed tomography, is indicated. In addition, radiography of both sides allows identification of bilateral pathology. Radiographs taken without a marker make it difficult to know which side is which when both sides of the skull are imaged. Radiographs should be taken with a right/left marker on the sensor indicating sensor placement. Proper labeling of the radiographs is essential for accurate interpretation. The American Veterinary Dental College (AVDC) convention of presenting the radiographs with “labial mounting” is used in conjunction with the American College of Veterinary Radiology (ACVR) terminology. Radiographs are presented so the viewer is looking at the images as if looking into the horse’s mouth. The right cheek teeth are presented with the horse’s nose on the viewer’s right. The dorsoventral views of the skull are presented with the nose down and the horse’s left side on the viewer’s right. Crowns of the maxillary incisors are pointed down and mandibular incisors are pointed up as if one were standing in front of the horse.

3. Results

Once appropriate diagnostic radiographs are acquired, systematic review of each radiograph taken should include assessment of the following:

- Is there motion artifact and is exposure appropriate to evaluate the image?
- Tooth numbers and anatomy: Is the entire arcade included on the image? Are individual teeth and roots visible with interproximal spaces and periodontal ligament spaces clear? Are there missing teeth or supernumerary teeth? Is evaluation of teeth for root blunting, resorption, hypercementosis, or malformation possible?

Fig. 3. Mandibular incisor images of the same horse. A, Motion artifact is present, no marker or orientation; the interpreter cannot determine left from right. B–D, Ventrodorsal and 15° to the left and right of midline; labeled and correctly oriented images free of motion allow assessment of all mandibular incisors and canines.
Alveolar anatomy: Can periapical alveolar bone radiopacity (sclerosis or lysis) be evaluated? Can the width of periodontal ligament space be evaluated? Can evaluation of alveolar margins and assessment of vertical or horizontal bone loss be performed?

Paradental sinuses: Are there fluid lines, soft tissue density, cystic structures? Are fine bony structures clearly visible or is the image overexposed?

Critically evaluating each radiograph will help the operator improve their technique. The practitioner will become accustomed to looking for specific changes that will lead to a diagnosis from quality images instead of guessing at nondiagnostic images. Consistency in positioning and technique improves the clinician’s ability to recognize normal and abnormal anatomy and allows identification of radiographic signs of dental and sinus disease.10 Figures 1–4 are provided to highlight the differences between nondiagnostic and diagnostic dental radiographs.

4. Discussion

Proficiency with the standard radiographic views of equine cheek teeth will allow the practitioner to obtain diagnostic images for immediate use in the field or for consultation. Dental disease diagnosis requires quality radiographic technique that is consistently applied in veterinary practice. There is significant age variation of radiographic appearance of cheek teeth and their apices, and an appreciation of normal variations is required for accurate interpretation of dental radiographs. By recognizing and preventing radiographic technical difficulties, image quality and diagnostic accuracy will improve. Becoming proficient with taking and interpreting dental radiographs will increase the level of service practitioners provide and thus improve the overall health care of their patients. It is important to understand the limitations of radiography to clearly diagnose dental disease in complicated cases. Additional advanced imaging with computed tomography, nuclear scintigraphy, or magnetic resonance imaging may be required to better characterize the extent and exact location of disease involving multiple teeth or sinus disease.

Acknowledgments

Declaration of Ethics

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

Conflict of Interest

The Author has no conflicts of interest.

References and Footnotes


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How to Recognize and Evaluate Periodontal Disease

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1. Introduction

Periodontal disease (PD) infers inflammation and infection involving the periodontium, which is collectively comprised of the gingiva, periodontal ligament, tooth cementum, and alveolar bone. In equids, PD is a common intraoral finding of variable severity and has been shown to occur at greater frequency with increasing age. Abattoir studies conducted during the last fifty years report an overall prevalence of 13%–60%. This is similar to results reported from more recent surveys of various patient populations showing a prevalence range of 13%–75%. Earliest stages of PD result in gingivitis without involvement of subgingival tissues; however, as the disease progresses, periodontitis ensues with deeper extension of inflammation and infection into subgingival supporting structures. Histopathological features of equine PD include gingival epithelial ulceration, leukocytic inflammation, gingival hyperplasia, cemental erosion, plaque formation, and bacterial infection. The practitioner must be cognizant that periodontal disease manifests in various locations in the equid hypsodont dentition. A very common occurrence of PD occurs due to calculus accumulation on mandibular canine teeth with gingivitis (redness, bleeding) in male horses, similar to PD that which occurs in dogs and cats. At the front of the mouth, gingivitis affecting the incisive region can occur due to local irritation when ingesting noxious plant material in hay or pasture, and focal gingivitis can also be seen in this area of the mouth during incisor eruption. In the back of the mouth, gingivitis is often noted along the cheek teeth arcades during tooth eruption of premolars and shedding of deciduous teeth, and mild gingivitis can also be seen with oral migration of Gastrophilus sp. larvae. The aforementioned conditions are typically limited to the supragingival region and likely cause mild to moderate irritation to the horse. Equine odontoclastic tooth resorption and hypercementosis (EOTRH) is an inflammatory process that results in significant periodontal disease of the incisor teeth, canine teeth, and less commonly the cheek teeth. In these cases, gingivitis, gingival recession, enlargement and distortion of alveolar bone, and gum boils are frequently noted in addition to typical radiographic lesions of EOTRH (tooth resorption lesions, alveolar bone lysis, and hypercementosis). Another painful manifestation of PD in equids occurs if roughage fibers become wedged between adjacent cheek teeth (premolar or molar teeth). Ordinarily tight apposition of the clinical crowns eliminates the possibility of food material entering the interproximal spaces; however, in some equids, a slim gap at the occlusal aspect of the interproximal space that allows entrapment of food material between teeth (the occlusal gap between the teeth is termed a diastema, plural diastemata). Diastemata may be congenital or acquired and in some instances have a sufficient occlusal opening allowing roughage to escape (“open” diastema); however, in some patients, the slim
opening acts as a one way valve to prevent release of the fibrous feedstuffs ("valve" diastema). In this event, roughage fibers are pressed through the occlusal aspect of the diastema during mastication, becoming wedged into deeper portions of the interproximal space, which leads to the pathologic cascade of PD (bacterial fermentation of carbohydrates → acid production → inflammation → necrosis). Untreated, diastemata result in periodontitis, pain, and progressive loss of tooth attachment (Fig. 1).

Potential symptoms of PD noted by animal caretakers are dependent on severity but can include halitosis, slow eating, frequent mouth rinsing, tilting the head when chewing, refusal to bite carrots/hard treats, quidding, or slobbering. In some instances, a patient affected with PD may present for dental procedures without any owner complaints of dental-related issues. As such, a thorough and systematic intraoral examination is necessary for appropriate recognition of PD, as well as radiographic examination to evaluate the teeth and supporting structures. Previous authors have reviewed periodontal anatomy extensively, and there is a growing abundance of research on anatomy and physiology of equine periodontium.

In practical terms, the reserve crown and root of the hypsodont tooth are anchored within the alveolus by diffuse subgingival attachment of periodontal ligament along the peripheral cementum of the tooth. These attachments span from the tooth across the periodontal ligament space and attach to the inner surface of the alveolar bone. The alveolar bone encasing the tooth is very compact and forms a subgingival alveolar crest in the interproximal region of the clinical crowns. The intraoral surfaces of the attached gingiva, unattached gingival margin, clinical crowns, and interproximal spaces are visualized in the mouth. The gingival epithelial sulcus depth along the inner surface of the gingival margin is normally measured about 3 mm in depth for incisor teeth and 5 mm for cheek teeth. The inner aspect of the attached gingiva is tightly adhered to the clinical crown and underlying bone, effectively blocking entry of oral contents from the gingival sulcus into the alveolus and periodontal ligament space. Continued eruption of hypsodont teeth is inevitably associated with constant remodeling of the periodontal ligament.

### Table 1. Index of Tooth Mobility Modified for Equine Dentistry

<table>
<thead>
<tr>
<th>Grade</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>No distinguishable sign of movement greater than normal.</td>
</tr>
<tr>
<td>Moderate</td>
<td>movement of up to approximately 3 mm</td>
</tr>
<tr>
<td>Severe</td>
<td>movement &gt;3 mm in any direction and/or is depressible.</td>
</tr>
</tbody>
</table>

### Table 2. Stages of Periodontal Disease

<table>
<thead>
<tr>
<th>Stage</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 Normal:</td>
<td>No attachment loss. Probing depth &lt;5 mm.</td>
</tr>
<tr>
<td>1 Gingivitis:</td>
<td>No attachment loss. Probing depth &lt;5 mm.</td>
</tr>
<tr>
<td>2 Early PD:</td>
<td>&lt;25% attachment loss and/or crestal bone loss around teeth.</td>
</tr>
<tr>
<td>3 Moderate PD:</td>
<td>25% to 50% attachment loss/bone loss &lt;50% around tooth roots.</td>
</tr>
<tr>
<td>4 Advanced PD:</td>
<td>&gt;50% attachment loss or bone loss &gt;50% around tooth root(s).</td>
</tr>
</tbody>
</table>

2. Material and Methods

- Sedation
- Dental halter or headstand
- Bright source of light
- Dental speculum
- Cheek retractor
- Dental mirror or oral endoscope
- Periodontal probe
- Dental scaler
- Pressurized oral irrigation instrument
- Right-angle periodontal forceps
- Radiographic instrumentation

Oral examination procedures are detailed in a different manuscript of this session. To evaluate the periodontium, all components of the dentition are examined to detect the presence of any of the following conditions of the oral cavity: abnormal tooth mobility, calculus, gingivitis, gingival recession, halitosis, enlarged juga, gum boils, periodontal pocketing, or diastemata. For complete evaluation, the veterinarian will need to visualize all gingival tissues associated with the lingual and labial aspects of the clinical crowns of the incisors, canine teeth, and cheek teeth. During this process gentle retraction of the lips, cheeks, or tongue is very helpful for visualization. Minimal instrumentation is required.
to accomplish the exam at the front of the mouth; however, complete evaluation of the periodontal structures of the cheek teeth is not possible without the use of a dental speculum, bright light, and dental mirror (or endoscope). Tooth mobility is assessed by attempting to wiggle the affected tooth with finger pressure or with the aid of a rigid dental scaler, and the movement can be estimated in millimeters to assign a Mobility Index13 (Table 1). Advanced PD is present in any tooth that is mobile > 3 mm and/or is depressible in the alveolus. Areas of the gingival margin that appear to be affected by bleeding, gingival recession, calculus, or roughage entrapment warrant further inspection. Entrapped roughage must be removed from periodontal pockets or interproximal spaces using high pressure irrigation and periodontal forceps. Removal of roughage allows visual assessment of the degree of gingivitis/gingival recession and allows probing. The periodontal probe is used to measure depth by sliding the tip alongside the tooth within the gingival sulcus. If a periodontal pocket is present, the probe will slide deeper within the sulcus in an apical direction alongside the tooth.16 Staging of PD helps define the extent of pathological change that is present.24 PD is progressive, meaning that gingivitis, gingival recession, probing depth, and tooth mobility will worsen without treatment. In general terms, PD Stage 1 (PD1) is comprised of gingivitis/bleeding without attachment loss, while cases with PD2, PD3, or PD4 are characterized by progressive worsening of gingivitis and gingival recession, increased sulcular probing depth, tooth mobility, and alveolar bone loss (attachment loss). The stage of PD present is ascertained via clinical examination and radiography. Dental radiographic techniques are covered by a different manuscript in this session and have been described extensively.25–27 In cases of PD, radiographs allow the veterinarian to observe the degree of bone loss involving the interproximal alveolar crest and alveolar margin in addition to other changes that may be present including sclerosis, widening of the periodontal ligament space, blunting of the tooth apices, and lytic changes of subgingival crown and roots.13 Radiographic findings are then correlated with clinical findings to determine the overall severity of attachment loss and stage of PD (Table 2). Intraoral images provide excellent detail of alveolar bone of the incisor teeth and mandibular canine teeth. Due to inherent superimposition of skull structures that occurs with extraoral radiography of cheek teeth, the author typically acquires an occlusal oblique with the mouth held open by a speculum to evaluate the crestal bone and clinical crowns and an apical oblique view to evaluate the alveolar bone margin, PDL space, and apices (Figs. 2 and 3). Computed tomography eliminates superimposition and provides exquisite detail of teeth and alveolar bone (Fig. 4).

3. Results and Discussion

Periodontal disease is a common affliction of equids. Using the techniques described here, the
veterinarian performing a complete oral examination in a properly sedated patient can effectively evaluate irregularities of the periodontal tissues. For PD affecting the cheek teeth, a dental mirror or endoscope is requisite for recognition. The use of high-pressure irrigation and periodontal instruments to remove entrapped roughage improves visualization of affected areas and allows detailed inspection of the gingiva for probing. In addition to clinical examination, radiography helps detect the degree of bone loss and other changes that help determine severity of PD and appropriate treatment planning.

**Acknowledgments**

**Declaration of Ethics**

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

**Conflict of Interest**

The Author has no conflicts of interest.

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24. Definitions of stage, grade, index. AVDC Nomenclature. https://avdc.org/avdc-nomenclature/
Equipment and Treatment of Periodontal Disease

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1. Introduction

Periodontal disease is a painful condition affecting young, middle aged, and old horses.1–3 Horses are stoic and oftentimes may not show outward signs of disease. This makes the oral examination critical in identifying this painful condition. Horses that do show outward signs may have a history of quidding, eating slowly, holding their heads sideways while chewing, or having a thin body condition.4 Periodontal disease begins as “food stasis”. Food stasis is the accumulation of feed material in the oral cavity adjacent to teeth, most often in the form of a diastema. This decaying feed provides an acidic environment for plaque forming bacteria, leading to gingival recession, periodontal attachment loss, and, in severe cases, periodontitis.2,3 Left untreated, periodontal disease leads to tooth loss and systemic disease. These diastema, pleural diastemata, may be present in horses of any age, may affect incisors or cheek teeth, and may be congenital or acquired. They are often associated with a decreased angulation of the cheek teeth, missing teeth, fractured teeth, aging horses, and erupting cheek teeth. Prevention of disease is the gold standard of veterinary medicine. Routine oral exams and effective odontoplasty (teeth floating) are paramount in preventing abnormal food accumulation. The prevention of overlong teeth, excessive wear of teeth in opposing quadrants, steps, hooks, and other areas of inefficient mastication all play a role in avoiding periodontal disease.4 Reduction of these orthodontic abnormalities must be considered when forming a treatment plan.

2. Treatment Planning

Any treatment plan for periodontal disease must begin with proper staging/grading. Advanced cases with significant attachment loss and loose teeth require extraction. Another manuscript of this session5 discusses the stages of periodontal disease (Table 1) as well as a tooth mobility index (Table 2). A thorough oral exam coupled with diagnostic quality dental radiographs provide the information needed to stage the periodontal disease. Specifically, the evaluation of bone loss and widened periodontal ligament spaces are best visualized on open mouth oblique views. These radiographic projections and how to obtain them are the topic of another manuscript in this session.6 The focus of this paper will be those cases that have not advanced to the point of extraction and the efforts that may be made to prevent tooth loss through the treatment of diastemata. Diastemata are common in both the incisors and cheek teeth of horses. Treatment of periodontal disease associated with diastemata must include the following: 1. Reduction of any abnormal clinical crown overgrowths (steps, ramps, protuberant transverse ridges); 2. Complete cleaning of the
periodontal pocket, which in some cases may not be achieved without a diastema widening; 3. Possibly the placement of a periocentric; and, 4. Possibly sealing with a dental packing material. Not all diastemata are the same. Treatments will vary based on the presentation of an open, valvular, or senile diastema, orthodontic imbalances, missing teeth, and incisors vs. cheek teeth. The primary goal is that during mastication, the horse is able to cycle food between teeth using the tongue and water to prevent quidding and food stasis.

3. Materials and Methods

Many of the above treatment goals may be achieved by the attending veterinarian with access to appropriate equipment and treatment planning.

Restraint

Periodontal disease is very painful, and additional analgesic may be necessary to perform treatments of inflamed/infected gingival tissues. The author commonly uses a combination of detomidine hydrochloride and butorphanol with an anti-inflammatory such as flunixin meglumine or phenylbutazone given prior to treatment. In many cases with severe pain, the patient’s tongue movement and head tossing becomes too severe and prevents placement of probes, picks, and curettes. Rotary burs are used for diastema widening and should not be used in a patient that is awake, moving, or reactive. To fully examine and treat these tissues in those most painful cases, it may be necessary to provide local and regional analgesia with injectable anesthetics. Topical gels and solutions may be used with success in milder cases. The more severe cases most often require a deeper level of sedation as well as a regional nerve block. A mouth speculum is placed, and with a bright headlamp and mirror or oral endoscope, the examination is performed. This may be done in a stall, wash rack, or preferably a set of stocks when available. Though stocks are not a necessity, they do provide additional support for the more heavily sedated patients.

Incisors

Treatment of incisors may be achieved with hand scalers, dental curettes, and a toothbrush. These instruments are used to remove feed material and calculus from the interdental space, surface of the tooth, and subgingival tissues. Water picks are also useful in flushing feed between the interdental spaces. With a compliant patient, home care by the owner with twice weekly brushing may prevent additional gingival recession. If the diastemata are valvular, then widening of the interdental space will be necessary.

Cheek Teeth

First is removal of feed material and complete cleaning of the periodontal pocket. This may be achieved with dental picks, probes, periodontal grasping forceps, water picks, air abrasion units, and gingival curettes. These work well in open diastemata. Valvular diastemata require widening before they can be fully cleared of feed material (Figs. 1 and 2).

Diastema Widening/Burring

With the valvular diastema, a diastema widening is often needed to completely remove the feed material. The diastema widening is performed using a rotary bur on a right-angled hand piece, followed by cleaning and disinfecting the pocket. Water cooling is available on some dental units and is needed to protect against thermal pulpal damage to the teeth during burring. The rotary bur should be placed parallel with the interdental space such that the bur is extending towards the cheek with the shaft of the dental unit lingual/palatal. The use of a cheek retractor is helpful to prevent iatrogenic damage to the buccal mucosa. Endoscopic guidance allows for proper positioning of the bur within the interdental space (Fig. 3). There are a variety of carbide and diamond coated burs available for diastema widening with both conical and cylindrical shapes commonly used. The carbide burs, while effective at removing dental materials, generate more heat than the diamond coated burs. Frequent water cooling is advised. The widening of these valvular defects must extend from the occlusal surface of the crown apically to the gum line (which often has gingival recession). Using a mirror or oral endoscope to monitor the bur placement and progress during burring is crucial. Great caution must be taken to avoid iatrogenic damage to the pulp horns of the two teeth.

<table>
<thead>
<tr>
<th>Table 1. Index of Tooth Mobility Modified for Equine Dentistry</th>
</tr>
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<tbody>
<tr>
<td>• Normal: first distinguishable sign of movement greater than</td>
</tr>
<tr>
<td>• Modest: movement up to approximately 3 mm</td>
</tr>
<tr>
<td>• Severe: movement &gt;3 mm in any direction and/or is depressible</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table 2. Stages of Periodontal Disease (PD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 Normal: No attachment loss. Probing Depth &lt;5 mm.</td>
</tr>
<tr>
<td>1 Early PD: No attachment loss. Probing depth &lt;5 mm.</td>
</tr>
<tr>
<td>2 Moderate PD: 25% attachment loss and/or crestal bone loss around teeth.</td>
</tr>
<tr>
<td>3 Advanced PD: 25% to 50% attachment loss/bone loss &lt;50% around tooth roots.</td>
</tr>
<tr>
<td>4 Advanced PD: &gt;50% attachment loss or bone loss &gt;50% around tooth root(s).</td>
</tr>
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</table>
burs used are often as narrow as 3 mm in diameter and, when placed within the interdental space, should only remove a small amount of dental material from each tooth. After widening, the entrapped feed material can be removed with the aid of instruments, water picks, or air abrasion (Fig. 4). Then the cleaned pocket may be treated.

Treatment
A variety of materials have been placed in cleaned periodontal pockets to treat the underlying infection and inflammation. Treatment of these pockets has been the source of much discussion within the veterinary dental community. At this time, it is unclear if the use of antibiotics or other perioceutics in these pockets is absolutely necessary and if so, which is best used in equids or humans. Additional long-term studies are needed to evaluate efficacy. Some of the materials that have commonly been used in the periodontal pockets include doxirobe, metronidazole,
antibiotic impregnated plaster of Paris beads, and calcium hydroxide. The interdental space above these deep periodontal pockets is often covered with a vinyl polysiloxane (VPS) impression material (Fig. 5). This is placed to prevent additional feed packing and allow time for the pocket to be completely filled by this regenerative gingival tissue. VPS is available as both a flowable and putty material. The two-part putty materials are typically easier to handle and place into the more caudal aspects of the mouth. This is because the flowable VPS requires an application gantry and mixing tip that are difficult to place into the caudal mouth.

Follow Up

Recheck exams are performed in three months to monitor tissue healing. It is not uncommon for the VPS to be present at the time of the recheck exam, and it can be removed at this time. The previous dental pocket should be resolved. This means there should be no decaying feed present in the diastema, no probing depth, no gingival recession, and no inflammation/bleeding of the gingival tissues. Some cases are refractory, and repeated treatment may be necessary in a year or more if the periodontal pocket returns. Therefore, continued monitoring of these cases long term with accurate oral exam charting is key to a successful outcome.

4. Conclusion

Periodontal disease can be a reversible and treatable condition if caught early. With proper restraint, equipment and training, practitioners can effectively treat periodontal disease in a field setting. Dental radiographs must be used to determine the stage of periodontal disease and underlying tooth viability prior to choosing a therapy. Adding diastema widening as a skill will allow practitioners to offer treatment for a painful condition, stop the progression of disease, and prevent tooth loss in many patients. This treatment is not innocuous. There is potential for harm including but not limited to iatrogenic soft tissue trauma, pulp exposure, and pulpal thermal necrosis. Training on cadaver specimens with veterinary dental specialists and attendance at dental specific continuing education wet labs are strongly recommended prior to performing diastema widening in a live patient.

Acknowledgments

Declaration of Ethics

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

Conflict of Interest

The Author has no conflicts of interest.

References and Footnote


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How to Recognize and Manage Age-Related Dental Problems in Geriatric Patients

Cleet Griffin, DVM, DABVP-Equine Practice, DAVDC-Equine

1. Introduction
With longevity, most equids will gradually experience decreased masticatory ability. This can frequently manifest clinically as quidding of roughage and/or thin body condition. In addition to severe dental attrition and malocclusion, it is the author's clinical experience that affected patients also have significant senile atrophy of the muscles of mastication. Along with the negative effects of aging and dental attrition, a significant percentage of the geriatric population has coexisting dental problems that include missing teeth, displaced teeth, diastemata, and periodontal disease. From a dental perspective it is important to remember that stress associated with constant oral discomfort and inadequate digestion of nutrients may predispose to a variety of other problems. As a result, every geriatric horse should undergo thorough oral examination with regularity in order to address a variety of age-related concerns. In addition to an oral examination, it is important for the veterinarian to assess for the presence of coexisting contributors to poor health that are prevalent in older patients such as pituitary dysfunction, chronic lameness, hoof abnormalities, or ocular disturbances. With good husbandry, correct feeding of dietary components formulated for senior horses, proper dental care, and appropriate veterinary maintenance of various ailments, it is the author's experience that unreasonable debilitation and excessively thin body condition can often be avoided or improved significantly in older horses.

2. Materials and Methods

- Sedation drugs (alpha-2 agonist, butorphanol)
- Reversal drug for alpha-2 agonist
- Dental halter or headstand
- Bright source of light
- Dental speculum
- Padding for bite plates of speculum
- Cheek retractor
- Dental mirror or oral endoscope
- Flexible 1M upper airway scope
- Periodontal probe
- Dental scaler
- Chlorhexidine gluconate 2% solution
- Pressurized oral irrigation instrument
- Right-angle periodontal forceps
- Intraoral extraction instrumentation
- Local anesthetic solution
- Radiographic instrumentation
- Test tubes and specimen cups
- Dental chart
Pertinent history is obtained, and a physical examination is performed for initial assessment of the health status of the senior patient. The author typically obtains photographs to use for comparison of body condition score during subsequent visits. Certain details about an older patient’s husbandry routine are particularly important including deworming history, housing management (stall/dry lot/pasture), level of exercise, individual vs group feeding, quality of water source, and types/amount/quality of ration and roughage fed. If the patient is presented for evaluation of thin body condition and/or other history or symptoms of illness are detected then the author obtains samples for minimum database laboratory testing (i.e., CBC, serum chemistry panel, serum electrolytes, baseline adrenocorticotropic hormone (ACTH), fecal egg/gram count) and is routinely offered grazing and pellets to observe the horse’s appetite and chewing ability. Oral examination and sedation procedures are detailed in a different manuscript of this session, and all abnormalities should be recorded in the dental chart. As with any equid, the front of the mouth (incisive region, lips, and bars) can be examined in senior horses without sophisticated instrumentation. With older equids, the incisor teeth may be severely worn or partially/entirely missing (Fig. 1). If this occurs, the author typically pads the bite plate of the speculum with a small folded towel to protect the gingiva when the mouth is open. The mouth should be thoroughly rinsed and the cheek teeth arcades inspected visually with a mirror to detect absence of teeth, smooth teeth, abnormally positioned teeth, overlong teeth, or other malocclusions. Fractured teeth and diastemata can easily be overlooked, especially toward the back of the mouth without the use of a mirror or dental scope. Each row of teeth should also be examined with fingers to check for sharp areas and loose teeth. Roughage entrapped by diastemata must be removed with irrigation and periodontal forceps to allow adequate visual assessment and probing. Radiographic examination is typically performed in cases with suspected apical abscess or other apical disease such as equine odontoclastic tooth resorption and hypercementosis (EOTRH) and in cases with moderate to severe periodontal disease. Some older patients are presented with foul smelling unilateral nasal discharge: in these cases, detailed oral examination, upper airway endoscopy, and radiographic examination are important procedures to rule out secondary sinus involvement or the presence of an oroantral or oronasal fistula. After opening the dental speculum, it is typical for sedated older patients to be resentful and reactive toward intrar oral placement of hands and instruments; as such additional dose(s) of IV sedatives often become necessary to safely continue the exam and treatments. Without the stimulatory effects of the dental procedures, the senior patient often appears to become much more sedate and ataxic during the recovery period after the procedures are completed. When this occurs, it is important to support the patient’s head to prevent severe dependent nasal edema and dyspnea, to monitor the patient closely for safe recovery and withhold feeding until sedative effects have worn off, and to make sure the patient is on soft/padded footing in case of a fall. In very rare instances, the author has found it necessary to mitigate the effects of sedation by administration of an alpha-2-agonist reversal agent in these patients. Management of several conditions of interest are discussed in the following sections.

Malocclusion and Age-Related Dental Attrition
Cupping of the maxillary cheek teeth occurs as crown attrition progresses beyond the apical extent of the infundibula into the apical region of the reserve crown and roots. Clinically this change is first noticed as a concave appearance to the occlusal surface of teeth 109/209, along with the development of a very sharp enamel edge that causes abrasions to the adjacent cheek. In addition, an undulating wave malocclusion comprised of worn and overlong teeth is a common finding (Fig. 2). With continued attrition, the mandibular premolar teeth and remaining maxillary molars sequentially may become very smooth with the gingival margin. This pattern of age-related dental attrition results in diminished masticatory ability especially in regard to coarse roughage.5

The goal of treatment in senior horses is to perform odontoplasty of sharp enamel edges and overlong teeth in order to improve comfort during mastication and performance. It is not appropriate to attempt to restore normal occlusion of the cheek teeth in an old horse with severe wave malocclusion—to do this may

Fig. 1. Severe incisor attrition.
worsen masticatory ability by elimination of existing areas of occlusion. As such the author is typically very conservative with odontoplasty of wave malocclusion in senior patients.

Equine Odontoclastic Tooth Resorption and Hypercementosis

Most commonly EOTRH is a condition of older horses involving resorptive lesions and hypercementosis of the incisor teeth and canine teeth, and less commonly the cheek teeth. The syndrome is gradual in onset and often undiagnosed until extensive lesions are present. EOTRH is diagnosed radiographically and typical findings include irregular areas of tooth lysis affecting one or more teeth, fracture of the reserve crown, production of excessive cementum, widening of the periodontal ligament space, and alveolar bone loss. Pathological changes of the teeth and surrounding structures may result clinically in gingivitis/bleeding, gingival recession, distortion of alveolar bone, parulis lesions (gum boils), pathologic tooth fracture, and discomfort (Fig. 3). Extraction of affected teeth generally results in resolution of clinical signs. Although general anesthesia is occasionally required due to fractious behavior or inability to achieve sufficient standing analgesia, extraction of affected incisor teeth using a simple approach can typically be carried out in the standing patient with adequate IV sedation/analgesia, regional nerve block, or local gingival anesthetic injections. Radiographs should confirm complete tooth removal, and suturing of the gingiva will help decrease contamination of healing alveoli in the days following surgery.

Periodontal Disease

Diastemata of the premolar/molar teeth are very common in aged horses in part due to gradual narrowing of tooth circumference as the tooth is worn toward the apical region. Thus, over time a small interproximal gap occurs between neighboring teeth that allows entrapment of roughage (known as a senile diastema). Less severe cases exhibit gingivitis and halitosis. These benefit from odontoplasty of overlong teeth and tall occlusal transverse ridges, along with daily mouth rinses to help control gingivitis. An effective oral rinse solution can be prepared using a solution of dilute chlorhexidine gluconate 2.0% solution and water (10–20 mL of chlorhexidine sol. per gallon of water). Feeding soft diets comprised of fine grass hay, lush pasture, and/or complete feed pellets help reduce entrapment of coarse roughage within affected interproximal spaces. The author will commonly perform intraoral extraction of mobile cheek teeth in senior equids affected with advanced periodontal disease. These include teeth that are depressible within the alveolus, teeth that have mobility greater than 3 mm, and teeth that are abnormally positioned in association with severe valve diastema. Adequate IV sedation/analgesia, local gingival anesthetic, and regional nerve block allow the extraction to proceed with maximal patient comfort. In cases of diastemata exhibiting bleeding, odor, gingival recession, and < 2–3 mm of tooth mobility, the author typically performs radiographs to assess the apical region and ascertain the degree of alveolar bone loss. In the absence of apical abscess or other complicating factors, the severity, bleeding, odor, and discomfort in these cases generally improve substantially after odontoplasty and mechanical widening of the affected interproximal space. A small diameter but problematic oroantral fistula or oronasal fistula may develop in aged horses in association with diastema, fractured teeth, or severely worn teeth in the maxillary arcades. Chronic periodontitis in these cases allows progressively deeper involvement and destruction of alveolar bone resulting in fistula formation. Clinically an oroantral or oronasal fistula allows oral contents and roughage stems to enter the
ipsilateral maxillary sinus or nasal passage (Fig. 4). Either of these situations manifests primarily as unilateral nasal discharge with foul odor. For diagnosis, oral examination will identify a deep valve diastemata or deep periodontal tract alongside the offending tooth. Nasal endoscopy and radiography are indicated if an oroantral or oronasal fistula is suspected; however, CT imaging is highly rewarding in these cases in terms of identifying apically infected teeth and defining the severity, extent, and location of the fistula. Effective treatment of an oroantral or oronasal fistula in geriatric patients requires patience and owner commitment. In general terms, treatment entails fistula debridement, extraction of loose teeth or apically infected teeth, and temporary obturation of the oral side of the fistula until healing. Appropriate treatment of sinusitis is required in the management of an oroantral fistula, and for an oronasal fistula roughage stems can be removed from the nasal passage with long forceps under endoscopic guidance. A very important management principle is elimination of coarse-stem roughage from the diet until healing of the fistula occurs. In the absence of apical infection or loose teeth on either side of a diastema, it may be possible to obtain closure of the fistula in aged horses by treating concurrent sinusitis and sealing the oral aspect of the interproximal space with dental acrylic. There are a few clinical reports detailing successful surgical treatment of oronasal or oroantral fistulae in horses.

Oral Masses

An oral neoplasm originating from bone, soft tissue, or dental tissue should be included as a consideration if an oral mass is encountered upon examination (Fig. 5). In some cases the presence of a non-neoplastic mass such as granulation tissue or gingival hyperplasia may mimic the appearance of an oral neoplasm. If an oral neoplasm is suspected then referral to a specialty center for advanced imaging and potential treatment is suggested by the author. Appropriate initial workup of an oral mass includes biopsy of the lesion and histopathology, CBC-Chemistry panel, and radiography or CT study to evaluate underlying structures.

Feeding an Older “Thin Horse”

Nutritional counseling is an important aspect of veterinary dental care in senior patients. The author is not a nutritional expert but does attempt to keep a few simple rules-of-thumb in mind when making nutritional recommendations for otherwise healthy but thin senior patients:

- A thorough dental exam and appropriate laboratory workup should precede ration modification.
- Thin geriatric patients should be fed a commercial senior feed formulation. The author typically recommends to gradually build up to at least 1%-1.5% body weight per day divided into three to four feedings.
- If thin patients are unable to masticate high quality long-stem hay, then an alternate roughage source such as soaked alfalfa cubes or pellets should be fed in addition to the commercial senior ration. Fat supplementation can be helpful in these patients to increase caloric intake for improvement in body condition.
Some geriatric horses will tend to do fine with grazing fresh pasture where the high moisture content of the forage allows less mastication.\textsuperscript{17,18}

If ever in doubt, consult an expert or trusted colleague when formulating nutritional recommendations for thin patients or for those with disease conditions such as hepatic dysfunction, insulin resistance, pituitary dysfunction, etc.

3. Results and Discussion

The challenges associated with dental care in senior age equids can be somewhat unique from other age groups. Despite decreased chewing ability, older horses, ponies, and donkeys can have a good quality existence with proper intervention.\textsuperscript{17} Oral examination presents an opportunity for the veterinarian to create client awareness of the effects of age-related dental attrition and to discuss important dietary modifications that may be required.\textsuperscript{6} Along with identification and treatment of various age-related dental and non-dental abnormalities, the attentive veterinarian can capably assess the older patient’s nutritional status. Veterinarians should continually strive to equip themselves with proper dental knowledge and instrumentation in order to successfully manage problems in the particular age group under examination.

Acknowledgments

Declaration of Ethics

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Conflict of Interest

The Author has no conflicts of interest.

References


How to Refer: What to Obtain, Gather, and Share for a Consultation or Referral

Travis J. Henry, DVM, DAVDC (NSS), DAVDC (Eq)

The purpose of this paper is to provide the practitioner and specialist with practice tips in the process of referring and consulting on equine dental cases. Author’s address: Midwest Veterinary Dental Services, PO Box 466, Elkhorn, WI 53121; e-mail: drhenry@midwestvetdental.com. © 2021 AAEP.

1. Introduction
The past two decades in veterinary medicine have seen an increase in the number of specialty colleges. This has led to an array of board-certified specialists in many different disciplines. Board Certified Veterinary Dentist™ (BCVD) in equine is now a specialty that is recognized affiliated with the American Veterinary Dental College.¹ The European Veterinary Dental College² also has an equine dental specialty. With this progress, veterinarians now have the opportunity to refer and consult with a colleague with advanced equine dental training. The transfer of information is often a frustration for the practitioner trying to get help with the cases and the referral center. This presentation will discuss how to make this a smoother process for both parties to reduce frustration and increase the client’s satisfaction from both parties.³ Many examples will be given of the information that should be provided between the referring doctor (RDVM) and the BCVD.

2. Materials and Methods
The practitioner is often presented with a case that is challenging and wishes to seek advice on how to proceed. This is often done by contacting someone with an interest or specialty title in the discipline of the particular case. Dentistry is no different. Given the vast number of dental diagnoses and different etiologies that are seen related to the age of the horse, arriving at a treatment plan can be a complex process. The practitioner should always provide as much information as possible. This would include the following:

1. Signalment of the horse
2. Duration of the signs
3. Pictures of the clinical findings and, wherever possible, intraoral pictures
4. Examination findings
5. Diagnostic images correctly labeled of the affected and nonaffected side
6. Appropriate client information and horse’s name

The client’s expectations are to find out what treatment is necessary and then get an estimate of the cost for the procedures. The client generally has many factors to consider when making decisions. These include the cost, the amount of time the horse is not in performance, the competence of how the
information was portrayed, the geographical consideration of hauling the horse, and their time investment. Often, clients will have considerations that can seem remedial to the practitioner but can be a large factor in their decision. This could include the clients paying for training and board while the horse is recovering at a hospital. One must listen to all of the owners’ concerns and help them navigate an appropriate solution. The RDVM that is sending the information for consultation or referral must often ask these questions of the specialist for the owner either in an email or via phone call. One good solution is to have a form letter that the RDVM can fill out and send to the referral center. This makes a checklist of the information so that nothing is left out. Pictures can then be placed in the document and emailed directly to the specialist. The RDVM can then easily file this information for the future if the client chooses to use them for a procedure. It’s important to remember that many things can still be conveyed with a simple phone call between the two parties. This can include information about the personality of both the client and the animal. This helps prepare the referral center for the case and dealing with the expectations of the client. The referral center/specialist needs to act in a reasonable and expedient manner when they receive request for consults. This not only benefits the practitioner in their ability to help the patient but also builds loyalty between the RDVM and specialist. The specialist should not hesitate to call the referring doctor to speak to them personally if further information is needed about the case. The BCVD should also consider having an online portal or form letter for the RDVM to fill out and add pictures (Fig. 1). It is also a good idea to have some sort of storage modality that the specialist can organize and store the referral information. This way, if it is a significant amount of time from initial discussion to when the client schedules the procedure, they will still have the information in an organized manner (Fig. 2).

If the BCVD is not receiving the information in a manner that is conducive to providing an accurate diagnosis, then they should provide images or communicate their expectations to the RDVM so that they can learn from the interaction for future instances. One of the most important points in the relationship for any specialist is to never give the impression that the referring doctor has done or provided sublevel care or information. This is also never to be spoken of or conveyed to the specialist’s staff. This information can get back to either the client or the referring doctor which can often lead to much frustration and the need to apologize or potentially ruin the relationship altogether. There are times, however, when the specialist may need to convey information to the RDVM on how the case was not handled appropriately. This must be done in a professional manner and in a way that is constructive. Veterinarians generally have a great deal of pride in their work and are only trying to do their best. The specialist can use this situation to educate the RDVM on different approaches, which will enhance the relationship rather than create tension. The final important point is that the specialist should not attempt to get the client to switch to using their services exclusively. This is accomplished by providing the client with the feeling that the referring doctor is part of the decision-making process and has an important role in the aftercare of the patient.

3. Discussion

The process of referring a case to a specialist should be smooth and painless. Both parties must place
effort in the information exchange so that the patient and client can have a successful outcome. This can be a mutual relationship that will reap considerable benefits for the client and patient. The close relationship between referring veterinarian and specialist must always be protected and honored. Clients can at times may promote dissension by miscommunicating what was said by one or the other veterinarian. This must be quelled immediately and may require a phone call between the veterinarians. If all goes well, the referring veterinarian can get their client help that they cannot easily provide and also retain them as a client. The specialist should see the referring veterinarian as an extension and a part of their practice. This way, the RDVM feels as though they are part of the care and will be supported by the specialist.

4. Conclusion

The referral process can be a tenuous one if all of the necessary information is not conveyed by the referring doctor. Sending radiographs with no history or clinical findings is not appropriate. This leads to specialist support staff spending countless time trying to email and call the referring doctors for further information. This causes loss of valuable time to the client and patient. The specialist needs to also provide timely information back to the referring doctor so that the client can make appropriate decisions.

Acknowledgments

Declaration of Ethics

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Conflict of Interest

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References

1. Introduction

The diagnosis and treatment of sinus pathologies remain a challenge mainly due to their complex anatomy and the diagnostic limitations of traditional imaging techniques.1,2 Once drainage is identified at the nasomaxillary opening during upper airway endoscopy (50–100% of the cases), radiography and dental examination are commonly the next diagnostic steps used by equine practitioners to diagnose sinus pathology.1,2 Unfortunately, a definitive diagnosis is only reached in 40% of affected cases with radiography, and dental examinations are often unrewarding to identify apical pathology.1–3 Thus, required techniques to improve this diagnostic rate tend to be either expensive and/or unavailable (computed tomography [CT]) or invasive (traditional sinoscopy is performed through a 10-15-mm trephination) for most practitioners. The main objective of the study was to hypothesize that MIST would be simple to perform and that at least 2 approaches would be required to obtain a thorough evaluation of the sinuses.

2. Materials and Methods

To perform the MIST, a flexible and disposable 2-mm-diameter and 18-cm-long endoscope was designed in collaboration with a veterinary endoscopy company (Fig. 1). This endoscope is longer and flexible when compared to the traditional needle endoscope commonly used to perform standing arthroscopy in horses but is compatible with the same arthroscopic console. The endoscope lacks a control knob, a flush system, or an instrument channel; however, its price is significantly lower than a specialized 2-mm endoscope with the aforementioned features. In order to identify the ideal landmarks to perform MIST and to determine the thoroughness of sinus evaluation, cadaveric heads from horses older than 5 years old and of various common breeds were used. Then, the technique was carried out in healthy horses to determine the suitability of the technique in live horses and validate it. Last, MIST was performed in selected clinical patients either after primary recommendation by the attending clinician or when CT examination was not a diagnostic option for the owner.

Sinoscopic Landmarks

The traditional landmarks to perform sinoscopy/trephination were slightly modified (Fig. 2) to be able
to obtain a thorough sinus evaluation with the 2-mm endoscope.\textsuperscript{4-6}

1. To access the frontal sinus, the mini-sinusotomy was performed at 40\% of the distance from the medial canthus of the eye to midline and 2 cm caudal to the rostral aspect of the rostral lacrimal tubercle, with the needle inserted perpendicular to the bone. The rostral lacrimal tubercle is a bony prominence palpable just rostral and slightly dorsomedial to the medial canthus and serves as the insertion point for the orbicular muscle.

2. To access the caudal maxillary sinus, the mini-sinusotomy was performed 1 cm rostral and 3 cm ventral to the rostral aspect of the rostral lacrimal tubercle, with the needle inserted perpendicular to the bone.

3. To access the rostral maxillary sinus, the mini-sinusotomy should be performed 40\% of the distance from the rostral end of the facial crest to the level of the medial canthus and 1 cm ventral to a line joining the infraorbital foramen and the medial canthus, with the needle directed slightly upwards (approximately 30 degrees). This needle orientation facilitates advancement of the scope over the infraorbital canal.

MIST

The following structures were evaluated during the MIST (Fig. 3):

- From the frontal sinus approach (FS): ethmoid, caudal recess of the frontal sinus (FS “cul-de-sac”), caudo-medial aspect of the dorsal conchal sinus, fronto-maxillary opening, maxillary septal bulla (MSB), caudal aspect of the caudal maxillary sinus, infraorbital canal, entrance to the sphenopalatine sinus, and roots of maxillary teeth 110/210 and 111/211.

- From the caudal maxillary sinus approach (CMS): ethmoid, fronto-maxillary opening, MSB, infraorbital canal, entrance to the
sphenopalatine sinus, caudal aspect of the caudal maxillary sinus, and roots of maxillary teeth 110/210 and 111/211.

- From the rostral maxillary sinus approach (RMS): roots of maxillary teeth 108/208, 109/209, and 110/210, infraorbital canal, MSB, and ventral conchal sinus (VCS).

A grading score was assigned for each evaluated structure: 3 for complete visualization, 2 for subcomplete (approximately 51-99% of the structure visualized), 1 for partial or limited (approximately 1-50% of the structure visualized), and 0 for no visualization. Only during this cadaveric phase, and after enlarging the frontal mini-sinusotomy with a 5-mm Steinmann pin, the MSB was fenestrated under endoscopic guidance with a 14-G catheter trocar. The fenestration was made at the level of the infraorbital canal (medial to lateral direction) and as dorsal and rostral as possible in the segment of the MSB protruding through the fronto-maxillary opening. The flexible endoscope was then inserted through the newly created opening, and the RMS and VCS were evaluated.

**MIST in Healthy Horses**

1. Horses were premedicated with procaine penicillin (22 000 UI/kg IM) and phenylbutazone (2.2 mg/kg IV), sedated with detomidine (0.01 mg/kg IV) and butorphanol (0.01 mg/kg IV), and restrained in standing stocks.

2. The region overlying the paranasal sinuses was clipped and aseptically prepared. Skin and subcutis were anesthetized with 1 mL of lidocaine hydrochloride 2% at the selected landmarks (Fig. 1).

3. A 3-mm stab incision was performed with a number-11 scalpel blade through the skin and soft tissues until contacting bone. Next, a mini-sinusotomy/trephination was created by advancing a 3.8-cm-long, 14-G needle (2.1-mm diameter) through the frontal (FS approach), zygomatic, or lacrimal (CMS approach) and maxillary bones (RMS approach) with the aid of a mallet.

4. The needle was then removed with circular movements to allow enlargement of the mini-sinusotomy and thereby facilitate entry of the 2-mm flexible endoscope.

5. Sinoscopic evaluation through the FS, CMS, and RMS approaches was then carried out in each horse (Fig. 4).

6. After sinoscopy, the sinuses were flushed with approximately 500 mL of isotonic solution to prevent secondary infection, and an adhesive bandage was placed around the head for 2 days. The skin incisions were not closed.

7. The endoscope was only wiped with alcohol before each procedure.

Procedural time started at the time of the first incision and ended after sinuses were lavaged. Any intraoperative or postoperative complications were recorded. A Wilcoxon-signed rank test (Prism, GraphPad Software, San Diego, CA) was performed to determine the best approach to examine the CMS (FS versus CMS). A p value of less than 0.05 was considered statistically significant. For clinical patients, the procedure was carried out as aforementioned, although preprocedural antimicrobials and postprocedural sinus lavage were not used. In addition, the endoscope was cleaned and sterilized with cold sterilization before each procedure due to the higher risk of bacterial contamination during the procedure in clinical cases with sinusitis.

**3. Results**

**Cadaveric Specimens**

Breeds included during the cadaveric phase (10 horses) were 4 Standardbreds, 3 Quarter Horses, 1 Percheron, 1 Hanoverian, and 1 Welsh pony. A variety of breeds were included to be able to validate the technique in horses of most sizes. In cadaveric specimens (mean age of 12 years old and range of 6-20 years old), most evaluated structures (9/11 for FS and 8/8 CMS) received a median score of 3 (range 2-3) through the FS and CMS, which indicated a thorough sinus evaluation during these approaches. A median score of 2 (range 0-3 for both) was obtained for the cranio-medial aspect of the dorsal conchal sinus and the caudal cul-de-sac of the frontal sinus. As it occurs with traditional sinoscopy, manipulation of the endoscope within the RMS was limited, and scores obtained were lower (median and range of 3 and 0-3, respectively). Five out of the six structures evaluated had a median score of at least 2.5 (range 2.5-3); however, the median score for the visualization of the caudal tooth roots of 08s was 0 (range 0-3). Fenestration of the MSB to improve visualization of the VCS and RMS was attempted during the cadaveric phase. Unfortunately, the RMS and/or VCS was completely visible in only 3
specimens, and MSB perforation was abandoned in subsequent phases of the study.6

Healthy Horses

Results obtained from 6 medium-size healthy horses (mean age of 15 years old and range of 13-18 years old) were similar to the cadaveric phase for the FS and CMS approaches. Additionally, both study phases revealed that there was no difference for the visualization of the structures within the CMS when the FS and CMS approaches were compared (p = 0.32). Thus, the CMS approach was considered as optional for clinical cases. With regard to the RMS approach, a score of 3/3 was obtained for all structures, which significantly improved the results obtained in the cadaveric phase for the tooth roots of 08s. Thus, the technique was considered to be validated for its use on live horses. The procedure took 34.2 ± 5.8 minutes (all 3 approaches) to be performed, and all horses tolerated the procedure well. Two to three gentle taps of the mallet were generally enough to go through the bone with the 14-G needle to perform the 2.1-mm mini-sinusotomy. Fogging or darkening of the field of view was frequently encountered upon endoscope introduction on live horses. Nonetheless, this quickly resolved by either contacting an adjacent mucosal surface or by cleaning the endoscope tip with a wet gauze before reintroducing the endoscope within the sinuses. No personnel or equipment damage occurred. No intraoperative complications were noted during the procedures other than mild petechiation in the RMS mucosa in 3/6 horses. This was associated to contact of the endoscope with the sinus mucosa while cleaning the endoscopic lens. All horses developed mild (2-cm diameter or less) subcutaneous emphysema at the level of the mini-sinusotomies within a few hours of the procedure that lasted between 12 and 24 hours. In one horse, moderate emphysema (4-cm diameter around the RMS mini-sinusotomy) extended rostrally and ventrally after MIST and persisted for 72 hours. All incisions were macroscopically healed upon bandage removal 48 hours after the procedure, and the cosmetic appearance was considered excellent for all horses but one. This horse developed local thickening (1.5 cm × 1.5 cm) at the FS mini-sinusotomy, which resolved after 2 weeks. No signs of sinus or incisional infection were noted postoperatively.

Clinical Cases

To date, the MIST has been used in 8 clinical cases since its introduction to the clinic (Fig. 5 and Table 1). The technique has been considered useful for case management (confirmed lack of sinus involvement [2/8], obtained a definitive diagnosis [2/8], or guided surgical approach [3/8]) in 7/8 cases. In one case, severe and diffuse pus presence in all sinus compartments prevented appropriate sinus visualization with the MIST despite sinus lavage was attempted. However, the technique partially helped to decide the surgical approach (maxillary sinus flap) in this case. Regarding the MIST approaches performed in clinical cases, 5 horses received the FS and RMS approaches, while 3 horses received the FS approach only. No intra- or post-MIST complications were encountered other than mild self-limiting subcutaneous emphysema in at least 2 cases. Unfortunately, the self-limiting nature of the emphysema and the retrospective nature of the study prevented obtaining more information in this regard. In one horse presenting fluid accumulation in the rostral sinuses based on radiography, the FS mini-sinusotomy was enlarged with a 7-mm Steinman pin to allow guided perforation of the MSB while visualizing the area with the 2-mm endoscope. This approach allowed inspissated pus removal and lavage of the rostral sinus compartments with a sinusotomy half the size of a traditional trephination. Telephone follow-up information revealed neither recurrence from the original sinusal problem nor presence of complications associated to MIST or treatment in 7/8 cases (5/8 horses are alive and 2/8 were euthanized due to a severe colic episode months after MIST). Euthanasia during hospitalization due to poor progression was carried out in the case with the comminuted mandibular fracture.

4. Discussion

The MIST allowed thorough paranasal sinus evaluation in horses thanks to a novel 2-mm flexible endoscope introduced through a mini-sinusotomy performed with a 14-G needle (2.1 mm) in standing sedated horses. The mini-sinusotomy itself is easy to perform and does not require special expertise or instrumentation. In fact, this technique is routinely used in the author’s institution for sinus sampling or lavages. In contrast, appropriate knowledge of sinus anatomy is necessary to perform and interpret the sinoscopic images obtained with MIST. Sinus anatomy is complex, but recent open-access literature (Anatomy and Diagnostic Imaging of the Equine Paranasal Sinuses, Educational Resources, University of Georgia) and performance of anatomic dissections can greatly facilitate practitioners’ self-learning. Small-diameter flexible endoscopes with a
Table 1. Information from the Clinical Patients Where the Minimally Invasive Sinoscopic Technique (MIST) Was Performed

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Breed</th>
<th>Presenting Complaint</th>
<th>Diagnostics Performed</th>
<th>MIST Approach and Findings</th>
<th>Usefulness of MIST</th>
<th>Final Diagnosis</th>
<th>Treatment</th>
<th>Follow-up (time)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. 18</td>
<td>Quarter Horse</td>
<td>Right CMND</td>
<td>R, DE, NE</td>
<td>FS and RMS, Diffuse pus in CMS and unidentified structured covered by pus</td>
<td>Yes, guided surgical approach</td>
<td>Sinus bony sequestrum in the CMS</td>
<td>Maxillary sinus flap: sequestrum removal and sinus lavage</td>
<td>No complications or recurrence (32 months)</td>
</tr>
<tr>
<td>2. 8</td>
<td>Thoroughbred</td>
<td>Left CMND</td>
<td>R, DE, NE</td>
<td>FS and RMS, Pus in CMS</td>
<td>Yes, guided surgical approach</td>
<td>Chronic CMS sinusitis</td>
<td>Maxillary sinus flap: pus removal and sinus lavage</td>
<td>No complications or recurrence EUR (8 months)</td>
</tr>
<tr>
<td>3. 8</td>
<td>Warmblood</td>
<td>Right CMND</td>
<td>R, DE, NE</td>
<td>FS and RMS, CMS hematoma and pus in RMS</td>
<td>Yes, final diagnosis</td>
<td>CMS hematoma, RMS sinusitis and nasal mycosis</td>
<td>FS and RMS trephination: hematoma and pus removal, sinus lavage and nasal lavage/debridement</td>
<td>No complications or recurrence (34 months)</td>
</tr>
<tr>
<td>4. 11</td>
<td>Pony</td>
<td>Left nasal masses</td>
<td>R, DE, NE, CT</td>
<td>FS and RMS, No abnormalities (performed before CT)</td>
<td>Yes, ruled out sinus pathology</td>
<td>Nasal osteomyelitis</td>
<td>Mass removal through rhinotomy</td>
<td>No complications or recurrence (13 months)</td>
</tr>
<tr>
<td>5. 4</td>
<td>Appendix</td>
<td>Right CMND</td>
<td>R, DE, NE</td>
<td>FS, Maxillary sinus cyst</td>
<td>Yes, final diagnosis</td>
<td>Maxillary sinus cyst</td>
<td>FS trephination: cyst removal</td>
<td>No complications or recurrence (30 months)</td>
</tr>
<tr>
<td>6. 16</td>
<td>Quarter Horse</td>
<td>Left CMND</td>
<td>R, DE, NE</td>
<td>FS, Pus within FS and CMS</td>
<td>No, pus obscured visualization</td>
<td>CMS hematoma and secondary sinusitis</td>
<td>Maxillary sinus flap: hematoma and pus removal, sinus lavage</td>
<td>No complications or recurrence EUR (30 months)</td>
</tr>
<tr>
<td>7. 18</td>
<td>Canadian</td>
<td>Severe left periocular swelling</td>
<td>R, DE, NE</td>
<td>FS, No abnormalities</td>
<td>Yes, ruled out sinus pathology</td>
<td>Committed mandibular fracture</td>
<td>Conservative treatment</td>
<td>Euthanasia during hospitalization</td>
</tr>
<tr>
<td>8. 3</td>
<td>Quarter Horse</td>
<td>Left CMND</td>
<td>R, DE, NE</td>
<td>FS and RMS, Pus in RMS</td>
<td>Yes, guided surgical approach</td>
<td>Chronic RMS sinusitis</td>
<td>FS trephination: pus removal and sinus lavage</td>
<td>No complications or recurrence (9 months)</td>
</tr>
</tbody>
</table>

Abbreviations: CMND, chronic malodorous nasal discharge; R, radiography; DE, dental examination; NE, nasal endoscopy; CT, computed tomography; FS, frontal sinus; RMS, rostral maxillary sinus; CMS, caudal maxillary sinus; EUR, euthanized for unrelated reasons.
control knob, flush port, and instrument channel are currently available on the market. However, they are cost-prohibitive and prone to damage.\textsuperscript{7,8} Thus, The author's institution used a 2-mm endoscope that is affordable (approximately $600) and reusable. To date, the same endoscope has been used for all clinical cases without being damaged, although it was anticipated that they have a limited life span, and can be damaged if used forcefully. With regard to sterilization, sinoscopy is not a clean procedure due to the bacterial population normally present in the sinuses, and as such, endoscope sterilization may not be required. However, it is likely recommended to sterilize the endoscope with cold sterilization\textsuperscript{b} between procedures as most clinical cases where the endoscope will be used will present active infection. The reported landmarks should be respected to allow adequate manipulation of the endoscope within the sinuses and thereby exhaustive sinus evaluation. These modified landmarks were the result of information gathered during the cadaveric phase of the study and highlighted that precision is required when performing a smaller sinusotomy (2.1 vs. 10 mm) for sinoscopy. The flexible endoscope was easy to manipulate and allowed a rapid evaluation (approximately 30 minutes) of the paranasal sinus without significant complications. To facilitate introduction and manipulation of the endoscope within the sinuses, it is crucial to perform circular movements with the 14-G needle once the mini-sinusotomy has been performed, with the goal of enlarging it. It is recommended to use two hands to manipulate the endoscope. A hand close to the mini-sinusotomy portal is used to introduce or withdraw the endoscope, while the other hand is used by the camera piece to allow 360° rotation of the endoscope, which is needed to thoroughly explore the sinuses. It is also recommended to perform all the mini-sinusotomies first and then carry out the MIST through each portal. This approach will promote clotting at the sinusotomy sites and therefore minimize sinus bleeding, which may obscure the field of view during sinoscopy. Bleeding was not a problem in healthy horses or clinical patients even though the latter presented mucosal inflammation secondary to the pre-existing sinus pathology. As previously mentioned, there were no visualization differences between the CMS and the FS approach to explore the CMS in healthy horses. Thus, the CMS approach was not performed in clinical cases and should be reserved for cases where the FS approach does not allow thorough evaluation of the CMS (i.e., when there is a very prominent bulla of the MSB that reduces visualization of teeth 10 and 11). In fact, the FS approach allows a look into the CMS with perspective, which is beneficial in horses where the sinus is partly filled with pus. In contrast, the minimal invasiveness of this technique and the lack of a control knob in the 2-mm flexible endoscope does not allow routine perforation of the MSB to look with perspective into the RMS and the CVS. This is a limitation of the technique that was bypassed in healthy horses with the RMS approach. It was believed that the bone of live horses was softer than in cadavers and that this allowed further enlargement of the mini-sinusotomy and therefore better visualization of the roots of teeth 08. However, the experience with clinical cases has revealed that visualization of the small rostral sinus compartments in cases where there is significant pus accumulation could be challenging. In those cases, performing a sinus lavage through a 14-G needle to reduce the sinus contents before attempting MIST could be recommended, although it may be unsuccessful in cases with inspissated pus. Thus, selected cases may benefit from enlarging the FS sinusotomy and perforating the bulla at that stage as it was carried out for horse 8. Last, only performing the FS approach may be deemed required. This was the case in 3 clinical cases where the FS approach was enough to obtain a definitive diagnosis (1), to guide treatment (1), or to rule out caudal sinus pathology (1). No major complications were found during MIST, hospitalization, or follow-up. Complications such as wound infection, bone sequestrum, or suture extrusion, which are commonly associated with trephination for traditional sinoscopy, were not found and are unlikely to occur considering the reduced invasiveness of this technique.\textsuperscript{9} Postprocedural emphysema was absent or minimal in clinical cases when compared to healthy horses. This is thought to be associated to the mucosal inflammation present in the sinuses of affected cases, which may have sealed the mini-sinusotomy hole after the procedure and prevented air from leaking into the subcutis. Regardless, emphysema is transient and resolves within days if present. Gentle needle insertion (gentle mallet taps during insertion) while performing the mini-sinusotomy of the RMS is strongly advised. This will prevent iatrogenic damage to the infraorbital canal as it is located close to the maxillary bone at this level. The MIST was conceived as a diagnostic alternative to CT and traditional sinoscopy that could allow popularization of sinoscopy among practitioners due to its simplicity and reduce invasiveness and morbidity. The author believes that with minimal training, practitioners may become proficient in the technique. Nevertheless, CT should still be considered as the gold standard to diagnose sinus pathology, and traditional sinoscopy can be used for diagnostic and therapeutic purposes, while MIST is mainly conceived for diagnostic purposes. The reported technique should be especially useful for practitioners without access to CT, those working in an ambulatory setting, and/or those looking for a simpler sinoscopic technique. Furthermore, the technique has shown value as a screening technique. It ruled out sinus involvement in two cases where sinus affection by involvement was still considered possible after initial work-up and basic imaging (radiography and nasal endoscopy). In the institution where the study was performed (secondary referral hospital), the number of cases receiving MIST...
is increasing; however, it is worth mentioning that many horses presented for sinus disease still go directly for CT due to its superior diagnostic rate, the availability at the institution, and the chronicity and complexity of most of the cases admitted. This should not necessarily be the case for most equine practitioners or primary hospitals. The technique presents several limitations. First, the technique requires a practice investment to purchase the endoscopic console, although practices performing standing arthroscopy may already have it. Second, the flexible endoscope lacks a control knob and a flush system, although this can be bypassed by manually guiding the endoscope within the sinus and by directly cleaning the lens with a wet gauze or by contacting sinus mucosa when fogging is identified. Fogging was not a problem in clinical cases. Third, it requires some degree of training and a good knowledge of sinus anatomy. However, the learning curve is not steep. Last, more clinical cases are needed to determine the diagnostic capabilities of the technique for each of the different sinus pathologies. To summarize, MIST is a diagnostic alternative to CT and traditional sinoscopy that can be used in addition to traditional imaging. MIST offers the advantage of being portable, involves a less invasive approach, and is performed rapidly and with a lower cost to clients, although specialized equipment is required. Additionally, MIST could rule out sinus involvement or assist treatment by providing information regarding the ideal location for treatment portals (trephine or flap).

Acknowledgments

BioVision donated equipment for the development of the technique. The study was funded by the Equine Health Funds supported by Zoetis and the Centennial Funds of the College of Veterinary Medicine of the University of Montreal.

Declaration of Ethics

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

Conflict of Interest

The Author has no conflicts of interest.

References and Footnotes


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How to Identify and Extract Blind Wolf Teeth

Ashton Broman, DVM

1. Introduction

The first premolar (Triadan -05), otherwise referred to as a wolf tooth, is a vestigial tooth not typically associated with mastication. Traditionally, wolf teeth have been extracted, although the necessity of extraction has been debated. While wolf teeth are often positioned directly rostral to the second premolar (Triadan -06), they can be found anywhere along the bar (Fig. 1). Occasionally, a wolf tooth will develop in a more mesially (rostrally) tipped or horizontal plane, as opposed to the normal vertical orientation, within the alveolus, resulting in a blind or nonerupted wolf tooth. These can also be palpated anywhere along the bars; care should be taken in mares not to mistake nonerupted canines for blind wolf teeth. Depending on a horse’s use, blind wolf teeth can be associated with biting problems and can lead to head tossing, resistance to pressure with the bit, and general discomfort when being ridden. In these instances, the nonerupted wolf teeth require extraction, which can be difficult due to lack of visualization, abnormal positioning in the alveolus, and amount of tissue overlaying the tooth. At Rood and Riddle Equine Hospital over a 5-year period (2015–2020), wolf tooth extractions were performed on 1185 horses, and of those, 28 (2.4%) had blind wolf teeth. These horses were identified from a complete oral exam on a horse with only one visible wolf tooth or a history of biting problems. For those horses with reported biting problems who were later found to have a blind wolf tooth at the point of bit contact, extraction of the nonerupted tooth resolved the clinical signs.

2. Materials and Methods

It is important to obtain a detailed history from the client. The patient should be well sedated—the author uses detomidine hydrochloride (0.01–0.02 mg/kg IV)—and well restrained in stocks or in a stall. Once the patient is sedated, a dental speculum should be placed to maintain the safety of the practitioner and allow for proper visualization of the entire oral cavity. Using an appropriate light source, perform a complete oral exam including a visual inspection of the oral cavity and also manual palpation of the bars. This is especially important in horses with a history of biting problems to differentiate causes of biting pain such as a blind wolf tooth versus osteitis or bruising along the bar. If performing a routine wolf tooth extraction and only visualizing one erupted wolf tooth, always palpate along the opposing bar to determine the presence of a blind wolf tooth. Once a blind wolf tooth has been identified on palpation, it is important to obtain radiographs to show the positioning of the tooth within the alveolus. As stated above, these wolf teeth often are nonerupted due to a more
horizontal position within the alveolus. Analyzing the position of the tooth along the bar and within the alveolus via radiographs is important for planning the placement of the wolf tooth elevators (Fig. 2).

Extraction Supplies (Fig. 3)

- 2% lidocaine
- 10cc syringe (regular or luer lock tip)
- 21ga butterfly catheter
- #10 scalpel blade
- Wolf tooth elevators (the author uses long handled half circle offset wolf tooth elevators. The offset angle of the elevators corresponds to the width of the maxilla, allowing for a more direct approach to the wolf tooth.)

Extraction Procedure

1. Palpate the location of the blind wolf tooth.
2. Using a 10cc syringe with 2% lidocaine and an attached 21ga butterfly catheter, perform a local block of the mucosa directly adjacent to the blind wolf tooth, forming a small bleb with 2 to 3 mL of 2% lidocaine (Fig. 4). Infuse an additional 2 to 3 mL local anesthetic along the periosteum on the buccal aspect (Fig. 5).
3. Holding the scalpel in a bumper press method to control the depth of the incision, make a stab incision through the mucosa directly over the rostral aspect of the blind wolf tooth, inserting until contacting the tooth (Fig. 6).

Fig. 1. The 105 (black arrow) can be seen rostral to the 106. There is a swelling of tissue along the bar rostral to the 206 that corresponds to a nonerupted or blind 205 (yellow circle).

Fig. 2. A nonerupted 205 (yellow circle) can be seen rostral to the 206. It is mesially tipped, positioned in a horizontal plane along the bar.

Fig. 3. Extraction equipment from top to bottom and left to right: 2% lidocaine, 10cc syringe, 21ga butterfly catheter, #10 scalpel blade, long handled half circle offset wolf tooth elevators.

Fig. 4. Perform a local block of the mucosa adjacent to the rostral aspect of the blind wolf tooth forming a small bleb.
4. Place the wolf tooth elevator at the rostral and dorsal aspect between the tooth and alveolar bone at the appropriate angle as seen on the radiographs (Fig. 7). Applying steady gentle pressure distally, move the elevators in a dorsal to buccal arcing motion until the tooth is mobile. Once the tooth is sufficiently mobile, move the elevator in a larger arc from the buccal to the mesial aspect of the tooth to luxate the tooth out of the alveolus and through the stab incision. If the tooth is too large, the incision can be extended slightly to allow the tooth to be removed.

3. Discussion

Blind wolf teeth can cause extreme discomfort in the bitted performance horse and frustration for owners and trainers. Knowledge about the proper approach to identifying and extracting these teeth can give the general equine practitioner the tools necessary to address these problem horses. While radiographs are not required to manage these cases, they are highly recommended, especially when performing these extractions for the first time. Utilizing dental radiographs for extraction planning can reduce the risk of complications and allow the practitioner to be more efficient when performing this procedure. The above extraction procedure details the process for extracting blind maxillary wolf teeth as these are more common. The procedure is the same to extract a blind mandibular wolf tooth, manipulating the wolf tooth extractors from the ventral aspect of the tooth in a buccal to lingual motion. As with all extractions, there are risks of complications including fractured roots, laceration of the greater palatine artery, laceration of the mucosa, and fracture of alveolar bone. The lack of visualization and abnormal position of the tooth inherently make these extractions more complicated. Fracture of the tooth can occur if excessive force is applied to the tooth prior to appropriate loosening of the periodontal ligament or in mature horses in which ankylosis of the root is more common. If the root fragment is below the gingival margin and cannot be palpated, the fragment can often remain without complication. If the fragment extends beyond the alveolar bone, it can irritate soft tissues and cause even more discomfort when bitted, so all care should be taken to extract the remaining fragment. Laceration of the greater palatine artery can occur when extracting wolf teeth, especially when displaced palatally. It is important to maintain control of the tip of the elevator to prevent slippage off the palatal aspect of the bone or inadvertently if the patient moves during the extraction. This can be avoided by placing the elevator along the distal or buccal margins of the tooth and only moving the elevator in an arc from the buccal to the mesial aspects of the tooth until the tooth is appropriately loosened. The incision made through the mucosa should only be large.
enough to allow the tooth to luxate out of the alveolus. If the incision is larger than desired or the elevator has slipped during the procedure and lacerated through the mucosa, sutures can be placed across the incision to prevent excessive feed contamination of the extraction site. Lastly, after extraction, palpate the margin of the alveolar bone to ensure there are no loose pieces of bone that could result in a sequestrum and be sure to smooth any rough bony edges to prevent soft tissue irritation or pain post-operatively. Post-operative care is minimal; a nonsteroidal anti-inflammatory can be given for 2 to 3 days, and refrain from placing a bit in the mouth for 3 to 5 days to allow for healing. Antibiotics are not warranted unless a secondary complication arises.

4. Summary

Extraction of blind wolf teeth can be performed successfully in a farm setting on a standing patient with proper chemical and physical restraint, local analgesia, and appropriate extraction equipment.

Acknowledgments

Declaration of Ethics
The Author has adhered to the Principle of Veterinary Medical Ethics of the AVMA.

Conflict of Interest
The Author has no conflicts of interest.

References and Footnote

"Veterinary Dental Products, LLC., Elmwood, WI 54740."
Intravenous and Intramuscular Butorphanol Pharmacokinetics in Donkeys

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Butorphanol, as administered with equine dosing, does not appear to achieve similar plasma concentrations and pharmacokinetics in donkeys. Authors’ addresses: Lincoln Memorial University, College of Veterinary Medicine, Harrogate, TN 37752 (Ebner); SAGE Veterinary Centers, 7121 Amador Plaza Road, Dublin, CA 94568 (O); Department of Small Animal Clinical Sciences, College of Veterinary Medicine and Biomedical Sciences, Texas A&M University, College Station, TX 77843 (Simon); Department of Large Animal Clinical Sciences (Smith) and Department of Biomedical and Diagnostic Sciences (Cox), College of Veterinary Medicine, University of Tennessee, Knoxville, TN 37996; Institute of Veterinary, Animal and Biomedical Sciences, Massey University, Palmerston North, New Zealand (Lizarraga); e-mail: lisa.ebner@lmunet.edu. *Corresponding author; †presenting author. © 2021 AAEP.

1. Introduction

This study compared the pharmacokinetic and pharmacodynamic effects of butorphanol after intravenous (IVB) and intramuscular (IMB) administration in donkeys. Healthy donkeys were administered 0.1 mg/kg butorphanol IV or IM in a randomized, crossover design.

2. Materials and Methods

Blood samples, sedation scores, and physiological parameters were obtained at predetermined intervals for up to 24 hours (IVB) or 48 hours (IMB) after administration. Plasma butorphanol concentrations were determined by high-pressure liquid chromatography, and pharmacokinetic parameters were calculated. Sedation was recorded based on a 0-to-3 whole-point sedation score.

3. Results

Following IVB administration, mean (± SD) apparent volume of distribution, elimination half-life, total body clearance, and area under the plasma concentration time curve from time 0 to infinity (AUC0–∞) were 270 ± 92 mL/kg, 0.82 ± 0.37 h, 378 ± 235 mL/hr/kg, and 371 ± 263 h·ng/mL, respectively. After IMB administration, a maximum plasma drug concentration of 447 ± 435 ng/mL was reached at 0.40 ± 0.22 hr. The AUC0–∞ was 453 ± 208 h·ng/mL. The administration of IVB and IMB resulted in no significant sedation but caused a decrease in the amount of borborygmi auscultated.

Research Abstract—for more information, contact the corresponding author

NOTES
shortly after administration (15 minutes to 2 hours). Based on this study, IMB at 0.1 mg/kg is not recommended for sedation purposes in donkeys. Future directions include co-administration with an alpha-2 agonist or higher butorphanol doses.

Acknowledgments

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

Conflict of Interest
The Authors have no conflicts of interest.
Pharmacokinetic Modeling and Distribution of Doxycycline in Healthy Female Donkeys After Multiple Intragastric Dosing

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Doxycycline at 10 mg/kg PO q12h may not achieve therapeutic concentrations in donkeys. Authors’ addresses: Department of Clinical Sciences (Chapuis, French, Peterson, Little), Department of Biomedical Sciences (Toka), Ross University School of Veterinary Medicine, Basseterre, St. Kitts, West Indies; Large Animal Clinical Sciences, College of Veterinary Medicine, University of Tennessee, Knoxville, TN 37996 (Smith); e-mail: elittle@rossvet.edu.kn. *Corresponding author; †presenting author. © 2021 AAEP.

1. Introduction
Donkeys have a different metabolism, and dosage should not be extrapolated from horses. Doxycycline is administered to horses for the treatment of bacterial infections but has never been investigated in donkeys. The aim of this preliminary study was to describe the population pharmacokinetics of doxycycline in donkeys.

2. Materials and Methods
Doxycycline hyclate (10 mg/kg, PO, q12h, 5 doses) was administered to eight non-fasted, healthy, adult jennies. Serum, urine, synovial fluid, and endometrium were collected. Serum doxycycline concentrations were measured with ELISA. Non-linear mixed effects model was used to analyze serum concentration.

3. Results
A one-compartment model with linear elimination and first order absorption best described the available serum pharmacokinetic data. Final parameter estimates suggested that doxycycline has a high volume of distribution (108 L/kg) as well as high absorption (10.3 h⁻¹) in donkeys. However, results suggest that the concentration of doxycycline reached in all fluids and tissues analyzed would not result in therapeutic concentrations.
concentrations compared to the minimum inhibitory concentration (MIC) of common equine pathogens.

4. Discussion
This study indicates that doxycycline dosed at 10 mg/kg PO q12h in donkeys results in lower blood and tissue concentrations than reported in horses. As a result, concentrations effective for treatment of equine pathogenic bacteria may not be reached in the donkey. Further studies are necessary to confirm the findings and find a therapeutic and safe dosage regimen of oral doxycycline in donkeys.

Acknowledgments

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

Conflict of Interest
The Authors have no conflicts of interest.
How to Equip and Deploy a Regional, Integrated Veterinary Disaster Response Trailer

Claudia Sonder, DVM*; Wallace Liberman, DVM; Gary Hanes, DVM; and Chelsea Damon, CVT

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1. Introduction
Planning for the animal component of disaster response is federally mandated by the Pets Evacuation and Transportation Standards (PETS) Act passed in 2006. The PETS Act was written to address companion animals, and historically, standardized planning for equines has been poorly detailed in the literature. The size and scope of federally declared disaster events in California within the last 5 years have prompted enhanced planning for mass evacuation and sheltering of animals. Veterinary integration with local emergency operation centers and creation of memorandums of understanding (MOUs) for activation and deployment are an essential component to an integrated veterinary response. The objective of this paper is to describe a template for creation and deployment of a regional veterinary disaster response trailer.

2. Materials and Methods
The Northern California Association of Equine Practitioners (NCAEP) acquired a veterinary disaster response trailer in 2017 after the California Wine Country Complex Fires prompted the evacuation of over 1000 horses into local shelters. Access to an empty cargo trailer and donation of veterinary supplies by NCAEP educational partners facilitated a veterinary hub for member veterinarians to volunteer time and services to the sheltered equines. The success of that collaborative response prompted the NCAEP leadership to seek donation of a disaster response trailer for future deployments. Donors were solicited to procure an 18-ft-long × 8-ft-wide × 10-ft-tall bumper pull cargo trailer. The cost of the trailer was $16,000, which included approximately $4,000 worth of electrical components, shelving, strapping, and detailing upgrades to promote mobility and organization of supplies (Fig. 1). A roadmap to a functional, integrated disaster response trailer starts with establishing key relationships and volunteer protections in advance of disaster season. Key relationships include the following:

- Integration with local emergency operations center. In most cases, the animal component of response is handled by the local animal control...
office or county animal shelter. This is a good place to start with a call to investigate local support for an organized plan for horses and livestock. Counties may require incident command system (ICS) training and a swear-in process to certify volunteers as disaster service workers (DSWs). DSW certification ensures volunteers can receive worker’s compensation if injured while volunteering. If deployed, DSWs can request supplies through a formal ICS 213 request routed through animal services. In a federally declared disaster, the county can be reimbursed for these supplies. An MOU can be drafted to clearly define the roles and responsibilities of the county and the veterinary responders.4

• Integration with state veterinary medical reserve corps (VMRC). Each state will likely have a VMRC, and response veterinarians who wish to deliver mutual aid to surrounding counties can comply with required training that usually includes ICS training, response training, and volunteer orientation. VMRCs are activated when the county needs exceed local response capacity and mutual aid is requested at the state level.

• An organized notification system for veterinary volunteers and a central hub for volunteer information and training. Volunteers can complete a membership survey with contact information, species familiarity, and license information and be added to a group call-out system that provides incident information and access to volunteer shift sign ups. Ideally, this group can gather to train and attend continuing education relevant to disaster response.

• Establishment of 501(c)(3) status is valuable to garner donor support for the acquisition of a trailer, equipment, and supplies. Alternatively, a response group can request fiscal oversight from a local Veterinary Medical Association or equine 501(c)(3). Cost to establish nonprofit status is variable and can take several months to secure.

• Industry partner collaborations. Outreach to seek supply donation channels can be key to maintaining a stocked trailer. Early in disasters, access to supplies via ICS 213 requests can take several days to achieve. Industry partners benefit from their involvement in response and can bring stories of medical aid and

Fig. 1. NCAEP disaster response trailer deployed in Butte County for the North Fire in 2020. Treatment board, “add on” board, NCAEP lead veterinarian contact information, shelter map, and COVID protocols are displayed.

Fig. 2. Treatment board (A), add-on board (B), and shelter map (C) are displayed at the trailer to allow attending veterinarians to complete daily treatments and address new issues as they arise.
reunification back to their company outreach professionals. The goodwill generated through disaster support is tremendous. Trailer inventory sheets can be maintained and checked quarterly. Supplies nearing expiration can be donated to volunteer veterinarians serving equine nonprofit organizations.

Additional Considerations for Deployment of a Veterinary Response Trailer in Advance of Deployment Include the Following:

- **Standard operating guidelines (SOGs)**

  It is helpful for veterinary response groups to have a set of standard operating guidelines made available to member veterinarians to review ahead of response. Clear SOGs ensure a common understanding of roles and responsibilities. Various operating procedures should be detailed such as medical record completion, trailer setup and lockup, communications with organizations running the shelter, communications with horse owners, and general treatment protocols. The NCAEP has developed a set of SOGs that are available to veterinarians upon request. These are updated annually based on lessons learned from deployments and volunteer feedback.

- **Liability**

  Veterinarians should sign a volunteer liability waiver annually, which protects the organization deploying the disaster response trailer. They should be sworn in as DSWs upon completion of required training in advance of disaster season or on the day of their deployment. Community agents are deputized to perform legal swear ins, and in the age of COVID-19, they can be performed virtually if locally approved and the appropriate paperwork is completed. Access to incident swear-in opportunities is included in the standard MOU for trailer deployment.

Considerations During Deployment Include the Following:

- **Patient care**

  The recommended ratio of veterinarian/technician pairs to horses in the shelter is 1:50. Equine wildland fire shelter data suggest that approximately 20% of sheltered equines will need veterinary care, which renders approximately 10 patients on the treatment board for every 50 horses in the shelter. Evacuated horse owners are often encumbered with the disaster and cannot provide daily care for their animals. In the initial stages of a disaster, it is helpful to schedule one veterinarian/technician team to inspect incoming animals, in addition to the team performing treatments. Scheduling veterinarians with an experienced equine assistant to restrain animals and assist with treatments creates added efficiency and safety. In many cases, volunteer veterinarians schedule their own technicians to accompany their shifts. Allowing the horses to settle into their enclosure for 30 to 60 minutes prior to examination helps offset the influence of adrenaline on the intake exam and improves overall safety. Horses needing care are examined by veterinarians and are added to the treatment board and medical record system based on their intake number (Fig. 2A). A second board for “add-on” exams is maintained adjacent to the treatment board such that attending shelter care volunteers can report medical issues to the veterinary
gastrointestinal disturbances and colic. If diminished
Shelter conditions create the perfect storm for equine
medical colics, suture lacerations, examine eyes,
for all of the common problems. Equipment to treat
status. NSAIDs are the most prescribed medications
materials necessary to treat these conditions should
shelter. Ample supply of medications and bandage
mon problems encountered in the wildland
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"Veterinary team scheduling"
The NCAEP schedules two volunteer veterinarians
shifts daily spanning 8AM to 3PM and 2PM
to 10 PM, with each veterinarian scheduled with
a treatment technician or experienced han-
der. One hour of overlap allows for information
exchange. Alternatively, responding veterinarians
can cover both shifts if caseloads allow. Shift
demand is often highest in the first week of
ployment and during active evacuation win-
dows. Shelter managers or community animal
response team leaders should be provided with local
emergency contacts for after-hours coverage, which
should be arranged at the event’s outset. At the end
of each deployment day, pictures of treatment boards
should be e-mailed to the operations manager and a
new board created for the following day by the treat-
ment technician. Discussions of staffing and inven-
tory needs should occur daily, with a plan to address
unmet needs. Operations manager is a paid position
within the NCAEP. The treatment technician
should also inspect medical record entries to ensure
completion by shift veterinarians. Ideally, a system
for medical record completion and data collection and
transfer should be established to track response
statistics. This is best accomplished by assigning a
lead veterinarian for a 72-hour shift, with 8 hours of
overlap. This structure facilitates orientation of vol-
unteers and continuity of care. The lead veterin-
ian can be the main point of contact with the agency
overseeing the shelter such as a local community ani-
mal response team or animal control staff. They can
also serve as the primary communicator with clients
to provide updates and document permission to treat.

"Tertiary care relationships"
Collaborations with local referral hospitals are neces-
sary to triage horses with needs exceeding the field
shelter team’s capabilities. Virtual case consults can
be valuable tools to assist decisions in the field and
are especially important when decisions for unclaimed

1. Patient identification

It is crucial that patient ID and not pen numbers are
utilized to identify patients. It is usually the respons-
sibility of the agency overseeing the shelter to assign
and tag the equines with individual numbers that are
unique to that shelter. Pen numbers and letters
should also be unique and not duplicated on-
site. Each animal should have a patient care sheet
attached to the pen maintained by the agency running
the shelter. Patient care sheets should document
owner contact information and horse signalment and
track appetite, water consumption, manure output,
and demeanor at given time points. Failures of
horses to meet expected normal values on these sheets
will trigger requests for veterinary examination to the
“add-on” board (Fig. 2B). Ideally, shelter care vol-
unteers are trained on normal water consumption, ma-
nure output (i.e., 1 pile q3-4h), stance, and mentation.

2. Medical supplies

Recent shelter data gathered by the NCAEP suggest
that wounds and lacerations, gastrointestinal distur-
bances, lameness, and eye problems are the most com-
mon problems encountered in the wildland fire
shelter. Ample supply of medications and bandage
materials necessary to treat these conditions should
be kept on hand and monitored for expiration
status. NSAIDs are the most prescribed medications
for all of the common problems. Equipment to treat
medical colics, suture lacerations, examine eyes,
examine hooves, collect laboratory samples, and per-
form stall side lab analysis enhance care options. A
mechanism to legally label medications and store
them appropriately should be developed.

3. Feeding station

Shelter conditions create the perfect storm for equine
gastrointestinal disturbances and colic. If diminished

Veterinary team scheduling

The NCAEP schedules two volunteer veterinarians
shifts daily spanning 8AM to 3PM and 2PM to 10 PM, with each veterinarian scheduled with
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Tertiary care relationships

Collaborations with local referral hospitals are neces-
sary to triage horses with needs exceeding the field
shelter team’s capabilities. Virtual case consults can
be valuable tools to assist decisions in the field and
are especially important when decisions for unclaimed
horses are made. In all cases, consultation with the agency having jurisdiction over the animals (animal services or other) is a necessary step when care decisions are being made on unclaimed animals.

3. Results
The establishment of the relationships, training, volunteer capacity, and resources to respond to large-scale disasters improves equine welfare and community resilience. Since 2017, the NCAEP trailer has served over 1471 sheltered equines through six significant fires. It has been entirely donor supported and strengthened collaborative relationships with industry partners, member veterinarians, tertiary care facilities, and the horse community. The trailer was deployed for 52 days in 2020, with multiple deployment requests coming in simultaneously during large-scale fires. Additional trailers and response teams are needed to meet the demands during California’s extended fire season and elsewhere in the country where disasters strike.

4. Discussion
The lack of inclusion of horses in the PETS Act has resulted in poor regional community planning and preparedness for sheltering and evacuating equines. Failure to integrate the veterinary component of response into the local emergency action plan results in difficulties organizing veterinary care, procuring supplies, and protecting volunteers with DSW certification. The Wine County wildfires were a good example of what happens when there is no pre-existing plan for treating mass numbers of horses regionally. At that time, the NCAEP had not developed an MOU with the local emergency operations center or the state VMRC, and the lack of volunteer protections jeopardized the organization as a whole from a liability perspective. SOGs were developed (Fig. 3) on the fly, and there was no prior ICS training of veterinarians, which complicated communication and understanding of the chain of control of the horses. The Camp Fire in 2018 presented a similar situation where the state VMRC was deployed to the companion animal shelters, and the NCAEP was asked to support veterinary operations of the sheltered livestock, with the assistance of the University of California, Davis Veterinary Medical Response Team. Although SOGs and veterinary supply chains had been established, there was still no mechanism available to ensure the daily swear in of non-Veterinary Medical Response Team veterinary volunteers. After 3 years of responding to various incidents in multiple counties, the NCAEP has adapted its training, SOGS, and key relationship-building practices to optimize response capabilities in Northern California. Regionally, equine practitioners have benefited from having the ability to request mutual aid through their emergency operations center and deploy colleagues to the shelters to lighten the burden of responsibility. This kind of planning is key to practice continuity, disaster resilience, and equine welfare. The goal of this publication is to share lessons learned and offer a template for the development of regional resilience.

Acknowledgments
The NCAEP wishes to acknowledge its educational partners for their role in acquiring and supplying the NCAEP trailer. The NCAEP acknowledges the West Coast Equine Foundation and the Trailer Specialist Company for their contributions to the trailer purchase.

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

Conflict of Interest
The Authors have leadership roles and are board members of the NCAEP. These are volunteer positions. Chelsea Damon, CVT, is the paid operations manager of the NCAEP. There are no declared conflicts.

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Medical Care of Sheltered Equines During Three Large-Scale Northern California Wildland Fires

Claudia Sonder, DVM*; Lais Costa, MV, MS, PhD, DACVIM, DABVP; Chelsea Damon, CVT; Molly Northrup, MBA; Wallace Liberman, DVM; and Gary Hanes, DVM

It is estimated that 20% of horses sheltered in wildland fires will require some form of veterinary care. The most common issues encountered are wounds and lacerations, gastrointestinal disturbances, lameness, and ocular problems. Responding veterinarians should plan to have a cache of wound care materials and medications to manage expected caseloads. Authors’ addresses: Napa Valley Equine, 3198 Silverado Trail, Napa, CA 94558 (Sonder); University of California, Davis One Health Institute, School of Veterinary Medicine, Davis, CA 95616 (Costa); 6073 South Oak Canyon Drive, Holladay, UT 84121 (Damon); Northrup Consulting, 3650 Mt. Diablo Blvd., Suite 215, Lafayette, CA 94549 (Northrup); Panorama Equine Medical & Surgical Center, 10302 Old Oregon Trail, Redding, CA 96003 (Liberman); Briarwood Equine Clinic Inc., 4370 Alpine Road, Portola Valley, CA 94028 (Hanes); e-mail: csonder@norcalaep.org.

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1. Introduction

The last 5 years in California have suffered the largest wildfires on record for the state, with limited data available regarding equine shelter caseloads. The American Veterinary Medical Association is working to establish core competencies for veterinary disaster certification. This study describes veterinary components of equine wildfire shelter response and compares medical record data across three separate incidents.

2. Materials and Methods

Medical records were collected from the Northern California Association of Equine Practitioners disaster trailer following three wildfire shelter deployments. The total number of equines evacuated and the medical records of horses requiring examinations were tracked, coded, and analyzed.

3. Results

The percentage of sheltered horses receiving medical care was 20%. Wounds and lacerations comprised 34% of cases, gastrointestinal disturbances 26%, lameness 16%, eye 10%, other 9%, and parasites 5% of cases examined. NSAIDS were the most prescribed medications.

4. Discussion

Veterinary response teams should estimate treating 20% of wildfire sheltered equines. Although the
percentages of case types vary between incidents, advance planning for the treatment of wounds and lacerations, gastrointestinal disturbances, lameness, and eye issues is necessary. Burn care of sheltered equines was minimal in this study. Understanding veterinary caseloads relative to disaster types will help to inform future training and disaster certification.

**Acknowledgments**

**Declaration of Ethics**
The Authors have adhered to the Principles of Veterinary Medical Ethics of the AVMA.

**Conflict of Interest**
The Authors have no conflicts of interest.
How to Respond to Equine Trailer Crashes on the Roadside

Rebecca Husted, PhD

As a de-facto member of the emergency response component, it is important for the veterinarian and their staff to know how to safely, efficiently, and effectively function on 911 animal transport crash scenes (Fig. 1). The effectiveness of the practitioner will depend primarily on their ability to appropriately interact with the various types of responders to an equine transport crash. Major safety and security concerns are inherent to all emergency scenes, particularly on trafficked roads and roadsides. Essentially responders are reacting to two scenes: the motor vehicle crash and an equine medical incident where animal(s) are stressed, injured, unpredictable, and generally dangerous. The purpose of this paper is to provide guidelines and best practices to assist the responding equine veterinarian to function in an efficient, effective, and safe manner during equine transport crashes in their role as an advisor to the local emergency response Incident Commander on the roadway or roadside. Methodology and procedures for equine field response, first aid treatment, field euthanasia, and clinical medicine as often required on scene are well covered in other sources, thus will not be addressed in detail here. Author’s address: 1787 Georgia Highway 18 East, Macon, GA 31217; e-mail: delphiacres@hotmail.com. © 2021 AAEP.

1. Introduction

Equines are frequently transported via ground transportation, both domestically and internationally, for pleasure, show, performance, work, veterinary assessment, teaching, and breeding. Crashes with horse trailers occur during all phases of transport—loading, underway, and unloading. Horses are on national road systems in a multitude of transports including “bumper-pull” tag along trailers, “gooseneck” trailers, small and large (semi-trailer) commercial horse transports, boxes, floats, modified trailers, stock trailers, livestock liners, and even home-made horse trailers. Smaller trailers are commonly owned by individuals who are not required to get a Commercial Driver’s License and whose trailers may be subject to incorrect trailer hitching, inadequate maintenance, and lack of driver training (Fig. 2). Even professional commercial haulers have challenges with transport due to impaired driving (i.e., drunk driving, drug-impaired driving, drowsy driving, and distracted driving), human factors, or actions of other drivers—crashes have included fires on board, animals fallen out of transport, overturned trailers, and a variety of collisions. Horses are transported more often than any other type of livestock, since cattle and pigs are normally transported by professional commercial drivers just a few times in their lifetime; in contrast, horses may travel to shows, events, for breeding, racing, pleasure or
trail riding to hundreds or even thousands of separate transported events in their relatively long-life span (15 to 25 working years). Thus, the statistical probability of an individual horse being involved in a crash with a trailer is far greater than individual livestock. There is far more research on crash frequency (415 between 1994–2007), causality (driver error 85%), and outcomes for commercial cattle transport wrecks than for horse crashes. There is minimal published data available on the number of horse trailer crashes that occur in any country, and information on factors associated with horse injuries sustained during transportation in small trucks and trailers is limited. A 2016 study in South Australia surveyed 223 drivers transporting their horses to equestrian events—a total of 55 (24.7%) participants reported transportation-related injuries to their horses. Of these, 72% were described as horse associated (scrambling, slipping, and horse-horse interaction), 11% due to mechanical failure, and 6% due to driver error. There was a modest positive association between increasing trailer age and the number of injuries. The study highlighted the potential for horses to sustain transportation injuries (Fig. 3) and suggested further study to address this risk to horse welfare. Another 2016 Australian study featured an online survey with 797 responses to determine associations between transport management and transport-related injuries and diseases in horses. The survey looked at transport management strategies or procedures (before, during and after transportation) and transport diseases. Modeling explored associations between variables and possible transport-related diseases as outcomes: traumatic injuries, diarrhea, heat stroke, muscular problems, laminitis, transport pneumonia, and colic. Traumatic injuries were the most common transport-related problem, with a reported incidence of 45.0% in this study. The total number of crashes that occur annually are unknown and poorly reported except through local media, anecdotes from practitioners and owners, or documented on social media (e.g., Large Animal Rescue Facebook Study Group with 15,000+ global members). Crashes may be due to a single cause or a combination of causalities, including poor maintenance of the trailer and/or towing vehicle (Fig. 4), inexperienced driver or lack of trailer driving skills, incorrect response to the situation, impaired driving, or exhaustion. Crashworthiness research encompasses new and improved vehicle design, safety...
countermeasures, and equipment to enhance occupant safety, but is nonexistent in livestock and horse trailers in any country. The collective result is that horse transport crashes of various types (overturned, off-road, impacted, floor failure, separated from tow vehicle) are a common source of callouts for fire/rescue response services, and calls to the local equine practitioner. Every crash is different, thus there is no standard operating procedure that can be applied to every crash; however, best practices in equine technical rescue do exist—providing the basic concepts of safest approaches to these crashes.4 “Best practices” in emergency services refers to procedures that produce superior results at a particular type of crash; and constantly evolve based on knowledge and technology available at the time. Responders in field environments must consider many variables: safety (for the human responders and the animals), resources (people, equipment, mechanical, logistics), environment (rain, cold, heat, humidity), medical (stability of the patient, arrival time of medical treatment), and unusual situational concerns such as accessibility, stability, and structural integrity (e.g., Is the overturned trailer down an embankment? In the water? On fire (Fig. 5) or crushed by another vehicle?). Practitioners should involve 911 personnel from the very beginning in these crashes (trailer overturn, horse trapped over or under chest bar, hoof caught in divider, etc.) because it speeds up the overall efficiency of the response.5 Fire/rescue departments have the techniques and expertise to stabilize trailers and provide safety and security; after all, they do extrications of people from on-road vehicle crashes as a matter of course. Very few horse owners or practitioners will have the equipment, training, and expertise to attempt equine extrications safely on their own—it takes teamwork to achieve safety and efficiency for all on scene. In the past, human emergency responders devoted much effort to extricating animals without veterinary assistance. Sadly, minimal attention to the health concerns and status of the horse was paid. Ironically, this is opposite to their intense human medical training where the patient is always stabilized before extrication, with subsequent packaging for transport. Their incorrect assumption was that horses were big and strong—but several hours to days after a seemingly “successful” rescue, untreated equine victims often died “unexpectedly” (Fig. 6). Today, human responders are taught that they are fragile medically and need veterinary attention immediately, and to utilize the “golden hours” concept to remind responders of the steadily deteriorating medical condition (Fig. 7) of affected horses.6 The fundamental challenge on scene is to balance the need to extricate, safety for personnel, and providing medical care to the patient. Numerous fallacies related to equine transport are derived from the lack of scientific rigor available or contributed to inappropriate methods being used by responders on scene. Practitioners are de-facto members of the emergency response team on scene—their effectiveness will depend primarily on their ability to appropriately interact with the various...
responders on scene. There are two crashes—the motor vehicle/transport crash and simultaneous equine crash—that should be approached in an organized manner.

2. Materials and Methods

Callouts of emergency responders for equine transport crashes frequently result in a request for a local veterinarian through dispatch at the local 911 center. In this article, it is assumed that the veterinarian has decided to respond to local crashes, therefore, it is important that practitioners and their staff be prepared with a basic response plan before that 911 dispatch call comes. No two responses or scenes will be the same, some practitioners will go through their entire career and never respond to a live wreck on the road, while others will receive numerous calls for assistance. When it occurs, it is best to have some idea of how to proceed.

Example 1

When responding to an overturned cattle trailer crash with 50 horses on September 15, 2004, in Lawrenceburg, IN, one veterinarian and technician triaged all 50 animals, euthanized 12, verified nine horses dead, and provided treatment to the surviving 29 horses (http://www.thehorse.com/articles/14759/be-prepared-for-the-worst).

The attending vet was Dr. John Nenni, who described the scene as “a horseman’s worst case scenario”; until 2007, this was the largest equine mass casualty transport crash documented in North America. Although he had been a graduate of veterinary school for only three months and two days, he fully credited his Boy Scout training for his ability to organize an effective scene response with local firefighters, law enforcement, horse lovers, and other responders.

Example 2

On September 26, 2006, in St. Louis, MO, Dr. Stuart Robson was requested to respond to an overturned horse trailer crash with 42 horses on I-44 at 3 a.m., with Longmeadow Rescue Ranch, Missouri Emergency Response Service, and Humane Society of Missouri team members. They deployed to the scene, triaged all the animals, euthanized nine, verified 17 horses dead, and provided transport for 25 horses off scene to a secondary triage location by direction of the State Highway Patrol in coordination with Technical Large Animal Emergency Rescue, Inc. (TLAER) trained personnel to further technical veterinary treatment at a local humane society rescue ranch, which also sought custody of the animals. He was interviewed thusly: “They don’t prepare us in vet school for anything like this,” he said. “I’ve been a vet for 10 years and I don’t ever get too excited about any emergency I see, because I’ve seen most things, and most things you can quickly solve. But having 42 animals trapped in a trailer like this was your worst nightmare come true.”
Example 3

On July 30, 2015, a veterinarian was called to treat seven horses that survived a horrific multiple vehicle crash on a bridge on Interstate I-76. The driver of the towing vehicle, a popular polo player, was pronounced dead along with his dog in the pickup, hauling originally 10 horses in the trailer, immediately three were pronounced dead, but others were loose and running on the interstate. Emergency responders provided first aid, then caught and delivered the horses to local farms for examination and treatment by the veterinarian.7

Example 4

A stock trailer with 14 polo horses swerved in the rain to avoid a vehicle then flipped onto the left side of Interstate 75 south of Atlanta, GA, on September 8, 2012. Within minutes, local horsemen driving past the crash start a calldown of their saddle club asking for assistance, successfully marshalling a local equine veterinarian and her staff to the scene and six horse trailers within 30 min of the crash. The author happened upon the crash just minutes later by sheer chance and offered assistance to the Incident Commander, a state patrol officer with no horse experience. Although one horse was killed instantly in the overturn, the responders were able to safely open the back door of the trailer (Fig. 8) and cut or lead out the other 13 horses, to which the local veterinarian and her staff provided immediate triage and first aid on the scene (Fig. 9). Almost immediately, the horses were loaded on trailers and taken to her clinic for follow-up treatment and shelter. The scene was cleared, and traffic restored within 1 hr and 30 minutes.8

Frequency of Transport Crashes

There is minimal scientifically maintained or reported data available on the number of horse transport crashes, so collecting the actual number of these events is impossible at this time, there is no nationalized reporting system. No data is available from the Department of Transportation, the National Highway Traffic Safety Administration nor the Centers for Disease Control and Prevention, which are normal venues for communicating statistics and prevention information. A literature search reveals a small pool of information about frequency and treatment of transport injuries in horses and “in transport” behavior related to research into improving transportation methods, especially for slaughter horses, but is not relevant to nor intended for response to crashes. This is a gap in the knowledge and represents a rich opportunity for research and statistical analysis. Thus, much knowledge in response to transport crashes— or suggestions for better prevention and response—come from a combination of the author’s extensive collection of journalistic and anecdotal reports, unpublished data from colleagues, training events with full size trailers and transports placed into scenarios duplicating past crashes, professional firefighters sharing their career knowledge, actual owner accounts, social media postings/photos, and personal reports from veterinarians/technicians/nurses that have responded to these crashes.

Worst Case Scenario

The largest mass casualty equine transport wreck is the October 27, 2007, crash in Wadsworth, IL, of fifty-
nine draft horses being transported in a double-decker trailer designed for cattle. A total of nine animals died in the crash, seven were euthanized on scene, and 43 were moved to a humane rescue ranch. Ownership of the animals was relinquished for adoption. Later, the owner and the driver of the transport were arrested and charged with four misdemeanor counts of cruel treatment of animals, and one count of failure to provide humane care and treatment of animals. Practitioners can imagine the challenges with responding to such a large crash in a public area, with well-intentioned but untrained responders. Seven veterinarians and their staff were involved with the response, including the state veterinarian’s office.

Factors to Consider Before Responding

- **Time:** Crashes commonly occur at busy traffic times of the day, in the middle of the night, or during bad weather. Responding to a crash may cause the practitioner to miss their normal calls for part of the day. Does the practitioner have an understanding with dispatch that will provide a police escort to the scene?
- **Payment:** Will the veterinarian get paid for his/her services/medications or lose money? Emergency response services do not charge for the extrication/rescue. For the practitioner, this is a difficult question, and answers come after the crash. Do you write off their expenses, request a donation to a related equine charity, or charge a professional fee? Opinion from emergency management professionals is slowly changing to this reasoning. If humans get transported to a hospital and treated, someone pays for it; so animal owners should pay for the professional medical treatment of their animals in these scenarios.
- **Liability:** Check the animal Good Samaritan laws in your state and ask the State Veterinarian’s office for legal advice on liability and requesting payment, even just to cover costs. Practitioners cannot afford to respond to many of these scenarios if it continues to require their donation of time, effort, drugs, and expertise.
- **Distance:** How far is the practitioner willing to drive to a crash site? Within the county? Within a certain region? Across the state? If there are limitations, consider a memorandum of understanding with the local 911 dispatch office to provide escort services to the scene.
- **Training:** Do you have training in Incident Command System (ICS) protocols (utilized by all 911 professional emergency response services) and rescue procedures? Emergency field medicine and emergency field rescue are very different subjects and require training to handle the wide variety of scenarios encountered in horse trailer crashes. Human safety is paramount and roadside situations are some of the most dangerous working conditions that can be found.
- **Equipment:** The basic load of equipment needed by the practitioner will include field medical equipment, lengths of webbing for manipulations, and various tools to induce euthanasia. Responding 911 services rarely have specialty TLAER types of large animal rescue equipment (Rescue Glide sled or large animal relocation sked systems, Nicopolous Needle, etc.) However, a variety of useful extrication equipment used for a variety of rescue situations will be brought to the scene by fire/rescue services.

Risk Assessment/Animal Evaluation

It is important to perform a risk assessment of the crash before proceeding to determine the health status of the horses involved. Both the veterinarian and the IC should work together to decide at what point the horse(s) can be approached safely for evaluation. No one should attempt to enter any trailer (or enclosed space) with horses until there is safe egress established. Stepping over live horses is dangerous, they will react and can severely injure rescuers. Evaluation of the horse(s) should include the following:

- Primary triage, secondary triage
- Initial first aid and treatment
- Clinical assessment
- Transport to definitive care location for further treatment and observation
- Evaluate the potential for infectious disease outbreak (especially mixed loads of horses)

Veterinarians should consider wearing a minimum level of personal protective equipment (PPE; gloves, coveralls, helmet) before establishing contact with on-scene horses. Horses can go into shock and die much faster than a human in a similar scenario. Recumbent animals entrapped in an overturned trailer are subject to abnormal orientation, gravity impacting muscle causing ischemia, with subsequent hyperemia and reperfusion injuries. These factors, over a prolonged period (>4–6 hr) have been shown to cause localized and distant organ injury or death in the horse, and muscle death and ongoing injury after 6–7 hr especially in sedated and anesthetized horses (Fig. 10). These factors must be considered to provide appropriate and timely prophylactic treatment to the animal – treatment methods are well covered elsewhere in the literature.}

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MEDICINE II: RESCUE, REPAIR, AND REFLECTION
Interaction with the Owner/Bystanders/Good Samaritans

Familiarity with, or knowledge about human medicine does not qualify a person as a professional emergency rescuer of humans. That is the recognized specialty and function of trained emergency responders (fire department, rescue squad, paramedics, etc.). Similarly, familiarity with, or knowledge about horses or even equine medicine does not qualify a person as a professional equine emergency responder. However, horse owners and good samaritans will tend to become “instant rescuers” during a crash. Allowing horse owners or bystanders to take charge of a crash is dangerous and unsafe to everyone else on the scene. The presence of the owner is important as a source of information and for authorization about the victim’s disposition and treatment, but not as a rescuer. The veterinarian should maintain control of the situation as the most veterinary medically qualified person on scene, others will look to them for leadership and advice.

Animal Handler

If possible, the animal handler should be personnel with advanced horse handling expertise, or an equine technician/nurse. Remember, most police and fire officers do not have experience with horses – and animal handling skills for emergency situations are a specialty skill set. Lack of training may cause them to underestimate the extreme weight, strength, and reflexive speed of a terrified, trapped or injured horse. The Incident Commander will direct the handler to take over this job from emergency responders when they arrive on scene. The handler at the head of the animal (if it is a safe place to be) is in the best position to advise about the medical status, potential behavior or reactions of the animal, approach techniques, and body positioning. The importance of safe positions around the horse should be emphasized by the handler to operational personnel.

3. Results

A database of horse transport crashes in published press reports, social media and privately reported anecdotes has been privately maintained (with caution that many crashes never make the newspaper, which contributes to skewed data on reportable crashes.) Analysis shows an interesting trend – over half of horses in various types of overturns and collisions walk away with minimal injuries (if they survive the crash.) Results were collected from August 1978 through August 2006. Of 204 crashes reported, there were 564 horses involved, and 464 people involved. In over 20% of reported crashes, the number of animals in the trailer was not mentioned or the trailer was empty. These data also tend to be better reported in the media if animals or people died – thus the study recorded 71 deaths of people and 127 horses either killed in the crash or euthanized on scene by responders. Of the horses that did not die initially, 255 had some injury mentioned, over 75% of these were noted to be minor (lacerations, bruising, bleeding). Followup on their condition was impossible to find or poorly reported. Trends noted in this study demonstrate the following:

- Inappropriate following distance between vehicles is commonly due to smaller vehicles cutting in front of the rig. Alternatively, it can be due to unsafe following distances or if the driver cuts off another driver. Numerous crashes caused by vehicles behind the trailer slamming into the trailer (often noted were poor reflective markings and non-working lights on the trailer). In these cases, animals in the trailer often received severe injuries, or were able to get loose onto the road. This type, and crash types due to unsafe speed for traffic conditions and road conditions, are very common (23%). Although this crash type rarely kills the horses in the trailer, it does kill people, particularly in the vehicle ahead. Special concerns derived from this crash type are the rig
jackknifing on impact, then rolling over (Fig. 11), and/or becoming unhitched (7%).

- Many single rig crashes (16%) showed that the driver went off road from inattention or excessive speed for conditions, lost control, sleepiness, or alcohol involved (3%). This number of crashes due to inappropriate speed or response (driver error) and inexperience pulling trailers is approximately what is expected from surveys of standard motor vehicle driver injury and death statistics in general.

- Trailer tire blowouts are not the cause of crashes, for every tire blowout that causes a crash there are over 100 that do not, unless they occur on the tow vehicle (2%) or are combined with a tow vehicle that is not appropriate (4%). However, if two tires on one side blow simultaneously (due to road hazard, poor quality two-ply tires or retreads) a crash more often occurs.

- There are numerous examples (8%) where another vehicle fails to yield the right of way or runs a stop sign, which results in being side-swiped or T-boned by another vehicle. How much of this is due to poor reflective warnings on the side of many transports is unknown. This type of crash is often fatal or generates horrific injuries for the driver of the other vehicle because trailers stand up very well to side impacts, even if it is so hard a collision that the trailer is pushed or rolled by the impact. Horses seem to do okay in these types of crashes.

- Incorrect or nonexistent vehicle and trailer maintenance contributes to many crashes and increases severity of the wreck. It represents the single most important prevention area for transport owners. Non-working lights, lack of reflective tape, incorrect hitching, and no brakes contribute to crash occurrence, and may arise because the vehicle industry has minimal standards for trailers that weigh less than 10,000 lbs.

Federal commercial vehicle regulations do not apply to trailers smaller than 10,000 pounds. Gross vehicle weight is the greater of the manufacturer’s gross weight rating (GVWR), or the actual weight of the vehicle plus the load. If the transport vehicle consists of a truck and trailer, add the GVWR or actual weight of the truck to the GVWR or actual weight of the trailer to obtain the gross vehicle weight of the combination. Many gooseneck trailers and other combinations do weigh more than 10,000 pounds and by the book should be regulated, but most states seem to ignore horse trailers for this rule. Other recommendations from manufacturers, insurers, and safety advocates to prevent issues include purchase of heavier duty axles, wheels, and tires, having two spares for the trailer, using a minimum of two axles on trailers to match the load, and better matching of the towing vehicle—appropriate to trailer size and weight.

- In this study, unsafe hitching was associated with tag-along trailers (96% of hitching issues) and more rarely (4% of reported crashes) for the gooseneck type trailers. The chains of tag along trailers in combination with the emergency brake engaging mechanism are important to prevent roll-away trailers when the hitch comes off the ball. Standardization of the correct ball size, maintenance and checking for correct hitching (i.e., locking the hitch, tightening the nut on the bottom of the ball, hitch correctly attached to the frame of the tow vehicle, appropriate lubrication of the ball to prevent microcracks) would prevent this type of crash where trailers become separated.
(10%). Properly hitched gooseneck trailers have even survived horrific impacts from trains (1.5%), head on collisions (8%), and side impacts (2%) without becoming separated from the tow ball – although the horses, if still inside the trailer, fare poorly.3

Proactive Involvement

Practitioners can contact their 911 call center dispatch and ask if there is a local, regional, or state team or Fire Department/Rescue Squad that has had training in methods and procedures to safely rescue horses in transport crashes. These teams may be private ambulance services, may be associated with a veterinary clinic or school, or with a fire department. Practitioners are encouraged to provide their 24-hr contact information to their 911 emergency dispatch center in their jurisdiction so that they can be contacted easily (if they are willing to respond to this type of crash).

Examples

1. Horsemen in the St. Louis area of Missouri recognized this gap in training and equipment, with the goal of minimizing risk of injury to rescuers and animal; in 2002, a group of volunteer horsemen, veterinarians and firefighters was formed to create the Missouri Emergency Response Services (MERS) response team. The members of this team have undergone specialized training in large animal emergency response and how to safely approach crashes such as overturned and wrecked trailers or livestock haulers. Their training and equipment cache immensely contributed to the success and professionalism of the response to the St. Clair, MO, crash,1 and at least three similar situations (cattle trailer wrecks, horse trailer wrecks) since. Similar results with numerous teams in other states and countries demonstrates that prior coordination between agencies and equine practitioners contributes to efficient, effective responses for these crash types.

2. In 2012, Alberta Farm Animal Care (AFAC), Canada launched the Emergency Livestock Handling Equipment Trailer and Training Program. There are now 17 cached livestock response trailers throughout the province, housed at county offices and fire departments.12 Connected to 911 emergency dispatch, they can be called out as needed across the province in response to trailer rollover and other emergencies involving horses. They have become the “go to” for emergency response to livestock or equine emergency or disaster response.

Each trailer contains equipment that may be used in these situations including a generator, livestock panels, ropes, portable lighting, various cutting tools and saws, tarps, livestock handling items, hand tools, Large Animal Relocation Sked systems,1 Nicopolous Needles,10 Mud Lances,a Häst Becker Slings,b Rescue Glide sled,p various specialty equipment, and plastic snow fence for containment and guidance. AFAC and Lakeland College’s Emergency Training Centre developed the accompanying training program “Livestock Handling in Emergencies.” This course is fully mobile and is aimed at first responders, veterinarians, and others involved in rescue—teaching coordination, safe response methods, equine handling, and behavior.

3. Over the last 30 years an increasing number of professional emergency responders and veterinarians have received training in technical emergency and disaster rescue of large animals (TLAER) and animal search and rescue (ASAR) for equines both domestically and internationally. These courses are aligned with National Fire Protection Association (NFPA) Standards for Technical Rescue 1006, 1670 and 150—which includes teaching proper and safe tactics, procedures and techniques for equine transport crash response, extraction of live and dead animals, dealing with vehicles on fire, trailer stabilization and cutting methods for overturned trailers to firefighters.13

4. The Canadian Livestock Transport (CLT) Program is a comprehensive training course and support service for livestock truckers, shippers, and receivers. The program offers core content for all species and breakout modules for beef and sheep, hogs, horses, and poultry. AFAC developed the original CLT program with funding assistance from industry partners and the Alberta Livestock Industry Development Fund. As of 2013, the CLT course transitioned to a national home with the Canadian Animal Health Coalition and was renamed the Canadian Livestock Transport Certification Program.14

4. Discussion

The Role and Responsibilities of the Practitioner

The veterinarian or their technician/nurse should direct medical stabilization, but should not go into the trailer, pull on assist/extrication devices, or perform the actual extraction. Let the professional emergency responders do their job. By taking a management and advisory role, and a less hands-on approach for the extraction, the practitioner can plan and prepare for treatment options, advise the IC and the owner, and prevent injury to themselves by staying out of the danger zone. Different from clinical and field practice, there are many dangers on the side of the road or even field environment where the veterinarian is not the “authority having jurisdiction” (AHJ) over the scene (the AHJ will be the fire department or police officer that arrived first) and the practitioner is expected to fit into the working model of ICS on scene (see below). The IC is ethically and legally responsible for the safety and lives of all personnel and the victim on his/her scene. Firefighters and police officers will quickly arrive to control traffic around the crash and extricate people (and deal with bystanders and frantic owners), paramedics for human injuries, and finally the IC will consider assisting the animal(s) when the scene is stable and safe. A good
risk assessment should be done to evaluate the risk versus benefit of various strategies for extrication of the horse(s) and should involve the veterinarian’s opinion (Fig. 12; for example, if the animal is not savable, why risk anyone’s life to extricate it and why make it suffer? It should be euthanized first.)

The veterinarian will be crucial as an advisor to the IC for making decisions about the animal’s savability, extrication options, handling, and behavior – as well as bringing knowledge of physical and chemical methods of restraint that will be useful to the extrication effort. Animals are a secondary consideration for responders—while the veterinarian’s primary concern may be the animal victim—but he/she must work inside the constraints of the Incident Command System on scene for everyone’s safety.15 Many animal owners will be emotional on scene, while veterinarians have training to remain calm, make rational decisions, and handle the situation professionally. Close coordination with the owner can ensure better outcomes based on diagnosis and early treatment options, or at the least in euthanasia being selected for induction at a rational point. The owner (if present) should consult with the veterinarian for timely decisions as to savability, transport to definitive treatment, or decisions on euthanasia. Bystanders will offer suggestions that may or may not be helpful – the veterinarian will be needed to sort through this barrage of “help” that is offered.

**Incident Command Response Framework**

Whether a single crash (e.g., two horse trailer runs off on the road) or a large-scale disaster (e.g., commercial hauler with million dollar valued horses in a fiery Interstate crash), the veterinary practitioner is just one member of a large group of emergency responders. Regardless of crash size, all individuals and organizations respond under a common protocol known as the ICS. In both scenarios it is essential to know how to interact as part of the team with other emergency response individuals from county, state, federal, and private emergency response organizations. Lack of understanding of, or respect for the chain of command on crash scenes result in power struggles that detract from the response, and when combined with the fear of distressed horses inside the transport, are a recipe for injury of personnel and animal victims. Every equine practitioner responding to a crash or disaster of any size or type should have at least a basic understanding of ICS which will benefit them by:

- Knowing how to properly interact with emergency responders;
- Understanding the language used on emergency response scenes;
- Behave in the correct manner on scene for safety and security;
- Work as a team member; and
- Be considered an asset—instead of a liability—by the Incident Commander.

**Example**

In the Lawrenceburg, IN crash, responders included the county Sheriff department, EMS service, two local Fire departments, Police department, State Department of Transportation, and the State DOT Police Enforcement, which demonstrates that even in the presence of professional emergency responders, a crash is a controlled chaos environment. In this crash, Dr. John Nenni, the veterinarian on scene, made a call to the state veterinarian’s office to check the Coggins and health transport paperwork on these horses. He also checked the bill of sale paperwork for the horses to ensure that they were not stolen. It is always a good idea to contact the State Veterinarian and the USDA/APHIS Area Veterinarian in Charge for large and out of state transports. The basic principles of ICS are fully applicable to trailer collisions and include the following:

- Planning: A crash action plan must be developed (simple and verbal, or complicated and written) depending on the size of scene and complexity or danger.
- Team approach: Every responder is part of a team and knows their job.
• One coordinator: The IC coordinates the crash response; he/she is the leader and shoulders the responsibility for the entire scene. This is normally a firefighter or police officer that has extensive professional training, leadership experience and certifications in scene safety and management.

• Span of control: One supervisory person can only coordinate the activities of five to seven responders. They respond to the chain of command for tasks.

• Safety: Safety is priority on scene, for both the animal victim and all human rescuers.

• No freelancing: Individuals responding/acting on their own constitute a risk and a liability to others on the scene. The IC has the authority to forcibly remove them from the scene (practitioners should normally not be the IC unless they have extensive training in crash management and safety.)

When the Call Comes in to Your Office

The caller may be the 911 dispatcher, client, or an unknown horse owner or bystander, especially since it is common for horses to be hauled for sales, shows or trail rides. On-road crashes are inherently very dangerous; the veterinarian should not respond on their own. If the caller is a 911 dispatcher, ask them to send Fire/Rescue Department and Law Enforcement to the scene. If the driver/horse owner calls directly to the practitioner, they should also call 911 and request their assistance. Preliminary information to obtain during the call is below:

• Name and contact information of caller
• Exact location and clear directions to the crash
• Type of transport
• Brief description of crash (text photos if possible)
• Number of horses

If the crash occurred on a major road, there will already be a traffic backup in which case the 911 dispatcher will need to provide you assistance in reaching the crash (via police escort, etc.). Photos and short videos of the scene should be requested; they will allow you to develop some possible plans and coordination while responding to the crash location. At the scene, ensure you are properly parked off the road surface; employ use of flashers and lights before getting out of the vehicle. Horses accommodate quickly to the consistency of strobes; on active roadway human safety standard operating procedures require that these be used. A retro-reflective vest with bright colors is required to be worn by OSHA (Occupational Safety and Health Administration) on all crashes, best practice is a retro-reflective vest and helmet for personal safety in the chaos of a crash. Upon arrival to the scene, the veterinarian should contact the Incident Commander, and the person transporting the horses (agent/owner) to obtain information on the following:

• Origin
• Destination
• Contact information of home veterinarian (name, address, phone number)
• Health documentation (Coggins/health certificate)
• Manifest (large commercial transports must have a manifest) or bill of sale

Because of the mobility of horses across different regions of the country, an important role of the veterinarian during equine transport crashes is the assessment of possible biosecurity risks. (Veterinarians are encouraged to follow the Infectious Diseases Control Guidelines recommended by the American Association of Equine Practitioners (https://aaep.org/guidelines/infectious-disease-control/using-guidelines.)

From the Horse’s Perspective

A trailer wreck, from the perspective of the animal in the trailer, is very different from what humans perceive. Rubber mats, horse bodies, and divider gates each move differently throughout the changing velocity vectors through the active part of the collision, which will be influenced by the structural stability and current maintenance level of the trailer. Horses that appear to be laying calmly are actually very stressed. Recumbent and trapped horses often lay quietly for a few minutes due to exhaustion (Fig. 13), but because their instinct is “a down horse is a dead horse,” they will struggle. They do not comprehend that the humans are there to assist. Instead, they interpret new noises and activity as threats to their survival and may panic and

Fig. 13. Horse down in a trailer with head trapped in the door. While the horse appears to be lying quietly, any stimulation from sounds or sight will cause the horse to struggle violently. Photo Courtesy Becca Speer.
struggle harder. Horses can hear everything going on (voices, tools, vehicles, footsteps, extrication equipment) and outside the trailer they may be able to see shadows and reflections—thus your advice should be to limit loud sounds (sirens, cutting equipment) until necessary. There are certain apparatus that are extremely loud (air chisel, reciprocating saw, K-12 saw, and chain saws); others (hydraulic tools) are silent but cut very jagged edges. Once a tool is started operators should keep it running; most animals accommodate to the sounds and vibration if used efficiently. Generally, selection and use of cutting tools are the responsibility and expertise of the fire department since trailer construction materials run the gamut from wood to rubber to steel to fiberglass, and all require different strategies to overcome. More dominant equines in these crashes will continue to exhibit aggressive behaviors to less dominant animals within range of teeth, hooves, and threatening behavioral expressions—thus “horse on horse” injuries are common in trailers of all types. Their desperate attempts to get away from more dominant animals can cause further injury and panic inside a trailer, another reason for a good plan and efficient extrication strategy.

Make the First Assessment from the Outside

Veterinarians routinely get close to horses to do their jobs, and due to work-related exposure frequency (or complacency), they can get injured. Emergency services actively work to reduce the chance of injury to responders; thus it is preferred that no one go into the trailer to halt or treat a horse because it is a confined space. Often, the trailer must first be stabilized (Fig. 14) or moved to a safe area before the animals are extricated (down an embankment, hanging off a bridge, trapped in trees or sinking into water, etc.). In real crash scenes, there are very few situations where an overturned trailer should be turned back onto its wheels while live animals are inside—this only causes more injuries. Horse trailers can come to rest in left- or right-side lateral, dorsal, posterior or even anterior recumbence. Fire/Rescue will have equipment to safely stabilize the weight of such a large object, then to make access (cutting may or may not be required) large enough for the animals to self-extricate or be removed safely. Approach slowly while talking to the horses, evaluating their stress and orientation by looking in a window or other existing opening and make an assessment without opening doors or ramps to prevent escape. Horses are well known to attempt to egress through openings too small for their body; a panicked horse lunging towards the light is dangerous to itself and handlers. What is the orientation of the trailer? Use a ladder to access the windows or access points on the top of the trailer if necessary. Ask the fire/rescue personnel to provide lighting for the scene for everyone’s safety. A good assessment of the inside areas of the trailer should include the following: Are animals dead or alive? What are the obvious injuries? Are they haltered and tied in the trailer? Is one on top of the other? Standing or lying down? Are the dividers/gates/rubber mats intact or fallen? Can you assist the horse to extricate himself from the trailer? Is there a safe way to cut or release the trailer tie without crawling over the horse’s body or head? The firefighters can make better decisions for extrication based on this type of information. Ask yourself questions based on the scenario—is the trailer flat on the ground or balancing on tree roots or the guard rail? Trailers are lightweight, unstable, and easy to move when in an overturned configuration—they should be stabilized for approach and to minimize any chance of the trailer harming a person or rolling and moving as a horse attempts to unload. Difficult scenarios will require stabilization of the trailer and perhaps re-orienting it by a few degrees to prevent a slanted standing surface for the animals, to provide an egress path for extrication. In others, it may be safer to pull the entire trailer back onto the flat road surface prior to extrication. Access holes into the trailer should be minimum 1.5m wide and as tall as possible while providing structural integrity, while an egress lane for leading the animal from the trailer to a safe area off the roadway should be cleared. Generally, if a horse is able to get up, it will do so immediately after the collision; if it is still recumbent upon arrival, there is a reason. Slick floors, obstacles, and lack of leverage or space may contribute to a horse’s failure to rise as much as injuries and must be evaluated. The most frequent injuries reported are head, neck and lower leg injuries after wrecks. Horses tend to fight to stand if physically possible, then stand quietly (feed hay to keep them quiet while you evaluate your next steps). Slapping or stimulating a downed horse that is entrapped and not able to move is not helpful; for example, short trailer ties (especially ones that don’t break) will guarantee that the horse cannot get up. Breakable halters can break—try not to release a horse outside of the trailer without control of the
head or use secondary containment with fencing outside the trailer. Beware of bungee-type trailer ties, they are extremely dangerous to people and horses when loaded with weight. The three top scenarios with very high injury and mortality rates are trailer floor failure, horse being ejected from the trailer, and horse trailers hit by semi-trucks, trains, or similar catastrophic collisions. When animals are ejected (trailer disintegration or floor failure), the results are similar to human crashes and usually result in severe injuries with poor prognosis for survival (Fig. 15). Horses are capable of surviving catastrophic trailer collisions, as long as they stay inside the trailer. High-speed catastrophic collisions can cause horses’ bodies to cut through bulkhead walls, doors, and windows in a macabre equivalent of cartoon characters breaking through a brick wall, but with bloodier results. Animals are often in shock, endure severe injuries and pain, and rarely have relevant bone, muscle, or tendon anatomy left available to make a surgical attempt at recovery.

Best Practices Responding to Trailer Collisions

It is easiest and safest for responders to assist an animal to self-extricate. If possible, remove all obstacles, such as divider gates, equipment, “removable” tack rooms, ramps, and doors that obstruct the exit. Cover windows (which now are holes in the overturned trailer’s “floor”) with a backboard or rubber mat, so a leg does not step thru them. Prevent the horse(s) unintentional exit before responders are ready to manage them—secondary containment may be set up around the rear of the trailer with cattle panels, tarps, parked vehicles, or snow fencing to prevent a loose horse situation once extricated. Do not remove animals from trailers until secondary containment is set up and traffic is stopped, if animals get loose on the roadway, they may cause secondary collisions and harm themselves or humans. Have extra halters and lead ropes available to catch and control the horse (or use an emergency rope halter). Control of the head is crucial unless the animal is obviously not halter broke—in that case consider secondary containment using cattle panels around the back of the trailer and allow the animal to extricate itself, then drive into a waiting alternative transport. Unloading a horse on the side of the interstate under the best of conditions is hazardous, and always requires law enforcement officers to stop traffic during actual extrication and unload/reloading operations. When performed in the dark, the rain, or on steep hazardous terrain, these problems are compounded. Get scene lighting from fire/rescue early in the crash. Once a door or ramp is opened, it should be tarped to cover the opening, then tied off so that it cannot slam back into place. Often, gates, dividers and doors will have to be removed or cut even for standing animals to be safely extricated. Doors and metal pieces do not make safe ramps when in abnormal configurations. If the horse’s head is tied inside the trailer, it must be released before extrication is attempted. The safest way to release a trailer tie is to cut it without crawling over the horse’s body or head. No one should go inside the wrecked trailer for any reason without sedation or anesthesia, unless the animals are standing and have a clear way out; then it may be possible for a person with proper PPE to go in, cut the trailer tie and attach a lead, then bring the animal out safely. A seatbelt cutter or curved sharp knife secured to a long painter’s pole allows responders to stay in a safe position outside the trailer (a serrated knife can work, but the push/pull motion on the tie strap stimulates the animal and causes accidental stabs). People have endured broken arms and fingers by advancing them into trailers, thus the recommendation is to use a long pole. A pole syringe can be fashioned from a long pole and duct tape to induce the animal with sedation or anesthesia intramuscularly, and without entering the trailer.

Triage

Triage should be part of the plan for veterinarians willing to become involved in response to emergencies/disasters. The level of triage applied to the scene will vary with the amount of personnel available, equipment and supplies, and the efficiency of the extrication. Some transport crashes will be mass casualty crashes from two to 25 injured horses in semi-trailer commercial transports. Be familiar with basic triage procedures, conduct a cursory inspection of all animals, then salvage animals that are easiest to retrieve from the transport, have cooperative
dispositions, and present the least threatening injuries. Primary triage should be conducted in an efficient manner, secondary triage may be conducted at the clinic or an off-site field hospital for mass casualty crashes. Severely injured horses may not be transported to definitive treatment without appropriate methods for medical support (i.e., horses with a fracture should not be loaded on a trailer without a splint, analgesia, and combating shock). For animals that have a reasonable chance of survival, wait to euthanize until the owner can be contacted. Documentation of injuries with pictures or video of animals before euthanasia is wise practice. Bystanders will be very upset that some animals have to wait for triage by the veterinarian. In the Lawrenceburg, IN crash, bystanders offered to shoot both dying and surviving horses with their personal weapons - the sheriff on scene had to threaten to handcuff anyone with a gun. Expectant animals (ones that have the poorest prognosis) should be euthanized efficiently to prevent suffering - based on resources and personnel.

Euthanasia and Field Euthanasia
Before providing medical attention or euthanasia to a horse(s) at a crash, consult with the owner/agent about possible treatment/cost and prognosis. Exhausted, stressed, and dehydrated animals often lie down after being extricated; this is not always an indicator of a poor prognosis. Some owners refuse veterinary attention to horses even in cases where the prognosis is good or fail to authorize euthanasia. Welfare considerations in some jurisdictions allow the animal’s ownership to be transferred to Animal Control or a humane organization, which can authorize further treatment or euthanasia. Check local and state regulations that affect decisions about animal welfare and ownership. The practitioner must be prepared to perform field euthanasia at the site of a transport crash. Practitioners may be able to use euthanasia solution as performed under normal circumstances; however, if there are insufficient amounts of euthanasia solution, horses in shock, or venous access is impossible without a safe egress for the responder, the veterinarian will need to direct the performance of euthanasia with a firearm by a police officer with guidance to landmarks, proper bullet placement, and angle of introduction. The person performing euthanasia should be willing to follow the veterinarian’s instructions to ensure proper landmarks for placement of the projectile. Alternatively, the veterinarian or animal control officer may perform the euthanasia with a penetrating captive bolt gun, or a firearm. In the absence of projectile weapons and euthanasia solution, field euthanasia may include intrathecal injection of lidocaine into the atlanto-occipital space in sedated animals, or exsanguination (jugular vein or posterior vena cava per rectum) in unconscious animals. Whether as a result of euthanasia or immediate to the original crash, it is not uncommon to have dead horses after response to transport crashes, these are called “recoveries” in ICS terminology. Navy-blue king-size bed sheets or drop cloths can be used to cover dead horses after crashes — this shows respect and hides the distasteful sight from onlookers - preventing secondary crashes from rubbernecks.

Concerns Special to Technical Extrication for Equines
Horses entrapped in abnormal positions (dorsal, posterior, lateral recumbence) are subject to a variety of consequences based on efficiency of pre- and postextrication examination and treatment. Even when a trained large animal rescue team effects an efficient rescue and the on-scene veterinarian treats the animal immediately—the animal can still die. Failing to treat a horse for crush syndrome and accidental hypothermia in cold or wet situations immediately after the extrication is a common iatrogenic cause of death. Loading a horse is one of the most difficult and most dangerous activities to attempt for both the horse and human. Anecdotes abound where humans get crushed, kicked, dragged, and run over by horses; while horses acquire lacerations, rope burns, bang their head on the roof and metal obstacles, and de-gloving injuries struggling to avoid going into trailers. Always stay out of the way of the horse while loading and unloading. Training horses to load should not be the job of the practitioner on the side of the road; however, injured horses will need to be loaded for transport and the emergency responders will look to the practitioner and their staff for assistance in emergency loading techniques (Fig. 16).

Recommended Equipment on Hand
Practitioners should carry basic equipment and tools to facilitate extrication of a horse trapped or injured, either in or out of a trailer (Fig. 17). Trailer crashes are common emergency responses for practitioners, so it makes sense to be prepared.
Personal Kit

- Gloves (high dexterity)
- Boots (with or without steel toe)
- Protective headwear (helmet with chinstrap; OSHA approved)
- Jacket or vest (brightly colored and retro-reflective)
- Protective goggles or glasses
- Knife and/or multi-tool
- Ear protection
- Professional shirt/jacket or scrubs with your logo to identify yourself
- Primary and secondary ID badge/card (one for yourself on scene and second for “manpower accountability”) if you have prior coordination with local dispatch

In Vehicle Trailer Response Kit

- A road hazard warning kit, reflective tape on rear surfaces of vehicles, working flashers on vehicles, and a retro-reflective vest for personnel on the side of the road. (Responders will be more worried about human safety than the horse victim, roadsides are a very dangerous place to be.)
- Cell phone to call 911 immediately for scene safety assistance.
- A good first aid kit for both equines and humans.
- A sharp curved knife or seatbelt cutter to be used for emergencies—capable of cutting thru tie straps and rope that may be entrapping the head or legs.
- A battery powered reciprocating saw capable of cutting metal or wood that may entrap extremities.
- A piece of 8 cm wide webbing 7 m to 10 m long with loops at each end (tow strap) to control a leg; or around the chest and abdomen to maneuver the animal into a safer position (forward assist, backwards or sideways drag). The head, neck, and tail are never safe anchor points with which to drag an animal out of anything—they can be severely injured or traumatically amputated.
- An extensible cane or pole to manipulate webbing, induce sedation (via pole syringe), or cut webbing (via curved knife) without having to get too close. An aluminum boat hook or painter’s pole extendable to 4-m or pike pole (used with interchangeable accessories; e.g., cutters, knife, carabiner hold-open, hook, etc.)
- A hammer to drive the pins out of a chest bar or divider gate, especially with a horse on top or under it.
- A towel or blanket to cover the head and eyes of a recumbent horse to calm it.
- Good quality hay to allow trapped or extricated horses to eat and relax while waiting for assistance or transport.
- Emergency rope halters 12.5-mm kernmantle rescue rope or yacht braid rope 7 m to 10 m long
- Insulated horse blanket (heavy duty).
- Häst Head Protector™ or similar whole head protection (or human life vest, towel, sweatshirt, etc.) to protect the eyes.
- Large animal physical restraint (nose twitch, various halters, lariats, etc.)
- 33-m containment portable fencing (construction plastic fencing).
- Heavy duty tarps.
5. Conclusion
Local equine practitioners are the responder of choice when an on-road crash involves equine(s). Prior preparation and simple equipment will contribute to more professional results; prior coordination with local law enforcement and fire/rescue will lead to improved relations for responders on scene and successful outcomes of trailer related crashes. Use of best practices allows the responding equine veterinarian to function in an efficient and safe manner advising the Incident Commander on a live horse trailer crash scene on the roadway or roadside.

Acknowledgments

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

Conflict of Interest
The Author has no conflicts of interest.

References and Footnotes

"Methodology and procedures for equine field response, first aid treatment, field euthanasia and clinical medicine as often required on scene are well covered in other sources, thus will not be addressed in detail here."

"This publication uses "crash", "wreck", or "collision" instead of "accident"; most road safety research publications have moved away from "incident" or "accident" because it implies that nothing could be done to prevent the crash, promoting a perception of inevitability and lack of understanding that these crashes are preventable. New policy initiatives aim to educate the public that crashes are not inevitable and aspire to eliminate traffic fatalities and serious injuries."

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How to Develop an Equine Postmortem Examination Review Program

Alina Vale, DVM, MS*; Timothy J. Grande, DVM, MPH; and Forrest Franklin, DVM

Postmortem examination of horses dying while engaged in equine sport is important to understand. A review of those findings with trainers, veterinarians, and others involved in the care of horses undergoing postmortem examinations is an important educational tool for both those with direct care of the horses as well as event organizers and regulators. Authors’ address: California Horse Racing Board, 1010 Hurley Way, Sacramento, CA 95825; e-mail: amvale@ucdavis.edu; *Corresponding and presenting author. © 2021 AAEP.

1. Introduction

Every horse that suffers a fatal injury on the racetrack in training or in competition or that dies or is euthanized within an area of California Horse Racing Board (CHRB) jurisdiction undergoes a postmortem examination at a diagnostic laboratory to determine the injury or sickness that resulted in euthanasia or death. The postmortem program between the CHRB and the University of California, Davis was initiated over 30 years ago. The CHRB had been conducting informal fatality reviews on cases of special interest for several years. On July 1, 2020, CHRB Rule 1846.6 became effective, requiring a postmortem examination review to determine the circumstances of each equine fatality within a CHRB enclosure. This fatality review program is designed to be an educational tool for trainers and attending veterinarians, as well as a fact-finding tool, providing information relevant for developing strategies to prevent injuries in the future. Postmortem review is separate from a fatality investigation where potential violations of laws or regulations are investigated. The cluster of Thoroughbred racehorse fatalities at Santa Anita Park in 2019 that garnered widespread public concern and put horse racing in California at risk of losing its social license to operate is an example of an investigation. The Los Angeles District Attorney’s Office opened a criminal investigation into the equine fatalities and collaborated with the CHRB, which investigated the cluster of fatalities.1 2

2. Steps of an Equine Postmortem Examination Review

Assemble a Panel of Experts

These individuals should have knowledge of the rules and regulations of the equestrian discipline or racing jurisdiction and have several years of experience working in the industry. At least one panel member should be a veterinarian. The CHRB postmortem examination review panel comprises a member of the board of stewards, a safety steward, and the equine
Gather and Review Information

For the CHRB program, the safety steward interviews the jockey or exercise rider and submits an accident report. Video footage from the race, workout, or barn surveillance is reviewed. The trainer is requested to provide training records for the expired horse, which includes exercise, medication, and shoeing history for a minimum of 60 days prior to the date of death of the horse. Additional information is collected from various sources, including racing records (past performances, result chart), regulatory veterinarian examination records, official veterinarian’s list history, and timed workout history. Attending veterinarians provide a summary medical record covering a minimum of 60 days; however, 1 year or longer of medical records is generally requested and provided. This includes a history, physical examination findings, interpretation of diagnostic imaging and laboratory findings, and treatments and medications administered or dispensed. Copies of laboratory data and diagnostic imaging may be requested and require client authorization. The official veterinarian also reviews veterinary confidential reports (medication form submitted daily to the official veterinarian), the health and racing soundness record, and the final postmortem examination (necropsy) report. The official veterinarian identifies key training, veterinary care, and horse health information relevant to the injury event and creates a questionnaire. Most of the questions are shared with the trainer and attending veterinarian in advance so they can prepare for the meeting and provide the most accurate information.

Conduct Trainer and Attending Veterinarian Meetings

The CHRB protocol is that the safety steward contacts the trainer of the expired horse to appear before the postmortem examination review panel and shares the questionnaire and postmortem exam (necropsy) report. The attending veterinarian is interviewed by the entire panel or just the official veterinarian. Owners, racing managers, assistant trainers, additional veterinarians, previous trainers, or any CHRB licensee may be questioned at the discretion of the postmortem examination review panel. Interviews are conducted in person or remotely via video conference or phone call. Trainer interviews are recorded so the discussion can be reviewed if needed. When scheduling the meetings and again at the beginning of the meeting, it should be emphasized that the discussion is not an investigation nor interrogation, but rather an educational opportunity (to educate the trainer and learn from the trainer). During questioning, panel members must remember this point to avoid confrontations as the educational opportunity is minimized if the trainer or veterinarian is fearful of sharing information. After questioning, the trainer is presented with images from the postmortem examination as well as case examples clearly demonstrating similar pre-existing pathology (if appropriate to the case). Examples of diagnostic imaging that could detect such lesions are presented and discussed. Video footage may also be reviewed and discussed.

Submit a Report

Upon completion of all interviews and collaboration, the panel submits a written report to the CHRB executive director, which is then shared with the horse’s owner and trainer. The report contains findings and recommendations for improvements to prevent similar injuries. If there is a potential animal welfare violation or other concern, the case is referred for a full investigation by CHRB investigators (sworn peace officers). Redacted summaries of each postmortem exam review are posted on the CHRB website under the veterinary tab: www.chrb.ca.gov/postmortem_summaries.html.

3. Discussion

Over a 10-month period, 63 equine postmortem reviews were conducted by the CHRB. Pre-existing pathology at the site of the fatal injury is a re-occurring finding at postmortem examination; however, some of the pre-existing pathology is not readily apparent. The majority of trainers and attending veterinarians are agreeable and willing to actively participate in the review process and interviews. Initially, many trainers did not display good working knowledge of anatomy or grasp the significance of major pre-existing lesions (e.g., palmar/plantar osteochondral disease lesions). At the end of the interview, many trainers express gratitude for the educational opportunity despite the difficult conversation. One author uses the technique of motivational interviewing. This strategy utilizes compassion, open-ended questions, reflective listening, and clarifying statements to engage the trainer. This communication technique can assist in modifying the trainer’s behavior (related to equine care, treatment, and diagnostics) with the goal of preventing future fatalities. This approach can initially be difficult for some individuals in a regulatory role accustomed to an adversarial relationship with horsemen. Given the CHRB postmortem review program is viewed as a fact-finding tool for preventing future injuries, the current objectives include developing standardized questionnaires and performing statistical analysis for risk factor identification and hypothesis generation. This technique can easily be modified for horses in different types of competitions (endurance riding, 3-day eventing, barrel racing, polo, etc.) or other uses (carriage and working horses). Ideally, a postmortem examination (necropsy) is performed; however, a postmortem review
can still be beneficial in cases where this is not performed.

4. Conclusion
Equine fatalities associated with competition attract significant public attention. Equine post-mortem review programs will allow the equine industry to proactively acknowledge and learn from these tragic events.

Acknowledgments
The Authors thank the pathologists, CHRB executive director, investigators, stewards, safety stewards, and other staff that have contributed to the CHRB postmortem exam review program, as well as the trainers and attending veterinarians that have participated. The authors especially thank Drs. Rick Arthur, Susan Stover, and Monika Samol for their guidance and involvement in the program. The authors are employed as official veterinarians by the CHRB.

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

Conflict of Interest
The Authors have no conflicts of interest.

References
Effect of Transcutaneous Carbon Dioxide Therapy on Wound Healing and Skin Graft Acceptance in Horses

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The use of transcutaneous carbon dioxide therapy may be beneficial for the treatment of distal limb wounds healing by second intention. Authors’ addresses: Department of Clinical Studies, New Bolton Center, School of Veterinary Medicine, University of Pennsylvania, Kennett Square, PA 19348 (Gaesser, Torcivia, Stefanovski, Engiles, Levine); Department of Large Animal Clinical Sciences, College of Veterinary Medicine, Michigan State University, East Lansing, MI 48824 (Vanderbroek); Department of Medical Sciences, School of Veterinary Medicine, University of Wisconsin-Madison, Madison, WI 53706 (Kowalski); Department of Pathobiology, New Bolton Center, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA 19104 (Engiles); e-mail: agaesser@vet.upenn.edu. *Corresponding and presenting author. © 2021 AAEP.

1. Introduction

One proposed mechanism of exuberant granulation tissue formation is persistent hypoxia. Carbon dioxide therapy (CDT) is thought to cause the release of oxygen from hemoglobin, improving local oxygenation. The objectives were to evaluate the effect of CDT on wound healing and skin graft acceptance in the horse.

2. Materials and Methods

Three wounds were created on the dorsum of each metacarpus of six horses. One forelimb received CDT while the contralateral limb was the control. Twelve treatments were performed after wounding. Skin grafts were performed on the larger wounds on day 7, and graft acceptance rate was determined on day 28. Granulation tissue grade was determined by a blinded observer. Biopsies were obtained on days 7 and 14 and were evaluated for the degree of inflammation, epithelialization, angiogenesis, and fibrosis. Digital images were used to determine wound area over time. Statistical analysis was performed with mixed-effects logistic regression.

3. Results

For the larger central wounds, which increased in area initially, CDT treated wounds did not expand as significantly as control wounds ($p = 0.004$). There was no effect of treatment on granulation tissue grade or histologic scores. There was no effect of treatment on graft acceptance rate.

4. Discussion

Limitations include the two-dimensional nature of wound planimetry and that treatments were only
administered during the first 14 days. CDT treatment may be beneficial for the wounds healing by second intention by limiting expansion of granulating wounds.

Acknowledgments
This study was funded in part by Veterinary Transdermal, Inc.

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

Conflict of Interest
The Authors have no conflicts of interest.
The Effects of COVID-19 on Equine Welfare and the Equine Industry in 2020

Clara Ann Mason, DVM

1. Introduction

COVID-19 is a zoonotic infection. Research uncovered that the bat coronavirus (RaTG13) could not bind with receptors in humans or pangolins, but the pangolin coronavirus was able to bind to pangolin and human receptors. Though this research may suggest that the pangolin virus is involved in the transmission of coronavirus in humans, currently there is no confirmation as to whether the pangolin virus was a part of SARS-CoV-2's evolution to humans.1 Equine coronavirus is transmitted between horses through fecal-oral route when an unaffected horse ingests infected manure or has oral contact with a contaminated horse. Symptoms may include anorexia, fever, lethargy, colic, and diarrhea. Diagnosis is achieved for enteric coronavirus by testing fecal samples, and treatment includes supportive care such as fluid therapy and anti-inflammatories. Though frequency of this disease is low in horses, biosecurity remains the most preventative modality for denying a horse transmission of the coronavirus.2 As of spring 2021, 3 million people have died of COVID-19 worldwide during the pandemic of 2020. The havoc that COVID-19 wreaked on our professional communities will have an impact on the livestock and horse industries for years to come. The challenges in the equine industry caused by COVID-19 are mirrored with similar problems in the swine and poultry production business. During the spring and summer seasons of 2020, the swine and poultry industries faced challenges with labor and resource availability, especially since both sectors have an overseas market. Though contingency plans were in place prior to the declared COVID-19 pandemic to manage both poultry and swine farms during a crisis period, and despite industry efforts to mitigate risks for human safety and health, animal health and welfare, and supply chain continuity, many challenges and extreme circumstances simply overwhelmed the contingency plans that were established. Labor shortages, lack of feed, closed slaughter facilities, and no income for the producer led to devastating outcomes and production shortages both immediately and in the long term. Attempts to slow poultry and swine growth by feeding them a limited-calorie diet, thereby providing more time to get the animal from farm to slaughter, was a short-term solution to a long-term problem. Ultimately, depopulation became necessary, with 2 million poultry euthanized in the Delmarva (Maryland and Delaware) region in April 2020. Swine and poultry slaughter facilities were closed due to workers infected with COVID-19 and a personal distance of 6 feet between employees. Pigs intended for human consumption were intentionally rationed food and water to slow growth until slaughter facilities reopened. This tactic increased aggressive behavior among housed pigs and compromised the welfare of each animal, ultimately resulting in depopulation. Though horses in the United States are not typically intended for
human consumption as are livestock, similar to the meat industry, feeding, housing, physical labor, and the intended-use market were negatively affected by COVID-19.

2. Thoroughbred Racehorses

According to Dr. Scott Palmer, NY State Gaming Commission Equine Medical Director, after analyzing data on Thoroughbred fatalities from 2020, he determined that the COVID-19 pandemic could be considered a novel risk factor for fatal Thoroughbred injuries last year. Overall, there were 24% fewer fatalities per 1,000 starts in the region in 2020 as compared to 2019, but some shifts in the types of fatalities occurred. The number and percentage of overall fatalities that occurred during racing versus training decreased, which was to be expected since the pandemic resulted in fewer race cards in 2020 versus 2019. However, a change in the proportion of fatalities occurred in training, especially in juvenile runners, where there was a very unusually high number of fatalities in 2-year-old racehorses, especially at Saratoga Racetrack in 2020. It is assumed that when racing was cancelled and with uncertainty as to resumed race dates, more trainers maintained their horses at the resident farm rather than on the racetrack where daily fees to maintain a racehorse are incurred. Consequently, some 2-year-olds did not post their first official timed works until June, much closer to their racing debuts than usual. Out of the 18 2-year-old fatalities in 2020, 8 occurred in horses that had never raced. It is suggested that some 2-year-old racehorses were denied the usual time frame of training that permits the bone remodeling process, which is crucial to preparing the skeleton for the rigors of racing. Bone remodeling after track training and added workload is critical to preventing catastrophic racing injuries. Due to limited racing during 2020, many horses ran fewer times overall during a normal race calendar, and their skeletons were unable to respond to intense exercise before the next race.

3. Equine Rescues

When many people realized that the restrictions of the pandemic such as working remotely could become a more permanent option for their employment, many adopted pets and horses as a complement to their home life and mental well-being. During the spring of 2020, several of the equine rescue facilities were depleted of their rescue horses because of increased adoption rates. Numerous people selected to live off the grid and move to a more rural area where they could participate in hobby farming. This also increased adoption rates in the rescue facilities. Initially in early spring 2020, though adoption rates were up, there was the overall appearance that horse rescues and confiscations due to equine abuse and neglect were decreased in number during the pandemic compared to years previously. With the inability for law enforcement, shelter workers, and humane officers to visit on-site purported horse abuse, fewer horses were confiscated, resulting in skewed data. In later 2020, as unemployment rose and the cost of horse care increased, it was expected that the number of abuse cases will also rise in 2021 as shelter officers and employees return to work. The increase in number of cases of horse abuse may create an overwhelming scenario for rescue facilities and shelters in 2021. Court dates for abuse and neglect cases will be delayed, which will delay adoption and fostering, ultimately increasing the cost for the care and custody of a rescue horse. Many of the donated horses have health issues that preclude them from a career other than as a companion horse, and this poses a greater challenge for suitable adoption. Horses adopted in 2020 may be returned if family finances warrant an adjustment. Equine rescue organizations face difficult decisions as to which horses need rehabilitation and which are euthanized based on financial allocation. In order to maintain equine welfare, some of the assigned changes to rescue facilities include more volunteers rather than paid employees, increased turnout for horses to avoid increased bedding costs, construction of turnout sheds in pastures, feeding more round bales rather than square bales to mitigate labor costs associated with square bales, and pulling shoes to lower farrier costs.

4. Survey

To determine its effect on horse owners and horse welfare specifically, a group of British, Australian, and
U.S. researchers conducted a survey of more than 11,000 owners from late March to early April. Lead researcher and independent scientific consultant David Marlin, PhD, presented their findings at the International Society for Equitation Science’s virtual meeting in August. Dr. Jane Williams, Dr. David Marlin, and Louisa Taylor, MRCVS, veterinary surgeon with equine nutrition specialists Science Supplements, carried out the study on the effects of the COVID-19 pandemic on horses and horse owners. In summary, the study revealed that there is an emotional bond that is developed with a horse and owner that is the same as the bond that other people have with a dog or a cat. The horse is considered a member of the family. Lack of time spent between owner and horse resulted in a negative impact on the owner’s mental health and well-being. The common themes seen across all countries were horse health and welfare and horse owner well-being. The effect on people's mental well-being is a theme that in general has become more prominent throughout the COVID-19 pandemic. Impact on the horse included lack of funding for board, labor, feed, hay, and bedding. Financial grants and fundraising are needed to manage horses during the pandemic. Key findings of the study indicated that horses housed at home suffered little impact as a result of the pandemic. Horses that were boarded or partially boarded felt a major impact as a result of the pandemic. In the United Kingdom, survey respondents indicated that 66% are still riding their horse, while 45% adjusted their riding habits to include not jumping their horses, not working with young horses, and not breeding for a foal crop. Many of these constraints were implemented because the rider was a financial provider in their family. In North America, 44% to 84% of horse owners are still riding, while 28% to 37% of the riders have adjusted their riding habits, similar to the U.K. owners. Australians expressed no difference in their riding habits despite the pandemic. Owners in the United Kingdom reported the strictest measures being taken by equine establishments, with 41% of the facilities allowing essential visitors only. When asked if pandemic-related restrictions were affecting horse health, up to 60% of U.K. owners, 30% of North American owners, and 33% of Australian owners said yes. Fifteen percent to 22% of respondents said the pandemic had impacted their access to veterinarians and allied services, while 52% to 85% said it had not yet but they anticipated that it would soon. Given the recent drought and bush fires, Australia was more concerned about the future availability of feed. The North American horse owners were concerned about human well-being and economics as well as restricted time with and lack of purpose for the horse. The U.K. owners were more concerned about risks associated with increased stabling such as obesity and laminitis; peer pressure, especially via social media; and the pandemic’s ongoing influence on equine welfare.

5. Discussion

Two bills were passed into law that will assist the horse industry. As of May 2020, the unemployment rate was 13.3%.

   i. Benefits small businesses and provides payroll protection. Instrumental in maintaining equine facilities and providing extra financial incentive to assist in caring for the horse.
   ii. American Horse Coalition lobbied for bill passage into law.

b. Great American Outdoors Act (S.3422).
   i. Aimed at getting Americans outside and promoting the health of recreational sports and rides.
   ii. Some of the bill was incorporated into other legislative bills. Encourages equine sports outside to help eliminate COVID-19 stress emotionally and physically.

6. Conclusions

It is imperative that rescues and shelters appeal and vocalize to benefactors, the public, and previous donors for money, supplies, and volunteer assistance for the rescue. Transparency with the public for the need of additional resources is necessary for the survival of the shelters and the welfare of the horses. There are many unknowns, and the future for equine welfare and the equine industry has yet to be determined, but a plan must be in place to survive the continued pandemic. It is important that myths such as “you can catch COVID-19 from your horse” are immediately deconstructed. Fear will limit any contact with the horse at many levels of engagement.

Seek guidance on how to access financial support either by loans, grants, or donations. It is imperative that equestrian charities that are suffering a downturn of income are supported. Further studies on COVID-19 and equine welfare during 2021–2022 are needed to verify anticipated changes in the industry.

Acknowledgments

Declaration of Ethics

The Author has adhered to the Principals of Veterinary Medical Ethics of the AVMA.

Conflict of Interest

The Author has no conflicts of interest.

References and Footnote


Personal conversation with Julie Broadway, president of the United Horse Coalition, April 1, 2021.
What Do We Know About the Pathophysiology of Equine Asthma?

Laurent Couëtil, DVM, PhD, DACVIM-LAIM

1. Introduction

The term equine asthma was introduced recently to unify the terminology of chronic pulmonary inflammatory diseases and, more importantly, because of strong similarities between the syndrome in horses and certain phenotypes of asthma in humans. Another important benefit of the new terminology is that horse owners and trainers usually understand that asthma is triggered by exposure to irritants or allergens, and this basic knowledge greatly facilitates the discussion of treatment and management options. Respiratory and musculoskeletal diseases are the two most common causes of training interruption and poor performance in athletic horses. Among respiratory causes of poor performance, equine asthma is increasingly being recognized with signs ranging from mild to severe. Mild-moderate equine asthma (MEA), previously known as inflammatory airway disease, is a common cause of coughing and poor performance in young athletic horses. Studies estimate the prevalence of MEA based on evidence of excess tracheal mucus of around 13% to 22% in racehorses, 31% in sport horses, and 20% in pleasure horses. The prevalence of MEA based on the cytology of bronchoalveolar lavage fluid (BALF) is as high as 80% to 95% in Thoroughbred and Standardbred horses racing in Europe and the United States. Horses with MEA present with normal breathing at rest and occasionally cough, whereas horses with severe equine asthma (SEA), previously known as recurrent airway obstruction disease exacerbation, exhibit obvious increased breathing efforts at rest, cough frequently, and show marked reduction in performance. Severe equine asthma is the most common chronic respiratory disease of mature to older horses, with the prevalence estimated at around 14% in horses living in cool climate regions of the Northern Hemisphere.

2. Etiology of MEA

Infectious Agents

Bacteria

The likelihood of isolating bacteria from tracheal wash (TW) samples collected from racehorses in training is associated with the degree of tracheal inflammation based on cytology. Isolation of more than 10 colony-forming units of pathogenic Streptococcus spp. per ml of TW fluid is associated with coughing. Bacterial species most frequently isolated from TW are Streptococcus spp., Pasteurella /Actinobacillus spp., and Bordetella spp. Mycoplasma organisms, in particular Mycoplasma equirhinis, have been isolated in some horses with MEA. However, up to 54% TW samples from horses with MEA yield no
bacteria. Also, the trachea is not a sterile environment, and recent evidence suggests that horses with MEA harbor a unique airway microbiota. Results from uncontrolled studies suggest that 50% to 69% of racehorses diagnosed with MEA improve after a single course of antibiotic therapy (7-10 days). Therefore, studies demonstrate an association between tracheal microflora and MEA in a subset of racehorses, but the effect on performance is currently unknown. In addition, demonstrating a causal role of bacterial infection will require randomized, controlled trials of antimicrobial therapy to examine potential beneficial effects on respiratory microflora and clinical signs of MEA.

**Viruses**

Acute respiratory infections with equine influenza virus and equine herpes viruses (EHV-1 and EHV-4) in racehorses typically cause systemic illness, such as fever, lethargy, and decreased appetite. Horses with MEA do not show signs of systemic disease, but the potential role of respiratory viruses has been investigated using serological testing or detection of viral genome in airway secretions by PCR. Studies have shown a low incidence of equine herpesvirus, influenza virus, adenovirus, and rhinoviruses in horses with MEA based on serology or virus isolation. Detection of the viral genome by quantitative PCR is controversial, with some studies showing a link between MEA and respiratory infection with EHV-2, EHV-5, and equine rhinitis B virus, but others do not. These differences are due to the fact that the genome of minor respiratory viruses is commonly detected in nasopharyngeal (EHV-2: 30%-76%; EHV-5: 74%-91%; ERBV: 1%-8%) and tracheal (EHV-2: 11%-35%; EHV-5: 0%-55%; ERBV: 8%) secretions of healthy racehorses. These viruses are also commonly detected in nasal or tracheal secretions collected from healthy sport horses (EHV-2: 0%-18%; EHV-5: 0%-41%).

**Fungi**

The role of inhaled fungi and molds is well documented in the pathophysiology of SEA in horses. Their potential role in MEA is suggested by recent studies revealing an association between exposure to beta-glucan in respirable dust and mast cell proportions in BALF from racehorses and an increased risk of MEA (odds ratio = 2.1) in sport horses with fungal elements detected in TW cytology. In this latter study, clinical signs of MEA (nasal discharge, cough, and exercise intolerance) were more frequently detected in horses with positive mycology culture in TW fluid.

**Environmental Factors**

The role of dust exposure in the etiology of MEA in racehorses is suggested by several studies. Exposure to respirable dust (diameter ≤ 4 μm) and not inhalable dust (≤100 μm) measured in the breathing zone of horses is associated with eosinophilic airway inflammation in young racehorses entering training for the first time (1-3 years of age) and neutrophilic airway inflammation in mature racehorses (4 ± 1.4 years of age). Also, horses in training kept on straw bedding experience episodes of MEA that last longer than horses bedded on paper. In sport horses, straw bedding and hay feeding are associated with an increased risk of neutrophilic MEA as opposed to shavings and haylage. In racehorses, mastocytic airway inflammation based on BALF cytology was associated with exposure to beta-glucan, a marker for mold exposure. Atmospheric oxidants such as ozone have the potential to cause lower airway inflammation in horses, but levels encountered during natural exposure are unlikely to induce MEA in otherwise healthy animals. Nevertheless, horses exercising strenuously while exposed to ozone levels comparable to some previously reported ambient levels may develop histologic evidence of airway damage, and oxidant injury may play a role in the pathophysiology of MEA. Horses exercising in cold weather (−5 °C) have a mild increase in BALF neutrophil proportions. Transport of horses over long distances can also induce airway inflammation and colonization of the tracheobronchial tree by bacteria. Strenuous exercise results in colonization of the lower airways by large numbers of bacteria (10- to 100-fold compared to pre-exercise levels), suggesting that tracheal inflammation postexercise is expected, in particular an influx of neutrophils. However, the duration of this inflammatory response is currently unknown.

**Etiology of SEA**

Horses affected with SEA are hyperresponsive to inhaled spores, and exposure to an environment rich in molds such as feeding hay or stabling triggers clinical signs within a few hours to days. Clinical signs usually resolve within days to weeks of the horse being removed from the dusty environment depending on the chronicity of the disease. Studies have documented that traditional horse management practices expose horses to high dust levels originating mainly from forage and, to a lesser extent, from bedding. In addition, horses are exposed to higher levels of dust around the nose (breathing zone) than in the stable because of their feeding behavior. The optimal dry matter content of hay is 85%, and as moisture content decreases in molds such as feeding hay or stabling triggers clinical signs within a few hours to days. Clinical signs usually resolve within days to weeks of the horse being removed from the dusty environment depending on the chronicity of the disease. Fungal spores have a small diameter (<5 μm), allowing them to be inhaled deep in the lung where they may trigger an inflammatory reaction. Furthermore, BALF neutrophilia increases in a dose-dependent fashion as dust exposure rises.

Horses are also exposed to higher endotoxin levels in stables than on pasture. Inhalation challenges
with hay dust fractions showed that endotoxin and other substances (e.g., β-glucans) exert a synergistic effect with molds on neutrophil recruitment to the lungs of SEA-affected horses.52 There is a strong genetic predisposition to SEA in particular bloodlines of Warmblood and Lipizzaner horses.53 Two regions on equine chromosomes 13 and 15 have been associated with SEA, and several candidate genes and protein products have been identified.54,55 These findings strongly suggest that, as in human asthma, genetic susceptibility is an important factor contributing to the development of SEA.

Pathophysiology of MEA

The mechanism responsible for the influx of neutrophils, mast cells, or eosinophils in airways of horses with MEA is still unclear, with both innate and adaptive immune mechanisms potentially playing roles. Some studies report an increase in cytokines in BALF of horses with MEA associated with the innate response (tumor necrosis factor [TNF]-α and interleukin [IL]-1β), whereas others do not (IL-6 and IL-8).56–58 The differences in MEA phenotypes are likely due to differences in inflammatory cytokine gene expression,56,59 but this response is probably modulated by differences in individual horse genomics and environmental exposures. The effect of MEA on performance is dependent on the severity of the disease and the intensity of exercise. Pulmonary gas exchanges are the limiting factor to performance in fit horses exercising strenuously, as illustrated by the marked exercise-induced arterial hypoxemia and hypercapnia developed by healthy racehorses.60,61 During strenuous exercise, horses exercise at or above maximum aerobic capacity (VO2max). In this context, a relatively mild degree of MEA can significantly impair gas exchanges and result in decreased performance.62,63 Improvement in MEA is associated with increased VO2 peak.64 MEA is not likely to cause exercise intolerance in a horse exercising at less than 50% of VO2max until airway inflammation causes marked airflow obstruction or frequent coughing. Therefore, the clinician needs to select diagnostic tools and interpret test results based on the horse’s fitness level and type of activity. Mechanisms responsible for decreased performance in horses with MEA are mainly speculative at this point. A study of Standardbred racehorses performing a submaximal exercise test on a treadmill found that horses with MEA exhibited increased pulmonary artery pressure compared with healthy controls.65 The elevated pulmonary artery pressure was thought to result from increased vascular resistance. Peripheral airway obstruction can be detected in horses with MEA by using sensitive methods such as forced expiration, forced oscillatory mechanics, or rebreathing maneuvers.66–69 Athletic ability as defined by the lactate threshold (speed for a blood lactate of 4 mmol/L) is negatively correlated with BALF neutrophil proportions in fit racehorses with MEA.70 In healthy horses, the degree of arterial hypoxemia is more pronounced as the level of training increases.71 Therefore, assessment of the significance of exercise-induced hypoxemia and lactate threshold is dependent on control data matched for horse age and fitness level.

Pathophysiology of SEA

Horses with SEA during disease exacerbation exhibit pulmonary hypertension and arterial hypoxemia at rest, but blood gas values are not different from healthy controls during periods of disease remission.72,73 Pronounced ventilation-perfusion mismatching is mainly responsible for these gas exchange abnormalities during SEA exacerbation.74,75 During submaximal exercise, gas exchanges worsen, and these abnormalities are associated with decreased expired minute ventilation and increased work of breathing.75 These changes are likely secondary to increased oxygen consumption by respiratory and cardiac muscles, thereby reducing the amount of oxygen available for exercising muscles, resulting in exercise intolerance, as shown in humans with chronic obstructive pulmonary disease (COPD).76 The cascade of events leading to pulmonary dysfunction starts shortly after susceptible horses are exposed to allergens. Circulating neutrophils are recruited to the lungs within 4 hours of an allergen challenge and are detectable in BALF in 5 hours.50,77 The production of numerous inflammatory mediators is upregulated in respiratory secretions or blood of SEA horses after allergen challenge; however, the complex relationships between effector cells, inflammatory mediators, and clinical signs are still unclear.1,78,79 The majority of studies suggest a predominant T helper type 2 (Th2) lymphocyte response as in atopic asthma in humans, but other studies implicate a Th1 or Th17 bias.

3. Conclusion

Environmental factors, in particular exposure to small dust particles, are common triggers for both MEA and SEA. Horses with MEA tend to be young and exhibit mild clinical signs such as intermittent coughing and decreased performance that can easily be overlooked. Horses with SEA are usually older than 7 years of age and have a prolonged history of frequent coughing and increased respiratory efforts. Exercise intolerance is marked during acute exacerbation of SEA, but clinical signs resolve during disease remission. Both MEA and SEA affect many athletic horses, but the diseases can be controlled with appropriate environmental management or medical therapy.

Acknowledgments

Declaration of Ethics

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

Conflict of Interest

The Author has no conflicts of interest.
IN-DEPTH: RESPIRATORY DISEASES IN HORSES

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IN-DEPTH: RESPIRATORY DISEASES IN HORSES


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Exercise-Induced Pulmonary Hemorrhage—An Occupational Hazard of High-Speed Exercise

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1. Introduction

Exercise-induced pulmonary hemorrhage, or EIPH as it is better known, is defined as bleeding from the lungs during exercise. This hemorrhage results in the accumulation of varying volumes of blood in the pulmonary interstitium and airways. Veterinary thinking regarding EIPH has evolved greatly over the last 45-50 years. The advent of flexible fiberoptic tracheoendoscopy (TE) and the seminal work of Pascoe et al. introduced the term “exercise-induced pulmonary hemorrhage” and showed that the prevalence of EIPH in Thoroughbred racehorses was >50% based on a single post-race endoscopic examination. Previously, EIPH had been generally regarded as infrequent and characterized by post-exercise epistaxis. The initial study by Pascoe et al. was followed by a series of additional reports based on TE that indicated that the prevalence of EIPH after racing was similar in Standardbred and Quarter Horse racehorses. Horses were regarded as “bleeders” or “non bleeders” and EIPH was considered to be an abnormality or a disease. It was widely presumed that EIPH impaired performance and post-exercise TE became a common diagnostic test in horses that had trained or raced poorly.

Subsequent studies involving multiple post-race TE of Thoroughbreds and/or the counting of red blood cells (RBC) or visually evaluating the color from post-exercise bronchoalveolar lavage fluid (BALF) led to the recognition that the prevalence of EIPH was greater than had been thought when based on results of a single post-exercise TE. Additional reports of EIPH in horses engaged in other high speed activities such as polo, barrel racing, endurance racing, and steeplechasing supported the suggestion that EIPH is associated with any equine performance involving high-speed exercise. These publications and others documenting evidence of EIPH in the lungs of 18-22 month-old Thoroughbreds in early training and post-race in other 2-yr-olds have led to the realization that the fundamental cause of EIPH must be physiologic rather than pathologic. To think otherwise implies that all horses that exercise at high speed have some degree of pulmonary pathology, regardless of their age, when they first experience EIPH. Such a premise appears to be untenable, with no evidence to support it. However, the presence of blood in the airways and pulmonary interstitial tissues does have a pro-inflammatory effect, particularly in the face of repeated hemorrhagic episodes. In some cases this inflammation is markedly progressive and results in severe pathology that could be career ending or life threatening, with episodes of EIPH, when based on a grade or score reflecting severity, tending to become more severe as the number of lifetime race
starts increases. Under these circumstances, the affected areas of lung can justifiably be regarded as diseased and the presence of these inflammatory changes probably exacerbates the severity of EIPH. However, this does not mean that the underlying cause of EIPH per se is a disease.

While post-exercise TE remains the most widely accepted test for assessment of EIPH, it has been recently shown that it is a relatively insensitive test when compared to BAL. Based on a study of 102 horses that underwent both TE and BAL after a simulated race, about 40% of horses that had EIPH based on analysis of BALF were negative on TE; in other words, TE was associated with a large number of false negative diagnoses of EIPH. In contrast, the sensitivity of BAL approached 1.0, with 99/102 horses positive for EIPH based on a BAL red blood cell count > 992 cells/μl recovered BAL fluid and/or TE. This strongly suggests that EIPH is a ubiquitous event in Thoroughbred racehorses and that its underlying cause is likely linked to the physiologic responses to high-speed exercise that characterizes these events (i.e., EIPH is an occupational hazard for horses engaged in these types of sports).

2. The Pathophysiology of EIPH

The fundamental cause of EIPH is generally regarded as being pulmonary capillary stress failure. This occurs when the capillary transmural pressure (Ptm; the difference in pressure between the inside and outside of the capillaries) exceeds a threshold value. According to cadaveric studies this threshold lies between 75 and 100 mmHg in equine lungs, higher than the equivalent values in rabbits and dogs. Ptm recently measured during high-speed treadmill exercise was reported to exceed mean maximum values of 160 mmHg in some horses. One of the principal reasons that horses competing at high speed or maximal exercise intensities are such elite athletes is due to the volume of RBCs stored in the spleen while at rest and the ability of these animals to autotransfuse or “blood dope” themselves with these cells in response to sympathetic nervous stimulation. Their circulating blood volume increases by up to 50% as a result. This ability to increase circulating blood volume appears to be central to the ubiquitous nature of EIPH in equine athletes undergoing strenuous exercise. Pulmonary arterial hypertension always develops in horses exercising at high or maximum speed (multiple reports >120 mmHg with mean pressures >90 mmHg; summarized by West et al), with this hypertension widely proposed to be responsible for the intravascular pressures associated with pulmonary capillary stress failure. However, arterial pressures rarely, if ever, result in increased capillary pressure, whereas only mild increases in venous pressures can result in increased intravascular capillary pressures. One of the principal determinants of pulmonary venous and capillary pressure is left atrial (LA) pressure. Volume expansion-related increases in left atrial pressure, particularly end diastolic pressure, probably exert a greater effect on pulmonary capillary pressure than the direct effects of pulmonary blood volume expansion. Further insight into understanding how marked increases in LA and pulmonary venous pressures play a key role in EIPH can be gleaned from the pathophysiology of the left-sided cardiac failure. Increases in pulmonary venous and capillary pressures are a hallmark of classic left-sided diastolic heart failure due to the inability of the LA to fully accommodate blood volume during diastole (i.e., volume overload). This form of heart failure is characterized by increased LA diastolic pressure and left ventricular filling pressure, increased pulmonary venous and capillary pressure, and pulmonary arterial hypertension.

Horses galloping at high speed are clearly not in heart failure per se. However, the effects of the splenic contraction-induced rapid expansion of the circulating blood volume on the left heart could be equated with a transient left-sided diastolic failure condition. One of the causes of this diastolic dysfunction is volume overload secondary to a marked increase in circulating blood volume. The most characteristic clinical sign in these cases is a marked increase in LA diastolic and left ventricular filling pressures and pulmonary artery wedge pressure (PAW). PAW is an excellent, albeit indirect, measure of LA pressure. At rest, the LA of a horse typically has a mean pressure of 3–8 mmHg, while that of maximally galloping horses measures in the range of 50–70 mmHg. If the pressure in the LA is that high, then it is inherent that in order for there to be adequate blood flow through the pulmonary circulation, the pulmonary venous and capillary pressures must be higher than LA pressure. Under these conditions, Ptm exceeds the threshold for pulmonary capillary stress failure and EIPH occurs. Direct measurement of LA pressure in exercising horses is technically extremely difficult. Left ventricular filling or end diastolic pressure (LVED) is, however, a valid indicator of LA pressure. The left ventricle of equine athletes is relatively stiff and lacking in compliance, making it almost impossible for horses to accommodate a large increase in circulating blood volume at high heart rates without an associated increase in LVED. Horses galloping on a treadmill with heart rates greater than 200 bpm have marked increases in left ventricular diastolic pressures. Pressures in the latter study were significantly correlated with EIPH score, BALF RBC, PAW, and Ptm. When 14L of blood was acutely removed and horses exercised at the same speed, the LVED, PAW and Ptm decreased as did the severity of EIPH according to both EIPH score and BAL red blood cell number. Replacement of the previously removed blood was associated with a return of these respective pressures to their initial values with EIPH significantly more severe than following blood volume depletion. These data strongly suggested that
there is a close link between circulating blood volume and EIPH. Furthermore, because of the autotransfusing capability of the spleen and its importance relative to the ability to gallop fast despite the impact it has on LVED and P,

3. EIPH and Performance

As understanding of the factors responsible for EIPH grows, the critical question regarding the condition is moving from “Is it a bleeder or not?” to “How bad was it?” The degree or severity of EIPH is usually assessed by applying a widely accepted grading scale that was first published in 2005. This score is usually based on a single TE that almost always takes place 30–90 mins after the completion of exercise. There are 5 grades that can be assigned: 0, 1, 2, 3, and 4, with the prevalence of grades 3 and 4 EIPH being < 0.1 (10%) in any population of horses that exercises regularly at high speed. Several studies involving large numbers of flatracing horses have indicated that a horse’s performance will be adversely affected by grade 4 EIPH. The largest of the three studies also suggested that EIPH was more likely to reduce performance if the grade was ≥ 3, when a different statistical model was applied to the dataset. In this instance, horses with EIPH ≥ 3 were significantly faster in the early and middle sections of a race but then slowed as compared to those with EIPH ≤ 2, which tended to accelerate their average speed over the last 600 m of a race. It is also notable that there was considerable variability in the finishing positions of individual horses in all 3 studies, regardless of EIPH grade, meaning that horses with EIPH ≥ 3 can and do win races or finish in the placings. However, when horses with grades ≥ 3 are considered as a population, they are less likely to win. Whether they are also less likely to finish second or third is equivocal when the results of these three studies are compared. Applying a population finding to an individual is an inexact science. Horses with grade 0 EIPH based on TE can have a dark red appearing BALF with a very high BALF RBC. TE EIPH grade is only weakly correlated with BALF RBC, with about 1/3 of grade 0 cases being false negatives. This might not matter from the perspective of whether undetected EIPH could have affected performance, as it is unlikely that these false negative TE cases were severe enough, based on BALF RBC, to have adversely impacted performance. There is currently no BALF RBC scale that relates EIPH severity to BALF RBC. There is a wide range of BALF RBC numbers associated with any given EIPH TE grade, and it may be that while BAL greatly reduces the number of false negative diagnoses of EIPH, it is no better than TE when it comes to assessing the severity of EIPH. It is generally assumed that the severity of EIPH and the likelihood that it will interfere with performance is dependent on the amount of EIPH. However, there is still no accurate way to quantitate the volume of blood present in the airways and pulmonary interstitium following a bout of EIPH, as determining BALF RBC is, at best, semiquantitative. Instillation of 100 ml of autologous blood into the dorsocaudal region of each lung about 30 mins before a supramaximal treadmill run to fatigue reduced horses’ ability to perform, based on a decrease in maximum oxygen consumption and run time to fatigue. This was in contrast to another study in which infusion of the same area of just the right lung with 100 ml autologous blood failed to demonstrate any significant effect on the same parameters. Each of these studies involved 6 Thoroughbreds, and it may have been that this relatively small number of subjects meant that very large changes in measured variables were required before statistical significance was detectable. Extrapolations based on the number of erythrocytes in circulating blood at the end of exercise and the number of BALF RBC suggest that volume of blood collected by BAL is a lot less than 100 ml. In summary, there is little doubt that EIPH can affect performance in some horses. What proportion of horses might be affected is unclear although, based on the largest study, this appears to be < 10% of the total number of horses racing. The relationship between EIPH and performance is further clouded by the fact that the severity of EIPH in an individual horse can vary from start to start when based on TE EIPH grade. Therefore, it may be that even when EIPH does impair performance, it does so intermittently. The extent to which an occurrence of EIPH grade ≥ 3 in an individual horse is predictive of future episodes of equivalent or worse severity requires investigation, even though the severity of EIPH tends to worsen as the cumulative number of race starts increase when populations of horses are considered as a whole.

Acknowledgments

The Author has been a recipient of research funds from the Grayson-Jockey Club Research Foundation and some of the work supported by that organization is cited in this paper.

Declaration of Ethics

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Conflict of Interest

The Author has no conflicts of interest.

References


Treatment and Management of Mild/Moderate and Severe Equine Asthma

Renaud Léguillette, DVM, MSc, PhD, DACVIM, DACVSMR

1. Introduction
Equine asthma is the most recent nomenclature to define a general disorder of noninfectious lung inflammation associated with respiratory clinical signs of variable severity. Equine asthma is a broad term that encompasses (1) mild/moderate equine asthma (previously known as inflammatory airway disease), (2) severe equine asthma (previously known as “heaves”/recurrent airway obstruction), and (3) summer pasture-associated recurrent airway obstruction (now also referred to as severe equine asthma). The updated equine asthma terminology has been adopted since the pathobiology of asthma found in horses shares similarities with human asthma.1–4 Specific information about the definitions and pathophysiology of equine asthma can be found in the in-depth manuscript from Dr. Couetil that is paired with the present manuscript. This manuscript will focus on the treatment and management of equine asthma. It is important to convey the message to horse owners that although equine asthma can be effectively managed, there is no cure for this disease. Effective management involves a combination of medical treatments and measures to control exposure to environmental organic particulates. Both must be implemented together to be successful over the mid- to long-term period.

2. Medical Treatment of Severe Equine Asthma
The literature disproportionately assesses the effects of medical treatments in horses with severe asthma as compared to mild/moderate asthma. This is likely because it is easier to objectively quantify responses to treatments in horses with severe versus mild/moderate equine asthma since the standard lung function techniques are not sensitive enough to document any of the small treatment responses in horses with mild/moderate asthma. However, since the roots of the pathophysiology are thought to be quite similar between the two types of asthma, practitioners commonly use a similar therapeutic approach for mild/moderate and severe asthma. Equine asthma involves a triad of lung inflammation, bronchoconstriction, and mucus production. Although each of these entities could be treated separately with specific medication for each of these processes, it has been shown consistently that controlling lung inflammation with corticosteroids will also positively impact the other two mechanisms.

Corticosteroids
There is an abundance of evidence supporting the use of corticosteroids in the treatment plan of equine asthma.5–15 It should be noted that an inhaled corticosteroid has been recently approved for commercialization to treat severe equine asthma, which will now have an impact on the treatment decisions made by practitioners.
Dexamethasone is the systemic corticosteroid of reference in equine asthma and has been used as a benchmark in many studies assessing the efficacy of therapies.\textsuperscript{5–12,16–25} It has been evaluated in equine asthma studies with oral, intramuscular, or intravenous administration. All the studies except for three\textsuperscript{16,17,20} used horses with severe asthma. The most frequently measured outcomes were clinical signs, lung mechanics (lung resistance, elastance, and transpulmonary pressure), and bronchoalveolar lavage fluid (BALF) cytology. Systemic dexamethasone sodium phosphate is commonly used at a dose of 0.04 to 0.1 mg/kg IV once daily across studies, without side effects reported on the small number of horses studied\textsuperscript{6–10,12,23} Authors have also reported the effects of dexamethasone sodium phosphate at a dose of 0.04 to 0.05 mg/kg IM once daily without reported local reactions using a product available in Canada and Europe.\textsuperscript{6,16,20} Systemic dexamethasone-21-isonicotinate has also been used at a dose of 0.04 mg/kg every 3 days in a study in the United States.\textsuperscript{26} Studies have assessed the efficacy of oral dexamethasone at doses between 0.05 and 0.16 mg/kg once daily.\textsuperscript{7,9,22}

a) Lung function. Systemic dexamethasone positively influences the clinical signs and lung mechanics in horses with equine asthma in a way that is comparable to the responses obtained by turning horses outside on pasture in dust-free conditions. Systemic dexamethasone (IV and IM) has been shown to significantly improve the lung function of horses with severe equine asthma (9 horses enrolled in a crossover design) after only 3 days of treatment, even when the horses were kept indoors and without measures to decrease exposure to dust.\textsuperscript{12} One study reported that 7 horses with severe equine asthma kept in a dusty environment but treated with oral dexamethasone (0.05 mg/kg) reached the same level of improvement in lung function as when they were put on pasture for a long period of time.\textsuperscript{9} This level of efficacy is not yet matched by any other corticosteroids tested in horses. The rapid and reliable efficacy of dexamethasone justifies its use in equine asthma; however, it comes at the price of potential side effects that can be life-threatening in some cases (e.g., severe laminitis). The side effects are thought to be mainly related to the effects of dexamethasone on the hypothalamic-pituitary-adrenal axis and adrenal suppression. Many studies using dexamethasone in severe equine asthma have reported a marked reversible decrease in blood cortisol, indicating a transient but strong adrenal suppression effect at the dosage used.\textsuperscript{6,7,10,11} Systemic dexamethasone has also been showed to induce a decrease in serum total proteins in horses, likely secondary to some subclinical gastrointestinal tract ulcerations.\textsuperscript{7} Dexamethasone therapy in horses also affects their glucose metabolism, which translates into an increased resting lactatemia.\textsuperscript{27} Systemic dexamethasone IV also affects the lung microbiota of horses with mild/moderate equine asthma, which show an increased abundance of Streptococcus sp. in their airways.\textsuperscript{28} The significance of this finding is still unknown, but it shows that the relationship between bacteria and the horse immune system in the lungs is affected by systemic corticosteroids therapy.

b) Bronchoalveolar cytology. Except for one study,\textsuperscript{12} there is an agreement in the literature that if horses with severe equine asthma are kept in a dusty environment, dexamethasone treatment will not decrease the percentage of neutrophils found in the BALF cytology.\textsuperscript{7–9,11} A similar trend was found in one study on mild/moderate asthma; however, a larger sample population would be required to statistically confirm this finding.\textsuperscript{28} As discussed in further detail in the environmental control section, airway accumulation of inflammatory cells will not persist when exposure to organic particulates is prevented. In comparison, there is some evidence that dexamethasone decreases the accumulation of mucus in the large airways of horses with severe asthma,\textsuperscript{6} even when they are kept in a dusty environment.\textsuperscript{29}

c) Mucus. There is some evidence that dexamethasone decreases the accumulation of mucus in the large airways of horses with severe asthma,\textsuperscript{6} even when they are kept in a dusty environment.\textsuperscript{29}

Oral Prednisolone

When prescribing oral corticosteroids in horses, oral prednisolone should always be chosen over oral prednisone since the latter has been shown to have a poor bioavailability and would be unlikely to have clinical efficacy in horses with asthma.\textsuperscript{26} Oral prednisolone (2 mg/kg) has been shown to have some efficacy at improving lung function of horses with severe asthma, even when kept indoors, but was not found to be as effective as oral dexamethasone (0.05 mg/kg).\textsuperscript{9} It seems that prednisolone therapy can only reach some clinical efficacy comparable to dexamethasone if it is combined with management measures to prevent exposure to aerosolized organic particulates.\textsuperscript{6}

Isoflupredone Acetate

Isoflupredone acetate (0.03 mg/kg IM) has been commonly used in the past in large animal practice. This medication has been shown to quickly improve the lung function of horses with severe asthma and to have a lasting effect, on par with dexamethasone (0.04 mg/kg IV).\textsuperscript{10} Unfortunately, some significant hypokalemia was also noted in the treated horses, which has been associated with myopathies, recumbency, and death in cattle and humans.

Triamcinolone Acetonide

The long-acting triamcinolone acetonide was the first corticosteroid to be assessed in a controlled clinical trial in horses with severe asthma.\textsuperscript{13} It has been shown to improve the lung function of horses with
severe asthma up to 4 weeks after systemic intramuscular administration of this medication at a dose of 40 mg. Interestingly, it has been recently shown that intra-articular injection (40 mg total) of triamcinolone acetonide also improved the lung function of horses with severe asthma for 3 weeks. Practitioners should discuss the potential side effects (particularly severe laminitis) with the owners and avoid repeating the treatment without a long washout period to decrease the likelihood of side effects.

Inhaled Corticosteroids

Because systemic corticosteroids are associated with hypothalamic-pituitary-adrenal suppression, inhaled corticosteroids present the advantage of having a local airway anti-inflammatory activity, while potentially decreasing the systemic absorption. In simple words, the goal of inhaled corticosteroids is basically “treat the lungs only, instead of treating the horse, including the lungs.” Furthermore, although little evidence is available, the withdrawal times are likely significantly less for inhaled than for systemic corticosteroids. However, inhaled therapy presents its own technical challenges, notably to achieve small enough particles to be respirable and reach a good lung distribution of the active product, while limiting ingestion (which could be associated with adrenal suppression). There are basically 3 ways currently used to deliver inhaled medication to the lower airway: mechanical nebulizers (e.g., vibration and jet), metered dose inhalers (MDIs), and a soft mist inhaler (SMI). All 3 techniques have been used in horses to deliver corticosteroids to the lower airways using various devices. The mechanical nebulizers usually involve a mask and a powered device. The MDIs come ready to use in a canister, are procured from the human market, and are delivered using a specialized chamber. The soft mist is the most recent technology approved to treat severe asthma using a specialized delivery system. The size distribution of the droplets varies between nebulizers and affects the amount of medication reaching the deep small airways; the respirable particulates reaching the alveoli/gas exchange area of the lungs should ideally be < 4 microns diameter. However, particulates of much smaller size stay aerosolized in the lungs and are expired back outside.

Mechanical Nebulization: Dexamethasone (Sodium Phosphate)

Dexamethasone sodium phosphate was found to have a low bioavailability when nebulized with a mechanical device and although it did not induce adrenal suppression in a study on healthy horses, it did induce some adrenal suppression on a small number of horses with severe asthma in which only 5 mg of dexamethasone was nebulized. Although this low dose of nebulized dexamethasone was not reported to improve the lung function of horses with severe asthma, more studies are necessary to optimize the dosage for severe asthma cases. Higher doses have been shown to affect the bacterial composition of the upper airways of horses with mild asthma, but the treatment did not have as much effect on the composition of the fungal population in the airways as the environment did. The adrenal suppression induced with higher doses of nebulized dexamethasone (unpublished) decreases the potential benefits of this approach.

Metered Dose Inhalers (MDIs): Fluticasone Propionate, Beclomethasone Dipropionate, Budesonide

When comparing efficacy between studies using MDIs, it should be noted that there were some changes in the type of propellant used over the years, which affects the lung distribution and therefore dosage equivalence. The first controlled studies reporting treatments of equine severe asthma with MDIs used beclomethasone dipropionate. Several dosage regimens have been proposed and showed some overall efficacy to improve the lung function of horses with severe asthma. However, adrenal suppression was also reported, even when using the lowest doses of beclomethasone dipropionate. Fluticasone propionate has a greater affinity for the glucocorticoid receptor and may be slightly more potent than beclomethasone dipropionate. The earliest studies have tested its use in horses with severe asthma over a relatively short period of time, whereas the more recent studies have assessed its effects on lung tissues when used over a very long period of time (up to 1 year of treatment). During short-term treatment periods, fluticasone showed efficacy inferior to that obtained with systemic dexamethasone in horses with severe lung obstruction. As with fluticasone, inhaled budesonide improved lung function in horses with severe asthma, but it was also associated with some adrenal suppression effect.

Soft Mist Inhaler (SMI): Ciclesonide (des-Ciclesonide)

Ciclesonide is a pro-drug with a low affinity for the glucocorticoid receptor, low bioavailability, and a high first-pass metabolism. This means that the small amount of parent drug (ciclesonide) that would be swallowed during inhalation would mostly stay in the digestive system or undergo hepatic metabolism without reaching the systemic circulation. Ciclesonide is metabolized by esterases in the lung epithelium into desciclesonide, which increases its potency by 100-fold. The effects of various doses and inhalation devices on the blood cortisol concentrations in 8 horses with severe asthma maintained in a dusty environment and using dexamethasone as a reference have been recently reported. The results showed no decrease in blood cortisol (indicating no adrenal suppression), even when the highest dose of ciclesonide was used. Contrary to dexamethasone, the serum total protein level did not decrease with ciclesonide therapy. The clinical efficacy of inhaled ciclesonide
on horses with severe asthma was also demonstrated in this study at the dosage of 2700 mcg twice daily and 3712.5 mcg once daily.\(^4\) The lung mechanics and clinical scores were significantly improved by day 7 at the 2700 mcg twice daily dosage, whereas they were improved by day 14 at the 3712.5 mcg once daily dosage. The number of horses reaching complete resolution of lung airflow obstruction with the ciclesonide treatment was similar to the results obtained after administration of a potent bronchodilator control treatment (N-butylisocopamine) in these horses.\(^7\) Recently, a large-scale randomized, controlled, and blinded field study used clinical scoring as a measured outcome to test the efficacy of inhaled ciclesonide (vs. placebo; 2744 mcg twice daily for 5 days followed by 4116 mcg once daily for 5 days) in 224 horses with severe asthma.\(^4\) By design, environmental and treatment conditions were not strictly controlled, and a large number of horse owners and equine practitioners were included, which may explain how > 40% of the placebo horses improved. However, the response rate and improvement in clinical score were much greater in the treatment group with > 73% responders and score decreasing from a mean of 15.3 to 7.2. Interestingly, some horses were diagnosed and treated for pituitary pars intermedia dysfunction but did not show signs of laminitis with the ciclesonide treatment.\(^4\) The blood cytology and chemistry analysis also confirmed the safety of the treatment as it did not show any effect of the ciclesonide therapy.

Additional Therapies Targeting Lung Inflammation

Because of the side effects associated with corticosteroid treatment (other than ciclesonide delivered via SMI), other therapeutic options have been investigated targeting lung inflammation in horses with severe equine asthma.

Dietary Fatty Acid (Omega-3)

The absorption of polyunsaturated fatty acids that are precursors of omega-3 series of polyunsaturated fatty acids decrease the substrate available to produce proinflammatory eicosanoids and inflammatory cytokines. A controlled crossover study evaluating the effect of increased free fatty acid diet content in 9 horses with severe equine asthma using sunflower or seal blubber oil showed an absorption and integration of the fatty acids into the airways’ leukocytes membranes.\(^4\) Although the lung inflammatory cell count from BALF decreased with treatment, there was no improvement in the horses’ clinical response measured by histamine challenge hyperreactivity.\(^4\) Another randomized controlled study evaluated the effect of dietary supplementation of omega-3 polyunsaturated fatty acid for 8 weeks in conjunction with a switch to a complete pelleted diet in 35 client horses with mild/moderate or severe equine asthma.\(^4\) The visual clinical score improved in all horses (including placebo), supporting the benefits of a pelleted diet in horses with equine asthma; however, the clinical score improved to a greater degree for horses on the high fatty acid diet, showing some added benefits of this diet over simple management measures. Interestingly, the BALF neutrophilia decreased in the fatty acid diet group but not in the placebo group. This study shows that the dietary omega-3 fatty acid approach would only be recommended in combination with a low-dust pelleted diet over a long period of time.

Cytosine-Phosphate-Guanine-Oligodeoxynucleotides Nanoparticles

Cytosine-phosphate-guanine-oligodeoxynucleotides nanoparticles are single-stranded synthetic DNA molecules that have an immunomodulatory effect and can direct the immune system reaction away from the allergic Th2 pathway, for example. They have been shown to decrease allergen-induced airway neutrophilia in the lungs, and administration improved clinical signs in horses with severe asthma when delivered by inhalation using an equine nasal chamber device and a mechanical nebulizer.\(^4\) Although not available commercially yet, this may be a therapeutic approach that could have the potential to control horses’ airway neutrophilia in the future.

Tamoxifen

Tamoxifen is a nonsteroidal estrogen receptor modulator that was tested in vitro on equine neutrophils.\(^4\) It affects growth and survival of equine neutrophils and decreases the production of oxidative chemicals,\(^4\) which are favorable properties to control the neutrophilic lung inflammatory cascade observed in severe equine asthma. However, a blinded study evaluating tamoxifen treatment in horses with severe equine asthma did not report any changes in BALF neutrophilia and mucus severity and reported only a slight decrease in the lung resistance.\(^19\)

Hydrosoluble Curcumin

A modified hydrosoluble curcumin derivate, modified for inhalation delivery, has been tested in horses with severe equine asthma either when kept in a dusty environment or after induction of lung inflammation with lipopolysaccharide (LPS) nebulization.\(^48,50\) The curcumin derivative decreased BALF cellularity in the horses with severe equine asthma kept in the dusty environment, but not after the LPS challenge. The production of reactive oxygen products was nevertheless decreased in the latter case. Further studies are necessary to assess the value of this compound in the treatment of equine asthma.

Bronchodilators

It is important to not rely solely on bronchodilation when implementing a therapeutic plan for equine asthma since this approach has been shown to wor-
sen asthma control and increase mortality in people.\textsuperscript{51} Indeed, the use of bronchodilators without decreasing exposure to dust has the potential to increase the number of organic particles reaching the lower airways, triggering an increased inflammatory response.\textsuperscript{52} One study evaluating horses with severe equine asthma treated only with an inhaled bronchodilator (salmeterol) for 3 months while kept indoors reported that horses experienced disease exacerbations after 8 weeks of treatment.\textsuperscript{52} Bronchodilators should be used as a third therapeutic line after environmental changes and corticosteroids. The author only uses bronchodilators in combination with corticosteroids and only as a short-term rescue approach to relieve respiratory symptoms in the most severe equine asthma cases. Research trials assessing bronchodilators in equine medicine have primarily evaluated both relief of the bronchoconstriction and the duration of these effects. The beta-2 adrenergic agonists are available as short- or long-acting products and have been reported to have a rapid onset of action, whereas the muscarinic cholinergic antagonists have a slow onset of action (up to 1 hour). This manuscript will focus on bronchodilators relevant to equine practice and not cover the medications that are used in research or are not easily available.

**Beta-2 Adrenergic Agonists**

The most-studied systemic beta-2 adrenergic agonist in horses is clenbuterol. Its beta-2 adrenergic bronchodilatory effect was first shown in ponies with severe equine asthma,\textsuperscript{54} which was then confirmed using a clinical scoring in a large field study of horses with severe equine asthma treated with oral clenbuterol (0.8-3.2 \textmu g/kg twice daily).\textsuperscript{54} Systemic administration can be associated with side effects that are dose dependent and include sweating, tachycardia, and anxiety.\textsuperscript{54} Furthermore, the internalization of beta-2 adrenoreceptors decreases its bronchodilator efficacy over time.\textsuperscript{56} However, this down-regulation effect is reduced when dexamethasone is added to the treatment protocol in conjunction with clenbuterol.\textsuperscript{56} Oral administration of clenbuterol does not have the ideal pharmacokinetics to be used in horses with respiratory distress since it has a delayed onset of action. It is currently recommended that clenbuterol therapy be used for a short period of time in combination with corticosteroids and in conjunction with appropriate environmental management aimed at reducing aerosolized allergens. Short-acting beta-2 adrenergic agonists used in equine medicine primarily include albuterol and/or salbutamol. The duration of the bronchodilatory effects of these drugs are only 30 to 60 minutes.\textsuperscript{57,58} However, because of their rapid onset of action (maximal bronchodilation within 5 minutes),\textsuperscript{58,59} these drugs are effective for clinical cases in respiratory distress where a quick relief is indicated. Administration of albuterol using an MDI and two different devices has been shown to have a quite similar bronchodilatory response.\textsuperscript{59} Currently, it is recommended to use a commercially available delivery device (nasal application with a chamber\textsuperscript{10}) for delivery of albuterol using an MDI as this tends to have a better efficacy as reflected by the reduced number of “puffs” to reach 50\% maximal bronchodilation level.\textsuperscript{59} Using this commercially available device and an MDI with hydrofluoroalkane propellant, the dose necessary to obtain maximal bronchodilation is 540 mcg (6 puffs) on average. Levalbuterol is the R-enantiomer of the racemic albuterol and does not have the potential adverse effects of the S-enantiomer (at least in vitro), while providing a duration of effect of 120 minutes in horses with severe equine asthma.\textsuperscript{57} This is still a much shorter duration of effect than in humans, where bronchodilation lasts for 6 hours, and is still too short to be practical as the sole medical treatment for horses with severe equine asthma. Salmeterol is a long-acting beta-2 adrenergic agonist that showed some effective bronchodilation in horses with severe equine asthma for approximately 6 hours.\textsuperscript{60} However, the onset of action is slow, taking up to 60 minutes for effects to occur,\textsuperscript{60} making salmeterol less practical of an emergency treatment option for horses in respiratory distress.

**Muscarinic Cholinergic Antagonists**

The flagship of muscarinic cholinergic antagonists is atropine. However, because of the many side effects associated with systemic administration of this drug, inhaled formulations of modified atropine compounds are used instead. The only muscarinic cholinergic antagonist recommended for use in horses is ipratropium, which is available as an MDI. Commercially available options for human use include bare ipratropium solutions, MDIs, dry powder, and SFIs. Most of the studies on horses with severe equine asthma used the dry powder formulation, and it was quickly effective as a bronchodilator at rest. It did not provide any beneficial effect during exercise.\textsuperscript{61} This was also the case for healthy horses treated with this drug, likely because the horses were already at maximal exercise-induced bronchodilation and did not benefit from the ipratropium intervention.\textsuperscript{61-63} Some side effects (decreased gastrointestinal sounds and oral dryness) can be expected in horses after nebulization of ipratropium solution.\textsuperscript{64} This may not be the case when administration is done with MDIs, but it remains to be confirmed. The duration of action of ipratropium in horses with severe equine asthma has been reported to be between 4 and 6 hours, varying a great deal between horses.\textsuperscript{64} It is interesting to note that ipratropium seems to have a greater bronchodilatory effect on the larger airways than on the small peripheral airways.\textsuperscript{64} Revatropate is another muscarinic cholinergic antagonist that has been evaluated in horses with severe equine asthma with the aim to decrease the potential side effects associated with ipratropium solution nebulization.\textsuperscript{64} The improvements in lung function obtained with revatropate and an
ipratropium solution were similar, but the revatropate induced further clinical scoring improvement probably because it induced some more peripheral airways’ bronchodilation.\textsuperscript{64} Revatropate, however, is not commercially available.

3. Medical Treatment of Mild/Moderate Equine Asthma

As mentioned above, because few technologies are sensitive enough to objectively measure the lung function of horses with mild/moderate asthma, clinical trials evaluating medical therapy for this disease process are scarce. Most of the information described above for severe equine asthma is used empirically in mild/moderate equine asthma clinical cases. There are, however, a few exceptions, and a few controlled studies have addressed the clinical efficacy of some medications in mild/moderate equine asthma horses. Sodium cromoglycate, a mast cell stabilizer, improved the clinical signs in a controlled study with 12 racehorses assessed for poor performance and abnormal respiratory signs at a high dose of 200 mg nebulized twice daily.\textsuperscript{65} The effect of this treatment on the performance of those horses was not reported, but the study showed a potential beneficial effect for horses with increased mast cells in the BALF. It may be indicated in cases where a proportion of mast cells is seen degranulating at the BALF cytological analysis. Airway hypersensitivity and hyperresponsiveness are hallmarks of asthma and can be objectively documented using the response to standardized bronchoprovocative challenges (such as with increasing doses of histamine). One study using a randomized crossover design in 8 horses found that dexamethasone IM and inhaled fluticasone propionate both decreased airway hypersensitivity and hyperresponsiveness after 1 week of treatment, but without effect on BALF cytology.\textsuperscript{20} This confirmed that corticosteroids therapy has some value in the treatment of mild/moderate equine asthma. In another study, horses with moderate equine asthma that were kept in a dusty environment had an allergic immunological signature (shown by an increase in IL-17 expression in the BALF).\textsuperscript{20} As expected, systemic dexamethasone therapy effectively decreased the cytokine expression of lung cells from the horses; the BALF cytology, however, was not significantly affected.\textsuperscript{57} This shows that similar to severe equine asthma, a dusty environment impedes the clearance of inflammatory cells in the lungs of horses with mild/moderate equine asthma, even when treated with corticosteroids. The benefit of bronchodilators over corticosteroids as treatment for horses with mild/moderate equine asthma was not reported when measuring the horses’ aerobic capacity assessed by measuring peak oxygen consumption during exercise to fatigue (VO\textsubscript{2peak}).\textsuperscript{16} It therefore appears that once horses have improved the lung inflammation by environmental and corticosteroids therapy, bronchodilators would not add to their aerobic capacity, similar to what has been found in healthy horses.\textsuperscript{16,62}

4. Environmental Measures

Organic dust plays a key role in the pathogenesis of equine asthma (see in-depth manuscript from Dr. Couetil in this AAEP session). Clinical re-response to a moldy hay challenge has been used for decades and is included in the definition criteria that assist in distinguishing mild/moderate from severe equine asthma.\textsuperscript{66} Horses with severe equine asthma will show some bronchoconstriction, sometimes within hours, after exposure to moldy hay, which translates into clinical signs of labored breathing at rest.\textsuperscript{66} When a moldy hay challenge is repeated in horses with severe equine asthma, their lung function deteriorates in a predictable manner after each challenge,\textsuperscript{67} contributing to the wide use of the moldy hay challenge as a model in research for severe equine asthma. Horses with mild/moderate equine asthma will present with abnormal respiratory tract clinical signs at rest but with no increase in respiratory effort after the challenge. Environmental air quality, however, seems to be equally important for mild/moderate equine asthma.\textsuperscript{16} It has been known for a long time that bringing horses indoors in an environment without measures to limit dust exposure will trigger an influx of neutrophils in both healthy and severe equine asthma horses; the inflammatory response can reach extreme levels in research horses known to have consistently severe equine asthma (> 71% BALF neutrophils as compared to > 27% BALF neutrophils in healthy horses).\textsuperscript{68}

Improvement in air quality has an overriding effect over treatments with corticosteroids in horses with mild/moderate equine asthma,\textsuperscript{16} as well as in horses with severe equine asthma.\textsuperscript{69} Keeping horses with severe equine asthma indoors, but combined with measures to decrease exposure to hay and bedding dust, improves clinical signs in horses with severe equine asthma within 3 days.\textsuperscript{69} There is a strong agreement in the literature, however, that except for the particular case of pasture-associated severe equine asthma,\textsuperscript{70,71} several weeks of pasture will provide the lowest level of lung airway obstruction and inflammation.\textsuperscript{69,72–75} In areas where pasture-associated severe equine asthma is prevalent, horses pastured are also susceptible to have seasonal moderate and severe lung inflammation, with up to 87% of horses in a teaching herd having at least some mild lung inflammation during the winter.\textsuperscript{76} Assessing air quality is usually performed with measurements of airborne particulates, with particular attention to those of a size that can reach the lower airways (i.e., respirable dust). Aerosols can be divided into 3 categories regarding their deposition in the respiratory system, and based on human data, it is accepted that 50% of the particulates in each category will achieve the following distributions: the inhalable, the thoracic, and the respirable fraction. Fifty percent of the particulates with 100 microns diameter will be inhalable (deposit in the respiratory tract, extra-thoracic), 50% with 10 microns diameter will be thoracic (deposit in
conducting airways), and 50% with 4 microns diameter will be respirable (deposit in the gas exchange/alveoli of the lung). It was recognized early on that activities such as mucking or sweeping alleys in barns contribute to the concentration of total and respirable dust. It is important, however, to note that breathing zone concentration for particulates is more relevant than barn concentration and that there is no correlation between the two zones’ dust concentration. This is also emphasized since there is a great variability between horses’ exposure to dust when stabled as some horses may be pacing in their stall or have other habits that favor dust aerosolization. In addition to airborne dust concentration, attention should be paid to the composition of the dust and in particular its concentration of endotoxins and beta-glucan. Standardized hay dust suspension has been used to challenge horses, and although it triggered a lung neutrophilia in horses with severe equine asthma (and a significantly lower response in healthy horses), the challenge did not induce clinical signs associated with bronchoconstriction. Importantly, all dusts are not equal and there is a synergistic effect between organic dust concentration, endotoxin, and beta glucan content. A longitudinal study in a racing barn with moderate levels of airborne dust found that the concentrations in beta-glucans and endotoxins were not proportional to the total dust concentration; furthermore, there was an opposite seasonal increase between the two components as the beta-glucans were greater in the winter and the endotoxins were greater in the summer. Therefore, variability in those three critical dust components cannot be considered to be correlated. There is a clear agreement between studies that improving hay and bedding conditions will have a dramatic effect to reduce airborne dust. For example, when horses were switched from a conventional management with straw bedding and hay to a low-dust management with wood shavings and pelleted diet, not only did the area respirable particulates decrease by 50%, but more importantly, the breathing zone respirable fraction also had only 3% of the dust measured with the conventional management. However, between the hay and the bedding, the forage has a greater impact on the respirable dust and endotoxin concentrations in the breathing zone of horses than the bedding.

Hay

Fungi are found in hay and bedding and play a critical role in the pathophysiology of severe equine asthma; challenges with mold antigens (Microspora faeni, Aspergilus fumigatus, and Thermoactinomyces vulgaris) done almost 30 years ago to assess the bronchoconstrictive response of horses with severe equine asthma. A European retrospective study showed that horses were 2 times more likely to have mild/moderate equine asthma when fungal elements were observed in the transtracheal wash cytology. As mentioned above, endotoxins are also important, and haylage is an effective method to decrease endotoxin concentration in the air. However, it is not practical for all areas, and when hay is used, the type of forage and the timing of its harvest and storage conditions are also important; late-harvest, second-cut hay stored in a dry environment is recommended to decrease the dust and fungi content. In any case, hay nets should also be avoided as they have been shown to increase exposure to dust in racing stables, and respirable dust exposure is associated with lung eosinophilia. Many hay options have been tested regarding hay handling in barns, all aimed at decreasing the respiratory risk associated with feeding unprocessed hay. Simply immersing hay in water resulted in a 60% decrease in respirable dust concentration, but it seems that soaking hay for 30 minutes may be the most beneficial approach with a reduction by 90%. However, a detailed analysis of the dust composition was not provided in those studies. There is a clear consensus among studies that steaming hay decreases bacterial and mold content, however, its effect on endotoxin exposure has not yet been described. Steamed hay is appetent and increases the dry matter intake of the horses. There are few data on the clinical efficacy of hay steaming in severe equine asthma horses. A study using a very small group of horses with severe equine asthma reported some benefits on mucus scoring, inhaled fraction, and exposure to endotoxins; however, the study did not have enough power to definitely conclude that steamed hay improved clinical signs in severe equine asthma. Pelleted hay has been used as a reference in clinical trials, with an oil mixed hay system shown to have equivalent efficacy. Lung mechanics were improved after only 6 days and remained improved for the 93 days of the study, while mucus score improved after 13 days and airway neutrophilia improved after 65 days of the oil mixed hay system diet.

Bedding

Many studies have also assessed a variety of beddings to decrease ambient and breathing zone particulates in horses. It is usually accepted that wood shavings are a practical and effective method to decrease ambient dust in barns, however, it should be kept in mind that excellent quality straw can have similar benefits. Wood shavings are often more practical and easier to obtain than the best-quality straw. Wood shavings combined with grass haylage are effective at maintaining horses with severe equine asthma free of symptoms even when kept indoors. Newspaper and cardboard bedding have been proposed as horse bedding options. Cardboard bedding was effective at maintaining horses with severe equine asthma in remission while kept indoors on grass silage.
Ambient Temperature

Heat (25°C) in the barns may worsen severe equine asthma as compared to warm temperature (18°C), supporting the possibility that cold temperature could be considered to help with uncontrolled severe equine asthma cases.  

5. Conclusion

In conclusion, environmental management of dust is important to successfully maintain horses with severe equine asthma free of symptoms and free of lung inflammation. Although it may be difficult to convince horse owners that these environmental measures are more important than medication, such discussions are worth the veterinarian’s time since owners who try to apply some measures do perceive a clear improvement in clinical signs.  

Acknowledgments

Funding Sources

The Author would like to thank the Calgary Chair in Equine Sports Medicine at the University of Calgary for its support.

Declaration of Ethics

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

Conflict of Interest

Dr. Léguillette has been remunerated by Boehringer-Ingelheim for a presentation on equine asthma.

References and Footnotes


*Respirat™, Soft Mist Inhaler, Boehringer Ingelheim International GmbH, Germany.*

*Flexineb™, Nortev, Ltd., Co. Galway, Ireland.*


*Aservo Equihaler™, Boehringer Ingelheim Vetmedica GmbH, Germany.*
How to Perform an Equine Esophagram

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1. Introduction

Esophageal disorders occur with relative frequency in equine practice, the most common being esophageal obstructions.1 While most esophageal obstructions are transient and require no imaging diagnostics, in cases with a history of dysphagia, recurrent esophageal obstruction, or other esophageal disease, an esophagram may provide important diagnostic information. Esophagrams are particularly indicated in cases of recurrent obstruction since these are more frequently associated with underlying morphological or functional esophageal disturbances.2 These may include, but are not limited to, stricture formation, diverticula, and motility disturbances. Less frequently, obstruction can result from extra-esophageal sources such as neoplastic masses and abscesses. Clinical signs of esophageal disease may include nasal discharge containing saliva/food, signs of dysphagia such as quidding/dropping food and delayed eating, gagging, and coughing, although more vague presentations such as anxiety and anorexia may also be seen.3 Esophageal perforation and rupture secondary to both intraluminal obstruction and external trauma have also been reported within the literature.4,5 Indicators of esophageal rupture can include a painful ventral cervical swelling with associated crepitus, cellulitis, and potential drainage.4 Cases of esophageal perforation may present with colic signs and pleural effusion, and this should always be considered as a differential diagnosis for such cases.5 An esophagram can be performed safely and effectively and can often be acquired using portable x-ray equipment. It allows assessment of esophageal integrity and morphology and is a helpful complement to esophagoscopy in the investigation of equine esophageal disorders. Although fluoroscopic dynamic esophagrams are reported within the literature, because fluoroscopy is not available for most practitioners, this article will focus on the acquisition of a static radiographic esophagram.

2. Materials and Methods

Timing

Passage of a nasoesophageal tube and/or endoscope can cause esophageal dilation and optimally would not be performed immediately prior to the exam. If chemical restraint was used to facilitate prior nasoesophageal tube placement, the interval before the esophagram should be at least 30 minutes.6

Sedation

If possible, sedation of the patient for radiograph acquisition should be avoided due to reported effects on esophageal motility.5,8 If sedation is required, the authors have used low-dose xylazine (0.2–0.4 mg/kg)1 to safely acquire diagnostic images.
Survey Radiographs
Prior to contrast administration, standing right-to-left laterolateral radiographs should be obtained. A complete radiographic examination of the esophagus should include the pharynx, cervical, thoracic, and abdominal esophagus. Images should be centered on the cranial esophagus caudal to the mandible, mid-esophagus ventral to the cervical spine, thoracic inlet, thoracic esophagus dorsal to the heart, and abdominal esophagus at the level of the dorsal diaphragm (Fig. 1). For radiography of the esophagus in an adult horse, exposures in the region of 80 to 120 kVp and 4 to 60 mAs are required, dependent on the radiographic system used. Due to the dorsal position of the esophagus within the thorax, it is usually possible to perform diagnostic studies with portable radiographic systems. Acquisition of survey radiographs is important, first, since they may themselves reveal esophageal abnormalities including any pre-existing gas or fluid dilation and, second, because they enable practitioners to optimize radiographic technique before contrast administration.

Foals may be radiographed in left lateral recumbency for ease of restraint, although in such cases, contrast medium should be limited to barium paste to reduce the risk of aspiration (Fig. 2).9

Contrast Selection
Liquid barium is the most frequently used contrast agent for esophagrams. In the majority of cases at the authors’ institution, 60% w/v barium suspension2 is utilized. However, in cases where esophageal perforation is suspected, nonionic iodinated contrast media should be used in place of barium since the latter has been associated with mediastinitis. If liquid barium does not identify a lesion consistent with the clinical suspicion, or in horses in which failure of patient compliance precludes intubation, barium may be mixed with feed and offered to the horse. A slurry/mash can be made of pelleted feed and 350 mL 60% w/v barium suspension.

Contrast Administration
Liquid contrast can be administered via 60-mL oral dosing syringe or via nasogastric tube. The syringe method requires multiple administrations, often gets a smaller bolus, and can be particularly messy. In most cases, the nasogastric tube is the preferred route unless a very cranial abnormality is suspected. Radiography may be performed with the tube in situ to confirm tube location prior to contrast administration. Careful intubation is important since manipulation of the tube has been shown to induce esophageal dilation.6 Once the tube has successfully been positioned in the cranial esophagus, a bolus of 350 to 500 mL of barium is administered under gravity. As the barium is being delivered, personnel should position the x-ray machine and plate appropriately to acquire images in a timely manner.

Image Acquisition
Radiographs are obtained immediately following barium administration. The decision on where to begin radiograph acquisition may depend in part on the suspected lesion location—it is recommended to focus on this region initially to ensure that barium passage through this area is adequately demonstrated. If no specific lesion location is suspected, begin caudally and move cranially. Radiographs may be repeated every 1 to 2 minutes until the contrast medium has disappeared completely. This usually occurs within a few minutes.10 Repeat boluses may be administered if required. In one study, two boluses of barium suspension, each of 2.5 mL/kg, were administered without associated signs of patient discomfort.10
Follow Up

If an intraluminal obstruction is visualized via esophagram as failure of passage of the barium bolus, it is recommended to repeat the study following clearance of the obstruction to ensure that no underlying esophageal lesion such as a diverticulum or mass is present. It may also be desirable to perform thoracic radiographs in the few days following clearance of esophageal obstruction to look for evidence of aspiration pneumonia. This secondary complication has been seen in up to 44% of horses presenting with esophageal obstruction. Thoracic radiographs may be particularly valuable in horses with a longer duration of clinical signs since these cases have been shown to be more likely to develop aspiration pneumonia. Thoracic ultrasound should also be performed in suspect aspiration pneumonia cases.

3. Results

At the authors’ institution, over a 10-year period (2010–2020), 18 positive-contrast esophagrams were performed. For many of these studies (n = 8), multiple (≥ 2) sequential 350-mL boluses of 60% w/v barium were administered via nasoesophageal tube. For three studies, the patient consumed barium-impregnated feed; in two cases, barium was administered per os via dosing syringe; in one case, barium administration was via both dosing syringe and nasoesophageal tube; and in one, both barium-coated feed and nasoesophageal intubation were utilized. Information regarding route of barium administration was not available for three cases. The normal passage of the esophagus is outlined in Figure 1.

On precontrast survey radiographs, the normal esophagus is difficult to discern since it does not contain air. Occasionally, a contrast bolus may persist within the normal esophagus until a second peristaltic wave completes passage into the stomach, particularly if a nasoesophageal tube is in place. Common locations for this temporary bolus retention are the thoracic inlet, just cranial to the cardiac silhouette, and at the cardia. In one study, disappearance of a barium bolus at the thoracic inlet by 2.5 minutes following administration was seen in 80% of normal horses, and bolus disappearance in the thoracic esophagus occurred in less than 2 minutes in 90% of horses. If material persists in these locations for multiple sequential radiographs and beyond these time limits, esophageal dysfunction may be suspected. In a normal esophagus, small volumes of residual contrast medium may be seen outlining the longitudinal mucosal folds after the passage of the bolus (Fig. 3). Several studies, and a number of cases at the authors’ institution, demonstrated a roughly U-shaped conformation of the caudal cervical esophagus in some normal horses; therefore, this should not be overinterpreted as a diverticulum (Fig. 4). The esophagus was deemed to be radiographically normal in six of the esophagrams performed within the study period.

In two of the horses examined within the study period, generalized esophageal dilation was seen as a wide, gas-filled viscus in the region of the esophagus on plain films (Fig. 5) and as static contrast material within the dilated esophagus on sequential radiographs as part of the esophagram (Fig. 6).
particularly important to perform concurrent or subsequent thoracic radiographs on cases of generalized megaesophagus since these horses are at higher risk of aspiration pneumonia (Fig. 7).13

Peri-esophageal abscesses, tumors, or cysts may be appreciable as soft-tissue masses that might cause deviation of the esophagus and/or trachea. Figure 8 demonstrates a case of peri-esophageal abscess seen within the study period. Where esophageal perforation is suspected, secondary to either chronic obstruction with intraluminal necrosis or external trauma, gas and/or feed material may be seen within the peri-esophageal soft tissues. Intraluminal obstruction with radio-opaque food material may appear as an ovoid heterogeneous mottled gas and soft-tissue opacity, often located at the thoracic inlet, heart base, or cardia. If the obstruction is well visualized on survey radiographs, it may be prudent to forgo contrast administration due to the risk of reflux and aspiration of barium. No cases of either simple esophageal obstruction or perforation were seen within the study period at the authors’ institution, and food material was only seen within diverticula. Strictures resulting from intramural scarring secondary to previous obstruction or other esophageal trauma may be visualized as a smooth narrowing of the esophageal lumen with contrast retention oral to this region (Fig. 9).9 This abnormality should be demonstrated to persist over sequential radiographs so as to differentiate it from a normal peristaltic contraction or esophageal spasm.9 Strictures were suspected radiographically in four horses in the study period.

Finally, esophageal diverticula are visible as outpouchings of the esophagus in which contrast material will usually accumulate (Fig. 10). It can be difficult to distinguish a true diverticulum from focal dilation radiographically. Diverticula may be classified as pulsion, in which herniation of mucosa occurs secondary to muscularis injury—usually as a result of chronic impaction—or traction, resulting from periesophageal scarring. It is reported in the literature that traction diverticula are spherical with a wide neck, whereas pulsion diverticula have a flask-like shape on esophagrams.14 In addition to contrast, food material and gas may be seen within the diverticula. Diverticula were detected radiographically in eight horses within the study period.

Two horses within the study period were suspected of having a functional esophageal abnormality based upon delayed passage of contrast medium. Several horses had multiple concurrent esophageal abnormalities, such as stricture with oral diverticulum (Fig. 9).

In one esophagram performed during the study period, it was recommended to repeat the study due to ingestion of an inadequate volume of barium-coated feed. The only other reported complication associated with esophagram radiography was a single
instance of liquid barium aspiration in a 2-month-old foal (Fig. 2). In this case, barium administration via nasoesophageal tube was performed; however, the presence of a congenital stricture likely resulted in reflux and aspiration of contrast material. The foal was treated for pre-existing aspiration pneumonia and made a full recovery with apparent stricture resolution.

4. Discussion

Contrast radiography can help to distinguish between generalized megaesophagus and focal esophageal dilatations and their possible causes. Generalized megaesophagus usually reflects a functional abnormality and has been reported secondary to gastroduodenal ulceration, gastroesophageal reflux, cardiac sphincter anomaly, grass sickness (United Kingdom), congenital esophageal neural deficits, and a hereditary condition associated with muscular hypertrophy of the distal esophagus in Friesian horses. Focal esophageal dilatation and associated contrast accumulation may be secondary to either intra- or extraluminal obstructions, including foreign bodies, extraluminal masses, vascular ring anomaly, or stricture, or may indicate diverticula.

On consultation with radiologists at other referral hospitals, variations in preferred esophagram technique are apparent. Two institutions utilize a 1:1 dilution of barium suspension (60–105% w/v) with water administered via nasogastric tube. This information is valuable since it indicates that a smaller barium dose can be used in a similar bolus volume to successfully demonstrate esophageal abnormalities, thus reducing cost. Opinions regarding the success of barium-coated feed ingestion vary, with some individuals citing a frequent failure to tempt the horse to eat and others advocating for barium-impregnated mash as the primary technique utilized. At one institution, the favored approach is to begin with ingestion of a liquid slurry/mash and follow with increasingly dense barium-coated feed material to assess esophageal function with a variety of media. Where the administration of barium-coated feed has been attempted within the authors’
institution, a variety of coated feed types have been offered in order to maximize the chances of success, with the addition of sweet feed, treats, or cookies providing additional incentive. Fasting the horse prior to the study can also help encourage voluntary ingestion of barium. If it is possible to tempt the horse to eat the barium product, this does have the advantages of, first, avoiding the challenge of intubating a horse without sedation and, second, enabling visualization of bolus manipulation and swallowing in cases of dysphagia. In these patients, it is particularly important to assess for evidence of barium dorsal to the soft palate, or within the larynx or trachea, indicating pharyngeal dysfunction. Abnormal esophagram findings have been reported in cases of congenital anomalies such as cleft palate and postsurgically in cases of laryngeal hemiplegia.

No cases of simple obstruction were seen at the authors’ institution. This likely reflects the study population of a referral hospital, with most simple obstructions resolved in the field prior to or without need for referral. In cases that presented to the hospital with clinical suspicion of obstruction, these were either cleared before the esophagram was performed or identified as being secondary to diverticula on radiography.

Soft-tissue masses, whether extraluminal or within the wall of the esophagus, may cause compression or displacement of the esophagus, which will be readily visible on contrast studies. Ultrasonographic evaluation of the cervical esophagus/thoracic inlet and additional diagnostic procedures may be required to help differentiate abscesses, neoplasia, cysts, or other soft-tissue masses. Abscesses of the esophageal wall may be associated with irregularity of the mucosal surface since these may originate from internal trauma.

Esophagoscopy is a useful adjunct to esophageal radiography and can be used in combination with esophagrams to provide the most comprehensive clinical picture to facilitate accurate diagnosis, implement successful treatment regimens, and assist with prognostication. Esophagoscopy provides important information regarding the gross appearance and integrity of the esophageal mucosa, which may not be apparent radiographically.

Management and treatment of esophageal disorders identified on esophagram vary depending on the specific lesion type. Medical management of esophageal obstructions is common, and these cases, if isolated incidents without associated aspiration pneumonia, typically have a favorable outcome. Recurrent obstruction is more likely to be associated with an underlying morphological or functional abnormality, which may carry with it a poorer prognosis. Standing endoscopic balloon dilation of esophageal strictures has been reported with a long-term survival rate of 56%. Options for surgical correction of strictures include esophagomyotomy, resection and anastomosis, creation of a traction diverticulum, or patch grafting. Interventions for treatment of stricture should be delayed for 60 days after the original presentation since some studies have demonstrated an increase in luminal diameter over this time period following the inciting incident. Traction diverticula infrequently produce clinical signs requiring medical intervention; however, pulsion diverticula may undergo progressive enlargement, predisposing to recurrent obstruction and esophageal rupture, and may require surgical repair. Medical management is the only option for generalized megaesophagus due to congenital or acquired esophageal dysfunction.

In conclusion, the esophagram is a valuable tool for diagnosis and characterization of functional and morphological esophageal abnormalities in horses and can be readily performed with infrequent complications by the well-prepared equine practitioner.

Acknowledgments

The Authors would like to thank Dr. Nathan Nelson, Dr. Kate Wulster, Dr. Erin Porter, and Dr. John Griffin for their contributions toward this manuscript.

Declaration of Ethics

Retrospective case series (level-4 evidence-based medicine) performed in adherence with the Principles of Veterinary Medical Ethics of the AVMA.

Conflict of Interest

The Authors have no conflicts of interest.

References

Deep Digital Flexor Tendon Lesions in the Pastern Are Associated with the Presence of Distal Tendinopathy

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Deep digital flexor tendon (DDFT) injuries, particularly core lesions, in the pastern are associated with additional tendinopathy within the hoof capsule. Further assessment of the extent of injury is indicated when pastern lesions are found. Authors’ address: Colorado State University, College of Veterinary Medicine, 300 West Drake Road, Fort Collins, CO 80523; e-mails: elizabeth.acutt@colostate.edu; myra.barrett@colostate.edu. *Corresponding author; †presenting author. © 2021 AAEP.

1. Introduction

Correct diagnosis of DDFT lesions allows targeted treatment and improved prognostication. This study aims to assess the prevalence of, and characterize, DDFT lesions in the pastern with concurrent tendon injury distally. It is hypothesized that tendinopathy, particularly core lesions, in the pastern will be associated with distal lesions of the DDFT.

2. Materials and Methods

Cases with DDFT lesions in the pastern with magnetic resonance imaging (MRI) or ultrasound of the foot were evaluated retrospectively. Lesion location and type were recorded. Odds ratios were calculated to determine associations between more distal tendinopathy and the presence of different DDFT pastern lesion types.

3. Results

Thirty-four MRI scans of 33 horses and 64 ultrasound exams of 58 horses were analyzed. Lesion location and type were recorded. Distal DDFT lesions were found in 75% of total cases of pastern DDFT tendinopathy and in 97% of cases with core lesions. A core lesion in the pastern was significantly more likely (OR = 20.7, 95% CI [2.2, 191.0], p = 0.008) to be associated with injury in the foot than other types of pastern lesion.

4. Discussion

Pathologic changes in the DDFT, particularly core lesions, are typically not an isolated finding. Further imaging is optimal to characterize the extent of tendinopathy to direct appropriate treatment and improve prognostication.
Acknowledgments

Declaration of Ethics

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

Conflict of Interest

The Authors have no conflicts of interest.
Comparison of Bone Scintigraphy and Standing $^{18}$F-NaF Positron Emission Tomography for Imaging of the Fetlock in Thoroughbred Racehorses

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Positron emission tomography (PET) imaging had a higher interobserver agreement than scintigraphy for assessment of the racehorse fetlock and detected additional abnormalities, in particular in the proximal sesamoid bones. Authors’ addresses: School of Veterinary Medicine, University of California, Davis, One Shields Avenue, Davis, CA 95616 (Spriet, Arndt, Pige, Pye); Southern California Equine Foundation, 285 West Huntington Drive, Arcadia, CA 91007 (O’Brion); Equine Medical Center, PO Box 1491, Sierra Madre, CA 91025 (Carpenter); von Bluecher, Blea & Hunkin, Inc., 282 West Sierra Madre Blvd, Sierra Madre, CA 91024 (Blea); 400 Harvard Drive, Arcadia, CA 91007-2638 (Dowd); e-mail: mspriet@ucdavis.edu. *Corresponding and presenting author. © 2021 AAEP.

1. Introduction
A positron emission tomography (PET) scanner, specifically designed for use in standing, sedated horses has recently been introduced. The goal of this study was to compare the ability of standing PET to bone scintigraphy for the detection of abnormalities in the fetlocks of Thoroughbred racehorses.

2. Materials and Methods
Thirty-three horses (72 fetlocks) imaged with both PET and scintigraphy for investigation of lameness or performance issues were included in this study. Seven observers, including experienced racetrack practitioners and radiologists, independently reviewed all data for evidence of increased radiopharmaceutical uptake in 10 different regions of interest.

3. Results
The interobserver agreement was higher for PET (Kappa-weighted (K-w) 0.73 (0.51-0.84)) (median (range)) than for scintigraphy (0.61 (0.40-0.77)) (P < 0.0001). When scintigraphy and PET were compared, the agreement was only fair (K-w 0.29). Agreement between the two modalities
was higher for the palmar condylar regions (K-w 0.59) than for the proximal sesamoid bones (K-w 0.25). PET detected abnormalities in 23/144 (16.0%) of proximal sesamoid bones, whereas scintigraphy detected abnormalities in only 4/144 (2.8%).

4. Discussion
The high interobserver agreement for PET, despite the recent introduction of this technique, demonstrates the ease of clinical interpretation of PET scans. The higher number of lesions detected with PET compared with scintigraphy may be explained by the cross-sectional nature and higher spatial resolution of PET imaging.

Acknowledgments
This study was funded by the Dolly Green Research Foundation with support from the Stronach Group, and by the Grayson Jockey Club Research Foundation.

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

Conflict of Interest
The Authors have no conflicts of interest.
Longitudinal Monitoring of Fetlock Injuries in Thoroughbred Racehorses Using Standing $^{18}\text{F-NaF}$ Positron Emission Tomography

Jannah Pye, DVM, DACVS; Mathieu Spriet, DVM, MS, DACVR, DECVDI, DACVR-EDI*; Julie O’Brion, RVT; Ryan Carpenter, DVM, MS, DACVS; Jeff Blea, DVM; and Joe Dowd, DVM, PhD

Positron emission tomography (PET) findings in the racehorse fetlock were repeatable. Significant reduction in radiopharmaceutical uptake associated with the palmar condyles and medial proximal sesamoid bone was appreciated over a 3-month lay-up period. Authors’ addresses: School of Veterinary Medicine, University of California, Davis, One Shields Avenue, Davis, CA 95616 (Spriet, Pye); Southern California Equine Foundation, 285 West Huntington Drive, Arcadia, CA 91007 (O’Brion); Equine Medical Center, PO Box 1491, Sierra Madre, CA 91025 (Carpenter); von Bluecher, Blea & Hunkin, Inc., 282 West Sierra Madre Blvd, Sierra Madre, CA 91024 (Blea); and 400 Harvard Drive, Arcadia, CA 91007-2638 (Dowd); e-mail: mspriet@ucdavis.edu. *Corresponding and presenting author. © 2021 AAEP.

1. Introduction
A standing equine positron emission tomography (PET) scanner has recently been developed that permits serial PET imaging without the risks associated with general anesthesia. The goals of this study were to assess the repeatability of standing PET findings and to evaluate the ability of PET to monitor progression of lesions in the fetlocks of Thoroughbred racehorses.

2. Materials and Methods
Twenty-five horses (54 fetlocks) were imaged with standing $^{18}\text{F-NaF}$ PET on three occasions six weeks apart. Images were reviewed by a radiologist and an equine surgeon, and areas of increased radiopharmaceutical uptake (IRU) were graded using a previously validated system and quantified using maximal standardized uptake values (SUVmax). Statistical comparisons were made between scans to detect changes in lesion grade and SUVmax over time.

3. Results
Standing PET findings were found to be repeatable, with 131/149 (88%) areas of IRU identified on the initial scans seen again at the 6-week follow-up.
Overall, 65% of fetlocks demonstrated improvement in lesion grade during the 3-month lay-up period. Significant differences for SUVmax ratios between the different scans were reached for the lateral and medial palmar condyles and the dorsal aspect of the medial proximal sesamoid bone (P = 0.0006, P < 0.0001, and P = 0.0006 respectively).

4. Discussion
Standing PET scans may be used to monitor areas of the fetlock involved in catastrophic breakdown injuries in Thoroughbred racehorses.

Acknowledgments
This study was funded by the Dolly Green Research Foundation with support from the Stronach Group, and by the Grayson Jockey Club Research Foundation.

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

Conflict of Interest
The Authors have no conflicts of interest.
External Transcutaneous Ultrasound Technique in the Equine Cricoarytenoideus Dorsalis Muscle: Assessment of Muscle Size and Echogenicity with Resting Endoscopy

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Ultrasound imaging of the equine cricoarytenoideus dorsalis muscle (CAD) can be performed transcutaneously using a simple method. Authors’ addresses: 203 Mitsuishi Higashi-hourai, Hidakagun Shinhidakacho, Hokkaido 059-3105, Japan (Satoh); Hokkaido South Agricultural Mutual Aid Association, Mitsuishi Animal Medical Center, Hokkaido, Japan; 200 Mitsuishi Higashi Horai, Shinhidakacho, Hidakagun, Hokkaido 059-3105, Japan (Higuchi, Miyakoshi, Yoshimura, Kaido, Shimizu); e-mail: satou_masato@minami-hkd-nosai.or.jp. *Corresponding and presenting author. © 2021 AAEP.

1. Introduction
Recent studies have assessed the cricoarytenoideus dorsalis muscle (CAD) using transesophageal ultrasonography in equine recurrent laryngeal neuropathy (RLN). This paper assessed the CAD using the external transcutaneous ultrasound technique, which may constitute an easier method in horses.

2. Materials and Methods
The axial plane thickness, cross-sectional area, and echogenicity of the L (left) CAD and R (right) CAD were measured using transcutaneous ultrasonography in 237 horses. Assessments of LCAD were compared with those of RCAD. The LCAD:RCAD ratios in thickness and area were compared between control horses (resting grades 1 and 2) and horses with resting laryngeal grades 3 and 4 using the Havemeyer 4-point grading system.

3. Results
The LCAD:RCAD ratios for thickness and area were 0.70 and 0.69 in horses with resting grades 3 and 4, respectively; LCAD was more hyperechogenic than RCAD in resting grades 3 and 4. LCAD:RCAD ratios for thickness and area in grades 3 and 4 were significantly lower than those in control...
horses. Thickness and area of the LCAD were negatively correlated with resting laryngeal grade.

4. Discussion

Results of ultrasonographic assessments of the CAD using transcutaneous ultrasonography were similar to those obtained by transesophageal ultrasonography. This technique enables a simple, non-invasive, direct, and easy examination. Assessment of the CAD using transcutaneous ultrasonography may be a useful technique and a potential option for determining whether to perform nerve graft or laryngoplasty.

Acknowledgments

The Authors thank Dr. Norm G. Ducharme (Cornell University) for his advice on this ultrasound technique and preparation of this manuscript.

Declaration of Ethics

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

Conflict of Interest

The Authors have no conflicts of interest.
Comparison of Transrectal and Abdominal Transducers in Identification of Pathology in Horses Presenting with Colic

Hanna Haardt, DVM, MRCVS*; Alfredo E. Romero, DVM, DACVS; Søren R. Boysen, DVM, DACVECC; and Jean-Yin Tan, DVM, DACVIM

Compared with a low-frequency (abdominal) transducer, a high-frequency linear (transrectal) transducer can be used to identify all common lesions found using fast localized abdominal sonography of horses (FLASH) admitted for colic, except for identification of the stomach and left kidney. Authors’ addresses: Department of Surgery and Anaesthesiology of Domestic Animals, Ghent University, Salisburylaan 133, Merelbeke, Belgium (Haardt); Department of Veterinary Clinical and Diagnostic Sciences, Faculty of Veterinary Medicine, University of Calgary, Calgary, AB T2N 4Z6, Canada (Romero, Boysen, Tan); e-mails: haardt.hanna@gmail.com, Hanna.Haardt@UGent.be. *Corresponding and presenting author. © 2021 AAEP.

1. Introduction
Abdominal ultrasonography is a valuable tool in diagnosis of abnormalities causing colic. Shortened protocols such as FLASH enable practitioners with little experience to perform an ultrasonographic examination in an emergency. However, most practitioners do not own an abdominal transducer to perform such an examination, while they commonly possess transrectal transducers for reproductive exams. The purpose of this study is to compare identification of abnormalities using a transrectal and abdominal transducer for transcutaneous abdominal scans of horses presenting with colic.

2. Materials and Methods
Twenty-four adult horses undergoing FLASH for investigation of colic were included in the study. The same clinician performed the ultrasonographic examination with the transrectal transducer in all patients, whereas examination with the abdominal transducer was performed by another clinician. Using a Chi square, Fisher’s exact, or Wilcoxon tests, the incidence of identification of each abnormality was compared between both transducers.

3. Results and Discussion
The transrectal transducer achieves comparable identification rates with the abdominal transducer for all abnormalities found on FLASH presenting for colic, except those affecting the left kidney and stomach. Although not statistically significant, thickening of the colon wall was detected more frequently with the transrectal transducer. A main
limitation was that the images were captured by ultrasonographers with limited experience, which may have led to artificially low identification rates of pathologies.

**Acknowledgments**
This work was funded by the University of Calgary - Faculty of Veterinary Medicine Internship Research Fund.

**Declaration of Ethics**
The Authors have adhered to the Principles of Veterinary Medical Ethics of the AVMA.

**Conflict of Interest**
The Authors have no conflicts of interest.
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