EQUINE VETERINARY EDUCATION/AMERICAN EDITION VOLUME 35 NUMBER 12 DECEMBER 2023

EQUINE VETERINARY EDUCATION

American Edition | December 2023

BEVA

The official journal of the American Association of Equine Practitioners, produced in partnership with BEVA.

IN THIS ISSUE:

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DECEMBER 2023 • VOLUME 35 • NUMBER 12

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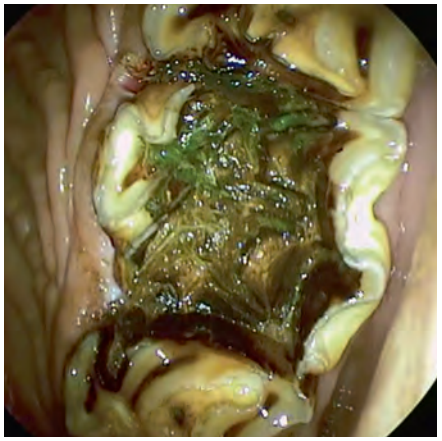

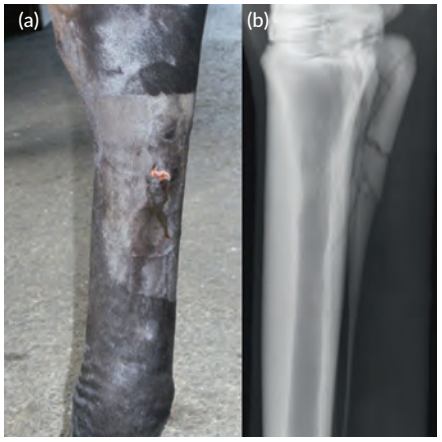

AAEP NEWS In this issue

Cover photo by Dr. Harry Werner.

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EQUINE VETERINARY EDUCATION
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Equine Veterinary Education is a refereed educational journal designed to keep the practicing veterinarian up to date with developments in equine medicine and surgery. Submitted case reports are accompanied by invited reviews of the subject (satellite articles) and clinical quizzes. Tutorial articles, both invited and submitted, provide in-depth coverage of issues in equine practice.

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All manuscript submissions for the journal should be submitted online at http://mc.manuscriptcentral.com/eve. Full instructions and support are available on the site and a user ID and password can be obtained on the first visit. If you require assistance, click the Get Help Now link that appears at the top right of every ScholarOne Manuscripts page.

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From the president: A horse doctor's reflection on 2023

By Rob Franklin, DVM, DACVIM

Dr. Rob Franklin

As I address you for the final time in my capacity as president, it is with a profound sense of gratitude and reflection. Serving at the helm of this esteemed association has been a privilege, and it is with immense pride that I reflect on the strides we've made together.

First and foremost, I extend my deepest appreciation to each and every one of you for your unwavering dedication to the horse and our noble profession. The tireless efforts of our volunteers and staff, the steadfast commitment of my fellow board members, and the diligence of our committee members continue to propel our association. The impact we've had on the equine veterinary field is immeasurable, and I am honored to have been part of this collective endeavor. Together, we have witnessed the resilience and strength of our equine veterinary community, reaffirming that being a horse doctor is not just a vocation, but a calling.

I am pleased to report that the AAEP has made a significant impact in various areas, particularly in our efforts to ensure the sustainability of our profession. Our Commission on Equine Veterinary Sustainability has been diligently working toward this goal over the past three years, emphasizing the need for a robust and thriving equine veterinary community. As native problem-solving practitioners, we have stopped assigning blame for the issues within the profession and started to make changes in our lives, practices and relationships that shape a brighter future for all stakeholders. These grassroots efforts are being amplified on a daily basis thanks to our ability to share what's working and what needs to be fixed. My travels on behalf of the AAEP this year have been a testament to this amazing change.

This initiative is about safeguarding the very essence of what it means to be an equine veterinarian. It involves ensuring that our practitioners are equipped with the knowledge, resources and support they need to flourish in their careers and that our profession remains vibrant and sustainable for generations to come. I hope you have begun to utilize the tools, best practices, case studies and other resources that the Commission has delivered over the past year. Resources will continue to be available on the Commission website as the subcommittees devoted to Students, Internships, Practice Culture, Compensation and Emergency Coverage finalize their work. Visit https://tinyurl.com/2v6e9z23 or scan the QR code to access.

As many of us returned home recently from our annual convention, we did so not just with new knowledge and insights acquired but with friendships rekindled and new connections formed. The camaraderie that permeates this event is a testament to the tight-knit community we have nurtured within the AAEP. Together we can create change for the betterment of the horse, the horse owner and our profession as a whole.

In addition to our convention, the AAEP continues to offer a multitude of resources and educational opportunities tailored to the needs of our diverse membership. From the virtual convention and online courses to publications and monthly web-based rounds, we are committed to providing you with the tools and knowledge necessary to excel in your practice. Our commitment to excellence extends to our advocacy efforts as well, as we work tirelessly to represent the interests of equine veterinarians on a national and international scale.

In closing, let us approach the future with the same determination and passion that have defined our association for decades. Being a horse doctor is a calling that requires not only skill but also a profound sense of responsibility. Together, we have demonstrated time and again that our profession is marked by resilience, compassion, excellence and an unwavering commitment to equine welfare and collegiality.

Thank you for entrusting me with the honor of serving as your president. It has been a journey marked by growth, learning, and a deepened appreciation for the incredible work each of you do. The personal insight I have gained this year largely comes from considering the multitude of perspectives of our over 9,000 members worldwide. We differ in many ways: in our backgrounds, geography, areas of professional interests and personal biases. We are similar in a small number of ways, but the similarities are perhaps more meaningful and create the ties that bind.

We are all horse doctors, a prestigious profession that has helped the world develop for thousands of years. We are all driven by that special bond between horses and people. Improving the welfare of horses is our goal, and one of the common outcomes when doing our job is that we can also improve the welfare of the people attached to that horse. Doing good by the horse does good by the people. This includes the owner; trainer; caretakers; farrier; feedstore workers; pharmaceutical, diagnostic, supplement and technology companies; and our colleagues across town or on the other side of the world.

It is a unique profession that can have such an impact on lives both lived and experienced. It is these commonalities that will create a bright future for all practitioners, old and young. It is these ties that bind that cause us to find motivation for another day in the saddle; to pause and show curiosity to a colleague's view different than our own; to show compassion to those that hurt; to exhibit kindness to all we encounter; to encourage those who feel burned out, left out or overwhelmed; to offer and receive forgiveness when needed; and to be generous with our time, talents and resources.

Together we are the light. Iron sharpens iron.

5 things to know about AAEP this month

1. Register for virtual convention at convention.aaep.org and earn CE from on-demand recordings of educational sessions from San Diego until Dec. 31, 2024.
2. Acquire 15 uniquely casual CE credits at the 25th Annual Resort Symposium in Costa Rica, Jan. 22–24, 2024. View the educational program and register at aaep.org/meetings.
3. Check out how your fees compare to national averages using survey data published by AAEP from Decade One and VMG 16 participants at https://tinyurl.com/37rkykuf.
4. Enjoy free access until Feb 22, 2024, to the *EVE* and *EVJ* articles highlighted during the Kester News Hour and cited in the Milne Lecture at https://bit.ly/3tTOzBq.
5. Access free and confidential support for personal or work-related stressors through the AAEP Healthy Practice Member Assistance Program. Seek assistance simply by calling (800) 633-3353.

Nominate a distinguished researcher for the 2025 AAEP Milne Lecture

Deadline to nominate is January 31

The Frank J. Milne State-of-the-Art Lecture is a traditional highlight of each year's annual convention, and you can help determine the 2025 honoree by nominating an accomplished researcher.

The Milne Lecture was created in 1997 to recognize an individual with a distinguished career in research and discovery, and who has presented and published their findings in a specific area of equine health. The lecture is intended to honor the accomplishments of the presenter and provide a meaningful learning experience to the AAEP membership. The lecture is a perspective on the state-of-the-art in the presenter's area of expertise.

The award recipient will be determined by a subcommittee of the AAEP Educational Programs Committee in February 2024 and will then be presented to the board of directors for approval. The selected individual will deliver their lecture and receive their award at the AAEP's 2025 Annual Convention in Denver, Colo.

Nominees should be an expert in their field with a track record of accomplishment and the ability to relate the topic to the audience. A nomination form must be completed and include qualifications and accomplishments of the nominee.

Dr. Susan Stover delivers the 2022 Milne Lecture on the topic of skeletal injuries in equine athletes.

A Milne Lecture nomination form may be requested from Carey Ross, scientific publications coordinator, at cross@aaep.org. Completed forms must be returned to her by Jan. 31, 2024.

Compare your fee structure against national data

Appropriate structuring of fees for services provided is an important part of building and maintaining a successful practice, and comparison pricing is one aspect for consideration when determining fees.

The AAEP has published fee survey data collected from equine veterinarians participating in Decade One and VMG 16. The data displays the low, high, mean and median prices for a comprehensive range of procedures, therapies and treatments, as well as emergency response and farm calls.

The data represents a broad cross-section of geographies and practice types and is approximately 12 months old, which is in accordance with U.S. antitrust guidelines. Going forward, the AAEP intends to conduct periodic fee surveys from a larger sample size so that pricing can be broken out by region or practice type.

See how your fees compare. View or download the equine fee survey data at https://tinyurl.com/37rkykuf.

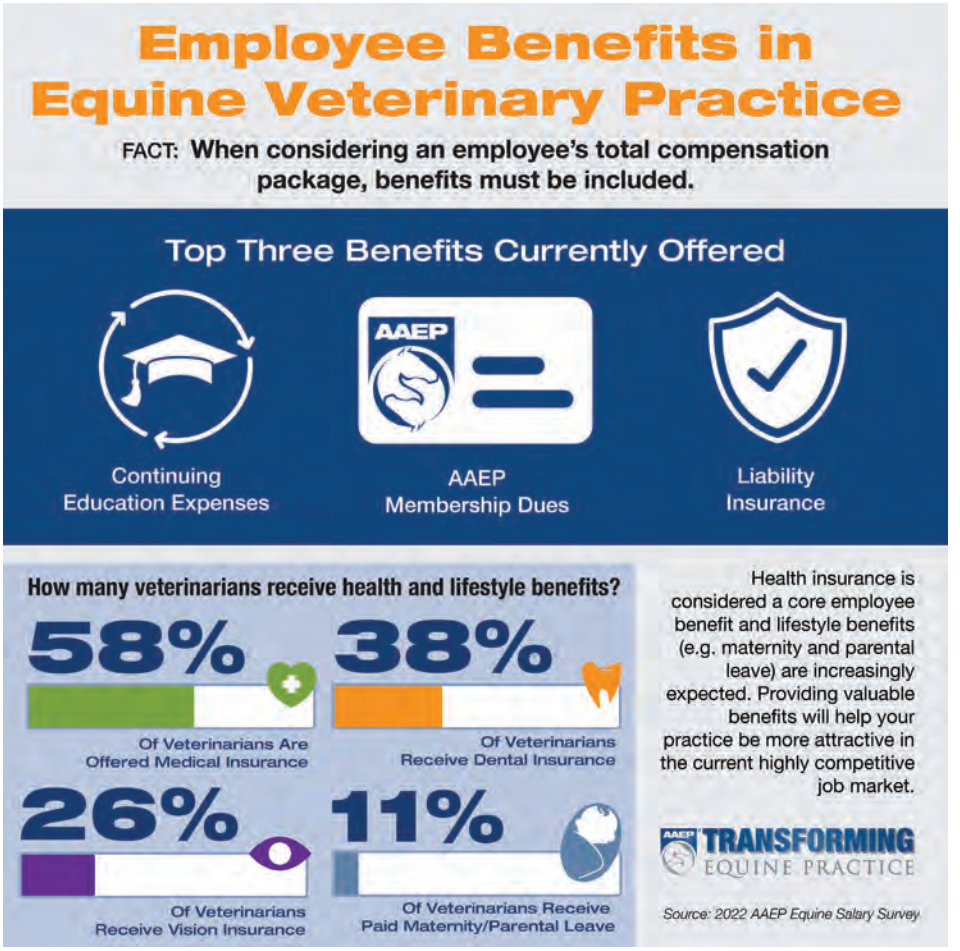
Sway job seekers by expanding your benefits package

The 2022 AAEP Salary Survey indicated that the top three benefits offered to equine veterinarians as part of their compensation are continuing education expenses (89%), AAEP membership dues (82%) and liability insurance (82%).

Interestingly, just over half of equine veterinarians are offered medical insurance, and significantly fewer receive dental and vision insurance as well as paid maternity or parental leave.

Health insurance is considered a core employee benefit and one that is highly valued by job applicants. Lifestyle benefits like maternity and parental leave are increasingly expected. Providing additional benefits beyond CE expenses, dues and liability insurance will help your practice stand out in our current highly competitive job market.

For additional survey insights, visit aaep.org/compensation.



CONTINUING EDUCATION

Couldn't be there in person? Register for virtual convention

Give yourself the gift of yearlong learning this holiday season by registering for the virtual convention.

With virtual registration, you receive indefinite access to on-demand video recordings of all educational sessions from the AAEP's recent annual convention in San Diego along with the ability to acquire CE from these sessions until Dec. 31, 2024. This means you can "learn and earn" at your pace and on your schedule for the next 12 months.

Register for virtual convention and view the complete educational program at convention.aaep.org. As a reminder, if you attended the convention in San Diego, you receive the virtual option at no additional cost.

ASSOCIATION

Help equine rescue and retirement facilities apply for free vaccines

AAEP members affiliated with 501(c)(3) equine rescue and rehabilitation facilities in the United States should work with those facilities now to complete the application for complimentary vaccines from the Unwanted Horse Veterinary Relief Campaign (UHVRC) by the Feb. 1 deadline.

The UHVRC is a non-profit partnership between Merck Animal Health and the AAEP to safeguard the health and facilitate the adoption of rescue horses. Since its inception in 2008, the UHVRC has provided more than 53,000 doses of core vaccines valued at over $2 million to protect horses in need.

The UHVRC provides qualifying equine facilities with vaccines to protect against Eastern and Western equine encephalomyelitis, equine rhinopneumonitis (EHV-1 and EHV-4), West Nile virus, equine influenza, tetanus and rabies. Eligible facilities must coordinate an application with an AAEP-member veterinarian and adhere to the AAEP Care Guidelines for Equine Rescue and Retirement Facilities.

Visit aaep.org/horse-owners/unwanted-horse-veterinary-relief-campaign to download the application and equine vaccine order form.

UHVRC
Unwanted Horse Veterinary Relief Campaign

Tap the potential of telemedicine with latest Practice Life podcast

Acquire tips and advice on implementing telemedicine in your practice by listening to the October episode of the AAEP's Practice Life podcast.

Host Dr. Mike Pownall explores successful integration of the service into daily practice with Dr. Erica Lacher, owner of Springhill Equine Veterinary Clinic in Newberry, Fla.; Dr. Cris Navas, assistant professor of cardiology/ultrasound and internal medicine at the University of Pennsylvania School of Veterinary Medicine; and Dr. Kelly Zeytoonian, owner of Starwood Equine Veterinary Services and Starwood Veterinary Consulting Inc. in Redwood City, Calif.

"Know and have the confidence that you are providing a service that does have value, and you shouldn't feel bad about charging for it," Dr. Zeytoonian said when encouraging colleagues to embrace the practice and price it appropriately. "You should feel really good that you are bringing that level of accessibility and care to your clients."

Topics discussed include how each uses telemedicine, which types of cases/appointments work best for telemedicine, the logistics of a telemedicine appointment, how each charges for a televisit and advice for others.

Download or listen to the 37-minute episode on iTunes or at podcast.aaep.org.

Share your research and knowledge at the 70th Annual Convention in Orlando

Deadline to submit an educational paper is March 15, 2024, 3:00 p.m. ET

AAEP members and others are invited to submit papers for consideration for presentation during the AAEP's 70th Annual Convention in Orlando, Fla., Dec. 7-11, 2024. Eligible for consideration are scientific papers, "how-to" papers, review papers, abstracts, and Business and Practice Life papers.

Submitting your paper

- All papers must be submitted by March 15, 2024, 3:00 p.m. ET at https://tinyurl.com/jes67nkh. The system will shut down after this time.
- Be sure to familiarize yourself with the submission process well in advance of the deadline. You can set up your profile with paper and author information in advance and then upload your paper when it is complete.
- Since the review process is blinded, make sure your paper does not include author or institution names.

A few key points

- Products and equipment must be identified by chemical or generic names or descriptions and footnoted.
- Due to the length and complexity of the process, all deadlines are strictly enforced.
- Submission of a paper represents a commitment to present this paper at the meeting if it is selected.
- Selected papers will be printed in the 2024 AAEP *Proceedings* and presented at the 2024 Annual Convention. The presenting author will receive complimentary registration and an honorarium.

Ethical Considerations

- 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 submitted paper or with companies that manufacture or sell competing products.
- If your presentation references the use of a compounded pharmaceutical, ensure that you are familiar with the FDA guidelines on the use of compounded pharmaceuticals and that the product you reference is in compliance.
- All AAEP papers submitted for presentation should cite levels of evidence-based medicine.

Types of papers accepted

All paper presentations are limited to 15 minutes plus 5 minutes for Q&A.

Scientific papers should be a minimum of 600 words. Special attention will be given by the Scientific Review & Editorial Committee to material with practical content or new information.

How-to papers should describe and explain a technique or procedure used in veterinary medicine or the equine industry. The technique should be relatively new or not widely understood or used in practice. There is no word limit for how-to papers.

Review papers should update the membership on a new subject or gather information that may be conflicting. Although a review paper does not necessarily contain original data, it is anticipated that the presenter will have considerable experience in the field.

Abstracts ≤ 250 words may be submitted by authors who intend to publish in a refereed journal. A full paper conforming to the AAEP guidelines to authors must also be submitted (for review purposes only) to allow the reviewers to assess the experimental design, materials and methods, statistical analyses, and results (with graphs, tables, charts, etc.) and to discuss the results as they pertain to interpretation and conclusions.

Journals differ in what they consider to be "prior publication"; some allow an author to submit an abstract up to 1,000 words, whereas other journals allow only 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.

Business and Practice Life papers may cover any business management or wellness topic that can help the practitioner improve the quality of their practice and/or life. The theme for 2024 is "Preparing for the Practice of the Future."

Need help submitting a paper?

As an aid to private practitioners, first-time authors or members seeking guidance with their submission, AAEP offers a mentorship program in which experienced presenters are available to provide advice and direction. However, mentors are not responsible for rewriting or selecting material.

Contact Carey Ross, scientific publications coordinator, at cross@aaep.org for a list of available mentors or with questions concerning the annual convention and educational paper submission.

A strong practice culture demands an ethical foundation

By Lisa Fultz DVM, MS, DACVIM, and Jamie Pribyl, DVM

Dr. Lisa Fultz

Dr. Jamie Pribyl

We have all heard the saying, "Being a horse doctor is a lifestyle, not a job." Long days and hard work are unavoidable, and a lot of it is "heart work" not just "hard work." But when the realities of burnout, complex communication dynamics, appropriate compensation and compassion fatigue go unrecognized, a practice can become more toxic than the most bacteria-ridden culture plate. Building a strong and ethical practice culture is beneficial to everyone in a practice. So, what is practice culture and how can we strive to build and maintain a culture with an ethical foundation?

The AAEP has recognized the importance of culture as part of the Commission on Equine Veterinary Sustainability. And the need is dire: A 2019 survey of 647 veterinarians revealed that 28.4% cited "culture of my practice" as a major contributor to leaving equine practice.

After experiencing a similar trend of decreasing satisfaction in the industry in the first 5 years of practice, our small animal counterparts established American Animal Hospital Association's Culture Initiative to identify practice culture characteristics that help employees thrive. In the AAHA 2020 study, "actively managed" cultures in small animal practices were 2.5 times more profitable, had a 30% increase in innovation and a 40% increase in employee retention.

But equine medicine has unique aspects that prevent direct application of other industries' culture initiatives. We have an army of solo practitioners that may not have staff, and ambulatory medicine presents unique situations where the facilities, assistants and hands-on-deck are often changing and out of your control. Most days, just getting the day's work done before the sun goes down is hard enough, let alone actively managing a practice culture.

With those unique challenges in mind, breaking down practice culture into subsets can help us ask ethical questions that can help us build a healthy and profitable environment.

Microculture

Micoculture involves the day-to-day regulars—the intimate team within a practice. Healthy connections, positive energy and each person's individual ethics are key to success at this level.

When the ethics of veterinarians within a practice don't align, it has negative effects on culture. For example, Associate A refuses to fill an Rx without a valid VCPR, but Associate B readily dispenses Rx medication for horses he hasn't seen. This mismatch creates friction between the veterinarians and trickles down to staff. Additionally, it damages the relationship with clients because there is an inconsistent message that will eventually result in a disappointed or disgruntled client.

Every member within a practice should take an inventory of their own personal ethics, and practice owners and managers should establish policies to minimize ethical dilemmas. Questions to consider include: Are you asking your associates and team for feedback? What cases does your associate prefer to see, and is there a way you can help grow that caseload? Are you providing adequate equipment and support to all staff members?

Bridge Culture

This subset involves the group work where different parts of the practice overlap.

Competition between associates or teams within the practice can affect the culture as a whole. A colleague recently shared that there is competition among partners for certain clients within their practice, and it has contributed to a toxic culture within the team.

Ethical dilemmas can arise if some staff members disparage other staff members. By establishing a shared vision of best outcome for the horses and clients, some practices may improve their culture by focusing on a larger "we" vision of a practice where clients consider themselves clients of the practice instead of clients of a particular vet. This could create a supportive environment and foster career growth and professional longevity of newly hired veterinarians within the practice.

The ethics of reporting errors is an important consideration at this level of practice culture. Could an employee be tempted to omit or falsify records rather than be honest with management for fear of retribution?

Macroculture

Lastly, culture can be viewed as more than a sum of the parts, which encompasses a practice's purpose of work and values.

continued on next page

AAEP ETHICAL PRACTICE Every Day-Every Time

Dr. Fultz practices with Paddock Equine Veterinary Services in Wellington, Fla. Dr. Pribyl is an equine professional services veterinarian with Boehringer Ingelheim Animal Health and member of the AAEP's Practice Culture Subcommittee. Both serve on the AAEP's Professional Conduct and Ethics Committee.

University of Florida student receives inaugural $50,000 Ellen DiBella Memorial Scholarship

Hannah Quail, a University of Florida student pursuing a career in academia practicing equine emergency and internal medicine, is the first recipient of the $50,000 Ellen DiBella Memorial Scholarship. This new scholarship rewards an exemplary third-year veterinary student at one of four veterinary colleges: Auburn University, University of Florida, Louisiana State University or Purdue University.

The Foundation for the Horse presented Quail with her award on Nov. 30 during the AAEP's 69th Annual Convention. The award will be paid over a five-year period as long as the recipient remains active in equine veterinary medicine.

Drs. Karen and Frank Wolfsheimer of Micanopy, Fla., created this scholarship opportunity within The Foundation to encourage veterinary students with a passion for horses to pursue the practice of equine medicine and surgery; and to honor the memory of Ellen DiBella, whose influence on equestrian sport and the Morgan Horse industry earned her the U.S. Equestrian Federation's Lifetime Achievement Award in 2019. Ms. DiBella passed away in August 2023.

"Our hope is to see young equine practitioners' passion strengthened as they learn of other passionate equestrians like Ellen DiBella, who felt that all horses should be valued and cherished," said Dr. Karen Wolfsheimer, AAEP member and 1978 veterinary graduate of Auburn University. "We also hope that this award, in its design, will keep recipients engaged in the wonderful world of equine medicine."

Hannah Quail

If you are interested in establishing a scholarship endowment or supporting The Foundation's scholarship efforts with a gift commitment, please contact The Foundation's development team at (859) 233-0147 or info@foundationforthehorse.org.

ETHICS

A strong practice culture, continued

Every practice member should question their ethics if what occurs within the course of the day spills over into personal lives and how our staff, clients and patients interact with the world.

Do you prioritize the safety of your employees and patients over "getting the job done"? This could include using proper biosecurity and proper preparedness for procedures (sedated versus unsedated procedures, use of protective equipment during radiography, etc.) Are contagious diseases appropriately reported? Numerous veterinarians on anonymous veterinary discussion boards mention being ethically challenged when they learn of a colleague knowingly writing a health certificate for a horse with signs of disease. This can put other horses at risk and upset the cultural foundation of a practice.

The social media presence of an organization and its members can also influence the microculture. Establishing rules on appropriate post content (both client-related and personal) facilitates a healthy culture. Maintaining positive outward comments online and in the real world is vital. A healthy culture would refrain from disparaging other veterinarians and practices to current or potential clients.

In conclusion, active management of a practice's culture requires establishment of ethical guidelines. In the ethics feature published in the April 2023 issue of *EVE*, the authors proposed "promoting a practice culture that encourages open modeling of our humanity." The essence of such humanity inherently involves ethics as part of our daily lives in equine practice. Although it is not easy or straightforward, practices and the industry are at risk without a strong ethical foundation.

AAEP HEALTHY Practice MEMBER ASSISTANCE PROGRAM

Services provided by MYgroup McLAUGHLIN YOUNG

The member assistance program is an AAEP-sponsored benefit that offers the support and resources you need to address personal or work-related challenges and concerns. It's confidential and free to you and your household family members. Two types of services are provided: counseling and consultation sessions and online resources.

Accessing Services

Call 800-633-3353 to speak to a qualified clinician, 24 hours a day, 7 days a week. No password or special access information is required to utilize this service.

To access online resources, visit mygroup.com * Click on My Portal Login * Select Work-Life option * Enter Username: aaep & Password: guest

Download MYgroup App

You can also conveniently access services through the MYgroup app, available for download in the Apple and Google Play stores. The login credentials are the same as they are for the mygroup.com website.

Assessment and Counseling

Reasons to use the member assistance program include relationship difficulties, depression or anxiety, stress, grief and loss, parenting concerns, and alcohol and drug abuse and addiction. The program provides short-term, solution-focused therapy.

When an AAEP member or family member calls, you will be offered face-to-face, telephonic, or virtual counseling sessions in which a thorough assessment can be conducted by a licensed, experienced clinician in your area. AAEP members and immediate family can utilize the member assistance program for an unlimited number of issues per year, with up to three counseling sessions offered per issue. There is no cost to you.

Legal Services

- Free telephonic legal advice
- Free 30-minute appointment for legal consultation with a local attorney
- In most cases, 25% discount on ongoing legal services
- Legal forms available to download on mygroup.com, such as wills, request for death certificate, etc.
- Please note: Legal services through this program do not cover disputes or actions involving the member's employer or issues related to business.

Financial Services

- Free financial counseling appointments
- Issues addressed include bankruptcy, buying a home, loan repayment and retirement planning.
- 40 financial calculators available online at mygroup.com.

Online Services

- 7 content divisions: Parenting, Aging, Balancing, Thriving, Living, Working, and International
- Monthly online seminars with certificates of completion
- Over 100 streaming audio files and 100 video files covering a range of health topics.
- Savings Center and Relocation Center

ACCESSING SERVICES

Toll-free: 800-633-3353

Website: www.mygroup.com > My Portal Login > Work-Life

Username: aaep
Password: guest

The Member Assistance Program is offered to U.S. and Canadian members only at this time due to variances in available providers outside of North America.

Members in the News

ARCI appoints Dr. John Chancey as committee chair

Dr. John Chancey has been appointed as the new chair of the Drug Testing Standards and Practices Committee of the Association of Racing Commissioners International.

Dr. Chancey, a veterinary graduate of Oklahoma State University, is executive director of the Oklahoma Horse Racing Commission.

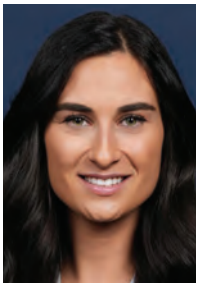
Courtesy ARCI

Dr. John Chancey

Three students awarded in case study contest

The AAEP's Educational Programs Committee selected three winners of the 2023 case study contest: Jordan Marsh, a fourth-year student at Colorado State University; Shelby Rivkin, a fourth-year student at Michigan State University; and Alexandra Bettencourt, a third-year student at Texas A&M University.

Jordan Marsh



Shelby Rivkin

Alexandra Bettencourt

Their case studies, listed below, are available as educational resources at aaep.org/case-studies.

- Marsh: "West Nile Virus in Equids: A Look at the 2023 Colorado Landscape"
- Rivkin: "Antemortem Diagnosis of Renal Hemangiosarcoma in a Warmblood Gelding"
- Bettencourt: "Equine Degenerative Myeloencephalopathy"

As winners of the contest, which was open to veterinary students and first-year graduates, each received complimentary registration for the AAEP's 69th Annual Convention and Extended Student Program in San Diego as well as $500 to support travel to the meeting. The AAEP thanks the American Board of Veterinary Practitioners for its sponsorship of the student case study travel stipends.

INDUSTRY

AAEP Educational Partner Profile: American Regent Animal Health

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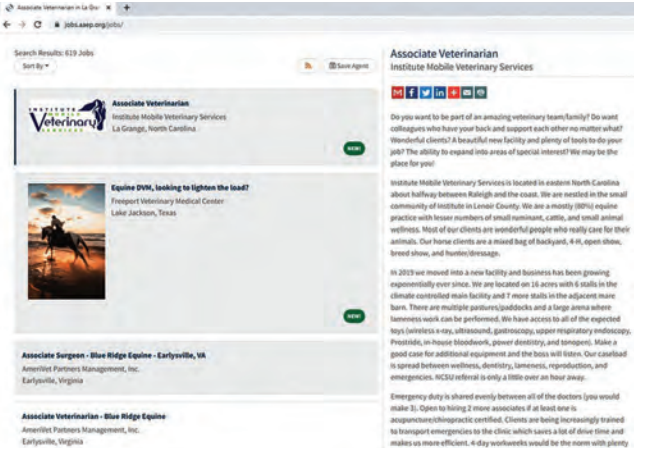

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Equine Veterinary Education

RESEARCH HIGHLIGHTS

Highlights of recent clinically relevant papers

ABNORMAL MARE BEHAVIOUR

This study by Lauren Huggins and co-workers in the United States examined the incidence of abnormal behaviours and their association with concentrations of ovarian hormones associated with a granulosa cell tumour (GCT).

A total of 2914 hormonal profile samples submitted with the words behave, behaviour or behaving in the submission history were analysed. The association between reported abnormal behaviours and concentrations of testosterone, anti-Müllerian hormone (AMH), inhibins and inhibin-B were assessed.

Overall, 2506/2914 (86%) cases did not have any of the measured hormones reach GCT-like concentrations. The remaining 408 cases had either one (63%), two (25.5%) or three (11.5%) hormones with concentrations consistent with those from confirmed GCT cases. Testosterone had the lowest per cent of GCT-like values among the cases (7.7%), compared with AMH (9.4%), inhibins (9.6%) and inhibin-B (8.7%). Stallion-like behaviour was significantly associated with increased concentrations of all four hormones. In contrast, aggression, oestrous and other abnormal behaviours were significantly less likely to be associated with increased concentrations of the hormones.

CURRENT ANTIMICROBIAL USE

In this study, Meagan Rockow and co-workers in the United States surveyed the current clinical use of antimicrobials in horses undergoing exploratory celiotomy to understand how recent literature and changes in microbial resistance patterns may have impacted antimicrobial selection practices.

Veterinary professionals within the American College of Veterinary Internal Medicine (ACVIM) and the American College of Veterinary Surgery (ACVS) were sent an electronic survey; 113 completed surveys were returned. Practitioners reported antimicrobials were most frequently given 30–60 min preoperatively (63.1%). Two antimicrobial classes were typically administered (95.5%), with gentamicin (98.2%) and potassium penicillin (74.3%) being the most common. Antimicrobials were typically not re-dosed intraoperatively (78.6%). Factors that affected overall treatment length postoperatively included resection (81.4%), bloodwork (75.2%), enterotomy (74.3%), fever (85.0%), incisional complications (76.1%) and thrombophlebitis (67.3%). The most common duration of antimicrobial use was 1–3 days for non-strangulating lesions (54.4% of cases) and inflammatory conditions such as enteritis or peritonitis (50.4%), and 3–5 days for strangulating lesions (63.7%). Peri-incisional and intra-abdominal antimicrobials were used by 24.8% and 11.5% of respondents, respectively. In summary, antimicrobial usage patterns were highly variable among practitioners and, at times, not concordant with current literature.

ELECTROHYDRAULIC SHOCK WAVE THERAPY

In this study Ahmed Khairoun and co-workers in the United States examined the effect of the number of electrohydraulic shock wave therapy (ESWT) treatments in the management of superficial digital flexor tendinitis (SDFT) and proximal suspensory desmitis (PSD) injuries and compared short- and long-term outcomes.

Medical records were reviewed. Horses were separated into two categories: group 1: ≥3 ESWT treatments; group 2: <3 ESWT treatments. For group 1, lameness scores between the first and third treatments were significantly reduced in both PSD and SDFT horses. However, neither the PSD nor SDFT's ultrasound findings were significantly different at the end of the third treatment. Horses with PSD showed a significant improvement in forelimb lameness between the first and third treatments compared to hindlimb. In the multivariable ordered logistic regression model, only time (months of follow-up) was significantly associated with a positive outcome and there was no difference in short- and long-term outcomes between groups 1 and 2. Chronicity of injury was negatively associated with improvement of lameness.

SUBCHONDRAL LUCENCIES

This study by Frances Peat and co-workers in the United States and New Zealand aimed to (1) identify the prevalence of medial femoral condyle (MFC) subchondral lucencies (SCLs) on sales repository radiographs in yearling and 2-year-old Thoroughbreds; (2) identify any association between grade of MFC SCLs and future racing performance and (3) monitor changes in MFC SCLs grades between yearling and 2-year-old sales.

Radiographs from 2508 yearlings (5016 stifles) and 436 2-year-olds (872 stifles), obtained from a 2016 yearling sale and five 2017 2-year-old sales, were included in the study. MFC SCLs of grades 1–3 were observed in 242 (9.65%) yearlings and 49 (11.2%) 2-year-olds. Bilateral MFC SCLs of grades 1–3 were observed in 54 (2.2%) yearlings and 12 (2.8%) 2-year-olds. Grade 1 MFC SCLs in

yearlings either remained unchanged (14/31), progressed to a grade 2 (6/31) or resolved (11/31) by the 2-year-old sale. Grade 2 MFC SCLs in yearlings remained unchanged (6/10), progressed to a grade 3 (2/10) or improved to a grade 1 (2/10). Yearlings with grade 3 MFC SCLs had a 78% probability of starting a race (95% CI 58.2%–89.6%), compared with an 84% probability of racing for grade 0 yearlings (95% CI 82.7%–85.8%). Six of the seven yearlings with axial MFC lucencies raced.

Grade 1 MFC SCLs were the most common type seen in yearling and 2-year-old sales horses. The majority of yearling grade 1 MFC SCLs resolved or remained static by 2-year-old sales. It was also possible for grade 2 and 3 MFC SCLs to improve one grade between sales. Fewer sales yearlings with a grade 3 MFC SCLs raced, but in those that did race, there was no evidence of worse performance compared to unaffected peers. Axial MFC lucencies did not affect racing performance.

STRESS RESPONSE IN LAMINITIS

This study by Alexandra Moss and co-workers in the United States evaluated parameters associated with stress response in horses with laminitis and compared these to healthy horses and horses with gastrointestinal (GI) disease.

Thirty-eight adult horses presenting for non-medical conditions, GI abnormalities or clinical laminitis were prospectively enrolled. Horses were assigned to disease groups (healthy, GI disease and laminitis) and had blood drawn on presentation to the hospital. Samples were analysed for plasma endogenous adrenocorticotrophic hormone (eACTH), serum cortisol, serum thyroid hormone and plasma histamine.

Stress hormone concentrations were significantly different between horses in the laminitis and GI disease groups. Plasma histamine levels were highest in horses with laminitis, compared with GI disease and controls. Horses with laminitis and GI disease had increased plasma eACTH compared to healthy horses. Horses with GI disease had higher serum cortisol concentrations than horses with laminitis or controls. Serum T4 was lower in horses with GI disease than in horses with laminitis and controls.

Horses with laminitis had relative increases in both plasma histamine and eACTH concentrations. Serum T4 and cortisol concentrations of horses with laminitis did not differ significantly when compared to healthy horses. The role of stress hormones in equine disease warrants further investigation.

ANALYSIS OF FACIAL EXPRESSIONS

This study by Su Min Kim and Gil Jae Cho, based in Korea, aimed to prove that deep learning can be effectively used for identifying various equine facial expressions as welfare indicators.

A total of 749 horses (586 healthy and 163 experiencing pain) were investigated. A model was developed for recognising facial expressions based on images and their classification into four categories (resting horses, horses with pain, horses immediately after exercise and horseshoeing horses).

The normalisation of equine facial posture revealed that the profile (99.45%) had higher accuracy than the front (97.59%). The eyes–nose–ears detection model achieved an accuracy of 98.75% in training, 81.44% in validation and 88.1% in testing, with an average accuracy of 89.43%. Overall, the average classification accuracy was high; however, the accuracy of pain classification was low.

These results suggest that various facial expressions in addition to pain may exist in horses depending on the situation, degree of pain and type of pain experienced by horses. Automatic pain and stress recognition could greatly enhance the identification of pain and other emotional states, thereby improving the quality of equine welfare.

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Equine Veterinary Education BEVA AAEP

CASE REPORT

Presentation and management of advanced occlusal caries affecting the maxillary cheek teeth of four horses

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SUMMARY

This case series describes the management of advanced occlusal caries of the maxillary cheek teeth in four horses. All horses presented with grade 4 caries, that is affecting cementum, enamel, dentine and the integrity of the tooth. Three Warmblood horses aged 10, 12 and 15, and one Norwegian Coldblooded Trotter aged 10 are presented in this case report. The main findings in these horses are the presence of caries mainly affecting the occlusal surface and less the peripheral cementum. Two horses presented with occlusal caries on only maxillary cheek teeth, and two horses also presented with occlusal caries on maxillary and mandibular cheek teeth. Maxillary cheek teeth were more severely affected than mandibular teeth in these horses. All horses were fed with haylage and concentrates. Treatment of the horses in this case series was debridement of affected dental surfaces with a high-speed handpiece and a diamond-coated round burr with water cooling. The occlusal surface of affected teeth was cleaned and dried, then a single-stage self-etching bonding agent (Adper Prompt L-Pop, 3M™) was applied, and light cured according to the manufacturer's instructions. A layer of flowable resin composite (Starfill™, Zest Dental Solutions) was placed on the occlusal lesion. Finally, odontoplasty was performed to smoothen surfaces of the restorative material and remove any sharp edges. In two cases, nonvital pulpitis were found on maxillary cheek teeth affected by occlusal caries, and after performing a radiographic examination, these teeth were treated endodontically. In one horse, infundibular restorations were performed. The purpose of occlusal debridement and fillings described in this paper was to prevent food packing in occlusal areas where there was extensive loss of enamel, dentine and cementum. Follow-up examinations of the horses presented here were performed for 7 months to 5 years. Two horses showed improvement of carious lesions, one horse showed complete resolution, and one horse showed the same findings 8 months after treatment.

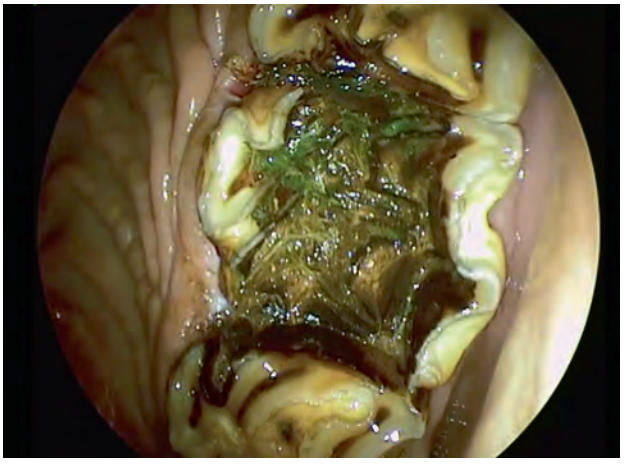

FIGURE 1 Occlusal caries on a maxillary cheek tooth Triadan 209. The peripheral cementum is macroscopically intact on the buccal and palatal side of the tooth.

KEYWORDS
horse, dental disease, dental pathology, equine dentistry, occlusal caries, peripheral caries

Key points

- Maxillary cheek teeth seem to be more commonly affected by occlusal caries than mandibular cheek teeth.
- In occlusal caries, cementum, enamel and dentine appear to be destroyed at similar rates, in contrast to peripheral caries.
- Occlusal carious lesions can be focal, sometimes affecting only parts of a tooth or one tooth in an arcade.

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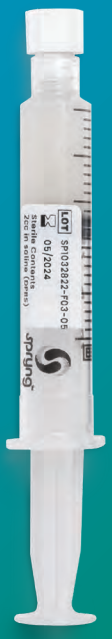

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CLINICAL COMMENTARY

Equine dental caries and restoration

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Summary

Peripheral, infundibular, and occlusal caries can result in significant dental disease affecting the quality of life of the horse. Recognition of dental caries and understanding the grading/classification and treatment options are vital for providing optimal equine oral health care. Accurate grading of dental caries and the recognition of the importance of location on the tooth impacts treatment planning that may or may not include restoration. Restoration of teeth can be performed to reduce dental decay and improve patient comfort. Restoration of teeth requires an armoury of dental restorative equipment and materials, and it is technique sensitive requiring the practitioner to have a deep understanding of dental materials and restorative dentistry. This commentary reviews the types and grading of equine dental decay, case selection for restoration, and the treatment steps for restoration of equine caries.

KEYWORDS
horse, caries, infundibulum, occlusal, peripheral, restoration

INTRODUCTION

Veterinary dental caries is defined as a progressive demineralisation of the inorganic matrix of dental tissues secondary to bacterial fermentation of carbohydrate substrates (Blood & Studdert, 1999; Pearce & Horbal, 2022). Caries develop from the bacterial fermentation of carbohydrates which releases acidic by-products. It is the acid produced by bacteria that demineralises dental hard tissue leading to the destruction of both hard and soft tissue structures. In general, carious lesions tend to occur more commonly on cheek teeth although incisors and canine teeth can be impacted. Lesions can occur on all surfaces of the tooth and even develop deep within the reserve crown.

DESCRIBING CARIOUS LESIONS

Carious lesions have been described by location, hard tissue involvement, and anatomical dental structure. It has been suggested that the G.V. Black classification system of dental lesions based on the location can be applied to the horse (Caiafa & Visser, 2018; Earley, 2022; Wiggs & Lobprise, 1997), but the lack of an incisal edge to equine teeth and the depth of the infundibulum makes the use of this classification system awkward clinically. Currently, the terms infundibular, peripheral, and occlusal have been used to describe the location or anatomic structure involved in the decay and classification systems have been applied to further describe the extent of decay.

Infundibular caries are carious lesions that originate within the infundibulum that is impacted with feed. The acidic bacterial by-products of feed decay slowly leading to demineralisation of dental hard tissue. The prevalence of infundibular caries has been reported to be 45–100% in horses (Fitzgibbon et al., 2010; Horbal et al., 2019; Veraa et al., 2009; Windley et al., 2009). Maxillary caudal cheek teeth are predisposed to infundibular caries with the rostral infundibulum of the maxillary first molar tooth being the most common location. Decay starts centrally localised along the path of the vascular channel remnant and creeps peripherally into the surrounding layers of dental hard tissue. See Table 1 and Figure 1 for the modified Honma Classification system as proposed by Dacre and further amended by Pearce (Dacre, 2005; Honma et al., 1962; Pearce & Horbal, 2022). It has been proposed that vascular channel remnants greater than 1 mm in diameter indicate incomplete filling of the infundibulum with

cementum, infundibular hypocementosis, which predisposes the tooth to the development of infundibular caries (Suske et al., 2016). The type and extent of infundibular lesions as imaged by computed tomography has been described and classified (Windley et al., 2009) (Figure 2). In this classification system, it can be seen that infundibular lesions can be shallow or very deep, extending the full length of the infundibulum. An understanding of the variation in depth and shape of infundibular lesions within the crown is of utmost importance if restorations are attempted.

Peripheral caries are carious lesions located on the periphery of the tooth originating from the peripheral cementum that is coated with thick dental plaque. The bacteria within the plaque create an acidic environment that slowly demineralises dental hard tissue (Borkent et al., 2020). The prevalence of peripheral caries varies from 51 to 91% depending on the geographical region and type of study performed (Borkent et al., 2017; Lee et al., 2019). Peripheral caries may be located on only one facet of the tooth or it may completely encircle a tooth. Decay progresses inward through dental hard tissue, and a modified Honma classification system has been developed to describe the extent of decay (Borkent et al., 2017; Honma et al., 1962) (Figure 3). A second grading system to describe active vs. inactive peripheral carious lesions has been proposed, but it is beyond the scope of this article (Jackson et al., 2021). Lesions are commonly located on the caudal three cheek teeth bilaterally with mandibular lesions reported more (Borkent et al., 2017). Concurrent dental disease (e.g. infundibular caries, fractured teeth, diastema) was associated with the development of peripheral caries (Borkent et al., 2017).

The term occlusal caries is relatively new (Pearce & Horbal, 2022) and it has not been well-described in the literature. *Occlusal caries* are carious lesions that are isolated to the occlusal surface of teeth, which may develop from the bacterial decay of feed retained on the occlusal surface of teeth (Pearce & Horbal, 2022). Occlusal caries is less common than peripheral and infundibular caries. The article by Schneider et al. (2023) is the first published article to report and describe more fully occlusal caries. In this article, the authors report that maxillary cheek teeth were more severely affected than mandibular teeth. In addition, cementum, enamel, and dentin appeared to be destroyed at similar rates. In this paper, the authors utilise the modified Honma Classification system as proposed by Dacre (2005) to describe the extent of occlusal caries.

It is not uncommon for teeth to have more than one of these carious lesions (e.g. peripheral and infundibular) at the same time.

TABLE 1 Modified Honma caries classification as described Dacre (2005).

Grade of carious lesion	Description of carious lesion
Grade 0	No evidence of caries at the macroscopic level but may include cemental hypoplasia.
Grade 1	Caries only affecting cementum. Can be divided into grade 1.1 and 1.2. Grade 1.1 has only pitting decay of cementum. Grade 1.2 has extensive decay or complete loss of the cementum.
Grade 2	Caries extends beyond the cementum into the adjacent enamel.
Grade 3	Caries includes cementum, enamel and dentin
Grade 4	Caries has progressed to affect the integrity of the entire tooth (e.g. tooth fracture, apical/endodontic infection)

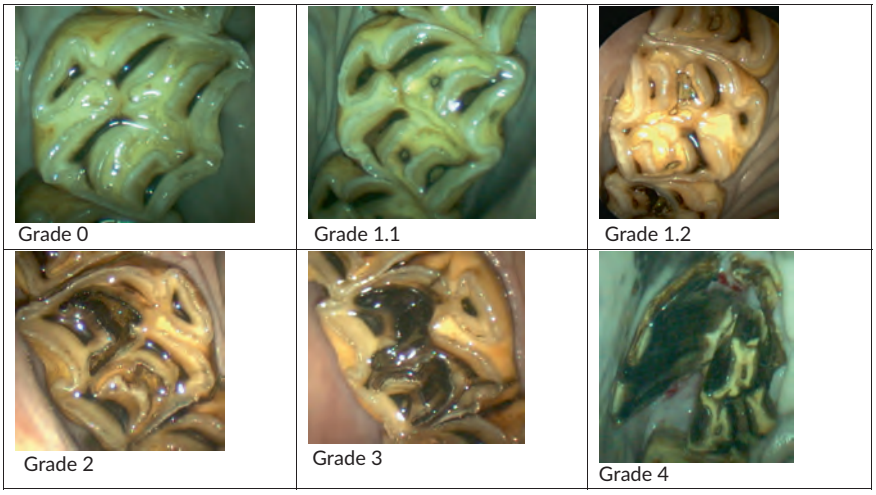

FIGURE 1 The Modified Honma Classification scale for infundibular caries. Grade 0: no sign of caries macroscopically. Grade 1.1: small degree of decay isolated to the cementum adjacent to the infundibular vascular channel. Grade 1.2: more extensive decay of infundibular cementum. Grade 2: Caries impacting infundibular cementum and enamel. Grade 3: Caries affecting infundibular cementum, enamel, and dentin. Grade 4: Caries has led to loss of tooth integrity as seen by dental fracture through the infundibulum.

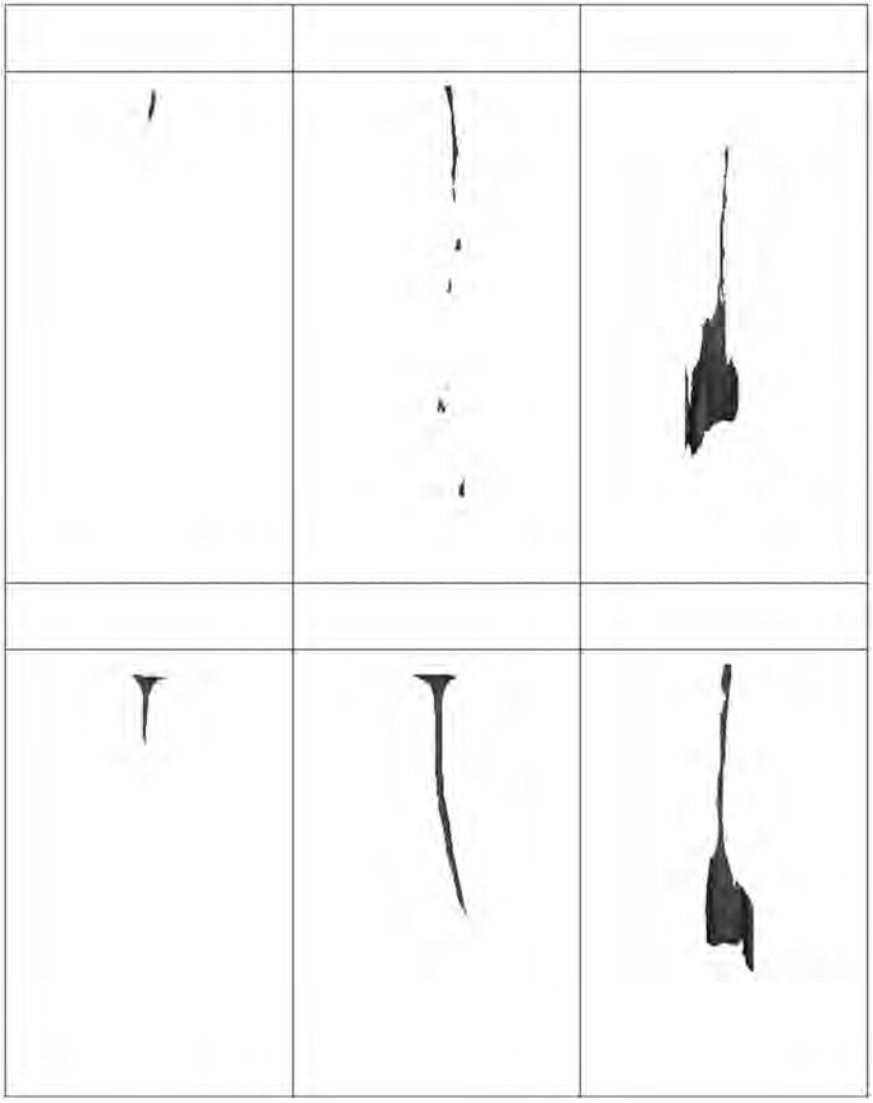

FIGURE 2 Windley et al. (2009) classification of infundibular lesions using computed tomography to demonstrate the cemental defects within the infundibulae of the crown. The black represents the cemental defect within the crown. The occlusal surface of the crown is located at the top of the image. Classification A0 has no defects within the crown (not imaged). Classification A infundibulae have no evidence of infundibular caries. Classification B infundibulae had evidence of infundibular caries. Classification A1 and B1 have abbreviated infundibular defects extending only slightly past the occlusal surface. Classification A2 and B2 have cemental defects along the length of the infundibulum. The defects may be continuous or broken up. Classification A3 and B3 have defects that continue apically from the occlusal surface along with an apical expansion of the infundibular defect. (Image from Windley, Z., Weller, R., Tremaine, W.H. & Perkins, J.D. (2009) Two- and three-dimensional computed tomographic anatomy of the enamel, infundibulae and pulp of 126 equine cheek teeth. Part 2: Findings in teeth with macroscopic occlusal or computed tomographic lesions. Equine Veterinary Journal 41(5), 441–447 with the permission of Equine Veterinary Journal).

Accurately identifying the type and extent of caries is critical to making clinical decisions regarding treatment planning and prognosis for individual teeth.

CASE SELECTION

Moderate to severe untreated carious lesions can lead to dental fracture, pulpitis, endodontic non-vitality, dental overgrowths, mastication dysfunction, quidding, hyporexia, and oral pain with diminished quality of life. Using restorative techniques, carious lesions can be cleaned, debrided, disinfected, and filled to prevent further accumulation of feed and deterioration with the overall goals of protecting the pulp, restoring the tooth to function, and rebuilding the structural integrity of the tooth. Case selection is a critical step in determining which teeth are good candidates for restoration. The following is a list of criteria that should be considered prior to any restoration:

1. Radiographs should demonstrate that the apical region is free of infection and disease that would otherwise necessitate an extraction. Apical radiolucency, apical osteosclerosis, blunting of the tooth roots, wide periodontal ligament spaces, reserve crown/root hypercementosis, and regional nodular hypercementosis can all be radiographic signs of endodontic infection.

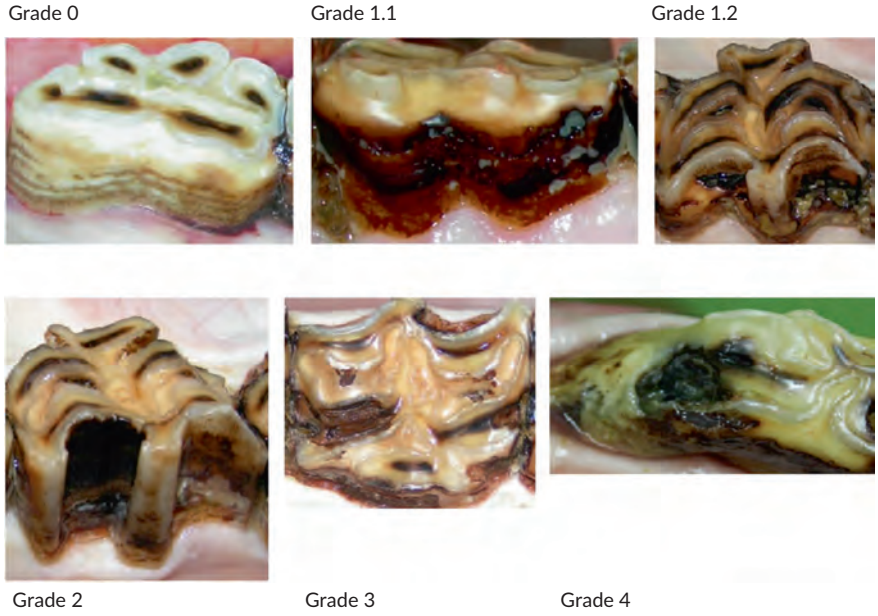

FIGURE 3 The Modified Honma Classification scale for peripheral caries as reported by Borkent et al. (2017). Grade 0: no sign of caries macroscopically. Grade 1.1: pitting decay/partial loss of peripheral cementum. Grade 1.2: total loss of peripheral cementum. Grade 2: caries impacting cementum and enamel. Grade 3: caries affecting cementum, enamel, and dentin. Grade 4: Caries has led to loss of tooth integrity as seen by involvement of pulp horn. (Image from Borkent, D., Reardon, R.J.M., McLachlan, G., Smith, S. & Dixon, P.M. (2017) An epidemiological survey on the prevalence of equine peripheral dental caries in the United Kingdom and possible risk factors for its development. Equine Veterinary Journal 49(4), 480–485 with the permission of Equine Veterinary Journal).

2. There should be no nasal discharge or sinus disease on the ipsilateral side of a questionable maxillary cheek tooth.
3. There should be no clinical signs of endodontic disease, dental fracture extending into the reserve crown, or tooth resorption. Intraoral clinical signs of significant infection include open feed-packed pulp horns, mucogingival fistulae, and/or extensive gingival recession.
4. No open pulp horns indicating endodontic infection/non-vitality of the tooth should be present. If noted, an endodontic procedure can be combined with restorative therapy, but open pulp horns should not be ignored and covered up with restorative material. An in-depth discussion of endodontic therapy is beyond the scope of this paper.
5. The owner should be committed to scheduled rechecks to monitor restoration(s) and carious lesion(s).
6. The location and depth of the carious lesion should be sufficiently deep to allow for creation of a cavity preparation site that can retain restorative material. In horses, there is no published adequate depth of cavity preparation to retain restorative material, but it is the author's experience that 5 mm at minimum is sufficient.
7. Operator experience and a restorative/endodontic equipment arsenal is necessary to perform restorations and possibly an endodontic procedure (indirect or direct pulp capping or vital pulp therapy) if pulp is exposed during the debridement phase of restoration. Pearce and Horbal (2022) provide a comprehensive list of equipment. A dental oroscope is necessary for performing restorations.
8. A horse's temperament plays a part in treatment too. Horses that are resistant to sedation or have excessive mouth motion during sedation may not be good candidates for restoration.

Further guidance has been provided regarding the grade of caries and restoration. In general, up to 90% of maxillary cheek teeth have some degree of infundibular hypocementosis and up to 90% of cheek teeth demonstrate grade 1 or 2 infundibular caries (Pearce & Horbal, 2022). The majority of these teeth never develop severe infundibular caries and do not require treatment (Pearce & Horbal, 2022). Aged horses with shallow or expired infundibula and young horses with shallow occlusal defects also do not require restoration (Pearce & Horbal, 2022). In addition, Pearce and Brooks (2021) described grade 3 infundibular caries with a minimum infundibular defect depth of 10 mm and grade 2 infundibular caries with a minimum infundibular defect depth of 10 mm and a contralateral tooth having grade 5 infundibular caries as suitable candidates for restoration. Finally, occlusal, infundibular, and peripheral caries can all be present on one tooth; therefore, the clinician should be prepared to perform restoration in any anatomic location of the tooth as indicated by the site and grade of decay.

RESTORATION OF CARIES

Restoration of dental caries is a technique-sensitive procedure. Without good visualisation and a thorough understanding of tooth anatomy, dental materials, and restorative dental principles, healthy dental structures can be damaged and long-term success diminished. The steps and equipment used for infundibular restorations has been described in detail (Horbal et al., 2017; Pearce, 2015; Pearce & Brooks, 2021; Pearce & Horbal, 2022). The following is an abbreviated list of steps to restore an infundibulum:

1. All necrotic, impacted feed material needs to be removed from the infundibulum. This requires the use of dental curettes and picks, a high-speed dental drill with carbide burs, a low-speed dental drill with Lindemen and other assorted dental burs, and Hedstrom and K-flex endodontic files.
2. Once the feed is removed, all loose and decayed dental material (cementum, enamel and dentin) is debrided. This requires the use of the endodontic files and drills with burs. Clear visualisation via an oroscope is required for this step because it carries the highest risk for pulp exposure. Avoid removing healthy dental material!
3. Once debrided, the infundibular cavity is flushed with an air-water syringe (part of the dental unit that can spray water, compressed air, or both together) and inspected. Disinfectant solutions such as EDTA, dilute sodium hypochlorite, and/or chlorhexidine are flushed into the cavity preparation. If sodium hypochlorite solution is utilised, suction should be used to prevent contact of the solution with the soft tissues of the mouth. Once the disinfectant solution rinse is complete, the cavity and surrounding tissues are copiously flushed with water and dried using the air-water syringe. It is imperative that the cavity be completely dry (not desiccated) to allow for proper bonding of restorative materials.
4. A layer of a single step-etch bonding product is applied to the walls of the cavity with an applicator to create a thin layer of product. A light flow of air is used to thin the material further to avoid clumping. The material is then light cured for 20 seconds.
5. The cavity is filled with a dual-cured flowable resin composite. The cavity should be filled in incremental layers with thin tight regions of the cavity filled apically first followed by apical to coronal filling of the wider portion of the cavity. Periodic light curing of the composite is necessary to stabilise the composite and prevent the flow of material coronally due to gravity. This incremental light curing will prevent the creation of voids within the restoration and provide for optimal bonding to dental hard tissue.
6. A round diamond dental bur is used to smooth the occlusal profile of the restorative materials. The occlusal profile should be even with surrounding dental hard tissue or located 1–2 mm below the occlusal surface to prevent abnormal contact and excessive wear with the opposing teeth. Rinse and dry the restorative material with the air-water syringe.
7. A final layer of unfilled resin material is applied and cured to seal exposed dentinal tubules and create a smooth finish.

Although the sequence of steps may seem simple enough, the level of skill required to execute all steps is high. Restricted oral access, dental anatomy, patient motion, modified dental equipment, and restorative material properties create a challenging work environment with a high level of skill needed for procedure execution. Practice on cadavers prior to a first attempt at restoration in a live horse is strongly recommended.

Occlusal caries restoration employs a similar series of steps to infundibular restoration. Although the removal of feed from the carious region may be easier for occlusal caries, the debridement of carious dental hard tissue still carries a significant risk of pulp exposure. Care should be taken during the debridement phase to avoid pulp exposure and the removal of healthy dental tissue. Occlusal debridement should also be performed in a manner that will result in the creation of a depression or cup (if not already present) to provide mechanical support for the restorative material. Composite material should fill the cavity preparation similar to infundibular restorations but not protrude above the occlusal surface.

Restorative techniques to address peripheral caries are not available in the literature. To date, oral hygiene and oral anti-septic regimens have been recommended for the treatment of peripheral caries. In addition, the primary dental problem leading to plaque accumulation and feed stasis on the periphery of the tooth (e.g. fractured tooth, severe infundibular caries, malpositioned/malerupted tooth, periodontal disease) needs to be addressed, if present.

In conclusion, the restoration of carious lesions can be quite successful in the horse. A recent study reported that infundibular restorations have a 97% success rate (Pearce & Brooks, 2021). Despite the high success rate reported, clinicians interested in performing restorations need to be prepared and experienced. Cadaveric practice is strongly recommended prior to work on the live horse because pulp exposures and poorly restored teeth can lead to pain and diminished quality of life for the patient. With this mind, clinicians who are willing to invest in their learning and equipment will be rewarded with the ability to address dental decay and significantly improve the comfort of their patient's oral cavity.

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Equine Veterinary Education

CASE REPORT

Proximal segmental ostectomy under standing sedation for treatment of open comminuted axially displaced fractures of the fourth metatarsal bone in two horses

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SUMMARY

Two horses with wounds of suspected traumatic aetiology over the proximal aspect of the fourth metatarsal bone that occurred 10–14 days previously were presented. Both were 4/5 lame (AAEP lameness scale) on the affected leg. Based on the clinical and radiographic findings, a diagnosis of an open comminuted axially displaced fracture of the fourth metatarsal bone was made in both cases. Initially, patients were managed with local wound therapy, external stabilisation, box rest and medical therapy. Subsequently, a proximal segmental ostectomy of the fractured part of the fourth metatarsal bone was elected in these cases, because this surgical technique avoids the use of implants and eliminates the impingement of the suspensory ligament. The procedure was performed under standing sedation and regional anaesthesia. An Esmarch bandage was placed proximal to the tarsus. The surgical incision extended from 1 cm proximal to the level of the tarsometatarsal joint to 1 cm distal to the fractured area at the plantarolateral aspect of the proximal metatarsus. The fractured proximal third of the fourth metatarsal bone was transected from its ligamentous attachments and all fracture fragments were removed (Figure 1). The proximal end of the remaining distal portion of the fourth metatarsal bone was rounded with a mallet and chisel and Luer bone rongeurs. A negative pressure wound therapy system was applied for 1–2 weeks and a distal limb Robert Jones bandage was placed for 7–8 weeks. Antimicrobial and nonsteroidal anti-inflammatory therapy was administered. Stall rest was recommended for 3–4 weeks before the progressive introduction of in-hand walking. Proximal segmental ostectomy of open, axially displaced fractures of the proximal third of the fourth metatarsal bone under standing sedation and regional anaesthesia is an appropriate treatment option, which can be performed as an alternative to open reduction and internal fixation. The procedure was well tolerated by the horses and owners were satisfied with the outcome.

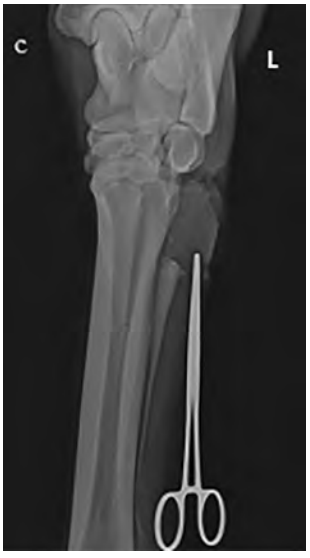

FIGURE 1 Intra-operative radiograph of Case 2. Dorsolateral-plantaromedial radiographic projection of the left proximal metatarsus immediately after proximal segmental ostectomy.

KEYWORDS
horse, open fracture, proximal ostectomy, splint bone, standing surgery

Key points

- Surgical ostectomy of the proximal portion of the splint bone provides a less invasive surgical option compared to complete splint bone extirpation.
- By avoiding internal fixation, the risk of implant infection is eliminated.
- Performing the procedure standing rather than under general anaesthesia is expected to minimise the risk of peri-operative surgical complications, although the frequency of postoperative complications with this technique merits ongoing evaluation.

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Equine Veterinary Education

CLINICAL COMMENTARY

Comminuted fractures of the proximal third of the fourth metatarsal bone: Treatment strategies

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GENERAL REMARKS CONCERNING SPLINT BONE FRACTURES

Fractures of the vestigial metacarpal and metatarsal bones, which are commonly referred to as splint bones, can occur anywhere along the length of the bone and are very common in horses of all ages (Jackson et al., 2007). Although small and vestigial in nature, the metacarpal/metatarsal bones are integral parts of the supporting and stabilising structures of the equine limb. They serve as a site for important carpal and tarsal ligament insertions (Jackson et al., 2005) such as the strong collateral ligaments, which attach to the proximal part of the bone (Figure 1). The proximal aspect of each metacarpal/metatarsal bone articulates with the carpal/tarsal bones and provides axial support to these structures. The metacarpal/metatarsal interosseus ligament differs substantially among horses and may start to ossify at an early age. Robust fascia (Figure 2) covers the tendons in the proximal region of the metacarpus and is attached to the splint bones (Jackson et al., 2005).

Splint bones are predisposed to injury because of their anatomic location, the unpredictable nature of horses and certain equine management practices (Derungs et al., 2004). Kicks from other horses are probably the most important cause of splint bone fractures (Figure 3), but fractures may also occur spontaneously during exercise. Splint bone fractures can be open or closed, simple or comminuted and localised in the proximal, middle or distal part of the bone. Injuries such as kicks or falls often result in open fractures of the proximal part of the splint bones. Lameness is usually severe in horses with open proximal fractures and moderate in those with distal fractures. The interval between the injury and the time of presentation also profoundly affects the severity of lameness. In addition to lameness, an open wound is present near the splint bone (Figure 4). The degree of swelling, pain and heat is directly related to the extent of soft tissue damage. Radiographs are required to confirm the diagnosis and to rule out other complications. It is very important to take several views, and the proximal articulation should always be included. Ultrasonography is essential for the assessment of the suspensory ligament. When available, computed tomography is a valuable tool in the diagnosis of difficult cases. Complications, including nonunion, osteomyelitis and the formation of a sequestrum or excessive callus are common when the fracture is not treated

FIGURE 1 Illustration showing the insertion of the collateral ligament on the lateral splint bone of the hind limb.

appropriately (Jackson et al., 2007). Interestingly, sequestrum formation occurs frequently and septic osteitis is not uncommon in splint bone fractures. Involvement of the cannon bone affects the prognosis negatively.

TREATMENT OF PROXIMAL SPLINT BONE FRACTURES

Proximal splint bone fractures are the most difficult to treat and a variety of management techniques including surgical intervention have been described. Although some authors suggest surgical fixation of open proximal splint bone fractures in an attempt to minimise complications and residual chronic lameness, the use of implants is not indicated in open splint bone fractures because of the high risk of implant infection. Horses with open multi-fragment proximal splint bone fractures are candidates for standing surgery; a combination of sedation and local anaesthesia allows thorough wound debridement to remove loose bone fragments and necrotic tissue (Jackson et al., 2007; Jackson & Auer, 2019). The wound must be kept open and flushed regularly. Postoperative management includes antimicrobial and anti-inflammatory drugs in conjunction with a support bandage. Follow-up radiographs are taken 2 and 4 weeks later. If the wound has healed and the radiographs show that the splint bone is stable and the fracture is healing, no further treatment is necessary (Figure 5). If the radiographs show instability of the proximal

FIGURE 2 Illustration showing the metacarpal interosseus ligament and distal ligament of the splint bone.

FIGURE 3 Illustration of a kick injury to the splint bone.

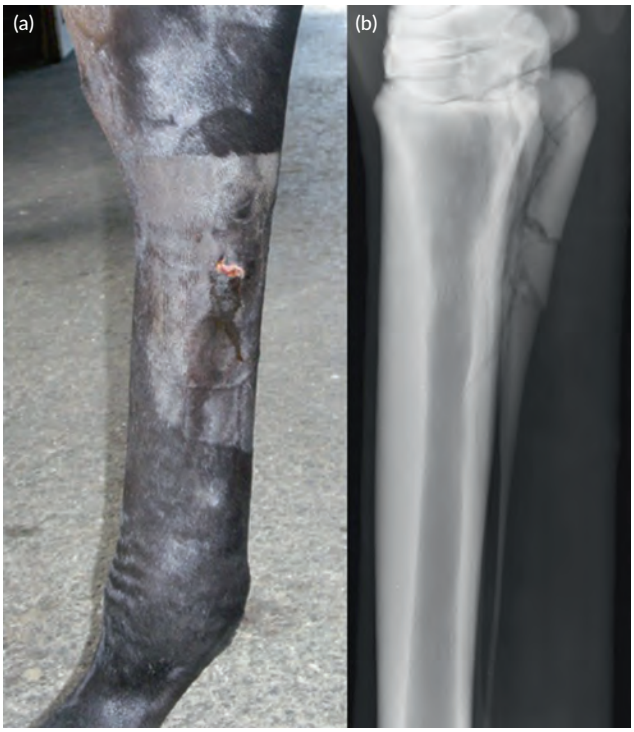

FIGURE 4 Typical clinical picture of a horse with a fracture of the splint bone after a kick injury. (a) wound over the lateral splint bone; (b) oblique radiograph showing a comminuted fracture of the splint bone.

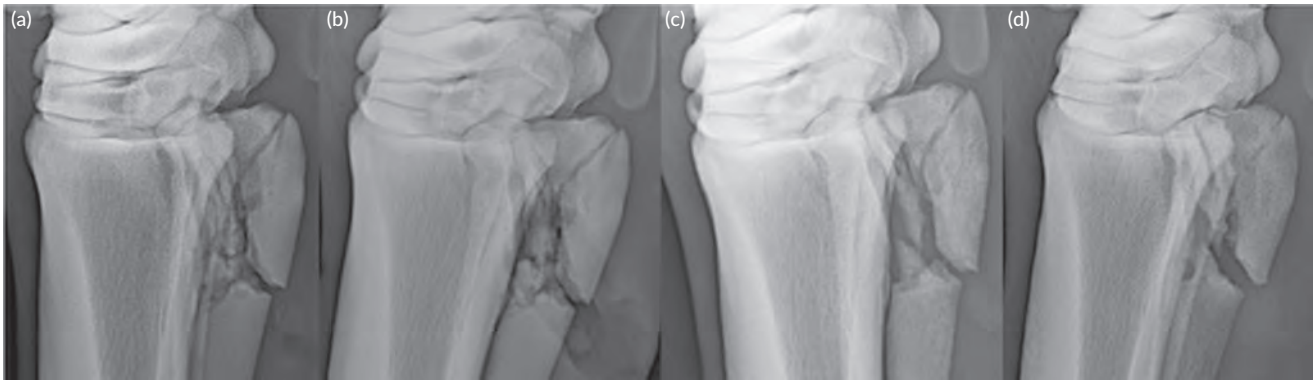

FIGURE 5 Radiographs showing a proximal splint bone fracture that was operated on using a standard technique and was considered healed 5 many months later. The necrotic and infected tissue was removed, but the proximal stump was left in place. (a) immediately after the injury; (b) after wound debridement; (c) 1 month postoperatively; (d) 5 months postoperatively.

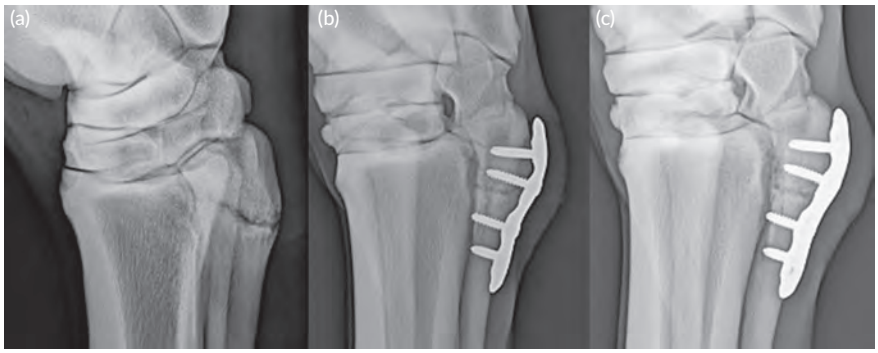

FIGURE 6 Radiographs showing an open proximal splint bone fracture: (a) nonunion after 2 months of conservative treatment; (b) fixation with a 4-hole 3.5 mm LCP; and (c) 2 months postoperatively.

fragment, internal fixation must be considered as soon as all signs of infection have resolved. Postoperatively, the horse should be kept in a box stall for 1 month followed by 2 months of hand-walking or turning out in a small paddock. In one report, 12 of 14 horses with open comminuted splint bone fractures returned to athletic function after this type of treatment (Walliser & Feige, 1993). In another study, healing occurred in the majority of horses that were treated with wound debridement alone; fracture stabilisation or splint bone removal was not done (Jackson et al., 2007). Removal or fixation of a splint bone can always be carried out later if conservative treatment fails (Figure 6). In some cases, a segmental ostectomy can be undertaken in horses with complicated fractures composed of multiple small fragments (Jenson et al., 2004). Contaminated and necrotic tissues are debrided as well as loose bone fragments, osseous callus and sequestra. Oblique ostectomy of the remaining proximal and distal portions of the splint bone is carried out using an osteotome or oscillating saw. Complete removal of the affected splint bone should only be considered in cases with extensive comminution and infection of the proximal part of the bone, or infection and sequestration that occur after conservative treatment (Baxter et al., 1992). However, complications such as instability of the carpal and tarsal joints (Figure 7) are common after the removal of an entire splint bone. According to Baxter et al., only the lateral splint bone of the hind limb should be removed. The other splint bone is required for axial support of the carpal/tarsal joint. However, in the hind limb, strong collateral ligaments insert on the lateral splint bone, which if completely removed can lead to severe instability of the tarsal joint. In our experience, removal of a splint bone is rarely necessary and can be avoided in almost all splint bone injuries.

Vajs and Haltmayer (2023) describe the successful treatment of an open fracture of the proximal splint bone in two horses. The proximal fragments were removed with the horse standing and sedated, and the nerves were blocked with local anaesthetic. Treatment of splint bone fractures in the standing horse has proved to be successful over the last few decades and general anaesthesia for operations on the lateral splint bones is seldom carried out. Although surgery in the standing horse is feasible, it is important to note that the proximal part of the splint bones in the hind limb is connected to the tarsometatarsal joint, and the collateral ligaments are inserted into the splint bones. It is, therefore, generally accepted that the proximal part of a splint bone should be preserved whenever possible (Jackson et al., 2005). Only loose, devitalised and infected bone fragments should be removed and the remainder of the splint bone left in situ.

Veterinary surgeons must realise that the tarsometatarsal joint will be open after removal of the proximal part of the splint bone,

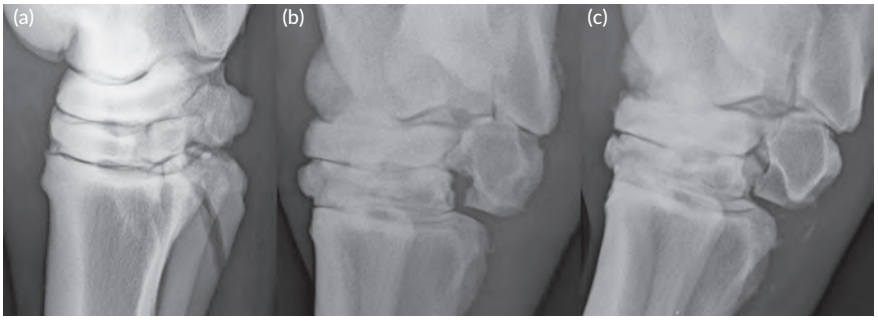

FIGURE 7 Radiographs showing a proximal splint bone fracture in which the proximal stump was removed. The resulting instability led to the development of significant osteoarthritis in the proximal and distal intertarsal joints as well as the tarsometatarsal joint. (a) acute proximal splint bone fracture; (b) after removal of the proximal stump; (c) development of osteoarthrosis.

and more importantly, instability of the tarsal joint may occur. Mild joint instability and arthrosis constitute the least severe complication, while subluxation or complete dislocation of the tarsometatarsal joint represents the most problematic. For these reasons, we would have chosen a different surgical technique and removed only the infected bone fragments in both cases. This surgical technique has also been described in a standard textbook on equine surgery (Jackson & Auer, 2019). We have used this technique to treat many horses and the outcomes were good and without complications in most. We have also rarely observed suspensory ligament problems after open fractures of the splint bone. Although the fragments are usually displaced axially by a kick injury, suspensory ligament problems are rare. This is in contrast to distal splint bone fractures, where irritation of the suspensory ligament has sometimes been observed.

However, the proverb 'all roads lead to Rome' is perhaps applicable. Although their surgical technique was somewhat risky, Vajs and Haltmayer (2023) achieved success with only slight lameness 17 months postoperatively in one case. In the other, telephone consultation rather than a veterinary examination revealed a good outcome. Nevertheless, we would not endorse this choice of treatment but would instead follow the recommendations described in the literature (Jackson et al., 2005). The proximal stump of the splint bone should be preserved whenever possible.

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CONFLICT OF INTEREST STATEMENT

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ETHICS STATEMENT

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Equine Veterinary Education

CASE REPORT

Surgical treatment of cervical (C7-T1) instability caused by discospondylitis in a horse

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SUMMARY

A 3-year-old Quarter Horse gelding presented for an approximately 4-week history of generalised stiffness and reluctance to move. Upon arrival, the horse was quiet, alert, and in poor body condition (3/9). Its rectal temperature, pulse and respiratory rate were within normal limits. He had a stiff gait and appeared by body posture, similar to that of a horse with laminitis. A neurological examination of the cranial nerves showed no abnormalities and proprioceptive deficits were not observed. The horse had been examined and radiographed by multiple veterinarians, without a conclusive diagnosis. EPM testing yielded a negative result. Serum Amyloid A (SAA) and complete blood count (CBC) were performed. The SAA was 3 (reference range 0-20 mg/l) and the CBC showed some abnormalities such as a haematocrit of 22.4% (reference range 30%–47%) and a haemoglobin of 8.5 g/dL (reference range 10.7–16.5 g/dL). A CT and a CT/myelogram of the cervical spine were performed under general anaesthesia. The most important findings observed in the CT images were the vertebral endplates moderately to markedly irregular with poorly defined and irregularly shaped regions of lysis at C7-T1 (Figure 1). These findings were consistent with discospondylitis at the level of C7-T1 leading to secondary instability of the intervertebral disc space. Based on the CT and myelogram findings, it was decided to perform a ventral intravertebral body fusion of C7-T1 using cortical screws in lag fashion and a 4-hole locking compression plate (LCP). The patient was discharged from the hospital with marked improvement. Four months after the procedure, the horse continues to show improvement in mobility and range of motion of the neck.

KEYWORDS
horsecervical fusion, discospondylitis, surgical treatment

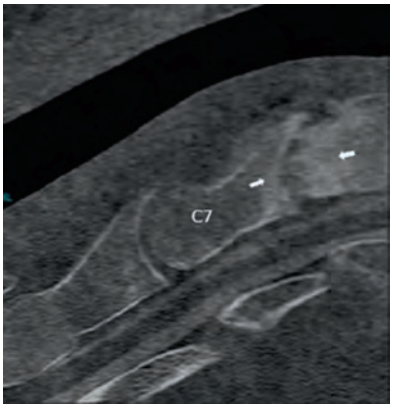

FIGURE 1 Presence of a moderate to marked sclerosis and regions of lysis of the opposing endplates at C7-T1 (arrows).

Key points

- Discospondylitis is an uncommon disease in horses and the clinical signs are associated with neck stiffness and forelimb ataxia.
- Affected animals are usually treated systemically with antibiotics combined with nonsteroidal anti-inflammatory drugs (NSAIDs). An inadequate response to conservative treatment, or if there is evidence of spinal cord compression on myelography or MRI, or if severe, progressive neurologic problems occur, surgical intervention should be considered.
- The use of the LCP combined with transvertebral screws proved a good alternative for cervical vertebral fusion of C7-T1 in horses.

[Correction added on 2 September 2023, after first online publication: The author Timothy B. Lescun has been added as the 5th author in this version.]

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Equine Veterinary Education

CLINICAL COMMENTARY

C7-T1 ventral interbody fusions: Opportunities, nuances and expectations

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Santos et al. (2023) describe an interesting case of discospondylitis at the level of C7-T1 in a young horse that was managed surgically. This is the first report of surgical treatment for discospondylitis at this level in the horse, and the authors should be commended for their efforts and surgical modifications to provide a successful outcome to the patient. Caudal cervical vertebrae are becoming a more recognised source of pathological conditions that clinically result in pain, lameness, ataxia, inferior performance or any combination of these clinical signs (Dyson, 2011).

The aetiology of discospondylitis in horses remains unclear but infection and trauma are often cited as the inciting cause (Moore, 1992). Horses affected with discospondylitis can develop degradation of the intervertebral disc, the collapse of the intervertebral space, dorsal protrusion of the intervertebral disc into the spinal canal, intervertebral foramen narrowing and/or endplate osteolysis of the affected vertebrate (Figure 1).

Clinical signs of discospondylitis include cervical spine pain, stiff gait and varying levels of ataxia that can originate from intertwined pathological processes. Horses with caudal cervical pathologic lesions often have uni- or bilateral atrophy of the pectoral muscles, atrophy of muscles where the neck and shoulder muscles tie in together and antebrachial muscle atrophy. Interestingly, in this case, cervical spine and forelimb stiffness were present without any ataxia. Cervical spine pain results in a limited cervical spine range of motion and consequently a stiff gait. The pain can be severe enough that a facial grimace and/or 'wide-eyed' appearance can be appreciated when the horse moves its head and cervical spine. Cervical spine pain can be attributable to instability caused by vertebral endplate osteolysis, loss of intervertebral disc and collapse of the intervertebral space; pain can also arise from vertebral endplate cartilage degradation resulting in subchondral bone and nerve exposure. A stiff gait associated with discospondylitis not only comes from cervical spine pain but may also originate from caudal nerve root neuritis and/or nerve root compression. Caudal cervical vertebral spine (C6-T1) instability secondary to pathological changes associated with discospondylitis may result in intervertebral foramen narrowing causing nerve root compression and a stiff forelimb gait. Ataxia that occurs in horses with discospondylitis can be multifactorial. One explanation is the instability caused by vertebral endplate osteolysis and loss of intervertebral disc causing varying degrees of vertebral kyphosis resulting in cervical vertebral stenotic myelopathy. A second explanation for ataxia associated with discospondylitis would be the dorsal protrusion of the intervertebral disc into the spinal canal because of the collapse of the intervertebral space.

Horses with C7-T1 pathological lesions can be separated into two different classes. Class 1 patients present with a varying degree of neurological deficits that originate from dorsal and/or lateral spinal cord compression. Class 2 patients usually present with moderate-to-severe forelimb lameness that cannot be eliminated with forelimb peri-neural anaesthesia or forelimb intra-articular anaesthesia; these cases are often associated with intervertebral foramen stenosis of the caudal cervical spine or some level of intervertebral disc disease (Ricardi & Dyson, 1993).

Imaging the C7-T1 region can be a challenge as this area is covered by heavy muscle and the scapulohumeral joint often superimposes this region. Diagnostic modalities used to assess the caudal cervical spine include cerebral spinal fluid analysis, standing survey radiographs, nuclear scintigraphy, ultrasonography, cervical myelography, computed tomography (CT) and magnetic resonance imaging (MRI). In this case report, serial complete blood cell counts and serum amyloid A measurements were used to help decide that an infectious process was under control. Cerebral spinal fluid culture and sensitivity along with cytology could have been viable options to help assess the presence of infection as well. Fan-beamed CT myelography was performed in this case providing great detail about structures at C7-T1 which included collapsed intervertebral disc space, irregular-shaped regions of lysis on vertebral endplates, ventral periosteal proliferation on vertebral endplates, mild-to-moderate spinal cord compression. Multiplanar CT reconstruction and three-dimensional imaging using fan-beamed CT technology is consistently attainable at the C7-T1 level with the horse under general anaesthesia allowing for thorough examination of dorsal and lateral contrast dye columns,

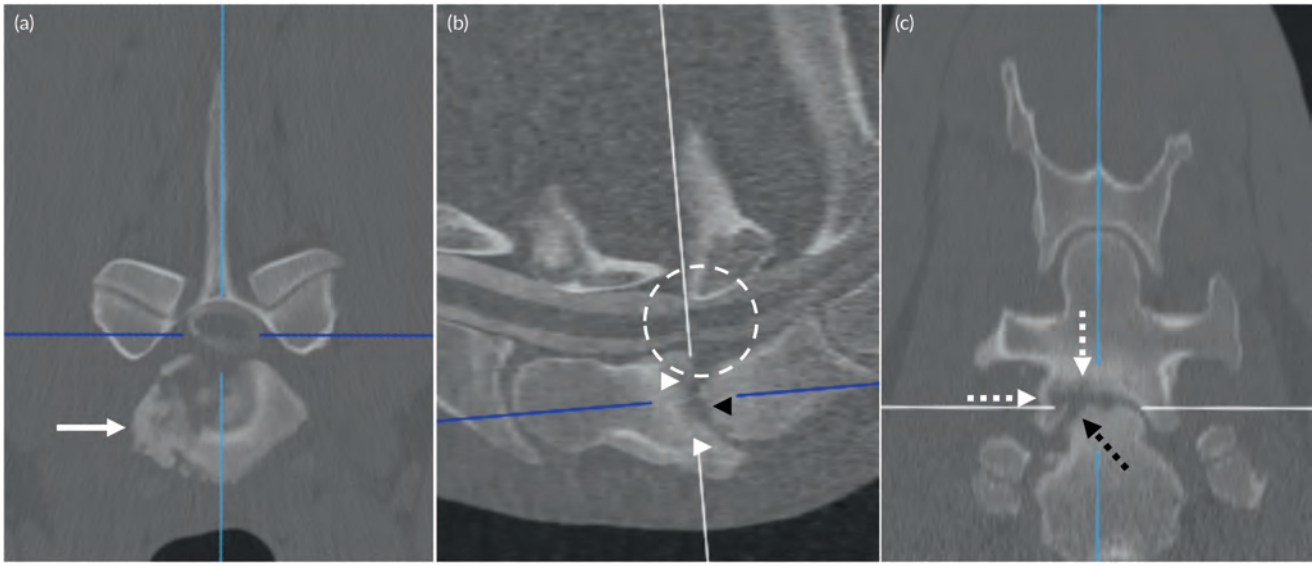

FIGURE 1 CT imaging centred on C7-T1 in the same horse. (a) Coronal view showing endplate lysis (white arrow) and ventrolateral spinal cord compression. Moderate irregular periosteal proliferation is present along the right lateral and ventrolateral aspect of C7 vertebral body. (b) Sagittal view showing endplate lysis of caudal C7 (white arrowheads) and cranial T1 (black arrowhead). The dashed circle is focused on dorsal protrusion of soft tissue into the ventral spinal canal and dorsal extradural spinal compression resulting in overall reduced dural diameter. The intervertebral disc spacing is markedly narrowed. (c) Axial view showing endplate lysis of caudal C7 (dashed white arrows) and cranial T1 (dashed black arrows).

assessment of articular facet sizes, shapes and associated pathologic lesions, permits for determining intervertebral foramen stenosis and detection of disc degeneration. CT myelography should be considered the current gold standard for caudal cervical spine diagnostic imaging and pre-surgical planning.

Surgical fusion of the equine cervical spine was first reported by Wagner et al. (1979) using the Bagby basket which has been modified over the past 40 years; currently, titanium fully threaded, or partially threaded Kerf–Cut Cylinder packed with cancellous bone graft is available for ventral interbody fusion and globally remains the mainstay for spinal fusions. Vertebral fusion using a locking compression plate for cervical vertebral stenotic myelopathy was first reported in a 3-month-old Warmblood filly (Reardon et al., 2009), which was followed by a biomechanical study comparing the locking compression plate to the Kerf–Cut Cylinder (Reardon et al., 2010). Results of that study indicated that the locking compression plate had higher biomechanical properties than the Kerf–Cut Cylinder; however, in order to obtain complete vertebral arthrodesis, a large portion of the intravertebral disc needs to be removed, which is difficult when applying a locking compression plate. The use of a polyaxial pedicle screw and rod construct was first reported in a proof-of-concept study (Aldrich et al., 2018) followed by a retrospective study using the polyaxial pedicle screw and rod construct that included 10 horses over a 4-year period (Pezzanite et al., 2022). In that study, two horses were euthanised within the first year. In 6 of 8 horses with ≥1-year follow-up, ataxia improved by 1–3 grades, with an average improvement of 1.25 grades. In four horses, ataxia improved to grade 0–1. In two horses, the gait was unaffected but the neck comfort improved (Pezzanite et al., 2022). Recently, an implant system consisting of a 3-D printed plate, cancellous screws and a titanium cage was designed from a 3-D rendering of the equine cervical spine to contour along the ventral aspect of the cervical spine. Clinical outcomes of this cervical spine implant system are favourable (Rossignol, 2022). Excluding the Kerf–Cut Cylinder, all aforementioned techniques have substantial limitations to arthrodese C7-T1. Ventral interbody fusion of the C7-T1 articulation using the Kerf–Cut Cylinder and specialised long-handled instrumentation has been performed for the past 10 years and is indicated when dorsal dye column compression is present on myelography and/or CT myelography, lateral spinal cord compression is present on CT myelography, intervertebral foramen stenosis is present on CT myelography, or when intervertebral disc disease is detected on CT myelography. Ventral interbody fusion of C7-T1 can be successfully performed in mature equine patients and results of an unpublished study by Grant et al. (2023) suggest that C7-T1 ventral interbody fusion has a good prognosis to improve comfort level, resolve lameness and/or reduce the neurological grade that is safe for riding or provides a good quality of life (Figure 2).

The success of C7-T1 vertebral arthrodesis, in this case, using a 4-hole locking compression plate along with two transvertebral 5.5 mm cortical bone screws placed in lag fashion, can be contributed to the light weight of the horse (weight was not reported, but a 3/9 body condition is reported) and the lack of an intervertebral disc, and intervertebral collapse. Although not reported in this case report, the use of cancellous bone grafting could have been used within the intravertebral disc space to help form a more solid C7-T1 arthrodesis. Access to the C7-T1 region requires retraction and securing the

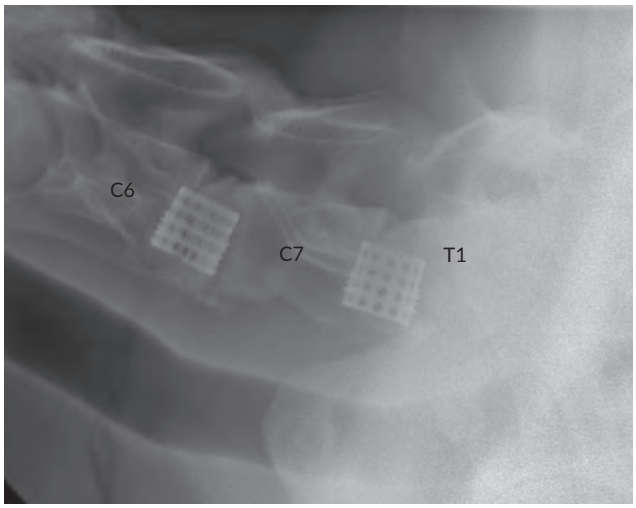

FIGURE 2 Radiograph obtained one year after ventral interbody fusion surgery for cervical vertebral stenotic myelopathy. Partially threaded titanium Kerf–Cut Cylinders packed with cancellous bone graft had been placed at C6-C7 and C7-T1.

forelimbs caudally before the area is aseptically prepared and draped. The surgical approach to C7-T1 is no different than when performing ventral interbody fusion in the remaining cervical regions, but there are some noteworthy anatomical variables. Gaining access to and visualisation of the C7-T1 area is a challenge because this site is deeper and partially obstructed by the cranial sternum. The truncus bicaroticus is the common trunk of the left and right common carotid arteries. The truncus bicaroticus lies dorsal to the caudal deep cervical lymph nodes and the bifurcation typically occurs directly over the C7-T1 articulation. The surgeon should be prepared for variable positions and lengths of the truncus bicaroticus. Careful dissection is necessary to allow for the common carotid trunk to be retracted from the drill site without damaging the vagosympathetic trunk. C7 does not have a ventral spinous process but does have two small chevron-shaped bony tubercles on the ventral midline in the mid-body area. High-quality intra-operative radiographs are necessary during surgery for measurement purposes and proper implant placement at C7-T1.

The only reported complication after surgery in the report by Santos et al. (2023) was the development of Horner's syndrome. This complication most likely arose from applying too much pressure on the right vagosympathetic trunk. An anaesthetic recovery complication associated with vagosympathetic trunk damage includes laryngeal spasm or right recurrent laryngeal nerve paralysis. This complication most likely occurs from inadvertent pressure on the recurrent laryngeal nerve during retraction for instrumentation at C7-T1. If the left recurrent laryngeal nerve was dysfunctional before surgery, then laryngeal spasm or complete laryngeal collapse can occur and be fatal. This complication can be reduced by performing a pre-operative endoscopic exam; if the left arytenoid is not abducting properly, then left recurrent laryngeal neuropathy is assumed and the surgical approach should be performed on the left side of the trachea.

It is important for veterinarians to be aware that diagnostics and treatment of varying pathological processes at the C7-T1 level are possible with a good prognosis. It is very important to assess C7-T1 when examining the cervical spine in horses. The advancements in CT myelography can help pre-operative planning and prognosticate for the owners. The Kerf–Cut Cylinder continues to be the most common implant used for cervical spine fusion; however, innovative surgical options and new implant systems are imperative to continue to improve the area of cervical spine surgery. The current case report by Santos et al. (2023) highlights a unique way to perform ventral interbody fusion for discospondylitis at C7-T1 and adds another tool to our toolbox for cervical spine orthopaedic surgery.

AUTHOR CONTRIBUTIONS

Sole author manuscript.

CONFLICT OF INTEREST STATEMENT

No conflicts of interest have been declared.

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ETHICS STATEMENT

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Equine Veterinary Education

ORIGINAL ARTICLE

The prevalence and changes over time of equine glandular gastric disease in a teaching herd population

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Summary

Background: The natural progression of equine glandular gastric disease (EGGD) in the absence of treatment has not yet been described in the literature, nor has the prevalence in a teaching herd population been reported.

Objectives: The aims of this study were to determine the prevalence of disease in a teaching population over the study period (2019–2021) and to observe the changes over time in disease severity of naturally occurring diseases (not experimentally induced) without medical intervention.

Methods: Twenty-one horses underwent an initial gastroscopy and a repeat gastroscopy between 14 and 731 days later. Gastroscopy data were graded quantitatively and described qualitatively. Prevalence and 95% confidence intervals (CI) were calculated. The changes over time were determined by comparing initial and repeat gastroscopies.

Results: The prevalence from initial, repeat and total number of gastroscopies was 62% (95% CI: 40.8–79.3), 71% (95% CI: 50.0–86.2) and 67% (95% CI: 51.6–79), respectively. The changes over time included worsening of disease in 29% of horses (95% CI: 13.8–50.0), improvement of disease to a lower grade in 24% (95% CI: 10.6–45.1), no change in grade in 38% (95% CI: 20.8–59.1), and complete resolution of disease to grade 0 in 10% (95% CI: 2.7–28.9).

Main limitations: Limitations included a maximum of two gastroscopies per horse given COVID-19 restrictions on data collection, and highly varied interval times between initial and repeat gastroscopies.

Conclusion: In conclusion, there is a high prevalence of disease in this teaching herd. The changes over time in naturally occurring diseases without medical intervention might include worsening, improvement, no change or resolution of disease.

KEYWORDS
horse, EGUS, gastroscopy, pylorus, stress

INTRODUCTION

Despite the current gaps in knowledge regarding equine glandular gastric disease (EGGD), it is clear the risk factors, signalments, pathogenesis, lesion distributions and types of EGGD are distinct from those of squamous disease (Rendle et al., 2018; Sykes, Hewetson, et al., 2015; Sykes, Sykes, et al., 2015). The risk factors currently described in the literature include breed, use (i.e. showjumping), frequency of exercise, the use of high-dose phenylbutazone, having multiple riders and handlers, and stress (Sykes, Hewetson, et al., 2015; Sykes, Sykes, et al., 2015).

There is a clear breed predisposition in Warmblood horses (Mönki et al., 2016), with a 72% prevalence documented in Canadian Warmblood showjumpers specifically (Pedersen et al., 2018). There is also a clear predisposition in horses that are in training and/or competing (Paul et al., 2021), with one study concluding that horses exercised more than 5 days per week are 10.4 times more likely to develop EGGD (Sykes, Hewetson, et al., 2015; Sykes, Sykes, et al., 2015).

Lastly, stress is suggested to be associated with EGGD, however, this association is yet to be proven. Horses with EGGD have exaggerated and more rapid elevations in cortisol levels in response to exogenous adrenocorticotrophic hormone (ACTH) (Banse & Frank, 2019; Sauer et al., 2018). The causal relationship is not yet established; that is, whether EGGD causes exaggerated cortisol responses or if elevated levels resulting in loss of local prostaglandin production causes the lesions, or if both are linked to a third factor (Scheidegger et al., 2017).

Non-exercise-related stressors such as an increased number of caretakers and/or number of riders have been associated with an increased risk of EGGD (Mönki et al., 2016). Changes in herd dynamics have also been considered a risk factor (Busechian et al., 2021). Horses in group housing had higher post-stimulatory cortisol levels compared to those housed individually in one study (Sauer et al., 2019).

Overall, these findings support the theory that psychological stress increases the risk of EGGD development. These studies developed the proposed pathogenesis of the stress-cortisol-ulcer complex (Sauer et al., 2018; Scheidegger et al., 2017).

It is unlikely that a single aetiopathogenesis can explain all EGGD lesions (Rendle et al., 2018). Nonetheless, given these risk factors, it is unsurprising that there is a high prevalence of disease in sport horse populations reported in the literature, for example a prevalence of 64% in a sport horse population ($n = 493$) in the United Kingdom (Hepburn, 2014). Australian Thoroughbred racehorses were found to have an EGGD prevalence of up to 65% in one study (Sykes, Hewetson, et al., 2015; Sykes, Sykes, et al., 2015). The prevalence in high-level endurance horses has been described in two separate studies as 27% and 33% during the competition season (Nieto et al., 2004; Tamzali et al., 2011). Interestingly, there is also a high prevalence of disease described in pleasure horse populations, for example a prevalence of 54% of 191 horses not undertaking intense exercise (Hepburn, 2014). The prevalence of EGGD in teaching horse populations has not previously been described in the literature.

A post-graduate continuing education course involving gastroscopy was conducted at The Animal Hospital at Murdoch University (TAHMU) in 2019 (T3165/19). Eight horses from the teaching herd were used, and six gastroscopies were diagnostic. EGGD was detected in 63% of horses (unpublished data). This result instigated this project and the six diagnostic gastroscopies conducted during the education course are included in this study as initial gastroscopies for those horses. The project continued for 3 years and two (initial and repeat) gastroscopies were attained for a total of 21 teaching horses throughout the study period.

The objectives of this study were to determine the prevalence of naturally occurring EGGD in a teaching horse population at Murdoch University (Western Australia) where minimal risk factors were present and to evaluate the changes over time in disease prevalence and severity. A secondary objective was to describe the teaching use of the horses in the month prior to gastroscopy and their potential relationship to disease.

MATERIALS AND METHODS

This study was conducted at The Animal Hospital Murdoch University (TAHMU). Given the number of horses with evidence of gastric ulceration on the education day, gastroscopies became part of the routine veterinary care of the teaching herd. The intended interval for gastroscopies for routine care was every 12 months, or sooner if clinical signs developed.

The teaching herd were monitored for clinical signs by means of daily observation by TAHMU staff including an animal welfare worker and veterinary nurse. The clinical signs of EGGD are considered under the umbrella of EGUS (equine gastric ulcer syndrome), as there are currently no studies describing the clinical signs of EGGD specifically (Banse & Frank, 2019). These signs are vague and highly variable and can include inappetence, behaviour changes (including aggression or nervousness), weight loss, poor body condition and colic.

Whilst the staff were not assessing the horses for EGGD specifically, the horses were assessed for any signs of unwellness. Additionally, the horses are weighed approximately four times per year. The horses undergo physical examinations during practicals with veterinary students, and by veterinarians when utilised for research. No horses displayed clinical signs of EGGD that indicated removal from the study.

This study utilised the data collected from the gastroscopies attained between 2019 and 2021 to evaluate EGGD. As such, it formed part of the clinical record for each horse and ethics approval was not required. Whilst originally intended to attain three gastroscopies per horse (q12 months), COVID-19 restrictions prevented data collection for over a year, thus only two gastroscopies per horse were conducted.

Study population

The Murdoch University School of Veterinary Medicine teaching herd consists of 23 horses. The herd was housed on irrigated Kikuyu pasture, and hay was provided ad lib in the form of two oaten round bales per week. The horses were paddocked as a single herd or in multiple smaller herds.

Their use for teaching purposes includes practical sessions with first to fifth-year Murdoch University veterinary and animal science students. Teaching use details are included in Tables S1 and S2. Additionally, the horses are utilised for research by Murdoch University staff. Teaching and research purposes are covered by appropriate animal ethics permits.

Study materials

Gastroscopy was conducted in a standardised manner as described in the literature (Loftin et al., 2017). Two different gastroscope models were used during the study, including Olympus (GIF H180-300) and Karl Storz (60130PKSK/60130NKSK). Data from routine gastroscopies of the teaching herd horses between August 2019 and December 2021 were utilised for this study. Three to ten image stills per video were taken and coded for blind assessment by two equine internal medicine specialists (CJS and EJM). The observers were blinded to the identification of the horse, the year of the gastroscopy, and initial versus repeat gastroscopies. The images were focused on the antrum and pylorus for observation of EGGD (Rendle et al., 2018; Sykes, Hewetson, et al., 2015; Sykes, Sykes, et al., 2015). The study population was defined as the horses that underwent both initial and repeat gastroscopies ($n = 21$).

Data analysis

Evaluation occurred independently and interobserver variability was not considered. EGGD was graded quantitatively and described qualitatively using the systems outlined in Tables 1 and 2, respectively. The grading systems were selected based on recent recommendations (Sykes et al., 2019; Sykes, Hewetson, et al., 2015; Sykes, Sykes, et al., 2015). The use of two grading systems was utilised to improve robustness, as there is significant variation in grading methods in the literature. Qualitative assessment was conducted on all gastroscopies that had evidence of disease ($n = 13/21$ for initial gastroscopies and $n = 15/21$ for repeat gastroscopies) as displayed in Figures 1–8.

Included in data analysis was the use of the horses for teaching in the month prior to their initial and repeat gastroscope to observe whether disease was present or absent after use. 'Used for Teaching' was defined as horses used for research or teaching in the month prior to the gastroscope. 'Disease Present' was defined as horses that had any quantitative grade of disease above grade 0 and included any qualitative description of disease. Complete resolution of disease was defined as grade 0 disease (complete resolution).

A month prior to gastroscopy was the selected period to assess for disease mostly due to the frequency of teaching use, as the horses are utilised on average 0–2 times per month. Additionally, another study's inclusion criteria were horses that had travelled to at least one event in the month prior to gastroscopy (McClure et al., 1999). Furthermore, when determining the efficacy of Apolectol in the management of EGUS, EGGD was found to develop and worsen between initial and repeat gastroscopies, with repeat gastroscopies conducted between 24 to 27 days following initiation of treatment (placebo versus Apolectol/yeast/magnesium hydroxide combination) (Sykes et al., 2014).

TABLE 1 Quantitative glandular grading system modified from the EGUS council score (Sykes, Hewetson, et al., 2015; Sykes, Sykes, et al., 2015; Sykes and Jokisalo, 2014). Lesions are graded on a scale of 0–4 and hyperaemia is noted as present or absent.

Score	Explanation
Grade 0	The mucosa is intact and there is no appearance of hyperaemia
Grade 1	The mucosa is intact, but there are areas of reddening
Grade 2	Small single or multifocal superficial lesions ($n = <5$)
Grade 3	Large single or multifocal lesions or extensive superficial lesions (≥5)
Grade 4	Extensive lesions with areas of apparent deep ulceration
Hyperaemia	Explanation
Absent	No appearance of hyperaemia
Present	Appearance of hyperaemia

Statistical analysis

Prevalence and 95% confidence intervals (CI) were calculated using EpiTools (online epidemiological calculator; https://epitools.ausvet.com.au). Prevalence was defined over the total study period of 3 years (2019–2021). The presence of disease was considered at a single time point for the individual, and this time point varied depending on the date the gastroscope was conducted. For continuous data, population data were assessed for normality using the Shapiro–Wilk test and are presented as mean (±SD). A normal distribution was concluded.

RESULTS

The inclusion criteria were horses that underwent two gastroscopies during the study period (21/23 horses). The study population consisted mostly of Standardbreds ($n = 13$) and Thoroughbreds ($n = 6$), with 1 Australian Stock Horse and 1 Warmblood. There were 4 mares (19%) and 17 geldings (81%). The mean age was 14.0 ± 4.2 years old.

A total of 42 gastroscopies were conducted over a 3-year period (2019–2021), including an initial and repeat gastroscopy for each horse. The mean number of days between gastroscopies was 443 ± 259. Individual horse results are presented in Table 3. All 21 horses remained in the study throughout the study period. No horses received treatment for squamous or glandular gastric disease, or any NSAID treatment.

Prevalence

The prevalence in this study from all gastroscopies (initial and repeat; $n = 42$) conducted over the three-year study period was 67% ($n = 28$; 95% CI: 51.6–79). The prevalence from initial gastroscopies

TABLE 2 Qualitative glandular grading system modified from the European College of Equine Internal Medicine (Banse & Frank, 2019).

Severity	Mild, moderate, severe
Distribution	Focal, multifocal, diffuse
Appearance	Flat and haemorrhagic Flat and fibrinosuppurative Raised and haemorrhagic Raised and fibrinosuppurative Depressed ± blood clot Depressed and fibrinosuppurative Other (describe)
Location	Cardia, fundus, antrum, pylorus

Quantitative and qualitative findings

There were eight instances of discrepancies with qualitative findings in which a third observer (DB) blindly evaluated the scope images and the majority grade was concluded. For discrepancies with quantitative grade, the highest grade of disease was concluded.

The quantitative findings are presented in Figures 10 and 11. The highest grade of disease present on initial and repeat gastroscopies was grade 3/4. No horses had grade 4 disease.

The most common qualitative description from initial gastroscopies was mild in severity (77%), multifocal in distribution (54%) and flat and haemorrhagic lesions (54%) located at both the antrum and

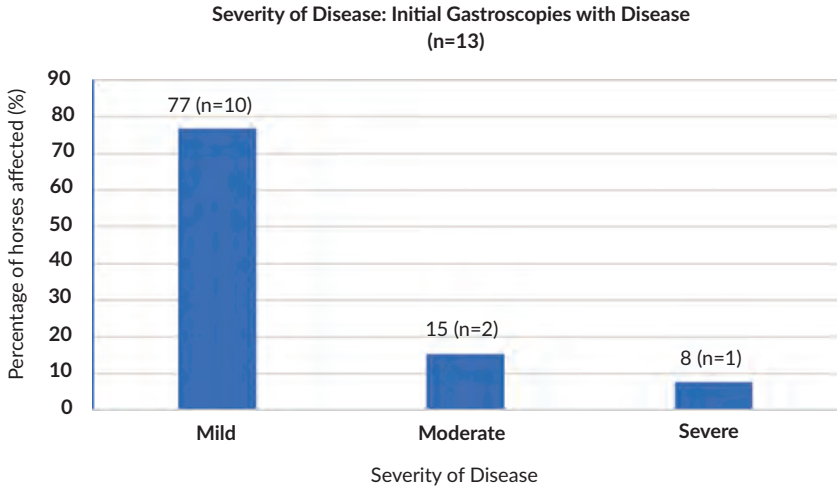

FIGURE 1 Qualitative analysis depicting percentage (%) of horses affected with mild, moderate or severe disease from initial gastroscopies with evidence of disease ($n = 13/21$) over the 3-year study period.

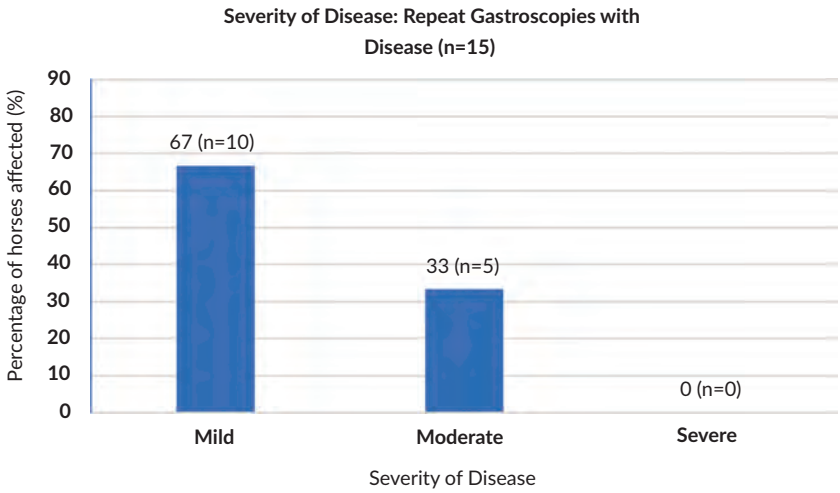

FIGURE 2 Qualitative analysis depicting percentage (%) of horses affected with mild, moderate or severe disease from repeat gastroscopies with evidence of disease ($n = 15/21$) over the 3-year study period.

($n = 21$) was 62% ($n = 13$; 95% CI: 40.8–79.3). The prevalence from repeat gastroscopes ($n = 21$), was 71% ($n = 15$; 95% CI: 50.0–86.2). Prevalence data is presented in Figure 9.

pylorus (38%). The most common qualitative description from repeat gastroscopies was mild in severity (67%), multifocal in distribution (80%), flat and hyperaemic (47%) or flat and haemorrhagic (47%)

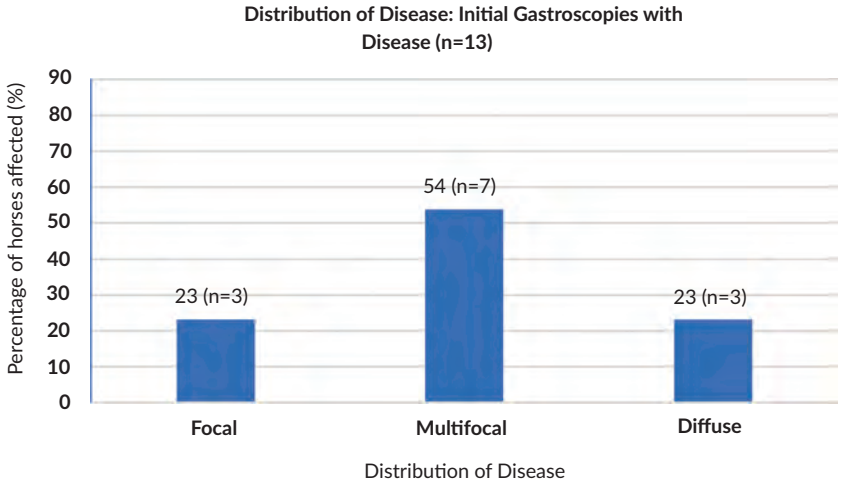

FIGURE 3 Qualitative analysis depicting percentage (%) of horses affected with focal, multifocal or diffuse distribution from initial gastroscopies with evidence of disease ($n = 13/21$) over the 3-year study period.

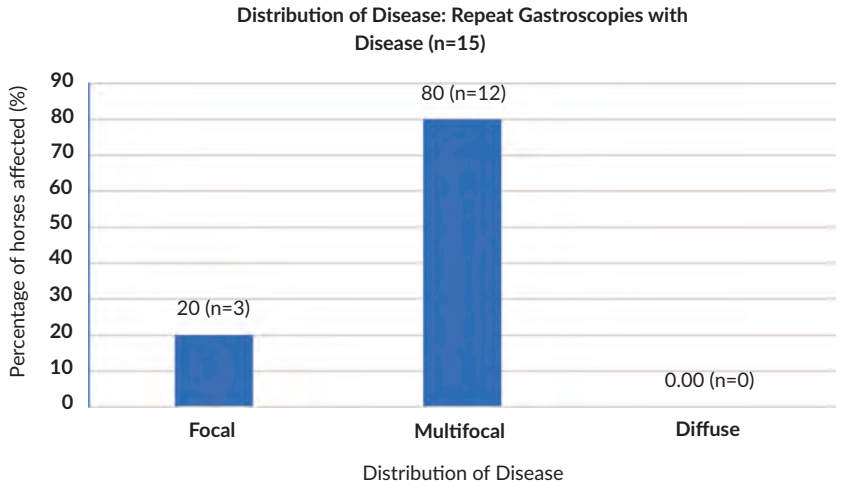

FIGURE 4 Qualitative analysis depicting percentage (%) of horses affected with focal, multifocal or diffuse distribution from repeat gastroscopies with evidence of disease ($n = 15/21$) over the 3-year study period

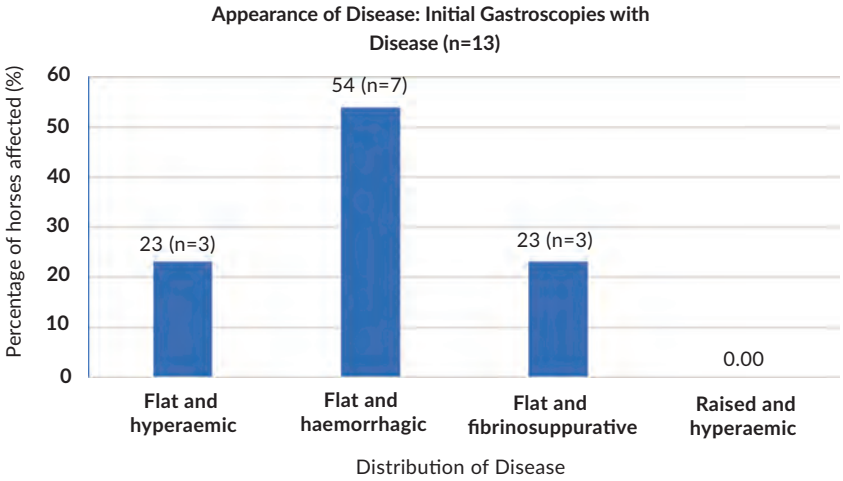

FIGURE 5 Qualitative analysis of percentage (%) of horses affected with each lesion appearance from initial gastroscopies with evidence of disease ($n = 13/21$) over the 3-year study period.

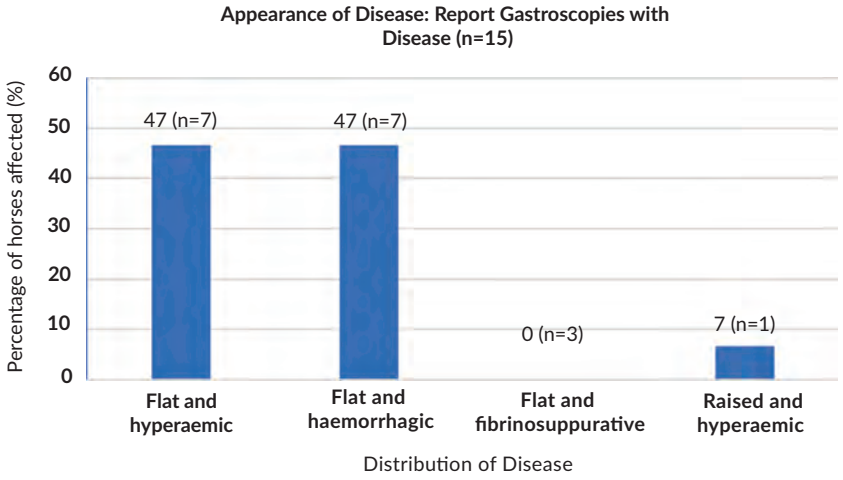

FIGURE 6 Qualitative analysis of percentage (%) of horses affected with each lesion appearance from repeat gastroscopies with evidence of disease ($n = 15/21$) over the 3-year study period.

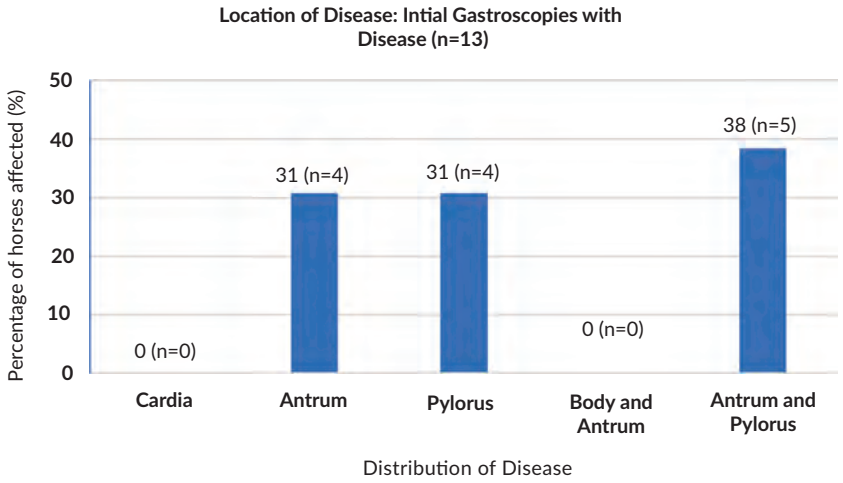

FIGURE 7 Qualitative analysis depicting percentage (%) of horses affected with disease in specific locations from initial gastroscopies with evidence of disease ($n = 13/21$) over the 3-year study period.

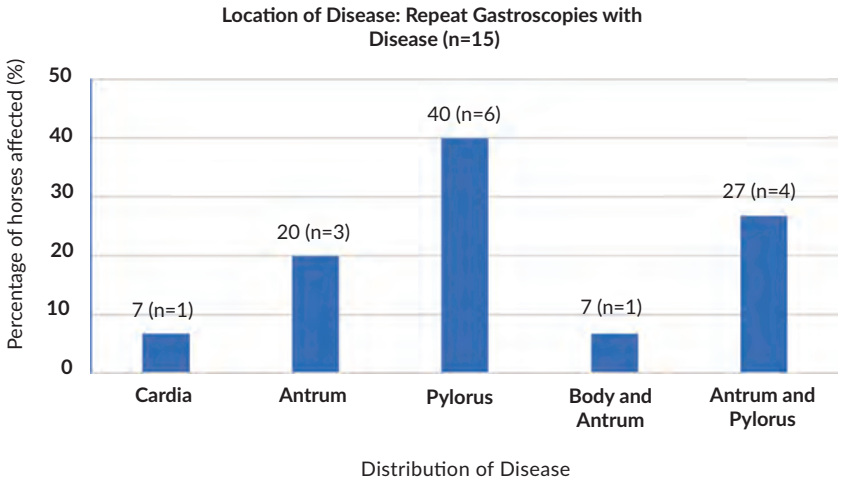

FIGURE 8 Qualitative analysis depicting percentage (%) of horses affected with disease in specific locations from repeat gastroscopies with evidence of disease ($n = 15/21$) over the 3-year study period.

TABLE 3 Signalment, quantitative and qualitative results from initial and repeat gastroscopies for all horses included in the study.

Horse ID #	Breed	Age (years)	Sex	Initial scope Quantitative (Qn) and qualitative (Ql) EGGD grade	Repeat scope Quantitative and qualitative EGGD grade	Interval between initial and repeat scope (days)
Horse 1	TB	20	Geld	Qn: G2, H+ Ql: Mild, multifocal, flat and haemorrhagic lesions at the antrum	No disease	181
Horse 2	SB	10	Geld	No disease	Qn: G1, H+ Ql: Mild, multifocal, flat and hyperaemic lesions at the pylorus	661
Horse 3	SB	19	Geld	Qn: G1, H+ Ql: Mild, multifocal, flat and fibrinosuppurative lesions at pylorus	Qn: G2, H+ Ql: Moderate, multifocal, flat and hyperaemic and raised and haemorrhagic lesions at the antrum and pylorus	511
Horse 4	SB	17	Geld	No disease	No disease	170
Horse 5	WB	17	Geld	No disease	No disease	665
Horse 6	TB	15	Geld	Not included in study		
Horse 7	TB	13	Geld	Qn: G2, H+ Ql: Mild, focal, flat and haemorrhagic lesions at the antrum and pylorus	Qn: G2, H+ Ql: Moderate, multifocal, flat and haemorrhagic lesions at the pylorus	678
Horse 8	SB	14	Geld	Qn: G3, H+ Ql: Moderate, diffuse, flat and haemorrhagic lesions at the antrum and pylorus	Qn: G1, H+ Ql: Mild, focal, flat and hyperaemic lesions at the antrum	266
Horse 9	TB	5	Geld	No disease	No disease	504
Horse 10	SB	8	Geld	No disease	Qn: G1, H+ Ql: Mild, focal, flat and hyperaemic lesions at the cardia	655
Horse 11	SB	15	Geld	Qn: G3, H+ Ql: Moderate, diffuse, flat and haemorrhagic lesions at the antrum and pylorus	Qn: G2, H+ Ql: Mild, multifocal, flat and hyperaemic lesions at the pylorus	612
Horse 12	TB	15	Geld	Qn: G3, H+ Ql: Mild, diffuse, flat and hyperaemic lesions at the antrum	Qn: G1, H+ Ql: Mild, multifocal, flat and hyperaemic lesions at the antrum	647
Horse 13	SB	15	Mare	No disease	Qn: G1, H+ Ql: Mild, multifocal, flat and haemorrhagic lesions at the body and antrum	719
Horse 14	SB	22	Geld	Qn: G2, H+ Ql: Mild, focal, flat and haemorrhagic lesions at the antrum and pylorus	Qn: G2, H+ Ql: Mild, multifocal, flat and haemorrhagic lesions at the pylorus	719
Horse 15	SB	17	Geld	Qn: G2, H+ Ql: Mild, multifocal, flat and haemorrhagic lesions at the pylorus	Qn: G2, H+ Ql: Mild, multifocal, flat and haemorrhagic lesions at the pylorus	708
Horse 16	SB	13	Mare	No disease	Qn: G2, H+ Ql: Mild, multifocal, flat and haemorrhagic lesions at the pylorus	124
Horse 17	TB	10	Geld	No disease	No disease	184

(Continues)

TABLE 3 (Continued)

Horse ID #	Breed	Age (years)	Sex	Initial scope Quantitative (Qn) and qualitative (Ql) EGGD grade	Repeat scope Quantitative and qualitative EGGD grade	Interval between initial and repeat scope (days)
Horse 18	SB	12	Geld	Qn: G1, H+ Ql: Mild, multifocal, flat and hyperaemic lesions at the antrum	Qn: G2, H+ Ql: Moderate, multifocal, flat and haemorrhagic lesions at the antrum and pylorus	263
Horse 19	TB	10	Geld	Not included in study		
Horse 20	SB	8	Mare	Qn: G3, H+ Ql: Severe, multifocal, flat and haemorrhagic lesions at the pylorus	Qn: G3, H+ Ql: Moderate, multifocal, flat and haemorrhagic lesions at the antrum and pylorus	731
Horse 21	SB	14	Geld	Qn: G2, H+ Ql: Mild, multifocal, flat and fibrinosuppurative lesions at the antrum	Qn: G1, H+ Ql: Mild, focal, flat and hyperaemic lesions at the antrum	33
Horse 22	TB	15	Geld	Qn: G1, H+ Ql: Mild, focal, flat and hyperaemic lesions at the pylorus	No disease	266
Horse 23	ASH	16	Mare	Qn: G2, H+ Ql: Mild, focal, flat and fibrinosuppurative lesions at the antrum and pylorus	Qn: G1, H+ Ql: Mild, multifocal, flat and haemorrhagic lesions at the antrum and pylorus	14

Abbreviations: –, gastroscopy not conducted; ASH, Australian Stock Horse; G, grade, where G1 is Grade 1; Geld, gelding; H+, hyperaemia present; Ql, qualitative grade; Qn, quantitative grade; SB, Standardbred; TB, Thoroughbred; WB, warmblood.

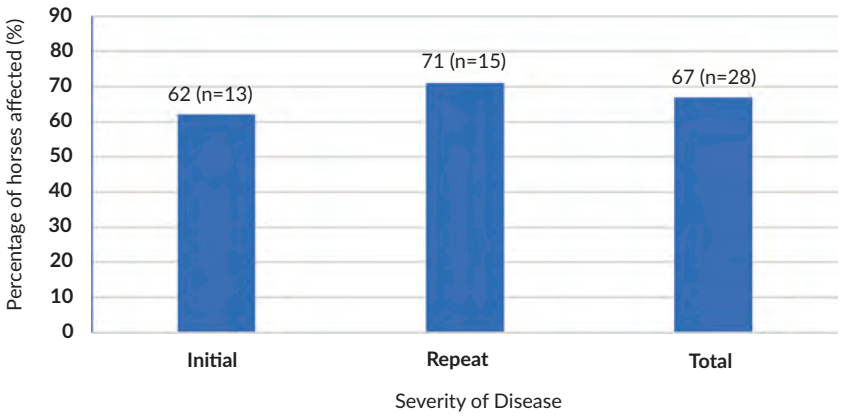

FIGURE 9 Prevalence of EGGD from initial ($n=21$), repeat ($n=21$), and total ($n=42$) gastroscopies for 21 horses over the 3-year study period.

and located at the pylorus (40%). Hyperaemia was present in 100% of cases with disease.

Changes over time

There was a higher grade of EGGD on repeat gastroscopy compared to initial gastroscopy in 29% of horses ($n=6$; 95% CI: 13.8–50.0). There was a lower grade of EGGD compared to the initial gastroscopy in 24% of horses ($n=5$; 95% CI: 10.6–45.1). In 38% of horses ($n=8$), there was no change in grade (95% CI: 20.8–59.1). In 10% of horses ($n=2$), the lesions observed in the initial gastroscopy had resolved on repeat gastroscopy (95% CI: 2.7–28.9). Nine horses had no disease on initial gastroscopy, and four of these horses developed disease during the study period (44%; 95% CI: 18.9–73.3).

Teaching use and EGGD

Data attained from all gastroscopies ($n=42$) were separated into four categories, as depicted in Table 4.

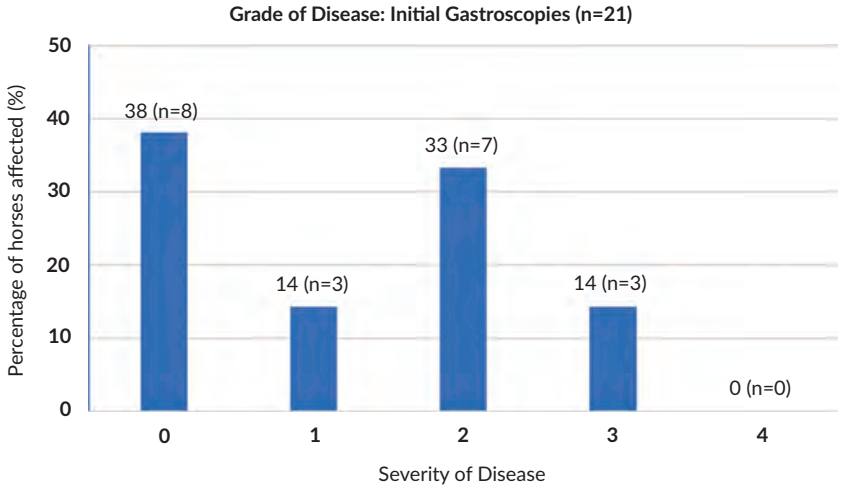

FIGURE 10 Quantitative analysis depicting percentage (%) of horses affected with each grade of disease on a scale of 0–4 from initial gastroscopies of 21 horses over the 3-year study period.

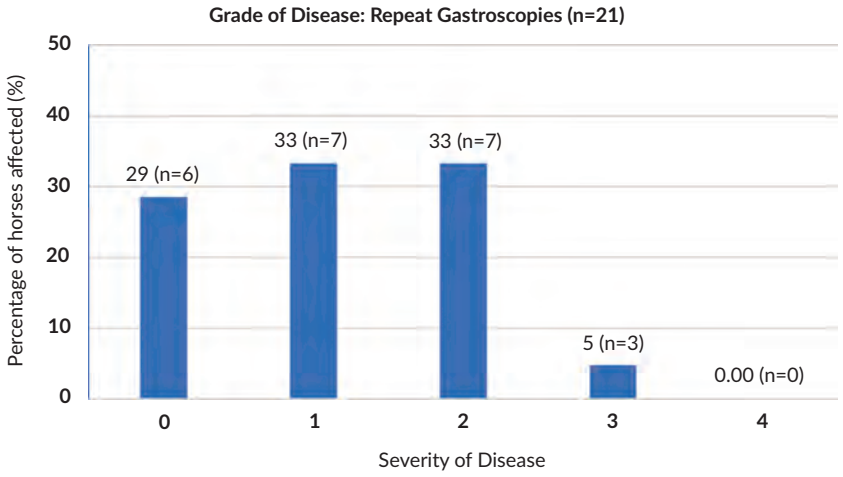

FIGURE 11 Quantitative analysis depicting percentage (%) of horses affected with each grade of disease on a scale of 0–4 from repeat gastroscopies of 21 horses over the 3-year study period.

TABLE 4 The category of use and the status of disease are organised into four categories based on all gastroscopy data ($n = 42$).

Category of teaching use and disease status	Percentage (%)	95% CI
Used for teaching, disease present on gastroscopy	33 ($n = 14$)	21.0–48.5
Used for teaching, disease absent on gastroscopy	14 ($n = 6$)	6.7–27.8
Not used for teaching, disease present on gastroscopy	33 ($n = 14$)	21.0–48.5
Not used for teaching, disease absent on gastroscopy	19 ($n = 8$)	10.1–33.3

DISCUSSION

The overall prevalence of 67% in this study is similar to that of sport horse and pleasure horse populations in the literature, for example 64% and 54%, respectively (Hepburn, 2014). The similar prevalence in this teaching herd was surprising given that few of the known risk factors for EGGD apply to this herd. These horses are retired from athletic use and thus do not undergo the stressors of intensive management and performance as sport horses do. Additionally, none of the horses received treatment with NSAIDs at any time throughout the study period.

There is no literature describing the changes over time in EGGD without medical intervention, thus the changes described in this study are novel. The data concludes that disease might improve, worsen or even resolve over time without medical intervention. Given the horses are housed and managed in the same way, risk factors at the individual level need to be acknowledged. The risk factors that can be attributed to this teaching

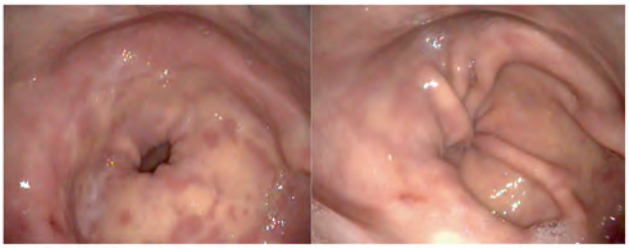

FIGURE 12 An example of a common gastroscopic appearance from an initial gastroscopy. Grade 2 with hyperaemia. Mild, focal, flat and haemorrhagic lesions are present at the antrum and pylorus.

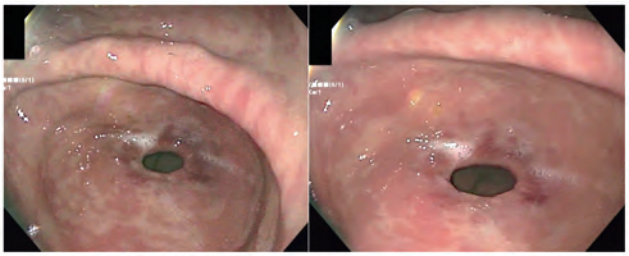

FIGURE 13 An example of a common gastroscopic appearance from a repeat gastroscopy. Grade 2 with hyperaemia. Moderate, multifocal, flat and haemorrhagic lesions are present at the pylorus.

herd include breed, number of handlers, hierarchy in the herd and stress.

An increased number of handlers is a known risk factor for developing EGGD (Mönki et al., 2016), which must be considered in this population given their interactions with numerous veterinary students and staff members. Changes in herd dynamics are also considered a factor in this herd (Busechian et al., 2021). Social stress and its contribution to medical issues is supported in other literature (Sauer et al., 2018). Although reorganisation of the herd is not a frequent occurrence, positive and negative social interactions for an individual will be impacted by their placing in the hierarchy of the herd. Lastly, the potential that stress might induce EGGD in a teaching herd population cannot be ignored.

Teaching horses are neither sport horses nor pleasure horses. Teaching might place unique psychological stressors on the horses that are not yet considered in the literature. These might include having multiple handlers including staff and students, separation from the herd during their use, stress as a procedure is being conducted, and uncertainty about the level of invasiveness of the procedure. An example of the latter is first year veterinary students using the horses to practice basic horse handling such as fitting halters, versus fifth years practicing venepuncture, nasogastric intubation and other invasive procedures. The link between psychological stress and stress ulcer development is well-documented in human literature (Rosenstock et al., 2015), and the potential for any factor that provokes stress to induce EGGD is supported (Scheidegger et al., 2017).

It is important to note that EGGD is likely a multifactorial aetiopathogenesis.

Most lesions described by qualitative description were mild, multifocal, flat and haemorrhagic lesions located at the antrum and pylorus. The site predilections were consistent with the literature (Sykes et al., 2019; Sykes, Hewetson, et al., 2015; Sykes, Sykes, et al., 2015). Examples of common lesions observed from initial and repeat gastroscopies are presented in Figures 12 and 13, respectively.

Limitations

The design of the study changed dramatically due to COVID-19, which restricted all data collection for over 1 year. Once restrictions had eased, the use of horses for teaching became the priority, which further delayed data collection to maximise welfare. Consequently, the timeframe between initial and repeat gastroscopies was highly varied between individuals (14 and 731 days). Ideally, initial gastroscopies for all horses would have been conducted over a few days with a set interval between initial and repeat gastroscopies to reduce the risk of other variables affecting the data. These variables might include changing herd dynamics, time of year, increased use for teaching during University semesters compared to semester break, etc. Consequently, there might be significant limitations in the observations and a stronger study design with a set timeframe for data collection is recommended for future studies.

Due to the highly varied presentation of EGGD, and the use of qualitative description, which is highly subjective, there might be variability in grading (Rendle et al., 2018). The differing clinical experience of equine practitioners might increase variability in grading however, each observer in this study was of similar qualification (registered equine internal medicine specialist). Furthermore, the use of different gastroscopes might alter the appearance of the glandular mucosa due to differences in light and colour settings, and picture quality (Rendle et al., 2018). The latter is a limitation of this study as two different gastroscope units were used.

The search for a potential relationship between psychological stress and EGGD was outside the scope of this study. Without objective stress parameters including cortisol levels, the stress-cortisol-ulcer pathogenesis cannot be concluded nor excluded in this population. However, the descriptive data on teaching use was included as this information might be hypothesis-generating. Similar studies in the future should obtain objective stress data including cortisol levels at the time of gastroscopy. This might aid in the search for a potential relationship between psychological stress and EGGD in teaching herds.

CONCLUSIONS

The prevalence of EGGD in a teaching herd with minimally described risk factors was greater than expected. Potential changes over time for naturally occurring disease without medical intervention include worsening of disease, improvement of disease, no change, or complete resolution of disease. The aetiology of disease

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in the herd is unknown, and teaching horses might have a unique set of risk factors.

AUTHOR CONTRIBUTIONS

D. Byrne contributed to the study design, study execution, preparation of the manuscript, drafted the paper, and critically reviewed and approved the final version of the manuscript before submission. C. Secombe and E. McConnell each conducted data analysis and contributed to its interpretation. Each author also critically reviewed and approved the final version of the manuscript before submission. K. Sharbine contributed to study design, study execution, conducted the data interpretation, and prepared the manuscript.

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CONFLICT OF INTEREST STATEMENT

No conflicts of interest have been declared.

FUNDING INFORMATION

No funding was received.

ETHICS STATEMENT

The data from this study was attained from gastroscopies conducted in accordance with the routine veterinary care of the teaching herd and thus the gastroscopy information formed a part of the clinical record of the horse and ethical approval was not required. The post-graduate continuing education course conducted at The Animal Hospital at Murdoch University (TAHMU) in 2019 which instigated this project required ethics approval (T3165/19). The use of the horses for teaching as described in this study required ethics approval (T3019/18).

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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Santos, M.M., Martinez, J., Mollenhauer, L., Schulze-Gronover, B. & Gudehus, T.H. (2023) Surgical treatment of cervical (C7-T1) instability caused by discospondylitis in a horse. *Equine Veterinary Education*; 35, 632, e731–737.

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Equine Veterinary Education

ORIGINAL ARTICLE

Single and double vaccination against *Lawsonia intracellularis* in foals: Investigation of the humoral immune response following different vaccination protocols

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Summary

Background: Equine proliferative enteropathy (EPE) is an enteric disease induced by the bacterium *Lawsonia intracellularis* that causes sporadically or endemically severe enteritis in foals.

Objectives: The current study aimed to describe the kinetic of serum antibodies against *Lawsonia intracellularis* by Immuno-Peroxidase-Monolayer-Assay (IPMA) before and after single or double intra-rectal vaccination of foals with the attenuated live vaccine Enterisol Ileitis® in comparison to non-vaccinated control foals. Additionally, we evaluated whether seroconversion after vaccination might provide information about the protection against EPE.

Study design: Prospective cohort study.

Methods: Five consecutive serum samples were evaluated by IPMA from all 210 foals: group 1 (single vaccination) = 70; group 2 (two vaccinations) = 69; group 3 (control group) = 71. The study period stretched from 2 weeks before to 6 weeks after the first vaccination.

Results: The analysis showed 944 (89.9%) seronegative samples. 45 samples of group 1 (single vaccination), 56 samples of group 2 (two vaccinations) and five samples of the control group were seropositive during the study period. A total of 63/210 (30%) foals seroconverted during the study period. In foals vaccinated once ($n = 70$), 32 foals seroconverted, in group 2 (two vaccinations, $n = 69$) 28 foals seroconverted and in the control foals ($n = 71$), three foals seroconverted. The vaccinated foals showed significantly more often seroconversion than the control foals ($p < 0.0001$). After the end of the study period, a total of six foals (two vaccinated foals, and four non-vaccinated foals) were diagnosed with EPE.

Main limitations: A longer study period might have added more information on the humoral response to vaccination.

Conclusions: Measurable *Lawsonia intracellularis*-antibodies can be detected after intra-rectal vaccination, but serological testing the time around the vaccination does not seem to be a reliable method to assess the efficacy of the vaccination.

KEYWORDS

horse, antibodies, equine proliferative enteropathy, immuno-peroxidase-monolayer-assay, live vaccine

INTRODUCTION

Proliferative enteropathy (PE) is an infectious enteric disease caused by the obligate intracellular bacterium *Lawsonia intracellularis* (Lawson & Gebhart, 2000). The bacterium infects the crypt epithelial cells and leads to hyperplasia and proliferation of these cells in the distal small intestine (Smith & Lawson, 2001). In horses, *L. intracellularis* causes equine proliferative enteropathy (EPE) and was first described in 1982 (Duhamel & Wheeldon, 1982). Today, there are worldwide reports of individual cases (Bihr, 2003; Cehak et al., 2007; McClintock & Collins, 2004; Williams et al., 1996) but also of herd outbreaks (Dohrmann et al., 2022; Lavoie et al., 2000; McGurrin et al., 2007; Merlo et al., 2009). EPE occurs mainly in foals and weanlings between 4 and 7 months of age. The most common clinical signs are weight loss, fever, lethargy, oedema, colic and diarrhoea and the most consistent clinicopathological findings are severe hypoproteinaemia, hypoalbuminaemia or both (Frazer, 2008; Lavoie et al., 2000). In many affected animals a thickening of the walls of the small intestine (>3 mm) is visible by transabdominal ultrasonographic examination (Pusterla & Gebhart, 2013).

The antemortem diagnosis of EPE can be confirmed by detecting the pathogen in faeces by quantitative real-time polymerase chain reaction (qPCR) or by serological testing for antibodies against *L. intracellularis* for example via Immuno-Peroxidase-Monolayer-Assay (IPMA) (Guedes et al., 2002; Pusterla & Gebhart, 2013). The treatment of EPE involves the use of antimicrobials and individual supportive care (Dohrmann et al., 2022; Sampieri et al., 2006).

In pigs, PE is mainly controlled by oral vaccination against *L. intracellularis* and the use of in-feed-antimicrobials (Guedes & Gebhart, 2003; Jacobson et al., 2010; Kroll et al., 2004). In horses, monitoring programmes are recommended for large breeding farms with the endemic occurrence of EPE in foals (Pusterla & Gebhart, 2013). Further, the protection of foals vaccinated with the live vaccine was demonstrated in an experimental infection trial (Pusterla, Vannucci, et al., 2012b).

The current study aimed to investigate the humoral immune response after single or double vaccination of foals against *L. intracellularis* and to evaluate whether seroconversion after vaccination provides information about the protection against EPE.

MATERIALS AND METHODS

Monitoring

The study took place at a large Warmblood stud in Germany during the 2019/2020 season. In the 2 years prior to the study, the farm had experienced several severe cases of EPE with the loss of seven foals after peracute small intestinal enteritis in 2018. A monitoring programme for EPE was first introduced in 2019, as well as the vaccination of foals against *L. intracellularis*. The monitoring included a daily observation for signs of disease, a weekly clinical examination including rectal temperature and evaluations of serum total protein and albumin concentrations every other week in every foal between 4 and 9 months of age.

Foals noticed with hypoproteinaemia (reference range: 50–75 g/L) or hypoalbuminaemia (reference range: 25–40 g/L) or with clinical signs of EPE were examined via ultrasonography for thickened walls of the small intestine (>3 mm) and if suspicion was confirmed by PCR from a faecal sample they were treated for *L. intracellularis*.

Only clinically healthy foals without any signs of EPE were weaned at 5.5 months of age.

Vaccination

As part of the study, vaccinated foals were randomly assigned to group 1 (single vaccination) or group 2 (two vaccinations). Only clinically healthy foals that did not receive antimicrobial therapy 7 days before to 7 days after vaccination were included in the trial. Group 1 ($n = 70$) received intra-rectally a single dose of the avirulent live vaccine (Enterisol Ileitis®, Boehringer Ingelheim Vetmedica GmbH). Group 2 ($n = 69$) received two vaccinations intra-rectally 4 weeks apart. The lyophilised vaccine was handled and re-constituted in accordance with label instructions. Thirty millilitres (mL) of the pure vaccine were administered intra-rectally using a PVC catheter (BRAUN, CH14), which was shortened to a length of 25 cm. After the application, the tube was rinsed with air and the foal was kept confined for about 5 min to avoid excretion of the vaccine. The interval between the last vaccination and weaning was approximately 2 weeks. In addition, 71 non-vaccinated foals, weaned at the same time as the vaccinated foals, were selected randomly as a control group (group 3). The foals were kept together in mixed groups of vaccinated and non-vaccinated foals.

Sample collection

Every other week a 4 mL and a 9 mL tube of whole blood were taken from each foal via jugular venipuncture. The 4 mL serum samples were used to determine total protein and albumin values at an external laboratory (Laboklin GmbH & Co. KG). The 9 mL whole blood samples were allowed to coagulate and centrifuged. The serum was separated and stored in individual tubes at −20°C.

Serological testing

The prerequisite for inclusion was a complete set of stored serum samples from 2 weeks before until 6 weeks after the first vaccination. Because the control foals did not receive any vaccination, the serum samples for these foals were chosen to be comparable in time with the samples from group 1 (single vaccination) and group 2 (two vaccinations). A total of five serum samples per foal were analysed (Table 1). In very few cases, the interval between two serum samples

varied between one and 3 weeks, as farm-specific processes did not allow a bi-weekly sampling in a few cases.

The stored serum samples were tested for anti-*L. intracellularis* specific IgG via IPMA as previously reported (Guedes et al., 2002). 250/1050 serum samples were analysed at the Equine Infectious Disease Research Laboratory (University of California, Davis, USA). The remaining samples were tested in a German laboratory (Labor Dr Böse GmbH). In both laboratories, the samples were analysed by the same person (JS) according to the same protocol, in order to avoid inter-assay variabilities. IgG serum antibody titres ≥60 were considered seropositive. Seroconversion was defined as an increase in the titre of at least 60. The highest dilution level performed was 1:960.

Statistical data analysis

Data were analysed using SAS Enterprise Guide 7.13 HF8® and SAS Software 9.4 (SAS Institute Inc.). Data were analysed by descriptive statistical analysis and tested for normal distribution using histograms and the Shapiro–Wilk test. To compare the age of the foals at different stages and the time between the first vaccination and seroconversion Kruskal-Wallis and Wilcoxon signed-rank tests were used. The influence of time and group on the antibody titres was modelled using a cumulative logit mixed model with the foal as random effect Logistic binary response models were used to compare the probabilities for seroconversion between groups, with Tukey–Kramer adjustment for post hoc test. *p*-Values ≤0.05 were considered statistically significant.

RESULTS

Population, weaning, vaccination

Data from 70 foals of group 1 (single vaccination), 69 foals of group 2 (two vaccinations) and 71 foals of group 3 (control group) were included. The foals were born between April and July 2019 and weaned between October and December 2019. The age at weaning, first vaccination and seroconversion are shown in Table 2.

Foals were vaccinated in August (10/139), September (44/139), October (83/139) and November 2019 (2/139). The Wilcoxon signed-rank test showed that foals vaccinated twice were significantly older (median: 125 days) at the first vaccination than foals vaccinated once (median: 124 days) ($p = 0.018$, Table 2). However, the difference in median age at first vaccination is only 1 day and thus has no biological relevance.

The second vaccination of group 2 took place 4 weeks after the first. In this context, 10/69 foals were vaccinated again in September, 31/69 foals in October and 28/69 foals in November 2019.

Results of serology

As part of this study, five consecutive samples from each of the 210 foals were tested for IgG serum antibodies against *L. intracellularis* via IPMA. In total, 944 (89.9%) samples were seronegative, and 106 (10.1%) samples were seropositive (Table 3). Figure 1 shows the number of each titre category among the seropositive samples per group.

In group 1 (single vaccination) 33 of the 70 foals showed at least one seropositive result. Thirty-two of these 33 foals seroconverted during the study period (Table 3). One foal showed a positive result 2 weeks before and on the day of the first vaccination (timepoint 1 and timepoint 2, respectively) and the subsequent samples were negative. Two foals seroconverted 2 weeks after the first vaccination and 15 foals seroconverted 4 weeks after the first vaccination. Eleven of these 15 were still seropositive 2 weeks later. Three of the foals showed an increase in titre between 4 weeks after vaccination and 6 weeks after vaccination. In three foals the titre decreased, and five foals remained at the same titre. Fifteen further foals seroconverted 6 weeks after vaccination (see Figure 2).

In group 2 (two vaccinations) 31/69 foals were seropositive and 28/31 foals were seroconverted during the study period (Table 3). In two foals all five samples were seropositive and, in another foal, only the first sample showed a positive result. Another foal was seropositive at all five timepoints but seroconverted only after the second vaccination. Seventeen foals seroconverted on the day of the second vaccination. Thirteen of these 17 foals were still seropositive 2 weeks later. In five of these foals, the titre increased, in three it remained the same and in five foals the titre decreased between just before and 2 weeks after the second vaccination. Ten further foals seroconverted 2 weeks after the second vaccination (see Figure 2).

TABLE 1 Timetable of vaccination (V) and blood sampling (S).

	Timepoint 1 week 0		Timepoint 2 week 2		Timepoint 3 week 4		Timepoint 4 week 6		Timepoint 5 week 8	
	S	V	S	V	S	V	S	V	S	V
Group 1	x		x	x	x		x		x	
Group 2	x		x	x	x		x	x	x	
Group 3	x		x		x		x		x	

Note: Group 1 (single vaccination; $n = 70$), group 2 (two vaccinations; $n = 69$) and group 3 (non-vaccinated group; $n = 71$).

TABLE 2 Age of the foals: Median (5th – 95th percentiles) in the three groups.

	Single vaccination ($n=70$)	Two vaccinations ($n=69$)	Non-vaccinated ($n=71$)
Age at weaning (d)	163[a] (152–177)	176[b] (165–192)	169[c] (155–188)
Age at first vaccination (d)	124[a] (121–128)	125[b] (121–129)	—
Age at seroconversion (d)	157 (137–170)	155 (150–170)	136 ($n=1$) 157 ($n=2$)
Time between first vaccination and seroconversion (d)	31 (14–45)	29 (25–44)	—

Note: Group 1 (single vaccination), group 2 (two vaccinations) and group 3 (non-vaccinated group). Different letters in a row show significant differences.

TABLE 3 Serological results: group 1 (single vaccination), group 2 (two vaccinations) and group 3 (non-vaccinated group).

	Single vaccination ($n=70$)	Two vaccinations ($n=69$)	Non-vaccinated ($n=71$)
Seronegative foals	37	38	66
Seropositive foals	33[a]	31[a]	5[b]
Seronegative samples	305	289	350
Seropositive samples	45	56	5
Seroconverted foals	32[a]	28[a]	3[b]
Non-seroconverted foals	38	41	68

Note: An antibody titre of 60 was used as minimum cut-off value. Seroconversion was considered as increase in titre. Different letters in a row show significant differences.

In group 3 (control group), five of the 71 foals had seropositive results, and only three showed a seroconversion (Table 3). Two of the five foals showed a positive result only at timepoint 1. One foal seroconverted at timepoint 3 and two foals showed seroconversion at timepoint 5 (see Figure 2).

The probability of having one seropositive sample was significantly higher in group 1 (single vaccination) and group 2 (two vaccinations) compared to group 3 (control group; adjusted $p=0.0038$ and adjusted $p=0.049$). The probability of having one seropositive sample was not significantly different between group 1 and group 2 (adjusted $p=0.5$).

A total of 63/210 foals (30%) seroconverted during the study period (Table 3). Of the vaccinated foals, 60/139 (43.2%) foals were seroconverted. In the control group (group 3), 3/71 (4.2%) foals were seroconverted. The probability of seroconversion differed significantly between the vaccinated groups and group 3 (adjusted $p<0.0001$, respectively). The probability of seroconversion did not differ between group 1 (single vaccination) and group 2 (two vaccinations; adjusted $p=0.8$).

The age at seroconversion showed no significant difference between the three groups ($p=0.8$, Table 2). The time between the first vaccination and seroconversion was a median of 31 days for group 1 (one vaccination) and a median of 29 days for group 2 (two vaccinations) and did not differ significantly ($p=0.9$, Table 2).

A comparison of the titres showed that as time went on an increasing number of foals vaccinated once or twice had higher titres. This trend was not observed in the control group. The overall time effect was statistically significant ($p<0.0001$). No significant difference between the groups was detected ($p=0.5$).

Clinical EPE

None of the foals developed clinical EPE during the study period. One foal from group 1 (single vaccination), one foal from group 2 (two vaccinations) and four foals from group 3 (control group) were diagnosed with EPE 27 to 59 days after the end of the current study. The affected foal from group 2 showed seroconversion 2 weeks after the second vaccination (titre 60) and 57 days before the diagnosis of EPE was made. One affected foal of the control group showed hypoproteinaemia 4 weeks and 6 weeks after the first vaccination of the other foals (46.1 g/L; 48.8 g/L, respectively), but all samples were seronegative. The other affected foals were seronegative and with physiological serum total protein and albumin values over the study period.

DISCUSSION

In the current study, the humoral immune response of 139 vaccinated foals was analysed over a period of 8 weeks around the time of vaccination against *L. intracellularis* (*LI*) by evaluating the specific antibody titres against *LI* by IPMA. Furthermore, these titres were compared with the titres of 71 unvaccinated control foals. To the authors' knowledge, this study is the largest field trial evaluating the humoral immune response in foals with different vaccination protocols against *LI* on a breeding farm with sporadic EPE in the former years and the year of the study.

Throughout the study period, 43.2% (60/139) of the vaccinated foals seroconverted compared to only 4.2% (3/71) of the foals of the control group. The time interval between the first vaccination and seroconversion was a median of 31 days in foals vaccinated once

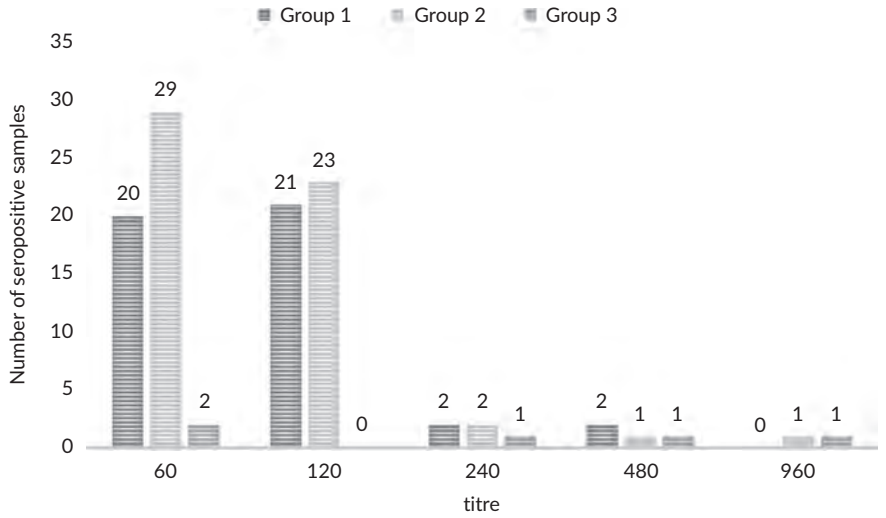

FIGURE 1 Number of each titre category among the seropositive samples per group. Group 1 (foals vaccinated once; $n=70$), group 2 (two vaccinations: $n=69$) and group 3 (no vaccination; $n=71$).

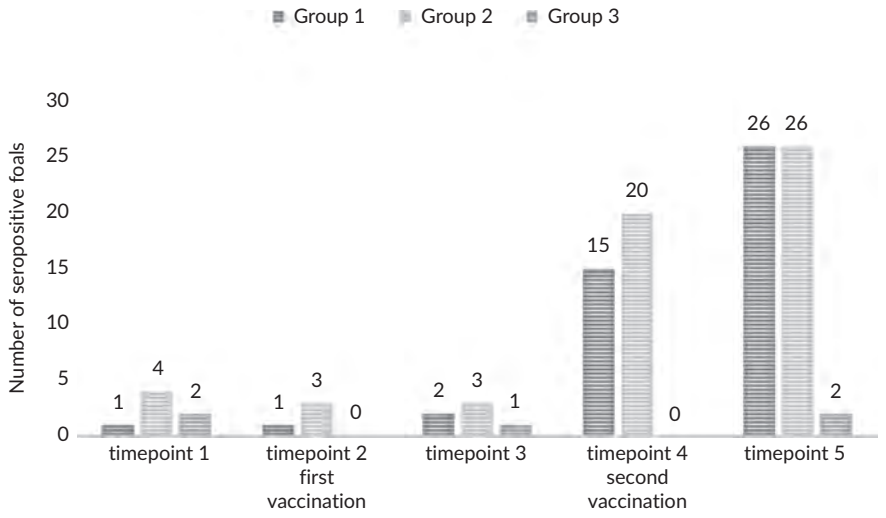

FIGURE 2 Number of seropositive foals per group at each timepoint. Group 1 ($n=70$) was vaccinated at timepoint 2, group 2 ($n=69$) was vaccinated at timepoint 2 and 4 weeks later (timepoint 4). Group 3 ($n=71$) received no vaccination.

and a median of 29 days in foals vaccinated twice which is similar to a previous study on 10 foals showing seroconversion 30 days after the first vaccination against *L. intracellularis* (Pusterla et al., 2010). However, evaluating the number of seropositive foals, there is a marked difference between the two studies: 7/10 (70%) of the foals showed a positive titre 30 days after the first vaccination while in the current study, only 25% (35/139) were seropositive at that time (Pusterla et al., 2010). In another study, seroconversion was reported in all four intra-rectally vaccinated foals already on day 21 after the first vaccination with a volume of 50 mL of a frozen–thawed vaccine (Pusterla et al., 2009). The higher volume of vaccine administered (30 mL in the current study), and the different vaccine formulations could be possible reasons for the faster generation of an immune response and must be considered when comparing these results. In contrast, in a smaller study on four foals even on day 30 after the second vaccination with 30 mL frozen–thawed vaccine intra-rectal no seroconversion was observed in any of the foals (Pusterla, Vannucci, et al., 2012b). Therefore, the volume of the vaccine might be a crucial issue for a proper humoral response. To the authors' knowledge, no studies have yet been published on the influence of the volume of the vaccine. In Germany only a lyophilised vaccine is available. Pusterla et al. (2010) compared 30 mL of the lyophilised vaccine and 30 mL of the frozen–thawed vaccine and showed no significant difference in terms of seroconversion and tolerability. Since another study by Pusterla, Vannucci, et al. (2012b) described effective protection of vaccinated foals with 30 mL of frozen–thawed vaccine, we decided to use 30 mL of the available lyophilised vaccine in this study design. Nevertheless, a study on the influence of different dosages of the vaccine on the humoral immune response would be very interesting in the future.

Humoral immune response after *LI* – vaccination was also reported in studies on vaccinated pigs. In a study on 10 pigs, all animals showed seroconversion 7–9 weeks after oral vaccination against *L. intracellularis*. The first animals seroconverted 5 weeks after vaccination (Guedes & Gebhart, 2003). In another study, no pig seroconverted until 21 days after oral vaccination. On day 21, the animals were experimentally infected with *L. intracellularis* and interestingly showed protection against disease (Kroll et al., 2004).

One should keep in mind that the pigs were vaccinated orally, and the period analysed was either shorter or longer compared to the current study (Guedes & Gebhart, 2003; Kroll et al., 2004).

As Guedes and Gebhart (2003) showed seroconversion mainly 7–9 weeks after vaccination, it would have been of great interest to evaluate the titres of the foals of the current study during a longer period. The increase in the number of seroconverted foals at the end of the study period suggests that more foals would possibly seroconvert in the following weeks. Furthermore, it is possible that a longer analysed period after the second vaccination would have revealed possible differences between single and double vaccination. In the study period of 8 weeks considered here, no significant difference between foals vaccinated once and foals vaccinated twice with regard to the probability of seroconversion or the level of the titres was observed. In contrast, in vaccinated foals, the probability of seroconversion was significantly higher at the last two sampling timepoints compared to non-vaccinated foals showing a humoral response to intra-rectal vaccination.

These significant differences suggest that the immune response observed in the vaccinated foals was hardly caused by natural exposure to the pathogen, but much more by the vaccine. Natural exposure to the pathogen would have caused a serological response in the control group as well since the foals were housed in the same groups and thus exposed to the same environment. It seems unlikely that seroconversion due to natural exposure occurs only in vaccinated animals. Nevertheless, it cannot be ruled out with certainty that natural exposure to the pathogen did not also lead to seroconversion in some of the foals.

During the study period, no clinical or subclinical EPE was diagnosed. Though three to 10 weeks after the end of the observation period, a total of six of the study foals were diagnosed with EPE. Only one of the six affected foals showed seroconversion (titre 60) 2 weeks after the second vaccination. But the diagnosis of EPE was made 57 days after this seropositive sample. The other affected foals were seronegative in all five samples. These results indicate that the humoral immune response the time around the vaccination does not provide any information about the efficacy or lack of efficacy of the vaccination. Kroll et al. (2004) also described seroconversion as not necessarily indicative of protection against disease in pigs. The label instruction of the vaccine (Boehringer Ingelheim, Vetmedica) also states that in pigs, seroconversion after vaccination cannot normally be detected and is not related to vaccination efficacy.

On the other hand, the systemic humoral immune response should probably not be the only aspect investigated in order to evaluate the immune response to *LI* – vaccination in foals as *LI* is an intracellular bacterium. The very variable detection of seroconversion after vaccination against *LI* was previously observed simultaneously to good protection of animals against disease (Kroll et al., 2004; Pusterla, Vannucci, et al., 2012b). This suggests that non-humoral factors might play a role in the development of immunity to *L. intracellularis*. This issue was investigated in pigs showing that vaccinated pigs had more IFN-γ producing cells than the control animals and that the number of IFN-γ-producing cells was highest in the group of *LI-infected* pigs (Guedes & Gebhart, 2003). The cellular immune response in foals experimentally infected with *L. intracellularis* has been investigated in an in-vitro study. It was shown that in foals that died after experimental infection with *LI*, peripheral blood stimulated in vitro with the bacterium produced significantly less IFN-γ than blood from animals that survived the infection (Page et al., 2011). With regard to the vaccination of foals against *L. intracellularis*, vaccinated foals showed significantly more IFN-γ gene expression than non-vaccinated control foals 60–180 days after vaccination (Pusterla, Mapes, & Gebhart, 2012a). However, if the amount of IFN-γ producing cells also provides information about the protection against EPE, this aspect should be investigated in further studies.

Furthermore, a specific local intestinal humoral response was also described in experimentally infected pigs by the detection of IgA titres in intestinal lavages and might also be a protecting factor in foals interesting to investigate in the future (Guedes & Gebhart, 2010).

CONCLUSION

Even if the humoral immune response in vaccinated foals in this study appears quite low, it is still higher compared to the non-vaccinated group. Based on the data presented here and on results described in previous studies it can be concluded that measurable antibodies can be detected after vaccination, but serological testing 6 weeks after the vaccination is not a reliable method to assess the efficacy or non-efficacy of the vaccination.

In the study period analysed here, the number of seropositive results did not differ between single and double vaccination of foals against *L. intracellularis*. Further studies with a longer study period are needed to compare the humoral immune response after single and double vaccination against *LI* in foals.

AUTHOR CONTRIBUTIONS

R. Wadephul, N. Pusterla and M. Venner contributed to the study design, study execution, data analysis and interpretation, and preparation of the manuscript. J. Straub contributed to the study execution. F. Freise contributed to data analysis and interpretation, and preparation of the manuscript. All authors gave their final approval of the manuscript.

CONFLICT OF INTEREST STATEMENT

No conflicts of interest have been declared.

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ETHICS STATEMENT

There is no ethical concern as the vaccine used is licensed for animals in Germany and scientifically proven to be helpful in preventing Lawsonia enteritis in foals. Informed consent was obtained.

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Equine Veterinary Education

CASE IMAGE

Atlanto-occipital subluxation in an adult Thoroughbred gelding

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Summary

A 13-year-old gelding was referred to the University of Liverpool Equine Hospital for further investigation of ataxia and neck pain following a suspected traumatic incident in the field 5 days prior. The following case report documents the clinical presentation, ultrasonographic, radiographic and computed tomographic (CT) findings of a right lateral atlanto-occipital (AO) subluxation. In brief, clinical presentation included abnormal head carriage, ataxia and cranial cervical swelling with associated neck pain. Radiography showed lateral deviation of the poll and subluxation of the right AO joint with significant widening of the left AO joint. CT was undertaken standing which confirmed lateral luxation of the right occipital condyle in relation to the right articular process of the AO joint such that the right articular process of the atlas was located medial to the right occipital condyle. The gelding was subjected to euthanasia and post-mortem the subluxation was resolved with a closed traction procedure. This case initiates discussion of diagnosis, management and outcome for this uncommon injury. The use of CT in this case gives previously undocumented detail on the nature of the subluxation and assisted in the management and post-mortem closed reduction procedure.

KEYWORDS
atlanto-occipital, computed tomography, horse, subluxation, traumatic

INTRODUCTION

The equine atlanto-occipital (AO) articulation is a paired ellipsoid joint, formed by the articular surface of the two convex condyles of the occipital bone and the corresponding two oval concave foveae of the atlas. The AO joint is stabilised by the dorsal and ventral AO membranes and the lateral AO ligaments (Gutiérrez-Crespo et al., 2014). The dorsal AO membrane extends from the dorsal border of the foramen magnum and occipital condyles to the cranial border of the dorsal arch of the atlas (Gutiérrez-Crespo et al., 2014) and is fused with the joint membrane. The dorsal AO membrane has two re-enforcing symmetric oblique long bands of fibres that cross, forming an X shape on the sagittal plane. The ventral AO membrane extends from the ventral arc of the atlas to the ventral border of the foramen magnum and is fused with the joint capsule (Gutiérrez-Crespo et al., 2014). The lateral AO ligaments are two short bands that are partially blended with the joint capsules (Gutiérrez-Crespo et al., 2014). The lateral AO ligaments attach cranially to the base of the jugular processes and part of the paracondylar processes of the occipital bone and caudally to the craniolateral border of the dorsal arch of the atlas; these fibres are also fused with the joint membrane (Wright et al., 2018). The medial AO joint margin lies adjacent to the lateral aspect of the dura mater and spinal cord meaning distention of the AO joint can result in spinal cord compression (Wright et al., 2018).

Subluxation occurs when a bone is partially displaced from its articulation, resulting in a portion of its articular surface remaining in the natural cavity or upon its edge. Subluxation can be congenital or acquired. Cranial cervical congenital subluxations include occipitoatlantoaxial malformation (OAAM), atlantoaxial subluxation (AAS)

and atlantoaxial instability (AI), but these conditions are uncommon and AAS and AI specifically relate to subluxation of the atlas and axis. Acquired subluxation in the cranial cervical region is also rare, traumatic in origin and usually occurs at the atlanto-axial articulation (Gerlach et al., 2012). AO subluxation case reports are limited and include three neonates (Griffin et al., 2007) and a foal with a concurrent atlantoaxial luxation (Licka, 2002).

Conventional two-dimensional imaging modalities such as radiography and ultrasonography are limited in the AO region due to the complexity of the anatomy, superimposition of osseous and soft tissue structures and difficulty in obtaining orthogonal views (Gough et al., 2020). Ultrasonography is further limited due to acoustic shadowing making assessment of deeper structures impossible. Multiplanar reconstruction, possible with computed tomography (CT) and magnetic resonance imaging (MRI), provides a more detailed assessment of the cervical region (Gough et al., 2020). CT has become the imaging modality of choice for the diagnosis of cervical vertebral pathology in horses (Lindgren et al., 2021). In canine and human orthopaedic trauma, CT is commonly used in the diagnosis of traumatic spinal cord injury and is considered the gold standard for the investigation of acute spinal trauma (Steffen et al., 2003).

We document the clinical examination, radiographic, ultrasonographic and CT findings of a lateral AO subluxation in a mature Thoroughbred gelding. To our knowledge, this injury has not been reported in a mature horse before and thus, advanced diagnostic imaging of this injury is unreported in equine veterinary literature.

CASE DESCRIPTION

Case history

A 13-year-old Thoroughbred gelding presented to the University of Liverpool Equine Hospital for investigation of dullness, ataxia, abnormal head carriage, neck swelling and neck pain following a suspected traumatic incident in the field 5 days prior. The referring veterinarian had identified low head carriage, bilateral soft tissue swelling in the poll region and a reduced lateral and dorsoventral range of motion of the neck. The gelding was treated with phenylbutazone (Equipalazone, 4.4 mg/kg bwt IV; Dechra Pharmaceuticals plc) and dexamethasone (Duphacort Q, 0.1 mg/kg bwt IV; Zoetis UK Ltd) initially and prescribed phenylbutazone (Equipalazone, 4.4 mg/kg bwt PO) twice daily and prednisolone (Equipred, 1 mg/kg bwt PO; Virbac Ltd) once daily. An initial improvement in the neck swelling and comfort of the horse was noted but after 4 days, when the prednisolone dose was tapered (Equipred, 0.5 mg/kg bwt PO), the gelding appeared more painful and was unable to elevate his head, prompting referral.

Clinical findings

On presentation, the gelding was quiet, but alert and responsive and all vital parameters were within normal limits. At rest, the horse stood with a low head carriage equally weightbearing on all four limbs. A left-sided soft swelling (~5 × 8 cm) was palpable dorsal to the vertebral column in the poll region with no associated heat, and an associated asymmetry of the cranial cervical region was observed when viewing the neck from dorsal (Figure 1a). A mild pain response was elicited on palpation of the cervical vertebral column. The left wing of the atlas was more prominent than the right wing with deviation of the head to the left of midline. This resulted in a palpable concavity on the right-hand side of the cranial cervical spine and convexity of the left-hand side of the cranial cervical spine (Figure 1b).

A targeted cranial nerve examination was considered largely unremarkable with pupillary light, dazzle and facial sensation reflexes all within normal limits. Menace and palpebral reflexes on the left side elicited a mild hyperreactive and myokymia response of the eyelid. Voluntary movement, range of motion and flexion of the neck were assessed by tempting the horse to prehend carrots in different

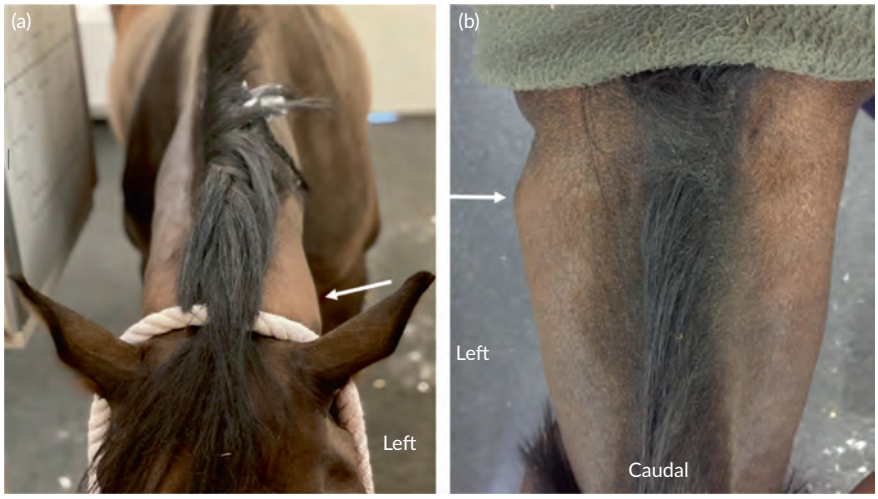


FIGURE 1 (a) Image taken from craniodorsal showing a left-sided swelling in the cranial cervical region (arrowed) and an asymmetry of the cranial cervical region. (b) Photograph of the cranial cervical region taken from dorsal to demonstrate the asymmetry in the region, the bottom of the image is the caudal aspect of the neck, and the convexity on the left side of the cranial cervical spine can be seen (arrowed).

directions. The cervical range of motion and left lateroflexion was good, while the range of motion and right lateroflexion was poor; this could be improved with gentle pressure. Dorsoventral flexion and extension were markedly reduced.

The horse was comfortable at walk and able to walk in a serpentine pattern without any lameness. Only very subtle intermittent proprioceptive deficits of the fore- and hindlimbs were apparent when the horse was walked in a straight line. When walked in tight circles to the right, the gelding pivoted on the front feet and demonstrated a reluctance to cross the hindlimbs but displayed no evidence of interference. When walked in a tight circle to the left, the same signs of ataxia were noted but more pronounced. The horse backed up normally and was able to walk up a gradual incline with a normal gait. When the head was held in an elevated position at rest, the horse showed discomfort including a wide-base stance and reluctance to go forward; at walk the horse showed an increased ataxic and hypermetric gait. The horse's ataxia was graded two out of five on the Modified Mayhew System.

Initial management

An intravenous catheter was placed in the left jugular vein and phenylbutazone (Equipalazone, 4.4 mg/kg bwt IV; Dechra Pharmaceuticals plc) and paracetamol (Paracetamol, 20 mg/kg bwt PO; Milpharm Ltd) were administered twice daily. The gelding was confined to a stable and carefully monitored.

DIAGNOSTIC IMAGING

Radiography

Radiographs were obtained the day after presentation. The gelding was sedated with detomidine hydrochloride (Detonervin, 0.1 mg/kg bwt IV; Animalcare Ltd.) and butorphanol (Torphasol, 0.1 mg/kg bwt IV; Animalcare Ltd). Standing laterolateral, lateral-oblique and ventrodorsal radiographs of the head and cervical spine were obtained using settings of 88 kV and 20mAs. The laterolateral radiograph showed asymmetry of the left and right side of the atlas with one side being dorsally displaced relatively to the occipital condyle. Additionally, there was widening of the AO joint space on one side suggestive of subluxation (Figure 2a). The ventrodorsal radiograph showed left lateral deviation of the atlas with asymmetric AO joint spaces (left vs. right) and marked widening of the left AO joint consistent with subluxation of the left and right AO joints. The right articular process of the atlas was displaced medially to the right occipital condyle and the left articular process of the atlas was positioned craniolaterally to the left occipital condyle (Figure 2b).

Computed tomography

Computed tomography examination of the cranial cervical spine and head was performed under standing sedation, with further sedation of acepromazine (Tranquinervin, 0.03 mg/kg bwt; Dechra Pharmaceuticals plc) and morphine sulphate (Morphine Sulphate, 0.1 mg/kg bwt; Martindale Pharmaceuticals). CT images were obtained using a 16-slice, 90-cm bore CT scanner (Canon Aquilion Prime 160; Canon Medical Systems Ltd), mounted on a sliding gantry system. Images were acquired using 16 row × 1.0 mm detector width, 1.0 mm slice thickness, 550 mm FOV, tube rotation time 0.75 s, collimator pitch 0.688, 120 KVp and 300 mAs. Bone and soft tissue reconstructions were performed. Images were viewed on a computer monitor, using proprietary DICOM software (HOROSTM; GNU Lesser General Public License, Version 3.0, LGPL 3.0) in single and multiplanar views using multiplanar reconstruction.

Computed tomography identified left lateral deviation of the atlas with asymmetric AO joint spaces, marked widening of the left AO joint and the right cranial articular process of the atlas was located medial to the right occipital condyle, within the right side of the foramen magnum (Figure 3a,b). The left cranial articular process of

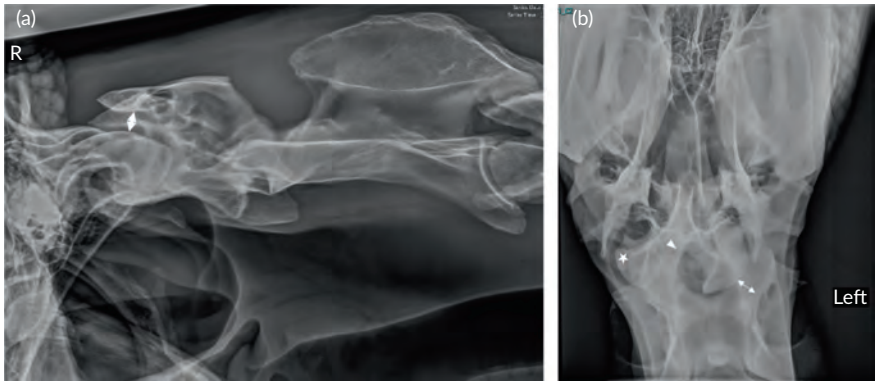


FIGURE 2 (a) Laterolateral radiograph of the cranial cervical region showing mild obliquity of the atlas with asymmetric atlanto-occipital joint spaces (arrowed). Cranial is to the left of the image. (b) Ventrodorsal radiograph of the cranial cervical region showing left lateral deviation of the atlas with asymmetric atlanto-occipital (AO) joint spaces (left vs. right) and marked widening of the left AO joint (double-headed arrow) The right cranial articular process of the atlas (arrowhead) is displaced medial to right occipital condyle (starred), consistent with subluxation of the left and right AO joint. The horse's left is displayed to the right of the image.

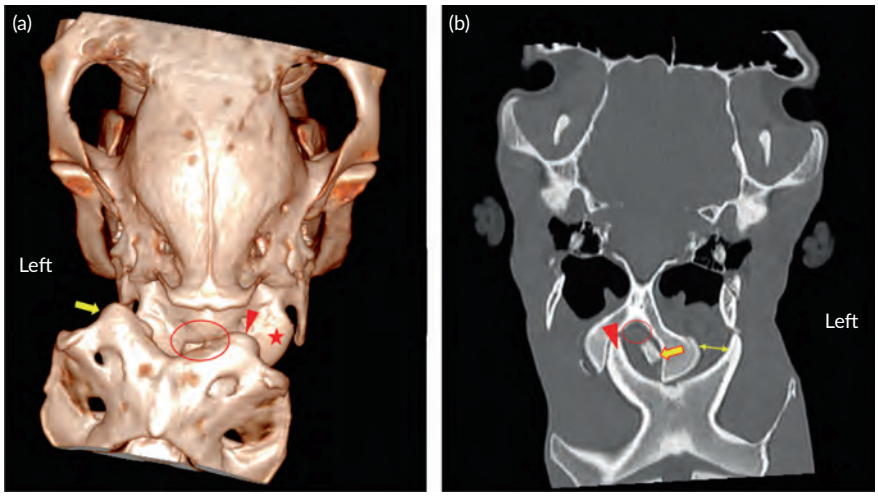


FIGURE 3 (a) 3D volumetric reconstruction of a computed tomographic study viewed from dorsal, demonstrating the left cranial articular process of the atlas (arrowed) displaced craniolaterally and the right cranial articular process of the atlas (arrowhead) located medial to the right occipital condyle (starred), within the foramen magnum (circled), consistent with a lateral atlanto-occipital (OA) joint subluxation. The horse's left is displayed to the left of the image. (b) Dorsal multiplanar computed tomographic image reconstructed with a standard bone algorithm, displayed in a bone window (window level 350; window width 1500) at the level of the AO joint demonstrating marked widening of the left AO joint space (double-headed arrow) and the abnormally positioned right cranial articular process of the atlas (arrowhead), located within the right side of the foramen magnum (circled). A displaced separate bone fragment (arrowed) is evident medial to the left occipital condyle. The horse's left is displayed to the right of the image.

the atlas was craniolaterally displaced resulting in a significant widening of the left AO joint (Figures 3b and 4a). Despite the abnormal bone placement, there was minimal compression of the dura at the foramen magnum; however, at the level of the atlas there was significant extradural compression resulting in right-sided displacement of the spinal cord. There was marked soft tissue swelling, dorsal to the left occipital condyle. The soft tissue swelling in the left side of the vertebral canal at the level of the AO joint created a mass effect on the spinal cord resulting in deviation of the spinal cord to the right (Figure 4b). Heterogeneous soft tissue attenuation filled the gap between the left articular process of the atlas and the left occipital condyle, consistent with organising haematoma and fluid. There was heterogeneous soft tissue attenuating material within the expected area of the AO joint capsule, consistent with haemorrhage and likely rupture of the joint capsule (Figure 4a). In addition to this, several osseous fragments approximately 3×4 mm were identified ventral and medial to the right occipital condyle and a smaller 2×3 mm osseous fragment was located extradurally within the vertebral canal at the level of the AO joint (Figure 3b). The CT confirmed AO subluxation with fragmentation and extradural compression.

Ultrasonography

Ultrasonographic examination (Logic S7 Expert; GE Ultrasound Korea Ltd) was performed following CT to assess this diagnostic imaging modality as a tool for ongoing assessment of subluxation and soft tissue trauma. This identified marked soft tissue enlargement at the level of the left AO joint and a loss of the normal relationship between the cranial fovea of the atlas and caudal surface of the occipital condyles on both sides of the neck. From a right dorsal ultrasonographic window, the bone contour of the right atlas could be appreciated located in an abnormally medial position to the bone surface of the right occipital condyle. This procedure was well tolerated with sedation alone.

Outcome

These findings were discussed with the owners; conservative management was considered inappropriate in this case and so closed reduction was offered. The owners opted for euthanasia with no gross post-mortem performed. The horse was humanely subjected to euthanasia with intravenous injection of secobarbital sodium (400 mg/mL) and cinchocaine hydrochloride (25 mg/mL, Somulose; Dechra Pharmaceuticals plc). Immediately after euthanasia, the horse was positioned in right lateral recumbency, and a 30×30×4 cm thick wooden block was placed under the horse's head to elevate the head, with the caudal margin of the block aligned to the caudal ramus of the mandible. A head collar was fitted tightly to the horse's head and the head was fully extended. A single person applied manual traction to the head in a cranial direction whilst a board-certified surgeon simultaneously placed a hand on the lateral aspect of the left atlantal wing and applied downward pressure in a short pulsing movement. A firm clunking noise was audible as the AO joint was successfully reduced. A ventrodorsal radiograph confirmed reduction.

DISCUSSION

This case adds detail to the clinical presentation and diagnostic imaging of the rare condition of traumatic AO subluxation in an adult

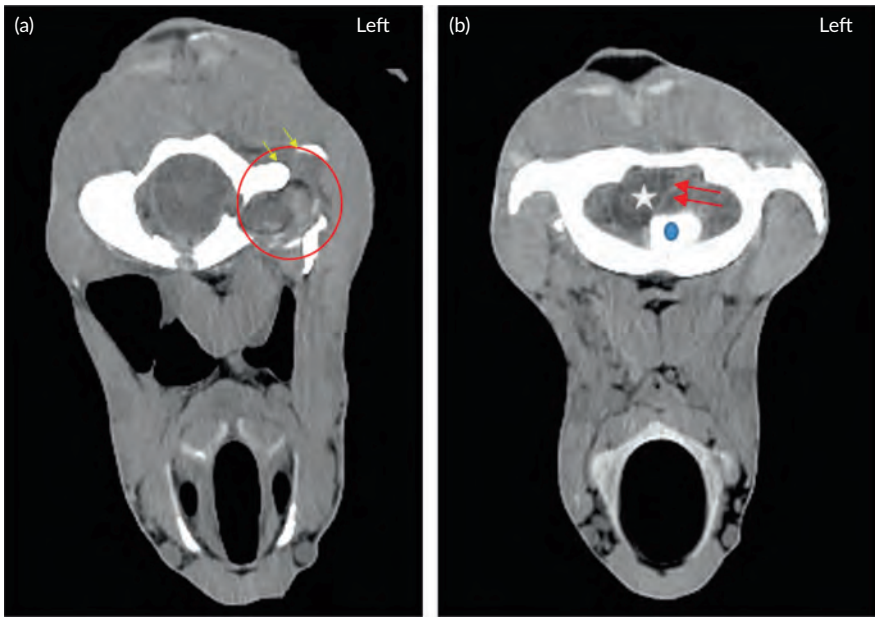
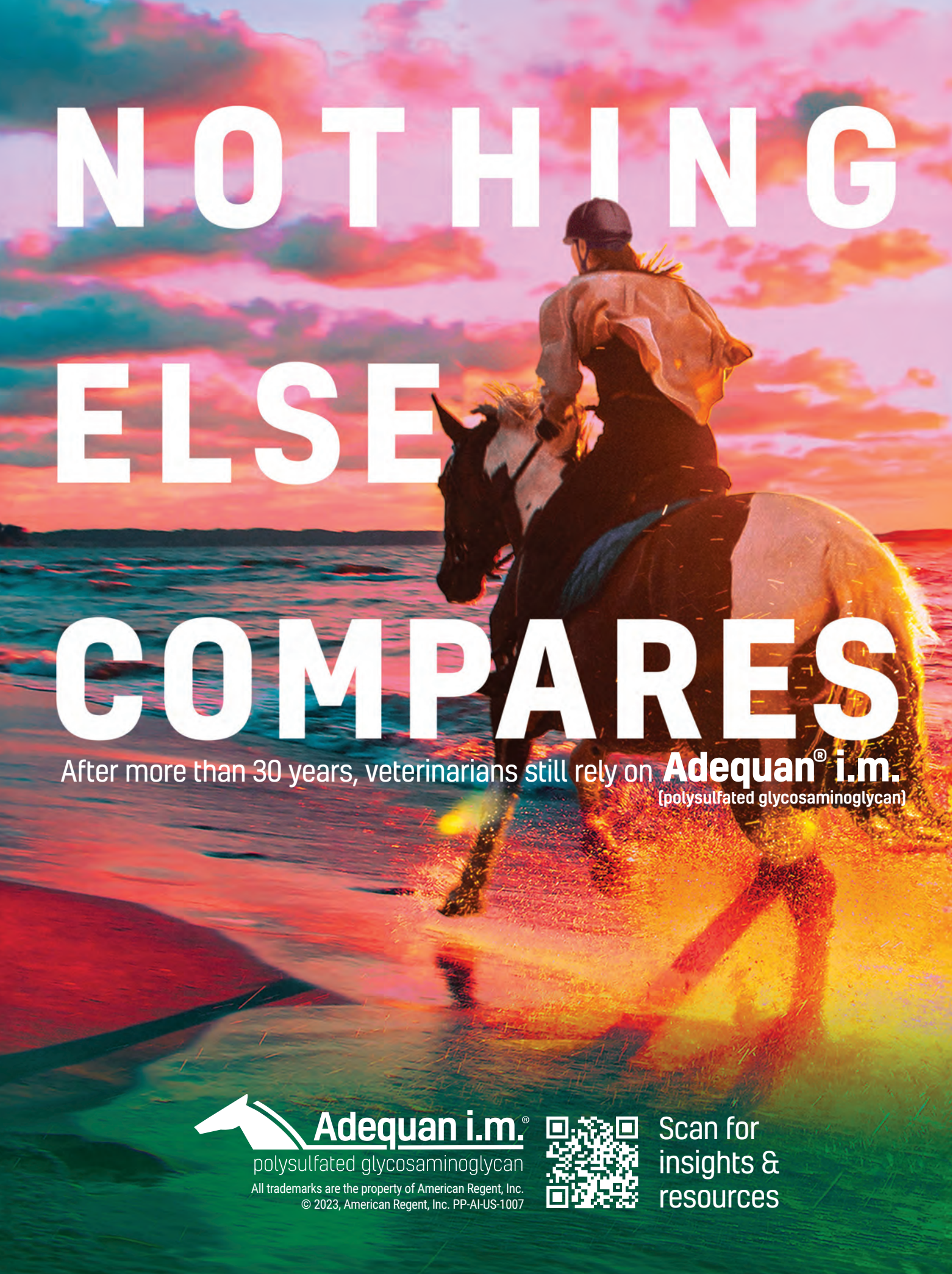


FIGURE 4 (a) Transverse multiplanar computed tomographic image reconstructed with a smooth soft tissue algorithm and displayed in soft tissue window (window length 350; window width 35) at the level of the atlanto-occipital (AO) joint. Only the left AO joint is evident due to the marked asymmetry and rotation of the atlas and the left AO joint is markedly enlarged with loss of congruency (arrowed). There is marked heterogeneously attenuating material within the area of the joint capsule, consistent with haemorrhage and suspected rupture of the joint capsule (circled). The horse's left is displayed to the right of the image. (b) Transverse multiplanar computed tomography image reconstructed with a smooth soft tissue algorithm and displayed in soft tissue window (window length 350; window width 35) at the level of the atlas. The left occipital condyle (blue circle) is abnormally positioned and evident caudal to its expected position and dorsal to the body of the atlas in the vertebral canal. There is marked soft tissue swelling consistent with haemorrhage, dorsal to the abnormally positioned left occipital condyle (red arrows). The abnormal soft tissue swelling in the left side of the vertebral canal is creating a mass effect on the spinal cord (white star), which deviated to the right. The horse's left is displayed to the right of the image.

equid. Whilst there are no reports of traumatic AO subluxation in the adult horse, neonate and foal luxations have been recorded; Licka (2002) reports a case of traumatic AO luxation and atlantoaxial subluxation in a 3-month-old Warmblood colt and Griffin et al. (2007) report a short case series of three neonates with congenital AO luxation. Traumatic luxation elsewhere in the cervical region in the adult horse has been reported, including a traumatic atlantoaxial luxation in a mature 500 kg Warmblood mare (Gerlach et al., 2012). Based on these cases (Gerlach et al., 2012; Licka, 2002), closed reduction was offered as a viable treatment option in this case and performed post-mortem to determine the feasibility of the procedure in a mature equid. The procedure was conducted as similarly described by Gerlach et al. (2012) with the horse in lateral recumbency and traction applied to the head by an assistant. Reduction was first attempted with the head in a flexed position but this was not possible. Manual traction with additional laterolateral force resulted in reduction of the subluxation with far less traction force required than if machine traction were to have been utilised. This is an important consideration as the strain exerted upon the spinal cord during manipulation of the foal was described as considerable and causative of the ataxia seen immediately after reduction (Licka, 2002).

Although closed reduction is considered a noninvasive or conservative treatment for AO subluxation, it should be strongly emphasised that any manipulation of the AO joint can result in deteriorating neurological status and even death (Griffin et al., 2007). The dorsal AO membrane is interwoven with large collagen bundles, and this forms the middle contact of the muscle–membrane–spinal dura mater connection (myodural bridging) linking the suboccipital musculature to the dura mater. We hypothesise that stretching of this membrane and myodural bridge either from the initial trauma or during traction applied in a close reduction procedure may result in dura mater and even spinal cord trauma. As the closed reduction in the present case was attempted, and achieved after euthanasia, it is not possible to predict what effect the closed reduction procedure would have had on this case.

In this case, the initial radiographs identified the AO subluxation, but CT provided useful additional information including spinal cord compression, soft tissue swelling within and outside the vertebral canal, deviation of the spinal cord at the level of the atlas as a result of the mass effect of soft tissue swelling consistent with haemorrhage and several separate osseous fragments within the vertebral canal at the level of the AO joint. Advanced cross-sectional imaging techniques, such as MRI and CT, have been shown to improve ante-mortem diagnosis of cervical pathology in equine cases (Griffin et al., 2007; Gutiérrez-Crespo et al., 2014; Lindgren et al., 2021). Additionally, advanced cross-sectional imaging techniques have been shown to improve ante-mortem diagnosis of AO joint pathology and treatment selection (Steffen et al., 2003).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Although myelography would have provided further information relating to the spinal cord, the procedure is contra-indicated in this case due to potential increased intracranial pressure (ICP). AO subluxation has the potential to obstruct cerebrospinal fluid (CSF) drainage from the cranium resulting in increased ICP. Whilst determining increased ICP in horses is challenging, the altered mental status of our case and the type of injury was enough that we considered a myelogram potentially fatal. If a myelogram is performed in a case with ICP, it is possible during the aspiration of CSF for brain tissue to herniate through the foramen magnum and for a Cushing-type reflex to be inducted which can lead to asystole (Bennell & Bardell, 2021).

CONCLUSION

Atlanto-occipital subluxation is a rare condition and CT imaging provided clinically relevant and useful information not ascertainable from conventional two-dimensional imaging. Extrapolating from the veterinary literature and the successful post-mortem reduction achieved in this case, closed reduction may be considered as treatment for AO luxation in the mature equine population but with all risks considered and communicated. The method of closed reduction described in this report shows a practical and feasible means of achieving reduction which could be trialled after appropriate case selection.

AUTHOR CONTRIBUTIONS

All authors contributed to case management and manuscript preparation. All authors have given their approval of the final manuscript.

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CONFLICT OF INTEREST STATEMENT

No conflicts of interest have been declared.

ETHICS STATEMENT

Ethical consent was applicable from the generic hospital consent form.

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Equine Veterinary Education

REVIEW ARTICLE

Advances in the understanding, detection and management of equine strangles

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Summary

Streptococcus equi subspecies *equi* (*S. equi*) is the causative organism of the upper respiratory disease of equids, strangles, characterised by pyrexia, lymphadenopathy and mucopurulent nasal discharge. Strangles was first reported over 750 years ago and continues to be of significance in equine populations across the globe. This review discusses how *S. equi* has adapted, the clinical manifestation of strangles, and how clinicians and caregivers can tackle the disease in the future. *S. equi* evolved from the commensal, and occasionally opportunistic pathogen, *Streptococcus equi* subspecies *zooepidemicus* refining its capabilities as it became host restricted. The success of *S. equi* can be attributed to its ability to cause both acute and persistent infection, the latter occurring in about 10% of those infected. In this carrier state, *S. equi* persists in the guttural pouch without causing clinical signs, intermittently shedding into the environment, and encountering naïve animals. Insight into the *S. equi* genome and lifestyle has led to advances in diagnostic assays and the development of a safe and efficacious recombinant-fusion vaccine, giving clinicians and caregivers the tools to better combat this infection. Alongside rigorous biosecurity protocols and pragmatic control measures such as screening new arrivals for exposure and carrier status, these new technologies demonstrate that strangles can be an increasingly preventable infection.

KEYWORD
horse, strangles, *Streptococcus equi*

INTRODUCTION

Strangles was first described in 1256 (Ruffo, 1256), although the disease and its causative organism *Streptococcus equi* subspecies *equi* (*S. equi*), first identified by Schütz (1888), are believed to have been infecting equids for much longer. In the 17th century, strangles was considered an inevitability; indeed, it was suggested that the disease was transmitted in utero due to the high numbers of horses that contracted the infection across varied backgrounds, genetic profiles and management systems (Paillot et al., 2017; Solleysel, 1664).

As was long suspected (George et al., 1983) and later confirmed (Newton, Wood, Dunn, et al., 1997; Timoney et al., 1998), *S. equi* persists in the guttural pouch, without causing clinical disease in a proportion of animals. *S. equi* survives in this low nutrient state, intermittently shedding into the environment, allowing the organism to spread to naïve individuals; indeed, its success as a pathogen can be attributed to the ability to cause both acute and persistent disease. Chronically infected equids rarely show clinical signs, presenting a major obstacle to the prevention and control of outbreaks (Verheyen et al., 2000). The challenges associated with detecting

carriers are a key reason for the perpetual spread of *S. equi* (Pringle, Venner, et al., 2020).

STREPTOCOCCUS EQUI

Pathogenesis

Contact with infected equids represents the primary cause of strangles infection; although, *S. equi* has been shown to persist in the environment for up to 34 and 13 days in wet and dry sites, respectively, and environmental persistence is an additional source of contagion (Durham et al., 2018). Equids become infected via the oronasal route, likely through ingestion of contaminated material (Boyle et al., 2018). Upon entry, *S. equi* attaches to the crypt cells of the lingual and palatine tonsillar tissue, before translocating to regional lymph nodes (Timoney & Kumar, 2008).

Virulence factors act to mitigate the effects of the host immune response: the hyaluronic acid capsule aids immune evasion (Woolcock, 1974), IgG endopeptidases are secreted to cleave antibodies (Lannergard & Guss, 2006) and antiphagocytic binding proteins such as Se18.9 are secreted (Tiwari et al., 2007). Additionally, SeM surface proteins block immune activity by binding to fibrinogen and immunoglobulin (Meehan et al., 2009; Timoney et al., 1997). High morbidity is achieved through this antiphagocytic activity, resulting in intra- and extracellular multiplication in tonsillar and lymphoid tissue, including regional lymph nodes (Timoney & Kumar, 2008). Additionally, *S. equi* can produce a microscopic biofilm with potential adhesive functions (Steward et al., 2017) that may play a role in persistence (Figure 1).

If visible lymph node abscessation occurs, it is not until 3–5 days after their infiltration, as large numbers of neutrophils are attracted to the site through the interaction of complement-derived factors and pathogen-associated molecular patterns such as peptidoglycan (Muhktar & Timoney, 1988). The ability of *S. equi* to import iron has been linked to its growth within these abscesses, with the secreted molecule equibactin facilitating this acquisition (Harris et al., 2015; Heather et al., 2008). If abscesses occur, they rupture into the airways, guttural pouches or through the skin 7–28 days after initial infection (Waller, 2014): abscesses of the retropharyngeal lymph nodes typically rupture into the guttural pouches, draining into the nasopharynx and subsequent nasal passages resulting in copious mucopurulent discharge.

Severity is dose-dependent with around 10,000 colony-forming units required to cause disease in a mature and immunocompetent equid (Boyle et al., 2018). Increasing the number of colony-forming units will result in more severe disease and a shorter incubation period, which can vary from 1 to 28 days (Boyle et al., 2018).

Shedding of *S. equi* typically commences 1–2 days after the onset of pyrexia and persists for 2–3 weeks; equids can remain infectious for over 6 weeks after purulent nasal discharge has resolved (Boyle et al., 2018).

Streptococcus equi evolution

Streptococcus equi is a host-restricted pathogen of equids, thought to have evolved from the opportunistic pathogen *Streptococcus equi* subspecies *zooepidemicus* (*S. zooepidemicus*) (Harris et al., 2015; Holden et al., 2009; Waller et al., 2011). *S. equi* and *S. zooepidemicus* are closely related, sharing over 97% of their DNA (Holden et al., 2009).

Streptococcus equi and *S. zooepidemicus* have many structural and functional differences (Bannister et al., 1985; Holden et al., 2009; Lindmark et al., 2001) but share a common phage pool; their divergent evolution is a result of functional loss, pathogenic adaptation and genetic exchange (Holden et al., 2009). The deletion of the clustered regularly interspaced short palindromic repeats (CRISPR) locus in *S. equi* is thought to have favoured the acquisition of genetic elements, at the expense of genome stability (Waller & Robinson, 2013). A notable difference between the two genomes is the presence of the equibactin locus in *S. equi*, involved in iron acquisition (Heather et al., 2008), which may have been the speciation event that distinguishes *S. equi* from *S. zooepidemicus* (Harris et al., 2015; Heather et al., 2008; Holden et al., 2009).

The *S. equi* genome is larger than that of *S. zooepidemicus*, partly because of its plasticity and the procurement of many mobile genomic elements, which have been crucial in the development of *S. equi* as a pathogen (Holden et al., 2009). The loss of genes not required to cause strangles has reduced the ancestral capabilities of *S. equi*, leading to host restriction (Waller et al., 2011); as a result, *S. equi* is only able to cause disease in equids, whereas *S. zooepidemicus* can infect a wide variety of mammalian hosts (Blum et al., 2010; Las Heras et al., 2002; Pelkonen et al., 2013; Pisoni et al., 2009; Priestnall et al., 2010; Salasia et al., 2004).

Streptococcus equi genome changes during persistent infection

Streptococcus equi has been characterised as possessing a dynamic genome with the ability to diversify and decay; mutations relating to metabolic streamlining and the loss of virulence have been noted in chronically infective isolates (Harris et al., 2015). The endemicity of *S. equi* can, in part, be attributed to its ability to persist in the guttural pouch following an infection, surviving in a low-nutrient state, yet intermittently shedding and thus exposing naïve animals to bacteria.

Genomic decay during persistent infection may reduce transmissibility and result in a lessened ability to cause severe acute disease, such as with the deletion of the equibactin locus, which is linked to the development of lymph node abscesses; although, the organism undoubtedly remains infectious (Harris et al., 2015). Individuals with residual immunity such as equids that are older or vaccinated, and foals with maternal antibodies can develop a milder form of disease, termed 'atypical' strangles (Prescott et al., 1982). This presentation may be caused by a reduction in virulence in isolates where deletions

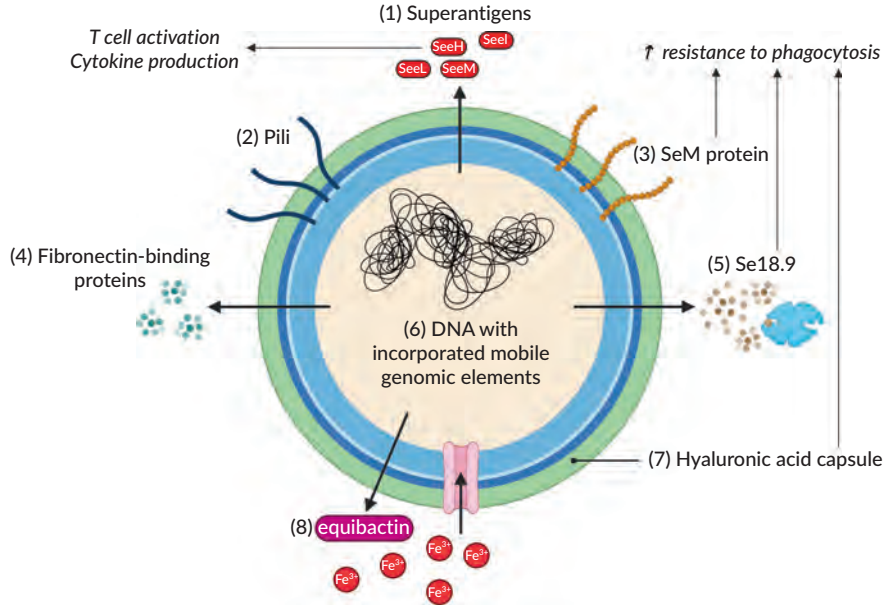

FIGURE 1 Virulence factors of *Streptococcus equi*. (1) Superantigens (SeeL, SeeH, SeeI, SeeM): Cross-link MHC class II and the T cell receptor generating an inappropriate activation of the immune response; (2) Pili: Longer pili compared to *S. zooepidemicus* to penetrate the capsule and bind to collagen more effectively; (3) SeM protein: Bind to fibrinogen and immunoglobulin, inhibiting C3b complement deposition. Targeted by the single and dual ELISA serological tests; (4) Se18.9: Secreted protein that binds to Factor H and interferes with complement activation; (5) Fibronectin-binding proteins (SFS, FNE): Assists with selectively binding to equine tissue and interferes with the attachment of competing pathogens such as *S. zooepidemicus*; (6) DNA and mobile genomic elements: The genome has acquired mobile genomic elements, such as prophage, increasing pathogenicity; (7) Hyaluronic acid capsule: Increased capsule depth compared to *S. zooepidemicus* enhancing resistance to phagocytosis, but reducing mucosal adherence; (8) Equibactin: Siderophore involved in iron acquisition, linked to the development of lymphadenopathy. Not present in *S. zooepidemicus* and lost in some carrier isolates. Created in BioRender.com by Luke McLinden.

in the genome are present (Waller, 2016), as with the outbreak described by Tscheschlok et al. (2018) where the strain of *S. equi* had a deletion in the SEQ_0402 gene, which likely attenuated it.

Morris et al. (2021) analysed the genomes of 35 *S. equi* isolates recovered from horses in Pennsylvania and Sweden to investigate changes that might explain long-term persistence in recovered horses. Whilst no consistent changes in the genomes of isolates from carrier horses were identified, the genomes of some individual isolates recovered from persistently infected horses were found to contain deletions in SeM and the citrate locus. Therefore, Morris et al. (2021) provides further evidence of genome decay in isolates from persistently infected horses that differs from one carrier to another, potentially reflecting the varied selective pressures that are exerted on *S. equi* as this organism persists within the guttural pouch.

A complex interplay between the host and causative agent is suggested (Harris et al., 2015; Morris et al., 2021) in which genomic plasticity could play a central role; this is an opportunity for further research with an emphasis on understanding host, as well as pathogenic, factors such as immunity.

Global endemicity

Equids are widely used and transported between geographic regions and strangles continues to spread as rapidly as ever (Leadon et al., 2008; Mitchell et al., 2021). Strangles is endemic worldwide, with only Iceland remaining free from the disease, due to a self-imposed import ban of equids and geographical isolation (Björnsdóttir et al., 2017). Population analysis of 670 isolates from 19 countries (Mitchell et al., 2021) revealed the extent of the international transmission that results in the endemicity of strangles across the world. The international transmission of *S. equi*, as demonstrated by Mitchell et al. (2021), is in accordance with the first criterion of the World Organisation of Animal Health listing of terrestrial animal diseases. The other three criteria are demonstrated elsewhere (Björnsdóttir et al., 2017; Boyle et al., 2018); therefore, it was recommended strangles be added to this listing (Mitchell et al., 2021).

CLINICAL MANIFESTATION

Acute *Streptococcus equi* infection

Strangles is characterised by sudden pyrexia, mucopurulent intermittent nasal discharge and the abscessation of the submandibular and retropharyngeal lymph nodes (Timoney et al., 1998). Less common clinical signs include respiratory signs, pharyngeal swelling, lethargy, inappetence, dysphagia, depression and the presence of chondroids (Rendle et al., 2021). Although strangles has a low mortality rate,

severe swelling of abscesses in the lymph nodes can lead to significant inflammation, asphyxia and, ultimately, death.

As abscesses form and subsequently rupture, empyema of the guttural pouch or upper respiratory tract can occur. Intermittent expulsion of this thick highly infectious pus is important for the resolution of the infection and removal of bacteria (Boyle et al., 2018); it results in mucopurulent nasal discharge and a cough, present in around half of equids with guttural pouch empyema (Judy et al., 1999). Abscessation and pharyngitis can obstruct the upper respiratory tract, resulting in dyspnoea and dysphagia, alongside potential temporary laryngeal hemiplegia (Boyle et al., 2018).

Systemic and mucosal immune responses are evident 2–3 weeks of post-infection, and this immunity wanes over time (Boyle et al., 2018). Hamlen et al. (1994) showed that 75% of foals exposed to *S. equi* 6 months after recovering from strangles were protected from severe infection, corroborated by historical and contemporary literature (Boyle et al., 2018; Todd, 1910), although no animals were completely protected from clinical signs. The use of antimicrobial therapy early in acute strangles has been demonstrated to interfere with the persistence of humoral immunity (Pringle, Storm, et al., 2020).

The term 'atypical' strangles is used to describe a milder presentation of the disease, in which clinical signs are lessened or absent. The disease presentation may be milder due to an attenuated strain of *S. equi* (Tscheschlok et al., 2018), the presence of residual immunity (Hamlen et al., 1994), a low infective dose or a combination of these factors. Strangles can present with a range of clinical signs (e.g. pyrexia, lymphadenopathy and nasal discharge) and with different severities; therefore, classification as 'typical' or 'atypical' is misleading. Crucially, the severity of disease is not predictive of infectivity, as an animal with a milder presentation can still cause more severe disease in other animals.

Complications of *Streptococcus equi* infection

Streptococcus equi has the potential to spread haematogenously, via lymphatics, septic focus or by direct aspiration of purulent material (Boyle, 2017). Common sites include the lung, mesentery, liver, spleen, kidney and brain (Boyle et al., 2018; Sweeney et al., 1987); additional clinical signs are dependent on the location of abscesses. This presentation is known as metastatic or 'bastard' strangles and has been documented since the 17th century (Solleysel, 1664).

Streptococcus equi infection is the most common cause of purpura haemorrhagica, but vaccination with M-protein-containing vaccines, other bacteria, viruses and neoplasia can similarly result in purpura complexes and vasculitis (Mallicote, 2015).

Myopathies can be seen with *S. equi* infection, with three predominant presentations (Boyle et al., 2018): muscle infarctions (Kaese et al., 2005) and rhabdomyolysis with either acute myonecrosis or progressive atrophy (Sponseller et al., 2005; Valberg et al., 1996).

Persistent *Streptococcus equi* infection

Once ruptured, abscesses of the retropharyngeal lymph nodes typically drain into the guttural pouches, resulting in guttural pouch empyema. Most strangles cases are cleared within 6 weeks, but some animals can enter a carrier state, continuing to shed *S. equi* following the apparent resolution of an acute infection (Newton, Wood, & Chanter, 1997; Newton, Wood, Dunn, et al., 1997). If the purulent material is not cleared and loses fluid, this can form chondroids over time; both empyema and chondroids can act as chronic reservoirs of *S. equi* (Judy et al., 1999; Newton, Wood, Dunn, et al., 1997).

An average of 10% of infected individuals in an outbreak develop into carriers (Boyle et al., 2018; Sweeney et al., 2005); although, this figure may be an underestimate and is highly variable between outbreaks, with detection rates being limited by current diagnostic sensitivity (Pringle et al., 2019). Carriers intermittently shed bacteria into the environment, leading to recurrence and perpetuation of strangles within their herd as well as transmission to naïve individuals (Mallicote, 2015).

There is no consensus on the question of what constitutes a carrier animal, and the answer can be argued from a number of different perspectives. The gross appearance of the guttural pouches demonstrates a spectrum of persistence, from a grossly normal but microbiologically active biofilm (Steward et al., 2017) to the presence of purulent material or chondroids harbouring *S. equi*. Likewise, the chronicity of infection and presence of an observable immune response on a serological ELISA might determine the risk of infection propagation (Ivens & Pirie, 2021). The polymerase chain reaction (PCR) status and associated cycle threshold number, indicative of bacterial load, is often used to identify carriers, but this may be too simplistic in some cases as little is inferred about the viability and infectivity of the *S. equi* identified.

The following definition is proposed: a strangles carrier is an equid in which a viable population of *S. equi* persists and continues to shed, intermittently or continuously, following the apparent resolution of infection. These carriers can be divided into categories based on gross signs of infection (e.g. the presence of chondroids within the guttural pouch), immune status, serological status, chronicity of infection (e.g. short-term carriers that have recently been exposed and long-term carriers that have harboured *S. equi* for months/years) and bacterial load. For clinical practitioners, a division based on gross signs of infection may be most appropriate with carriers being categorised as symptomatic or asymptomatic accordingly.

DIAGNOSIS OF STRANGLES

Diagnosis of acute *Streptococcus equi* infection

The diagnosis of strangles relies on a thorough understanding of an animal's history, in particular with respect to onset, management structures, and possible exposure, including the history of travel, or new arrivals to the equestrian facility (Boyle et al., 2018).

Clinical signs can be variable and nonspecific; indeed, not all animals develop clinical signs (Boyle et al., 2018; Tscheschlok et al., 2018). Nevertheless, they form a vital part of any clinical diagnosis, especially during an outbreak where the testing of all affected individuals may not be necessary (Rendle et al., 2021).

Pathogen identification historically relied on the culture of *S. equi* due to its low cost and wide availability (Waller, 2014). However, sensitivity can be as low as 30%–40% (Boyle et al., 2012; Lindahl, Båverud, et al., 2013; Pusterla et al., 2021), and other beta-haemolytic Streptococci such as *S. zooepidemicus* can complicate interpretation (Boyle et al., 2018). Low levels of bacterial shedding, the presence of host-produced growth inhibitors, sample site and poor sampling technique can also lead to false-negative results (Pusterla et al., 2021).

Advances in PCR (Baverud et al., 2007; Noll et al., 2020; Webb et al., 2013; Willis et al., 2021) and loop-mediated isothermal amplification (LAMP) assays (Boyle et al., 2018) have improved the sensitivity and specificity of the detection of *S. equi* and these assays are now regarded as the gold standard (Boyle et al., 2018). The first PCR assays for *S. equi* were designed to detect a part of the SeM DNA sequence (Timoney & Artiushin, 1997); however; a homologue of SeM is known to exist in *S. zooepidemicus* resulting in cross-reaction (Kelly et al., 2006). In addition, SeM is variably found within the *S. equi* genome, particularly in persistently infective isolates (Chanter et al., 2000; Harris et al., 2015), limiting the diagnostic value of assays that only target SeM. Other PCR assays detect non-SeM sequences (Baverud et al., 2007; Boyle et al., 2016) or have multiple gene targets such as the commercially available triplex assay, which targets two *S. equi* genes (SEQ_2190 and eqbE) and an internal control (Webb et al., 2013).

Polymerase chain reaction and LAMP assays detect DNA of live and dead bacteria indiscriminately; although efforts to determine the physiological state and viability of *S. equi* using molecular approaches show promise (Pusterla et al., 2018). Despite the potential for false-positive results, all positive PCR cases should be taken seriously, even if they are culture-negative (Boyle et al., 2018; Pusterla et al., 2018; Rendle et al., 2021; Waller, 2014). Identification of animals with clinical signs consistent with strangles, regardless of the PCR result, should result in strict movement restrictions and biosecurity protocols (Rendle et al., 2021; Willis et al., 2021).

Advances in diagnostics and surveillance are interlinked: techniques such as quantitative PCR (Webb et al., 2013), nested PCR (Noll et al., 2020) and LAMP assays (Boyle et al., 2021) are rapid and possess high sensitivities and specificities. These technologies allow for the creation of clinically valuable surveillance schemes (McGlennon, 2019), with both laboratory and veterinary contributors. Point-of-care assays have limitations in detection threshold, but have the potential to reduce diagnostic turnaround times and provide a simpler option to caregivers (Slovis et al., 2020). This would allow for the screening of high-risk animals, reducing diagnostic guesswork and ensuring well-timed enaction of biosecurity measures.

The successful identification of *S. equi*, whether through bacterial culture or molecular methods, is dependent on the stage of infection (Rendle et al., 2021) and the sampling site and technique used (Boyle et al., 2017). A single negative test result does not equate to the absence of infection and multiple different samples may be required to obtain a positive result (Boyle et al., 2018). *S. equi* is only present transiently on the nasal mucosa and is often undetectable in a nasopharyngeal swab or wash sample until the lymphoid abscesses rupture, which typically occurs 1–4 weeks after infection (Rendle et al., 2021). Similarly, guttural pouch washes will yield negative results in the initial stages of infection, until the retropharyngeal lymph node abscesses rupture (Boyle et al., 2018). Nasal swabs are only recommended when an equid has active mucopurulent nasal discharge (Lindahl, Båverud, et al., 2013). Aspiration of a mature lymphoid abscess can be used to confirm *S. equi* infection and is often optimal during this stage of the disease (Boyle et al., 2018).

Diagnosis of persistent *Streptococcus equi* infection

Carriers of *S. equi* do not differ clinically and cannot be diagnosed on the basis of inflammatory markers, including white blood cell counts and serum amyloid A (Christoffersen et al., 2010; Davidson et al., 2008; Pringle, Venner, et al., 2020); therefore, carrier status has little impact on systemic inflammation. There is conflicting evidence on the utility of endoscopy scoring and *S. equi* has been demonstrated to produce a microscopic biofilm, microbiologically positive but grossly normal, with potential adhesive functions (Steward et al., 2017). Many carriers have been shown to possess grossly normal guttural pouches at 6 months and beyond following an outbreak (Pringle, Venner, et al., 2020; Riihimäki et al., 2016). The timing of guttural pouch examination is likely to influence findings as Boyle et al. (2017) found distinct differences are visible in many carriers at a median time of 3 months after an outbreak.

Endoscopically guided guttural pouch lavage followed by quantitative PCR is recommended for the detection of persistent infections (Boyle et al., 2018). This technique provides visualisation of the guttural pouch, allowing identification of chondroids, inflammation or empyema; although, contamination of equipment can result in false-positive results (Svonni et al., 2020). Guttural pouch lavage has been validated as superior to a single nasopharyngeal swab or lavage (Boyle et al., 2017). However, nasopharyngeal lavage on three separate occasions has been demonstrated to predict freedom from persistent infection (Pringle et al., 2022; Sweeney et al., 2005), with repeated testing mitigating the possibility of false negatives. Serological testing is unreliable in identifying carrier animals (Davidson et al., 2008; Durham & Kemp-Symonds, 2021; Pringle, Venner, et al., 2020), and does not replace these other more invasive, expensive, and time-consuming methods of detection. Guttural pouch lavage combined with quantitative PCR is considered the best, albeit imperfect, method for carrier detection (Boyle et al., 2018; Rendle et al., 2021; Svonni et al., 2020); although, economic and practical implications mean it is not always applicable.

Serological testing

Indirect ELISAs detect antibodies generated by the host: they are used for screening animals (Craig, 2021), identifying exposure following an outbreak (Rendle et al., 2021; Robinson et al., 2013), and diagnosing the complications of strangles (Boyle et al., 2009). Commercially available ELISAs detect antibodies produced against the SeM surface protein, or both antigen A (SEQ_2190, a non-SeM target) and antigen C (a fragment of SeM) of *S. equi*, the so-called dual-target ELISA (Boyle et al., 2018; Robinson et al., 2013). Carrier status cannot be determined using commercially available ELISAs (Durham & Kemp-Symonds, 2021; Van Maanen et al., 2021); however, the identification of recently exposed animals can be helpful in identifying animals to perform further diagnostics or in biosecurity plans that do not allow for blanket guttural pouch lavage for economic and/or practical reasons (Rendle et al., 2021). Biosecurity plans are always a balance of benefit versus risk and serology can play a role although its limitations should be clearly understood.

SeM-based ELISAs can be used to aid in the diagnosis of purpura haemorrhagica or metastatic abscessation (associated with titres ≥ 12,800), as well as identify animals predisposed to developing purpura haemorrhagica (titre > 1:3200) (Boyle et al., 2009, 2018). They can also be used to indicate recent infection (≥4-fold increase in titre between paired samples taken 10 days apart) (Boyle et al., 2009, 2018), although a single reading does not provide a measure of protection or active infection.

Cross-reactivity with a SeM homologue in *S. zooepidemicus* (Kelly et al., 2006) combined with the failure of the SeM-based ELISA to detect *S. equi* strains not containing SeM (Harris et al., 2015) led to the development of the dual-target ELISA (Duran & Goehring, 2021; Robinson et al., 2013). Following an outbreak, it is advised to use the dual-target ELISA to identify horses exposed to *S. equi* (Boyle et al., 2018; Duran & Goehring, 2021). The dual-target ELISA is reported to have similar sensitivity but greater specificity than the single target SeM-based ELISA (Robinson et al., 2013). It can be used to identify recent exposure, from as little as 2 weeks of postinfection, and has been used to determine exposure in populations across the globe (Ling et al., 2011; Štritof et al., 2021).

CLINICAL MANAGEMENT

Treatment of acute *Streptococcus equi* infection

Most equids with acute strangles exhibit non-specific signs of generalised respiratory infection with presentation depending on challenge dose and host immunity, often responding well with only supportive and nursing care (Rendle et al., 2021; Whitelegg & Saunders, 2021). Acute disease can quickly deteriorate into severe cases, emphasising the need for regular monitoring (Rendle et al., 2021).

Nursing for an animal with strangles is vital; good provision should include an environment that encourages rest, appropriate nutrition, regular monitoring (TPR), abscess management and a quarantine protocol (Whitelegg & Saunders, 2021). A soft, calorific and palatable diet alongside water, to facilitate deglutition, both provided from a height, can help equids with profound lymphadenopathy; assisted nutrition may be indicated (Rendle et al., 2021). The experience of individual equids must be considered during a strangles outbreak, as small changes in diet and environment can aid in assuaging the effects of infection with *S. equi*.

Individuals with visible lymphadenopathies require good supportive and nursing care, with a focus on facilitating the maturation and subsequent drainage of abscesses (Boyle et al., 2018). Surgical drainage may be required if the abscesses are not spontaneously rupturing, although care must be taken to ensure the abscess is mature to enable maximal drainage (Boyle et al., 2018). Once open, abscesses should be lavaged with saline or antiseptic solutions, followed by daily flushing so long as discharge persists (Rendle et al., 2021). NSAIDs can be employed to provide analgesia and reduce pyrexia; it has been suggested that their use can slow the development of abscesses, but this claim lacks evidence (Rendle et al., 2021). Paracetamol has also been recommended since it does not inhibit inflammation but possesses antipyretic and analgesic actions, resulting in improved appetite and welfare (Rendle et al., 2021). Phenylbutazone or flunixin meglumine could also be considered (Boyle et al., 2018).

Antimicrobial therapy has a role in combatting *S. equi* infections but must be prescribed responsibly and only when clearly indicated, with careful consideration to minimise the development of antimicrobial resistance (Boyle et al., 2018; Jaramillo-Morales et al., 2022). For most strangles outbreaks, antimicrobials are not indicated or required for mature horses. Antimicrobials may be indicated between initial exposure and abscessation (Boyle et al., 2018), but this window is not always adhered to since abscesses can develop within days (Timoney & Kumar, 2008).

Penicillin is the drug of choice for *S. equi* infection; however, population analysis (Morris et al., 2020) revealed that pbp2x mutations are emerging. This mutation is in the penicillin-binding site and is associated with penicillin resistance in *Streptococcus pneumoniae* (Maurer et al., 2012; Nichol et al., 2002). Although penicillin resistance has been observed in *S. equi* isolates (Fonseca et al., 2020), it is not typically seen (Clark et al., 2008; Johns & Adams, 2015) and further work is needed to determine the clinical implications of these conflicting findings. It is important that resistance is monitored, and that unusual results (Fonseca et al., 2020) are followed up in accordance with international standards (CLSI, 2020; EUCAST, 2023).

Treatment of persistent *Streptococcus equi* infection

Persistent infections of the guttural pouch are typically treated with combination of topical antimicrobials in combination with lavage (Boyle et al., 2018); systemic antimicrobial therapy is only indicated in a proportion of cases (Rendle et al., 2021). Administration of penicillin systemically and an endoscopically guided gelatin-penicillin mix topically, has been regarded as broadly successful (Verheyen

et al., 2000). The use of a reverse thermodynamic gel with benzylpenicillin presents an easier alternative to using a gelatin mix, where antimicrobial concentration is maximised since the gel can be retained in the guttural pouch for over 72 h (Bowen, 2017; Rendle et al., 2021).

The removal of purulent material and chondroids from the guttural pouches is required for the elimination of the carrier state (Boyle et al., 2018). Standing endoscopic intervention is preferable to general anaesthesia surgical intervention due to inherent anaesthetic risks, surgical dissection around vital structures, and *S. equi* environmental contamination (Boyle et al., 2018). Topical application of 20% acetylcysteine (w/v) solution can facilitate drainage of non-inspissated mucopurulent material through the nasal passages by disrupting disulphide bonds, thereby reducing mucus viscosity (Boyle et al., 2018).

Specific treatment methods depend on the individual presentation and the type of material within the guttural pouches. Many carriers do not present with empyema or chondroids, and it has been reported that the carrier state can self-resolve without treatment (Pringle et al., 2019).

Outbreak prevention and management

Strangles was once considered an inevitability (Solleysel, 1664), but has since been demonstrated to be a very preventable infection (Rendle et al., 2021). Outbreaks can be prevented by limiting exposure to the infectious agent, through enacting rigorous biosecurity protocols, using appropriate quarantining and screening facilities, and understanding of the pathogenesis of *S. equi* (Boyle et al., 2018). Outbreaks of strangles are controlled through the cessation of movement to and from the equestrian facility, isolating animals that are infected and where infection is suspected. A tiered 'traffic light' system with segregation based on exposure and no mixing between groups should be adhered to (Boyle et al., 2018). Following the outbreak, all animals should be tested for exposure and persistent infection.

Long-term control strategies should consider the vaccination of unexposed animals, the identification and treatment of carrier animals, and caregiver education on clinical signs associated with acute disease (Duran & Goehring, 2021).

Vaccination

Vaccination as a tool for outbreak prevention has been limited by efficacy, safety, practicality, clashes with other vaccination schedules, geographical restrictions, differences in circulating *S. equi* strains and owner compliance (Boyle et al., 2018; Mitchell et al., 2021). Strangles vaccines should provide a high degree of protection against *S. equi*, a long duration of immunity, the ability to be administered intramuscularly safely, and permit the differentiation of infected from vaccinated animals (DIVA) (Waller & Jolley, 2007).

The first strangles vaccines were developed in the 1940s, using heat-killed bacteria, conferring a limited degree of protection but often resulting in adverse effects, including injection site reactions and pyrexia (Bazeley, 1940a, 1940b, 1942a, 1942b, 1943). Cell-free variations of this vaccine still exist (Waller, 2014), although the incidence of adverse reactions and the lack of DIVA capability have limited their use. A recent attempt to combine the *S. equi* bacterin and recombinant SeM protein in a vaccine yielded promising results in mice, with all demonstrating a humoral response (Rosa et al., 2021); evaluation of its safety and efficacy in horses is ongoing.

M-protein-containing extract vaccines have demonstrated some efficacy in reducing the frequency and severity of disease; although adverse reactions are common and they possess no DIVA capability (Hoffman et al., 1991). Commercially available options, although none are available in the UK, include Strepvax II (Boehringer Ingelheim), Equivac S (Zoetis New Zealand) and Strepguard (MSD Animal Health) (Duran & Goehring, 2021).

Live-attenuated vaccines have been at the forefront of strangles prevention since the early 21st century (Jacobs et al., 2000). The Equilis StrepE (MSD Animal Health) is administered submucosally, and the Pinnacle IN (Zoetis) is administered intranasally; they are commercially available in Europe and North America, respectively, as well as other countries intermittently (Duran & Goehring, 2021). Adverse reactions were reported upon intramuscular administration, and these live-attenuated vaccines possess no DIVA capability (Borst et al., 2011; Kemp-Symonds et al., 2007; Lanka et al., 2010; Livengood et al., 2016). Furthermore, the Equilis StrepE (MSD Animal Health) vaccine has been linked to *S. equi* replication, resulting in lymph node abscesses (Harris et al., 2015; Kelly et al., 2006; Kemp-Symonds et al., 2007; Mitchell et al., 2021).

Strangvac (Intervacc AB) is a recombinant fusion protein vaccine that is administered intramuscularly and has been shown to protect up to 94% (15 of 16) of ponies from clinical signs of disease, including the development of abscesses in the retropharyngeal or submandibular lymph nodes when challenged 2 weeks following third vaccination (Robinson et al., 2020). Strangvac has DIVA capability as the vaccine does not contain live *S. equi*, *S. equi* DNA nor the SeM and SEQ_2190 antigens that are targeted by culture, PCR, or ELISA diagnostic tests (Robinson et al., 2018). Future studies will be needed to evaluate the utility of Strangvac (Intervacc AB) in clinical practice.

Advancements such as the Strangvac vaccine represent a promising development, potentially allowing vaccination to become a more efficacious control measure. However, continued work is required from veterinary professionals to build trust with owners and caregivers over the use of any strangles vaccines due to past difficulties (White et al., 2021).

CONCLUSION

Understanding *S. equi* is crucial to combatting strangles, and much work has been carried out to characterise its evolution (Holden et al., 2009), genome (Harris et al., 2015), epidemiology (Mitchell

et al., 2021), survivability (Durham et al., 2018), resistance profile (Fonseca et al., 2020) and pathogenicity (Timoney, 2004; Timoney & Kumar, 2008). This increased understanding has enabled the development of more targeted diagnostic assays (Boyle et al., 2021; Noll et al., 2020; Webb et al., 2013; Willis et al., 2021), better outbreak prevention and management protocols (Rendle et al., 2021) and a safe and efficacious vaccine with DIVA capability (Robinson et al., 2020). These advances better equip clinicians and caregivers to treat and prevent strangles.

Further research is required to investigate the role of *S. zooepidemicus* as a primary respiratory pathogen in equids (Lindahl, Aspán, et al., 2013; Waller, 2017; Waller & Wilson, 2021), and to better understand the growing concern of antibiotic resistance in both *S. equi* and *S. zooepidemicus* (Fonseca et al., 2020; Johns & Adams, 2015). The success of *S. equi* as a pathogen can be attributed to the carrier state allowing infection to spread to naïve animals. Understanding the host and pathogenic factors that predispose equids to persistent infection and validating a gold-standard method of diagnosis will help prevent future outbreaks and safeguard animal welfare.

AUTHOR CONTRIBUTIONS

L. McLinden conducted the literature review, created the figure and prepared the manuscript. All authors contributed to the design and revision of the manuscript. The final manuscript was approved by all authors.

CONFLICT OF INTEREST STATEMENT

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