EQUINE VETERINARY EDUCATION
AMERICAN EDITION

AUGUST 2019 • VOLUME 31 • NUMBER 8

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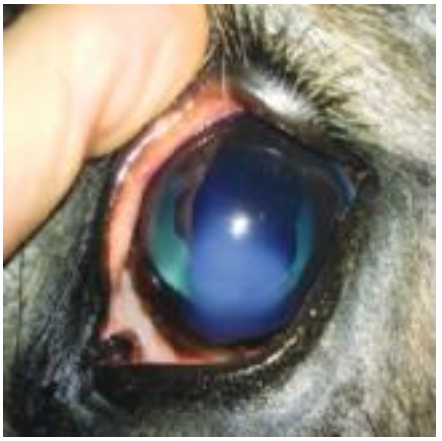

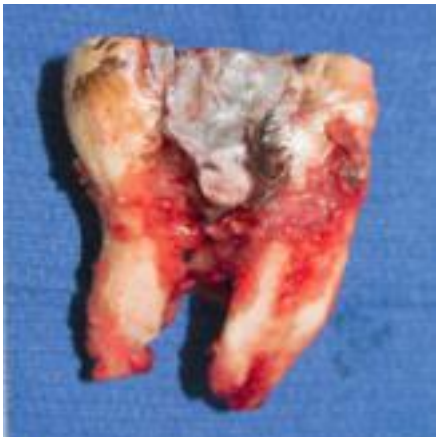

AAEP NEWS In this issue

Cover photo by Dr. Scott Palmer.

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Published monthly. Deadlines are the seventh of the preceding month.

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

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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.

Equine Veterinary Education (American Edition ISSN 1525-8769) is published monthly by the American Association of Equine Practitioners, an international membership organization of equine veterinarians. Office of publication is 4033 Iron Works Parkway, Lexington, KY 40511. Periodicals Postage paid at Lexington, KY and additional mailing office. POSTMASTER: Send address changes to: Equine Veterinary Education, 4033 Iron Works Parkway, Lexington, KY 40511.

Communications regarding editorial matters should be addressed to: The Editor, Equine Veterinary Education, Mulberry House, 31 Market Street, Fordham, Ely, Cambridgeshire CB7 5LQ, UK. Telephone: 44 (0) 1638 720250, Fax: 44 (0) 1638 721868, Email: sue@evj.co.uk.

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Canadian Subscriptions: Canada Post Corporation Number 40965005. Send change address information and blocks of undeliverable copies to IBC, 7485 Bath Road, Mississauga, ON L4T 4C1, Canada.

Printed by: Cenveo Publisher Services, Lancaster Division, Lancaster, PA.

The ethics and legalities of rebates to farm managers

By Kenton Morgan, DVM, DACT

As consumers, we have become accustomed to rebate programs, in which an after-purchase price discount is received at a predetermined time. You can receive a cash rebate after purchasing a new practice vehicle. Your distributor or pharmaceutical manufacturer may offer rebates for purchasing certain quantities of product. The rebated incentive is paid to the person or entity that made the original product/service purchase. (See "Ethics: Navigating your practice in light of animal health company incentives and rebates," *EVE*, Feb. 2016)

A question on the practice of veterinarians providing rebates came up during the ethics session at the 2018 AAEP Annual Convention. Based on that discussion, I recently posted this issue on the AAEP Ethics Talk Rounds/listserv and I would like to share some of that discussion.

It was reported there have been instances where large veterinary accounts, such as breeding or training farms, are offered rebates from veterinarians or their respective practice. These rebates are typically based upon the dollar amount of business transacted between the veterinary practice and the farm during the preceding year. Here is the scenario: (1) On these farms, there are many different horse owners. (2) The owners are billed directly from the veterinarian/practice, and these invoices are paid directly back to the same. (3) The expectation is that this farm will do business exclusively with this veterinarian/practice. (4) The rebate is paid directly to the farm manager or sometimes the farm owner. (5) The horse owners at these facilities are unaware of this arrangement with the farm manager/owner.

A potential problem arises when a farm manager or farm owner receives and then keeps the rebate. Since the horse owners have paid for the veterinary services throughout the year, they should receive an appropriate portion of the rebate—if one is paid. If this does not occur, then the rebate could be considered a kickback and construed by many to be unethical.

It was noted during the online discussion that, in some states, this arrangement violates the veterinary practice act and would be considered illegal. In those states, the answer is clear: Don't do it. In other states, it is not a violation of the practice act requirements. Check your own state practice act if you are considering anything resembling this type of activity.

The online discussion also raised the issue of transparency. Without the knowledge of this activity by horse owners (who are paying the bills), it begs the question of whether it is ethical?

I would like to share some of the input from contributors to this discussion:

Regardless of its legality, I believe it is patently unethical:

- *The real origin of the revenue stream is not the trainer/farm owner/farm manager but the owner of the horse; should not the owner then benefit from any rebate?*
- *If this is considered ethical practice, then why is the owner not informed of this business model? Transparency is nothing to be feared when ethical practices are employed.*
- *Does this not invite the trainer/farm owner/farm manager to prioritize the value of the rebate over the value of choosing the equine practitioner more experienced/competent in diagnosing and treating the horses' problems?*

From another member:
The question is not: is it legal? The question is: is it right?

I also liked the Rotary "Four-Way Test" shared by another member:
1. Is it the truth?

2. Is it fair to all concerned?

3. Will it build goodwill and better friendships?

4. Will it be beneficial to all concerned?

And lastly:
Do not worry whether or not an action is legal. Worry whether or not it is ethical. Ethical trumps legal—and anytime it does not, the fault lies with legal.

Good food for thought for all of us. If you have an ethical question or issue to discuss, please join the Ethics Talk Rounds at communities.aaep.org/home. We would appreciate your participation, and you can post questions or comments anonymously.

Dr. Morgan is managing veterinarian with Zoetis' Equine Technical Services group and chair of the AAEP's Professional Conduct and Ethics Committee.

5 things to know about AAEP this month

1. Don't miss the early-bird savings deadline for annual convention registration. Register by Sept. 15 at convention.aaep.org and save $200.
2. Renowned equine surgeon Dr. Dean Richardson will deliver the Milne Lecture at the 2019 AAEP Annual Convention.
3. Download a free copy of the AAEP Foundation-supported e-book *Ultrasonography of the Equine Pastern Region* at https://tinyurl.com/aaepfeb.
4. The AAEP Foundation in July brought together stakeholders for a planning session to develop strategies for improving the industry's disaster preparedness and response.
5. Save time on client education events. Download custom-designed PowerPoint presentations on numerous topics at aaep.org/dashboard/clienteducation.

Why using compounded toltrazuril for the treatment of EPM is outside the standard of care

By Jane G. Owens, DVM, DACVCP

Dr. Jane G. Owens

Despite the availability of three FDA-approved products for treatment of Equine Protozoal Myelitis (EPM), a number of compounding pharmacies are selling oral preparations of toltrazuril as a treatment for this serious disease.

Toltrazuril is not approved by the FDA for use in horses. Compounding pharmacies are offering this preparation at lower price points than the FDA-approved products. Veterinarians should understand that "cheaper drug cost" is not a valid reason to use compounded products when FDA-approved products are available for their patients.

Use of compounded products when approved options are marketed puts both the patient and practitioner at risk. We've seen this in the press a number of times in recent years. In 2014, a compounded product containing a combination of toltrazuril and pyrimethamine resulted in the needless death of four horses and serious injuries to six others[1]. This event prompted the FDA to issue a warning letter[2] to the pharmacy involved.

Three products to treat EPM are currently approved by the FDA. These are prescription drugs and can be used only by or on the order of a licensed veterinarian.

- Ponazuril is marketed as Marquis® Antiprotozoal Oral Paste by Boehringer Ingelheim Animal Health and administered once daily for 28 days either with or without a loading dose. Ponazuril is the active metabolite of toltrazuril and is often referred to as toltrazuril sulfone. This chemical similarity does not make these drugs equivalent and they are in fact two different drugs with differing safety and efficacy profiles.
- A combination of pyrimethamine and sulfadiazine Sodium is marketed as ReBalance® by Pegasus Laboratories as an oral suspension administered once daily for 90 to 270 days.
- Diclazuril is marketed as Protazil™ by Merck Animal Health as a pelleted, alfalfa-based top-dressing which is fed for 28 days.

The AAEP urges practitioners to use FDA-approved products, when available, to treat their patients with EPM. An excellent review of EPM diagnosis and treatment is available on the AAEP website[3]. For further reading on the ethical use of compounded products, please see Dr. Kenton Morgan's ethics feature in the February 2019 issue of *EVE*[4].

Dr. Owens is director of companion animal research at Elanco Animal Health in Greenfield, Ind., and chair of the AAEP's subcommittee on compounding. She is past president of the American Academy of Veterinary Pharmacology and Therapeutics and serves as president and founding member of the Veterinary Pharmacology Research Foundation.

References

[1] Pyrimethamine deaths: http://veterinarynews.dvm360.com/compounded-drug-may-have-killed-four-horses-fda-reports

[2] FDA warning letter: https://www.fda.gov/inspections-compliance-enforcement-and-criminal-investigations/warning-letters/wickliffe-pharmaceutical-inc-08142014

[3] AAEP EPM Ref: https://aaep.org/horsehealth/equine-protozoal-myeloencephalitis

[4] "Using compounded products within the standard of care," *Equine Veterinary Education*, February 2019: Kenton Morgan EVE article.

ASCEND
NEW HEIGHTS IN EQUINE PRACTICE

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Work through career challenges together at new 'Practice Life Conversations'

AAEP HEALTHY Practice

Let's face it: Successfully navigating the daily rigors of life in equine practice can be a challenge. During the AAEP's 65th Annual Convention in Denver, Colo., share your experiences and learn from those of your colleagues at new AAEP Practice Life Conversations.

This conversation series on Saturday, Dec. 7 from 3:00-5:00 p.m. is meant for group think and will be facilitated by veterinarians who have "been there and done that." Sessions will run concurrently on the following topics:

- Managing Emergency Coverage in a One- or Two-Doctor Practice
- Planning for Maternity Leave
- Creating a Culture of Wellness in Practice

No advance sign-up is necessary; just show up and plan to participate in these interactive conversations.

Dr. Dean Richardson to spotlight fracture repair possibilities during Milne Lecture

Dr. Dean Richardson

Renowned equine orthopedic surgeon Dr. Dean Richardson will impress upon practitioners the surgical opportunities available for fracture repair and the associated importance of improving emergency management when he delivers the Frank J. Milne State-of-the-Art Lecture on Monday, Dec. 9.

During his lecture, "The Tao of Equine Fracture Management," Dr. Richardson will encourage a shift in the industry's perception of fractures based on imaging advancements, technology developments and philosophical changes in recent decades that have resulted in successful outcomes for many seriously injured horses that previously would never have been treated. Practitioners will acquire a thorough understanding of and ability to communicate the treatment options available for horses with major bone and joint injuries.

Dr. Richardson is the Charles W. Raker Chair in Equine Surgery and Chief of Large Animal Surgery at the University of Pennsylvania School of Veterinary Medicine's New Bolton Center. His research and clinical interests have focused on improving fracture repair and joint surgery.

Dr. Richardson received his veterinary degree from The Ohio State University in 1979 and completed his internship at New Bolton Center, where he has been ever since. He is perhaps best known as leader of the New Bolton Center veterinary team that treated 2006 Kentucky Derby winner Barbaro following serious hind leg injuries sustained in the Preakness Stakes. Despite his administrative load, Dr. Richardson maintains a busy clinical practice, oversees a research laboratory and still greatly enjoys teaching veterinary students, interns and residents.

His accolades include numerous teaching awards as well as the 1997 Pfizer Award for Excellence in Research, the 2006 Special Eclipse Award from the National Thoroughbred Racing Association (as part of "Team Barbaro"), and a Special Commendation from the American College of Veterinary Surgeons in 2007.

The convention lecture, sponsored by Platinum Performance, is named for AAEP past president and distinguished life member Dr. Frank J. Milne.

FOUNDATION

My Mentor program giving exceeds $100,000

The AAEP Foundation's My Mentor Honor Program, which allows people to express gratitude to influential figures in their lives and careers, has surpassed $100,000 in gifts thanks to generous nominators and donors.

There are 13 mentors on the Honor Wall and another 22 nominees. To be listed as a nominee, $500 minimum in gifts must be reached. A nominee is added to the honor wall when $5,000 minimum in gifts is attained. The gifts are not required all at once; you may nominate a mentor and then find others to help honor your mentor.

My Mentor gifts can be designated among three endowments:

- Research Endowment
- Scholarship Endowment
- Benevolence Endowment (helping working equid programs, at-risk horses, and disaster preparedness and relief)

Visit aaepfoundation.org and click the "Giving" tab to learn more about how you can honor someone important to you.

WELLNESS

Healthy Practice: The slippery slope of alcohol use and abuse

By Kevin Foote, LMSW and Ted Fish, Ed.D., MBA

Kevin Foote

You always know from the tone.

The question I asked was simple: "Could you go without a drink?" And immediately she grew defensive and tense.

"Of course, I could," came the reply. "But why should I? It's the way I let off steam."

Alcohol lives on a slippery slope. There is the full-blown alcoholic who slips the canteen of whiskey underneath the seat; the high-functioning professional who likes a can of beer to get through the day; and the evening addict. This is the person who takes a glass of wine with dinner or a stiff drink after the meal. The one who makes the evening's drink a ritual in order to let off steam. The one like my client.

Each of these three has a problem. The clinical sign of a dependency on alcohol is when you suffer a mood change from either drinking or from not drinking. This is the point when the substance uses you, not the other way around.

Perhaps it is the long hours, the demanding clients, or the physical exertion. Perhaps it is the stress of always being on. Whatever the cause, self-medicating through alcohol is something I see regularly among equine vets.

Most would never admit there was a problem. Most would say as my client did: "It's just what I do to ease the stress."

What I ask is: "Can you leave it? Can you leave your drinking for a night, or a week, or even a month? Can you leave it without becoming irritable?"

I told my client that I was concerned. She was a brilliant doctor, completely devoted to preserving her health. She was in terrific shape, relying upon the sharpness of her reflexes to dodge those kicks. And she was a mother who was committed to modeling a good life for her daughter. She was violating her values and perhaps endangering her career. Additionally, I had heard from others in the practice that she was prone to fits of temper, one potential sign of alcohol abuse.

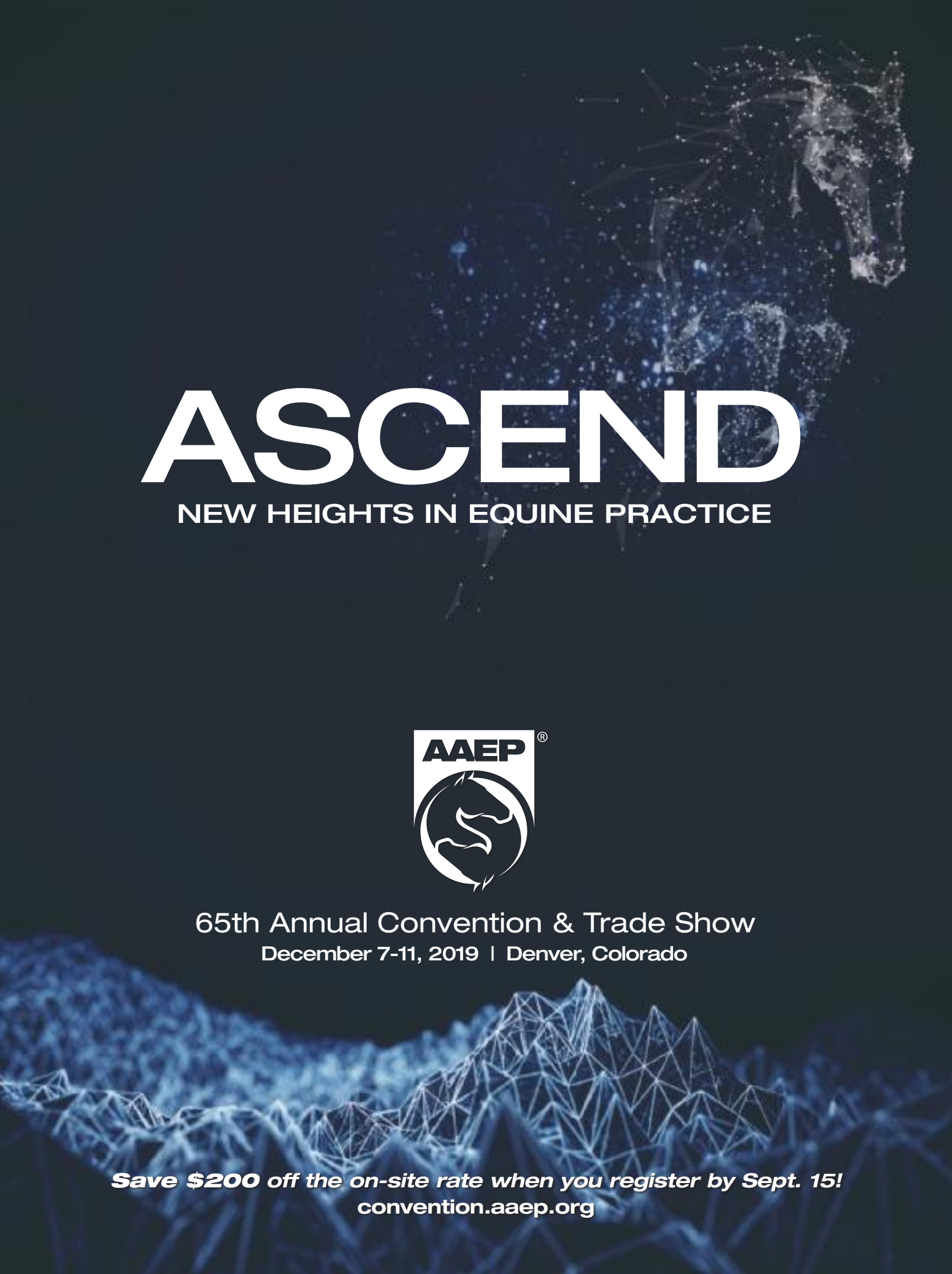
We spoke about her goals in life and her need to take those drinks, and the next week she made a decision: She took her addiction in hand, reducing her consumption by 75%. It was an act of courage and strength. The changes were immediate. Her blowouts at work got better, she had more energy for her job and she regained her self-respect.

My client was lucky, because we know there reaches a point of a full-blown alcoholic response in many drinkers. This is when changes are triggered in the brain, and a person cannot moderate their habit. When this occurs, in-patient or outpatient care, along with 12-step counseling, is a must.

As you stop reading this article, I want to ask: Where would you put your drinking on the scale? Could you leave alcohol today? Could you leave the beer or the wine for a week, or a month? Would it change your sleeping habits or your mood?

Be honest, and if you need, get help—it's out there.

Kevin Foote, LMSW, is President of Footeworks, a company in Mattituck, N.Y., that helps business owners and their staffs produce the results they want in their professional and personal lives. Ted Fish, Ed.D., MBA, formerly with Footeworks, is executive director of the Gardner Carney Leadership Institute in Colorado Springs, Colo.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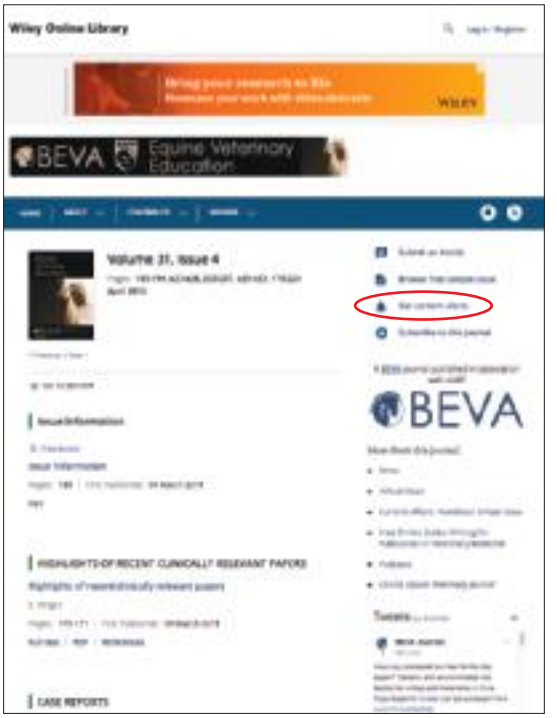

Wiley publishes the British version of *EVE*, which includes the same clinical content as the American version mailed to AAEP members. Issues are generally available in the Wiley Online Library several weeks prior to availability of the American version, which includes AAEP News.

To begin receiving content alerts, go to aaep.org/equine-veterinary-education and click on the link to the Wiley Online Library in the introductory copy near the top of the page. On the *EVE* landing page in the Wiley Online Library, click "Get Content Alerts" on the right side of the page (see image). If you have not already established a Wiley Online Library account, you will be asked to do so. There is no charge to establish an account, nor to receive content alerts.

You can also sign up to be alerted when papers are published on topics that match your pre-defined search criteria. Simply conduct a search on a particular topic and choose "Save Search" on the results page.

If you have questions about this benefit, contact John Cooney, publications coordinator, at jcooney@aaep.org.

UCalgary instructor shaping future horse doctors through care of area's needy horses

One equine veterinary instructor's commitment to Indigenous communities near Calgary is not only improving the health of horses in underserved areas, but also preparing her students to enter practice and planting in them the seeds of community service.

Identifying an opportunity to bridge both the clinical needs of students and the need for preventive veterinary care in nearby Indigenous communities, Dr. Jean-Yin Tan, an instructor of equine clinical sciences at the University of Calgary, implemented annual clinical skills labs at the Tsuut'ina Nation in early 2018. During these labs, second-year veterinary students perform physical examinations, deworming and vaccination of horses at no cost and with vaccines donated by Boehringer Ingelheim and Zoetis.

The labs revealed a broader need for primary care services. Over 17 months, Dr. Tan worked with university officials and members of the Tsuut'ina and Siksika Nations' equestrian communities to resolve funding, logistical and off-site communication challenges before receiving approval for a two-week rotation for fourth-year veterinary students at both Indigenous communities.

The rotation launched in June at the Tsuut'ina Nation with four students administering veterinary care to 65 horses under the supervision of Dr. Tan and another faculty member. Students performed physical exams, deworming, dentistry, castrations and lameness workups as well as higher-level services such as a full respiratory workup with bronchoalveolar lavage. Students also received plenty of practice in the important art of owner communication and relationship building. Between the labs and rotation, students have provided nearly $50,000 worth of veterinary services to 200 horses.

"It would be one thing for me to go out there and do this myself but being able to involve the next generation of veterinarians is more fulfilling," said Dr. Tan. "They not only learn the skills, but they also learn about the opportunities out there to do some good in our communities. That's a lot more powerful."

Dr. Tan, who joined the University of Calgary from private practice in 2015 for the responsibility and reward of shaping future equine veterinarians, hopes to expand the program to more than one rotation and to more students and other Indigenous communities.

The initial rotation made a strong impression on its participants.

"It was an amazing experience," said fourth-year student Erica Ward. "Every day we saw a varied case load that sharpened our hands-on and diagnostic skills. Throughout school we have been taught about First Nations Communities, but it is completely different to be in the communities and have meaningful conversations.

"To be a great community practitioner you need to serve the whole community, and First Nations Groups are an often-overlooked part of that. This was my first opportunity to use my clinical skills to serve the community and, now that I've experienced how fulfilling it is, I don't see myself stopping anytime soon."

Todd Korol

Dr. Jean-Yin Tan

Todd Korol

Dr. Tan oversees filing of a horse's teeth by rotation participant Jenn Brandon.

Throughout 2019, the AAEP's Good Works for Horses Campaign will spotlight AAEP-member practitioners whose volunteer efforts are improving the health and welfare of horses. To discover the Good Works of AAEP veterinarians or nominate a Good Works candidate, visit aaep.org/horse-owners/good-works-horses. For more information on nominating a veterinarian for this program, contact Giulia Garcia at ggarcia@aaep.org.

Welcome new members, and congratulations recent graduates

New Members:

Nathan Boyer, DVM, Prescott, AZ
Joseph Eastwood, DVM, Hugo, OK
Mari J. Good, DVM, Fort Lauderdale, FL
Lawrence Kramer Gopffarth, DVM, Glenwood Springs, CO
Michelle Immel, DVM, New London, WI
Samuel Jones, DVM, Raleigh, NC
Jessica Lauren MacDonald, DVM, Ashland, VA
Sheila Jean McDonald, DVM, Kelowna, BC, Canada
Linda Paul, DVM, Baton Rouge, LA
Sandra Marie Zetterström, DVM, Auburn, AL

Recent Graduates:

Ashley Allemand, DVM, Schriever, LA
Olivia Barr, DVM, Lexington, KY
Rebecca Barton, DVM, Blessington, Co. Wicklow, Ireland
Cassandra Baumgarten, DVM, Weatherford, TX
Erin Bentley, DVM, Ocala, FL
Madison L. Berger, DVM, Gainesville, FL
Emily Berman, DVM, Deep River, CT
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Nicole Bledsoe, DVM, Urbana, IL
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Alexandra Catalano, DVM, Grafton, MA
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Jillian Johnson, DVM, Lowell, MI
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Hannah West, DVM, Peculiar, MO
Katrijn Whisenant, DVM, Newport Beach, CA
Katharine White, DVM, Banff, AB, Canada
Marthe Wildsmith, DVM, Cochrane, AB, Canada
Marley Williamson, DVM, Sherwood Park, AB, Canada
Rebecca Wilson, DVM, Whitevale, ON, Canada
Emma Winstead, DVM, Ashland, VA

Members in the News

Dr. Mary Scollay

Dr. Mary Scollay to lead RMTC

After 11 years as equine medical director for the Kentucky Horse Racing Commission, Dr. Mary Scollay has been appointed executive director and COO of the Racing Medication and Testing Consortium.

Dr. Scollay has been involved in equine health, welfare and safety initiatives across the racing industry, and previously served on the AAEP's Racing Committee and Professional Conduct and Ethics Committee. She is a graduate of the University of Illinois.

Dr. Natanya Nieman

Dr. Stuart Brown II

Drs. Natanya Nieman, Stuart Brown elected to KTA-KTOB board

Dr. Natanya Nieman, resident veterinarian at WinStar Farm in Versailles, Ky., has been elected to a three-year term on the Kentucky Thoroughbred Association/Kentucky Thoroughbred Owners and Breeders board of directors.

Dr. Nieman, who received her veterinary degree from the Ohio State University, has been with WinStar Farm since 2002.

Relatedly, Dr. Stuart Brown II, a partner in Hagyard Equine Medical Institute in Lexington, Ky., has been re-elected to the KTA-KTOB board. A Tuskegee University graduate, Dr. Brown serves on the AAEP's Racing Committee.

Dr. Steve O'Grady

Dr. Steve O'Grady honored in South Africa

The South Africa Veterinary Association bestowed its 2019 Gold Medal upon Dr. Steve O'Grady during the 10th SAVA Veterinary and Paraveterinary Congress in Gauteng, South Africa. The medal honors outstanding scientific achievement of veterinary science.

Dr. O'Grady received his veterinary degree from the University of Pretoria in South Africa. He is the founder of Equine Therapeutic Farriery in Keswick, Va., and his accolades also include induction into the International Equine Veterinarians Hall of Fame and receipt of the AAEP President's Award.

Dr. Ernest Beier

Dr. Ernest Beier named to NJ State Board of Agriculture

Dr. Ernest Beier, owner of Beier Veterinary Services in Mickleton, N.J., has been elected to a four-year term on the New Jersey State Board of Agriculture.

Dr. Beier received his veterinary degree from Oklahoma State University. In addition to his veterinary practice, he has owned and operated Rattling Run Farm, a livestock management and hay production company, since 1980.

Dr. Jennifer Durenberger

Dr. Jennifer Durenberger named TJC Steward at NYRA

Dr. Jennifer Durenberger, chief examining veterinarian for the New York Racing Association, has been appointed as The Jockey Club steward at NYRA tracks effective Sept. 6.

Prior to her current role, Dr. Durenberger served as an association veterinarian for NYRA from 2003-2008 and as a commission veterinarian for the California Horse Racing Board from 2008-2010. She is a member of the AAEP's Professional Conduct and Ethics Committee and serves as secretary for the Racing Officials Accreditation Program board of directors.

Don Preisler

Dr. Gregory Ferraro

Dr. Gregory Ferraro appointed to CHRB

Dr. Gregory Ferraro, emeritus director of the University of California, Davis Center for Equine Health, has been appointed by California Governor Gavin Newsom to the California Horse Racing Board.

Dr. Ferraro, who earned his DVM from UC Davis and spent over 25 years in racetrack veterinary practice, served as director of the UC Davis Center for Equine Health from 1997-2011 and associate director of the UC Davis Veterinary Medical Teaching Hospital from 2011-2014.

AAEP Educational Partner Profile: Dechra Veterinary Products

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Equine vet. Educ. (2019) **31** (8) 394-395
doi: 10.1111/eve.13139

Highlights of recent clinically relevant papers

Honey and wound healing

This prospective, randomised clinical study by Hadar Mandel and colleagues in Israel aimed to evaluate the effect of intralesional medical grade honey (MGH) on wound infection and dehiscence following closure.

A total of 127 horses were included in the study and randomly allocated into the treatment (MGH; 69 horses) or control (58 horses) group. Neonatal foals, horses with major systemic illness, penetrating wounds requiring hospitalisation and eyelid lacerations were excluded from the study.

All wounds were first cleaned thoroughly with diluted chlorhexidine or diluted povidone iodine followed by a balanced sterile electrolyte solution. The MGH group had sterile MGH (L-Mesitran gel) applied directly onto the subcutaneous tissue prior to skin closure or after partial wound closure. Data relating to wound healing was subsequently collected from the 11 participating practitioners through questionnaires and telephone conversations.

No adverse effects of the MGH were recorded in any of the horses participating in the study. MGH-treated horses were significantly more likely to heal completely, to have no signs of infection and for the veterinarians to report some degree of satisfaction compared to control cases.

The authors concluded that intralesional application of MGH to lacerations prior to wound closure may help prevent wound infection and therefore dehiscence.

Per rectum fluid therapy

This randomised controlled crossover study by Adeel Khan and colleagues in Australia and the UK compared the use of rectally administered fluids with nasogastric and intravenous administration.

Six healthy horses each received each of three different fluid treatment protocols (intravenous Hartmann's, nasogastric polyionic solution and rectally administered tap water) at 5 mL/kg bwt/h and also underwent a control protocol (no treatment) while feed and water was withheld for 6 h. A minimum 2-week washout period was observed between each treatment.

Prior to administering rectal fluids the rectum was manually evacuated and a 24 Fr flush enema tube inserted approximately 50 cm into the rectum and secured to the tail. Fluid was delivered continuously by gravity flow. Packed cell volume (PCV), total solids (TS), albumin, electrolytes, lactate, urine specific gravity, vital parameters, gastrointestinal borborygmi and central venous pressure were measured every 2 h.

Rectal administration of fluid was well tolerated in all horses. PCV decreased over time with all fluid treatments but not with the control, and TS decreased with intravenous and rectally administered fluid. There was an increase in gastrointestinal borborygmi with rectally administered fluid.

Rectal fluid administration may offer an effective, inexpensive alternative or adjunct to intravenous fluid therapy, particularly when administration via nasogastric tube is not possible or contraindicated.

Chemotherapy for lymphoma

In this retrospective study Daniela Luethy and colleagues in the USA, Australia and Canada reported the long-term outcome of 15 horses with lymphoma treated with chemotherapy.

Fifteen cases with adequate data were identified through a search of medical records and an email call for cases on the ACVIM listserv for horses treated with chemotherapy for lymphoma.

Complete remission was achieved in five horses (33.3%), partial response was achieved in nine equids (60%), and stable disease was achieved in one horse. Overall response rate was 93.3% (14/15). Overall median survival time was 8 months (range, 1–46 months). Nine horses experienced a total of 14 adverse effects attributable to chemotherapy. Adverse effects were graded according to the Veterinary Cooperative Oncology Group common terminology criteria for adverse events grading system (grade 1 alopecia, n = 2; grade 1 neutropenia, n = 2; grade 1 lymphopenia, n = 3; grade 1 lethargy, n = 1; grade 2 neurotoxicity, n = 1; grade 2 colic, n = 1; grade 1 hypersensitivity, n = 1; grade 2 hypersensitivity, n = 2; grade 5 hypersensitivity, n = 1). Higher grade adverse effects most commonly were associated with doxorubicin administration (n = 4), including one horse that died 18 h post-administration.

The authors concluded that chemotherapy can be used successfully for treatment of horses with lymphoma. Adverse effects, most commonly mild, occurred in approximately two- thirds of treated horses.

Saddle fitting

This pilot study by Kathryn Nankervis and colleagues in the UK assessed the reliability of saddle fitters (SFs) to determine the position of the last thoracic vertebra of horses using palpation techniques.

According to published guidelines an English saddle tree should not extend beyond the 18th thoracic vertebra (T18). This study aimed to assess reliability of SFs to identify the T18 spinous process (SP). Part 1 investigated agreement between T18 (T18SF) as identified by three SFs using palpation and a veterinary surgeon (VS) using radiography (T18VS) in seven horses. SF1 and SF2 palpated the lumbosacral joint and counted cranially six SPs, whereas SF3 followed the rib curvature toward the dorsal midline. In part 2, SF1 and SF2 identified T18 by counting cranially five SPs in seven horses on two occasions. Agreement between SFs and VS was assessed using *t* tests and Bland-Altman plots. Interrater and intrarater reliability were estimated using intraclass correlation coefficients. In part 1, SF1 and SF2 found T18SF 4.3 cm (±4.1 and 4.0 cm, respectively) cranial to T18VS. Mean difference between T18SF3 and T18VS was 0.1 ± 4.9 cm (95% CI: −9.5 cm, 9.6 cm). When counting cranially five SPs, mean difference between T18SF1 and T18VS was −1.5 ± 3.4 cm (95% CI: −8.3 cm; 5.1 cm) and T18SF2 and T18VS was −0.3 cm ± 4.5 cm (95% CI: −8.8 cm; 8.5 cm). Interrater reliability was 'good' (ICC = 0.798). Intrarater reliability was

'excellent' for SF1 (ICC = 0.905) and 'good' for SF2 (ICC = 0.847).

These results indicate that counting cranially five SPs from the lumbosacral joint, when coupled with observation of the rib position and curvature should ensure a saddle is not placed beyond T18.

Behaviour associated with dental pain

In this study Jaana Pehkonen and colleagues in Finland investigated behavioural signs associated with equine periapical infection in cheek teeth (CT).

Owners of 47 horses whose CT had been removed because of periapical infection completed an internet-based questionnaire including 23 questions about eating behaviour, bit behaviour, and general behaviour observed before and after the operation. The number of signs exhibited by each horse before and after CT removal was compared using Wilcoxon signed-rank sum test. Before the operation, avoidance behaviours, such as evading the bit, difficulties in eating, and even asocial or aggressive behaviours were commonly reported by the owners. Removing the infected tooth significantly reduced the number of these behavioral patterns expressed by the horses, suggesting that they could be associated with dental pain. Half of the cases had been diagnosed during a routine dental examination, indicating that many owners did not realise that certain undesirable behavioural patterns of their horses might be associated with dental pain.

These findings highlight the importance of training owners to recognise behaviour potentially related to dental pain in horses and that routine dental examinations are essential for ensuring horses' well-being.

Equine coronavirus faecal shedding

In this study Macarena Sanz and colleagues in the USA evaluated equine coronavirus (ECoV) faecal shedding in hospitalised horses.

The objective of this study was to determine whether systemically healthy horses and horses with gastrointestinal disorders shed ECoV in their faeces at the time of admission to a referral hospital and after 48 h of stress associated with hospitalisation.

The study included 130 adult horses admitted to the Washington State University Veterinary Teaching Hospital for gastrointestinal disease (n = 65) or for imaging under anaesthesia (n = 65) that were hospitalised for 48 h. Faecal samples were collected at admission and 48 h later. Polymerase chain reaction (PCR) for ECoV and electron microscopy (EM) were performed on all samples.

Only one of 258 faecal samples was PCR-positive for ECoV. Electron microscopy identified ECoV-like particles in 9/258 samples, parvovirus-like particles in 4/258 samples, and rotavirus-like particles in 1/258 samples.

The prevalence of ECoV in faeces of hospitalised adult horses was low. Therefore, faecal samples that are PCR-positive for ECoV in adult horses that have clinical signs consistent with this viral infection are likely to be of diagnostic relevance. The clinical relevance of the viruses observed using EM remains to be investigated.

Effects of magnesium sulfate on headshaking

In this prospective study Shara Sheldon and colleagues in the USA investigated the effects of magnesium sulfate on six geldings with trigeminal-mediated headshaking.

Trigeminal-mediated headshaking results from low-threshold firing of the trigeminal nerve resulting in apparent facial pain. The authors of this study believed that magnesium may have neuroprotective effects on nerve firing that could dampen signs of neuropathic pain in affected horses.

Horses were controlled for diet and infused intravenously (i.v.) with 5% dextrose solution (DS; control solution at 2 mL/ kg bwt) and $MgSO_4$ 50% solution (MSS at 40 mg/kg bwt). Headshaking behaviour was recorded at times T0 (baseline, before infusion) and T15, T30, T60, and T120 min post-infusion. Venous blood variables such as pH, HCO^{3-}, standard base excess (SBE), Na^+, Cl^-, K^+, Ca^{2+}, Mg^{2+}, total magnesium (tMg), glucose, and lactate were measured; strong ion difference (SID) and anion gap (AG) were calculated for each time point.

Blood variables including pH, Na^+, Cl^-, K^+, SID, AG, lactate, Ca^{2+}, tMg, and Mg^{2+} had significant changes with MSS compared with DS treatment. Glucose, SBE, and HCO^{3-} did not have significant changes. A 29% reduction in headshaking rate occurred after MSS treatment but no change occurred after DS treatment.

Administration of MSS i.v. increased plasma total and ionised magnesium concentrations and significantly decreased headshaking behaviour in horses with trigeminal-mediated headshaking.

S. WRIGHT
EVE Editorial Office

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EQUINE VETERINARY EDUCATION
Equine vet. Educ. (2019) **31** (8) 396-397
doi: 10.1111/eve.13034

Editorial

Putting meaning into continuing professional development

It is accepted that a part of the social contract that gives legitimacy to the professions is the responsibility placed on individual members to keep up-to-date in the areas in which they serve the public (Thistlethwaite and Spencer 2008). Prior to our current era of scientific progress, this was not difficult. 'Standard texts' (e.g. Markham's Masterpiece), which were widely used, ran to multiple editions that spanned several generations (1610–1734), with little alteration (Poynter 1962). However, the late 20th and early 21st centuries have seen an acceleration in our understanding of health and disease and the choices available for animal health management and therapy. Therefore, for modern practitioners, in contrast to the past, it cannot be assumed that knowledge and skills learned in clinical school will be current even a few years later, never mind at the end of a career spanning at least 40 years (Van Hoof and Meehan 2011).

In the past, the relatively slow pace of knowledge and skills development meant that traditional lectures and approaches to updating clinicians worked – or at least up to a point. Certainly, information was transmitted, but this was not always learned and the lecture was a relatively poor way of turning knowledge into changed clinician behaviours and practice/client benefits (Davis *et al.* 1999). However, rapid progress of knowledge in all areas means that, as individual professionals, each of us needs (and only has time for) the specific new knowledge and innovations relevant to our own practice and this, together with the inefficiency of many individuals' approaches to continuing professional development (CPD), means that professional bodies and veterinary associations are encouraging more reflective and organised approaches to CPD; approaches that truly bring meaning to CPD (Wallace and May 2016).

For many equine and other large animal practitioners, practice is an ambulatory service, with large blocks of time spent alone and away from colleagues. Face-to-face CPD plays an important social function in reinforcing our collective endeavours and the standards we expect of one another. However, without thought, we often gravitate to lectures and workshops in our own areas of expertise (Schostak *et al.* 2010). We feel good about being able to confirm that we are largely up-to-date and fail to recognise the poor learning value in terms of the amount of time spent for only a few nuggets of new information – if that. We miss opportunities to attend alternative sessions on subjects about which we know less which could address knowledge and skills gaps that are hampering the progress of our personal practice and that of our businesses.

One of the most important RCVS day one competences and responsibilities of veterinary schools is that of independent, lifelong learning – taking charge of our own professional development. In order to undertake that, we need to be selfaware, recognising not only our many strengths but also the areas we need to improve. For that, we need to be able to reflect on how we are practising (May 2017). We need to be able to identify and recognise the required professional standards for the tasks we undertake, compare that with our current level of performance and, where there are gaps, source and undertake the learning necessary to close those gaps (Sadler 1989). Increasingly, that is a continuous cycle of self-audit that both assures and enhances the quality of the services we provide. Rather than being flattered by attending lectures or reading articles on a subject about which we know a lot, particularly as our professional lives develop, we find ourselves taking pleasure from relevant and meaningful learning from reading articles written by experts in a diverse range of new fields to us and, in some cases, discussions directly with them or attending their workshops. Where a session only partly addresses a learning need, or disappoints entirely, our reflective recognition of this allows us to plan for further learning and also to provide meaningful feedback on sessions that did not meet their authors' learning goals. Not only is our own learning of higher quality and more efficiently achieved but we ensure that CPD providers continuously improve their offerings to the benefit of all our colleagues.

As CPD evolves, models are emerging that provide genuinely continuous professional development in the workplace. Through supported reflection on our daily tasks and responsibilities in formal (such as the RCVS CertAVP) and informal systems, individuals can start to focus on their next areas in need of development, and share these with colleagues, at the same time as contributing in a reciprocal way to the development of these colleagues. This cascading of the development process is unusual in traditional CPD contexts, but has been recognised as an extension of personal and community-relevant CPD activity (May and Kinnison 2015). At their best, these integrated professional developmental activities, involving the whole workforce, are a part of the ethos and culture of what have been termed 'deliberately developmental organisations' where 'people's ongoing development is woven into the daily fabric of working life' and 'visible in the company's regular operations, day-to-day routines and conversations' (Kegan *et al.* 2014).

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EQUINE VETERINARY EDUCATION
Equine vet. Educ. (2019) **31** (8) 398-402
doi: 10.1111/eve.12874

Case Report

Actinobacillus capsulatus peritonitis and chyloabdomen in a Warmblood gelding

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Keywords: horse; abdomen; *Actinobacillus*; chyle; peritonitis; chyloperitoneum

Summary

Primary peritonitis in horses attributable to *Actinobacillus equuli* has been reported in Australia, New Zealand and North America. Published reports describe a rapid response to treatment with appropriate antimicrobial and supportive treatment and an excellent prognosis for survival. To our knowledge, this is the first reported case of *Actinobacillus capsulatus* in the horse. The case was complicated by the development of an idiopathic chylous abdominal effusion. The report highlights the importance of molecular methods in the correct identification of bacterial species. Prognosis for horses diagnosed with *Actinobacillus* peritonitis may be guarded given the atypical response to appropriate antimicrobial treatment in this case.

Introduction

Peritonitis, or inflammation of the peritoneum, in horses results from a number of infectious and noninfectious causes (Hall 2015). Most cases occur secondary to gastrointestinal disease (Elce 2006) or following urogenital trauma (Hall 2015). Primary peritonitis is uncommon in the adult horse (Gay and Lording 1980; Davis 2003; Tennent-Brown *et al.* 2010; Hall 2015) but is usually caused by *Streptococcus zooepidemicus*, *Streptococcus equi* subspecies *equi*, *Corynebacterium pseudotuberculosis* or *Actinobacillus equlli* (Stewart 2006).

Primary peritonitis in horses attributable to *Actinobacillus equuli* has been reported in Australia (Gay and Lording 1980; Golland *et al.* 1994; Matthews *et al.* 2001), New Zealand (Mogg and Dykgraaf 2006) and North America (Patterson-Kane *et al.* 2001; Stewart 2006; Tennent-Brown *et al.* 2010). Cases typically present with an acute onset of mild to moderate signs of abdominal pain, tachycardia, tachypnoea, fever, lethargy and inappetence (Gay and Lording 1980; Golland *et al.* 1994; Matthews *et al.* 2001, 2002; Mogg and Dykgraaf 2006; Watts *et al.* 2011).

Published reports of primary *Actinobacillus* peritonitis cases describe a rapid response to treatment with appropriate antimicrobial and supportive treatment (Gay and Lording 1980; Golland *et al.* 1994; Matthews *et al.* 2001; Mogg and Dykgraaf 2006; Watts *et al.* 2011) and an excellent prognosis for survival (Gay and Lording 1980; Golland *et al.* 1994; Matthews *et al.* 2001, 2002; Mogg and Dykgraaf 2006; Stewart 2006).

This case describes the diagnosis, treatment and atypical outcome of a 13-year-old Warmblood gelding that was referred to The Animal Hospital at Murdoch University for further investigation of peritonitis.

Clinical features

History

A 13-year-old Warmblood gelding presented to the referring veterinarian with acute onset of mild colic, lethargy and inappetence of less than 12 h duration. Abdominal ultrasound demonstrated a mild increase in free peritoneal fluid. Peritoneal fluid was a strawberry colour, with a total nucleated cell count (TNCC) of 19,400 cells/μL (predominately nondegenerate neutrophils [79%]) and a total protein (TP) of 26 g/L, consistent with a diagnosis of peritonitis. The gelding was referred for further investigation and treatment.

Initial clinical findings

On presentation, the gelding was tachycardic (heart rate: 80 beats/min), tachypnoeic (64 breaths/min) and febrile (rectal temperature: 39.4°C). Gingival mucous membranes were pink but tacky, with a normal capillary refill time. Intestinal sounds were present but reduced on auscultation. Abdominal ultrasound (**Fig 1**) and peritoneal fluid character (**Fig 2**) and cytology were consistent with that already reported. In addition, peritoneal fluid lactate was increased (7.3 mmol/L), glucose was 3.1 mmol/L, and pH of 7.203 and extracellular bacterial rods were observed. No abnormalities were detected on rectal palpation of the abdomen and no nasogastric reflux was present. Bloodwork performed demonstrated a leucopenia (WBC: 2.0×10^9 cells/L) and increased haematocrit (0.48 L/L). Venous lactate was within normal limits (1.7 mmol/L). A diagnosis of peritonitis was thus confirmed and peritoneal fluid was submitted for aerobic and anaerobic culture and sensitivity.

Treatment

As the gelding appeared relatively stable and was not actively colicking, medical treatment with broad-spectrum antimicrobials [benzyl penicillin (22,000 IU/kg bwt, i.v. q. 6 h), gentamicin (6.6 mg/kg bwt, i.v., s.i.d.) and metronidazole (25 mg/kg bwt, per os, b.i.d.)], anti-inflammatories (flunixin 1.1 mg/kg bwt i.v.) and intravenous fluid therapy (Hartmann's solution, approximately 100 mL/kg bwt/day) were instituted. Over the course of the next 24 h, the gelding remained tachycardic, although respiratory rate and temperature normalised. On repeat abdominal ultrasound the next day, there was no apparent change in the appearance or volume of free peritoneal fluid. Repeat rectal palpation was again considered within normal limits. Blood work confirmed that the gelding's hydration status was corrected and medical management was continued, although intravenous

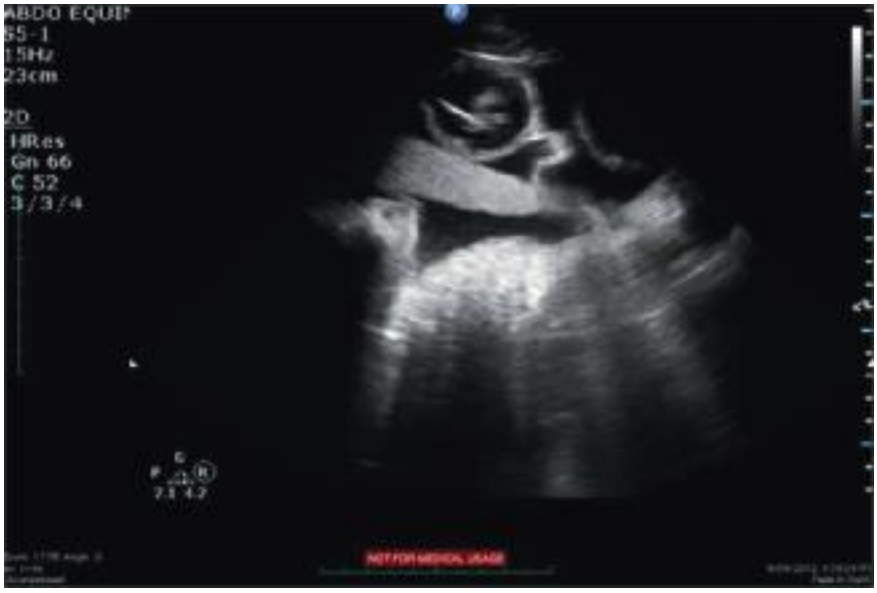

Fig 1: Transabdominal ultrasound image of left side of abdomen on initial presentation demonstrating an increase in free peritoneal fluid.

Fig 2: Gross appearance of peritoneal fluid obtained by abdominocentesis at presentation.

fluid therapy was stopped. Repeat abdominocentesis performed 48 h post admission demonstrated a decrease in TNCC (8.5×10^9 cells/L) but increase in TP (3.5 g/L). On microscopic examination, approximately 77% mildly degenerate neutrophils containing moderate numbers of bacilli were present. Peritoneal lactate had decreased to 3.6 mmol/L. Repeat haematology demonstrated a normal WBC count (9.9×10^9 cells/L) with a mild to moderate left shift (16% band neutrophils) and moderate toxic changes. Fibrinogen was within normal reference range (3 g/L) and serum biochemistry was unremarkable. Conservative therapy was thus continued.

Culture and sensitivity results on the sample collected at admission demonstrated the presence of *Actinobacillus* spp., sensitive to penicillin, gentamicin, ceftiofur, trimethoprim–sulphonamides (TMS) and tetracyclines. Antibiotic treatment was changed to TMS (30 mg/kg bwt, per os, b.i.d.) on Day 7 post-admission. The gelding was discharged from hospital 24 h later and a further 10 days of treatment with TMS and a larvicidal dose of an anthelmintic were recommended.

One month post initial presentation

One month after initial presentation, the gelding represented with similar signs of lethargy and inappetence. Ongoing mild colic signs were reportedly present since discharge from hospital. Despite a quiet demeanour, clinical examination was normal and routine bloodwork was unremarkable. Abdominal ultrasound revealed copious amounts of free peritoneal fluid. Seven litres of turbid, serosanguinous peritoneal fluid was drained from the abdomen. On cytology although TNCC had further decreased to 4.2×10^9 cells/L, an unusual profile of mostly lymphocytes with smaller numbers of nondegenerate neutrophils (11%), plasma cells and eosinophils was observed. No bacteria were seen on this occasion and bacterial culture was negative. Treatment with oral doxycycline (7.5 mg/kg bwt, per os, b.i.d.) was commenced and continued for 10 days given that the previously cultured *Actinobacillus* spp. was sensitive to tetracyclines. Once daily treatment with oral firocoxib (0.1 mg/kg bwt, per os, s.i.d.) was also instituted.

Clinical examination 5 days after discontinuation of doxycycline treatment was normal. Repeat abdominal ultrasound, however, revealed a persistence in the high volume of peritoneal fluid with a similar cell count (TNCC: 4.2×10^9 cells/L; TP: 31 g/L) and cytological profile. No bacteria were seen. These findings combined with an increased triglyceride concentration (3.5 mmol/L) were consistent with a chylous effusion (Meadows and MacWilliams 1994). Approximately 30 L of peritoneal fluid was drained. Blood work performed on this occasion was relatively unremarkable, albeit a mild anaemia (RBC:

6.81 × 10^{12} cells/L) and mild lymphopenia (1.33 × 10^{9} cells/L). Treatment with firocoxib was continued.

Two months post initial presentation

On repeat evaluation, 2 weeks later (approximately 2 months after initial presentation), the gelding appeared brighter and the volume of peritoneal fluid had decreased significantly although fluid cytology and triglyceride levels (5.6 mmol/L) remained consistent with a chylous effusion.

Three months post initial presentation

Approximately 1 month later, the gelding presented with acute onset of tachycardia, tachypnoea and pyrexia. A substantial accumulation of cellular peritoneal fluid was present on abdominal ultrasound (**Fig 3**). A decision was made for euthanasia due to a markedly increased peritoneal fluid TNCC (500 × 10^{9} cells/L) containing 88% degenerate neutrophils and intra- and extracellular Gram-negative rods. Peritoneal fluid collected ante-mortem yielded a moderate pure growth of *Actinobacillus* spp., identified as *Actinobacillus capsulatus* by PCR.[1] Searches for 16S rRNA sequences were performed with FASTA and BLAST.

Necropsy findings

Post-mortem findings were consistent with severe, subacute to chronic, generalised fibrinosuppurative peritonitis with peritoneal effusion and multifocal subserosal intestinal petechial and ecchymotic haemorrhage (**Fig 4**). There was no evidence of abdominal adhesions, vascular compromise or perforation of the gastrointestinal tract, urinary tract perforation or an abscess/neoplasia compromising intestinal integrity. A definitive cause of the chyloabdomen could not be located. A thorough search for nodular disease and/or dilated lymphatics, particularly in the region of the cistern chyli and within the root of the mesentery, was undertaken to no avail. The medial iliac lymph nodes and the root of the mesentery and adjacent connective tissues in the region of the cistern chyli were extremely oedematous. Histology of the root of the mesentery showed little change. Approximately 200 mL of turbid, pale orange fluid was also present in the pericardial sac, and this fluid also cultured *Actinobacillus* species, identified as *Actinobacillus capsulatus* by PCR.

Discussion

To our knowledge, this is the first clinical report of *Actinobacillus capsulatus* infection in the horse. *Actinobacillus capsulatus* is recognised as a primary pathogen of lagomorphs (Blackall *et al.* 1997; Meyerholz and Haynes 2005). The majority of previous reports of peritonitis due to *Actinobacillus* spp. have been accepted to have been due to *Actinobacillus equilli,* diagnosed either via positive culture (Gay and Lording 1980; Golland *et al.* 1994; Matthews *et al.* 2001, 2002; Tennent-Brown *et al.* 2010), or on the basis of characteristic clinical signs, abdominal fluid variables and response to treatment (Matthews *et al.* 2001, 2002).

The initial presentation of this horse, with signs of mild colic, lethargy, inappetence, tachycardia, tachypnoea and a fever, was consistent with the clinical presentation of horses in other reports of *Actinobacillus* peritonitis (Gay and Lording 1980; Golland *et al.* 1994; Matthews *et al.* 2001, 2002; Mogg and Dykgraaf 2006; Watts *et al.* 2011). The response to appropriate antimicrobials, however, was disappointing in this case, with persistence of clinical signs much longer than typically reported with primary *Actinobacillus* peritonitis (Golland *et al.* 1994; Matthews *et al.* 2001). Although prolonged treatment is sometimes required, reported survival from *A. equuli* peritonitis is excellent with appropriate antimicrobial treatment (Golland *et al.* 1994; Matthews *et al.* 2001; Watts *et al.* 2011), even in the case of an immunosuppressed filly (Tennent-Brown *et al.* 2010).

A major clinical difference between acute peritonitis associated with *A. equuli* compared to that secondary to intestinal catastrophes is the lack of clinical progression to

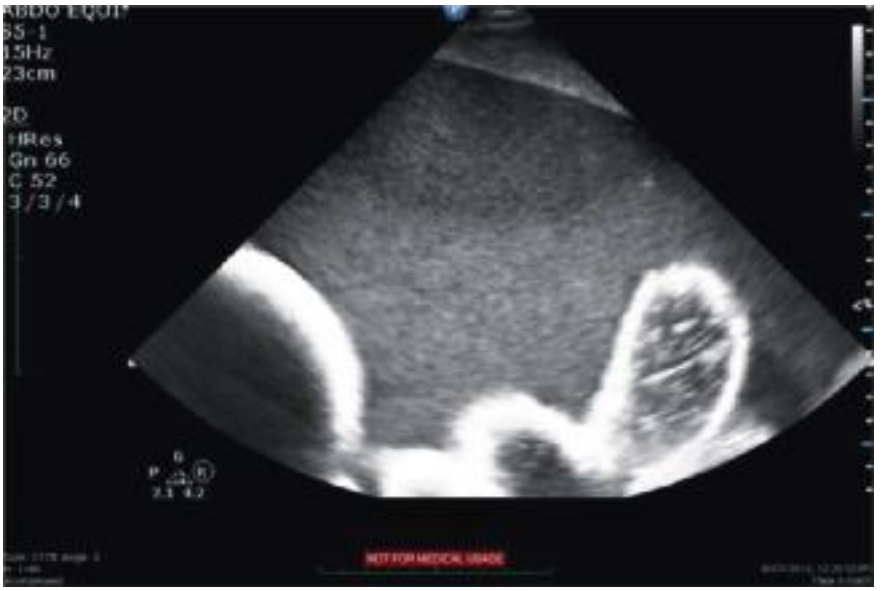

Fig 3: Transabdominal ultrasound image of left abdomen caudal to stomach at final presentation demonstrating a substantial accumulation of cellular peritoneal fluid.

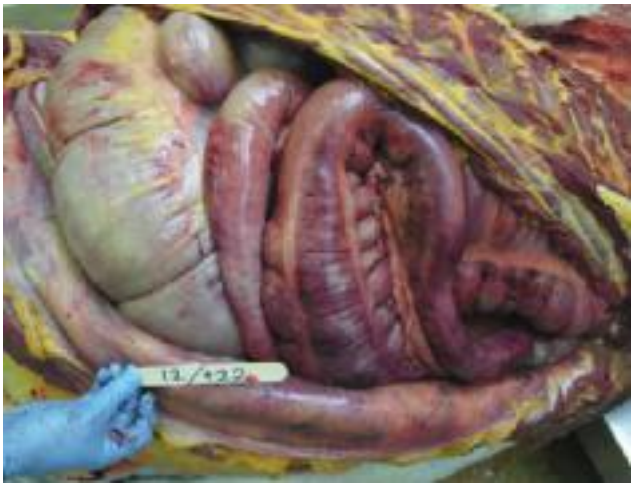

Fig 4: Post-mortem image of the right side of the abdomen with abdominal organs in situ demonstrating generalised fibrinosuppurative peritonitis.

SIRS and shock (Gay and Lording 1980). The cytological profile of peritoneal fluid of horses with *Actinobacillus equilli* peritonitis is characterised by the predominance of nondegenerate neutrophils (Gay and Lording 1980; Golland *et al.* 1994; Matthews *et al.* 2001; Mogg and Dykgraaf 2006; Stewart 2006; Tennent-Brown *et al.* 2010; Watts *et al.* 2011). This contrasts to the profile of peritoneal fluid in peritonitis cases due to an intestinal catastrophe, in which neutrophils demonstrate severe degenerative changes (Gay and Lording 1980), suggesting that *A. equuli* is of low toxicity. In our case, peritoneal fluid cytology at initial presentation was consistent with that of a classic *A. equilli* peritonitis, with a predominance of nondegenerate neutrophils. However, the peritoneal fluid findings of predominately degenerate neutrophils at final presentation were highly inconsistent to that of other reports of *A. equilli* peritonitis, despite yielding a pure growth of an *Actinobacillus spp*. The pertinent difference in our case was the identification of *Actinobacillus capsulatus* on PCR.

Historically, the classification of *Actinobacillus* species has been made on the basis of phenotypic or biochemical characteristics, which can be a difficult and unreliable task (Sternberg and Brandstrom 1999; MacInnes 2010). In an Australian study that re-examined previously diagnosed isolates of *A. equuli* using a more extensive range of phenotypic tests, 7 of 16 of the isolates were demonstrated to have been misclassified as *A. equuli*, one of which was re-classified as *Actinobacillus capsulatus* (Blackall *et al.* 1997). The family *Pasteurellaceae* and genus *Actinobacillus* have undergone a long series of taxonomic revisions (Naushad *et al.* 2015) and increasing emphasis has been directed to the division of bacteria into groups based on molecular methods (MacInnes 2010). Our case highlights the importance of such methods in the correct identification of species, as outlined by the identification of *A. capsulatus* in both peritoneal and pericardial fluid at post-mortem, as the prognosis of such cases in the future may be guarded. Unfortunately, we did not use molecular methods to further identify the species of *Actinobacillus* present at initial presentation and thus cannot definitively identify the initial species present.

This case was also complicated by the development of chyloabdomen, an uncommon cause of abdominal effusion in the horse (May and Good 2007; Cesar *et al.* 2010; Fish *et al.* 2015). Chyloabdomen develops when lipid-rich fluid from a mesenteric lymphatic vessel leaks into the abdominal cavity due to vessel rupture or increased intralacteal pressure due to obstruction. Differentials that could cause this include trauma, tears induced by intra-abdominal adhesions, intra-abdominal lymphadenopathy, primary or secondary lymphangiectasia, thoracic duct disease, cardiac disease, fungal disease, neoplasia or congenital anomalies of lymphatics (Nelson 2001; May and Good 2007; Cesar *et al.* 2010; Fish *et al.* 2015). The assumption in our case was that the gelding developed a primary peritonitis that may have led to adhesion formation within the abdominal cavity, which at some point may have damaged a lymphatic duct and ruptured into the abdomen, causing ongoing but improving chyloabdomen. The aetiology of chylous effusion in many cases can be difficult to determine and thus remain termed idiopathic (Meadows and MacWilliams 1994). Despite a thorough post-mortem examination, the definitive cause of chyloabdomen could not be found in our case.

Conclusion

To our knowledge, this is the first reported case of *Actinobacillus capsulatus* in the horse. The case highlights the importance of molecular methods in the correct identification of bacterial species. Failure of horses diagnosed with *Actinobacillus* peritonitis to respond rapidly to appropriate antimicrobial treatment should prompt further investigation and could result in a guarded to poor prognosis.

Authors' declaration of interests

No conflicts of interest have been declared.

Ethical animal research

Ethical review not applicable for this retrospective case report.

Source of funding

None.

Acknowledgements

Special thanks to Drs Aswin Beck and Eleanor Crispe for their contribution to veterinary care in this case.

Authorship

A. Cullimore managed the clinical case and prepared the manuscript. N. Stephens performed the post-mortem and contributed to the description of necroscopy findings. G. Lester managed the clinical case and edited the manuscript. All authors approved the final manuscript.

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EQUINE VETERINARY EDUCATION
Equine vet. Educ. (2019) **31** (8) 403-409
doi: 10.1111/eve.12815

Case Report

Primary corneal malignant melanoma in a horse

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Keywords: horse; malignant melanoma; superficial keratectomy; strontium-90 beta irradiation; mitomycin C

Summary

A 13-year-old Missouri Fox Trotter gelding of chestnut colour was referred for a 6-week history of blepharospasm and epiphora of the left eye. Due to the presence of irregular corneal masses, superficial keratectomy was performed along with adjunctive strontium-90 beta irradiation and subsequent topical mitomycin C chemotherapy. A diagnosis of poorly melanised malignant melanoma was made based on histopathological examination and immunohistochemistry. There has been no recurrence of the neoplasm over 10 months of follow-up. To the authors' knowledge, this is the first documented case of a primary corneal melanocytic neoplasm in a horse.

Introduction

Melanocytic tumours, which can be malignant or benign, are neoplasms originating from melanocytes. These tumours have been described in many domestic species, including horses. The most frequently diagnosed equine melanocytic tumours are those affecting the skin in older grey horses (Valentine 1995; Giuliano 2010; Phillips and Lembcke 2013). In contrast, equine ocular melanocytic neoplasms are rare, but have been described in adnexal, orbital, epibulbar and intraocular tissues (Ramadan 1975; Murphy and Young 1979; Moore *et al.* 2000; McMullen *et al.* 2008). In horses, neoplastic extension into the cornea has been described from conjunctival malignant melanoma (Moore *et al.* 2000) and epibulbar malignant melanoma (McMullen *et al.* 2008). To the authors' knowledge, there are no confirmed equine cases of primary corneal melanocytic tumours. One case report in 1975 described a primary ocular melanoma in a horse with possible corneal origin; however, due to the advanced state of the tumour infiltrating most of the globe, the origin could not be confirmed (Ramadan 1975).

Primary melanocytic neoplasms of the cornea are rare in all species, but have been reported in human patients (Naseri *et al.* 2001; Romaniuk *et al.* 2002; Uçakhan-Gündüz *et al.* 2012) and one dog (Bauer *et al.* 2015), and can be either pigmented or amelanotic with variable clinical appearance. These tumours are often treated with surgical excision by superficial keratectomy; adjunctive treatment, such as topical chemotherapy, can be administered to help decrease recurrence (Naseri *et al.* 2001; Uçakhan-Gündüz *et al.* 2012). Metastasis is rare, but recurrence and local invasion requiring enucleation is reported (Uçakhan-Gündüz *et al.* 2012).

The equine cornea can be affected with other neoplasms that have the potential for local invasion and metastasis, with the most common being squamous cell carcinoma (SCC) (Lavach and Severin 1977). Corneal SCC typically appears as raised, white-pink, fleshy lesions or as nonraised lesions infiltrating the corneal stroma (Clode 2011). Definitive diagnosis is made via histopathological evaluation of biopsy specimens; characteristic features of SCC include sheets, cords and whorls of malignantly transformed epithelial cells, intercellular bridges, individual cell keratinisation and keratin pearl formation (Grahn *et al.* 2013). Other corneal tumours that have been reported in horses include vascular tumours, mast cell tumours and lymphosarcoma (Clode 2011). Surgical excision of corneal tumours with an adjunctive therapeutic modality tends to be the most effective treatment to eliminate the tumour and prevent recurrence (King *et al.* 1991; Clode 2011). Adjunctive therapies that have been reported for equine ocular neoplasia include cryosurgery, topical chemotherapy, radiofrequency hyperthermia, carbon dioxide laser ablation and beta irradiation (English *et al.*1990; King *et al.* 1991; Schoster 1992; Rayner and Van Zyl 2006; Plummer *et al.* 2007; Malalana *et al.* 2010; Clode *et al*. 2012).

In this report, we describe a case of a primary corneal malignant melanoma in a horse that was diagnosed based on histopathology and immunohistochemistry, and was treated with surgical excision, adjunctive strontium-90 beta irradiation, and subsequent mitomycin C topical chemotherapy.

Case presentation

History and initial examination

A 13-year-old Missouri Fox Trotter gelding of chestnut colour was referred to the Iowa State University Lloyd Veterinary Medical Center (ISU-LVMC) for a 6-week history of blepharospasm and epiphora of the left eye (OS). The horse was initially treated by the referring veterinarian for suspected corneal scar tissue with a topical steroid, and a few weeks later was treated for a superficial corneal ulcer with a topical antibiotic. The corneal ulcer healed with treatment, but blepharospasm and epiphora persisted.

A complete eye examination including rebound tonometry (TonoVet®)[1], slit-lamp biomicroscopy and indirect ophthalmoscopy was performed. On initial examination, the horse had mild blepharospasm and epiphora OS. Intraocular pressures were 19 and 26 mmHg in the right eye (OD) and OS, respectively. No fluorescein stain uptake was noted on either eye. Ophthalmic examination OD was unremarkable. Slit-lamp biomicroscopy OS revealed two adjacent irregular pale pink corneal masses rising minimally from the dorsal corneal surface (**Fig 1**). The masses were approximately 4 mm and 5 mm in diameter. Superficial corneal

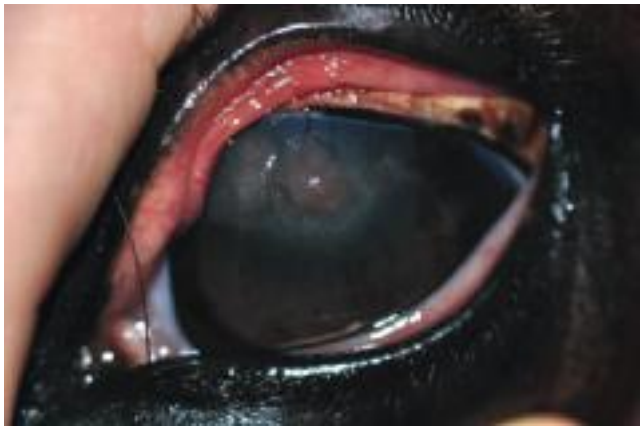

Fig 1: Photograph of the affected cornea at initial presentation. There are two irregular pale pink corneal masses rising minimally from the dorsal corneal surface. There are pinpoint areas of faint pigmentation present throughout the entire cornea.

vascularisation was present extending from the limbus with multifocal regions of anterior stromal haze and pinpoint areas of faint epithelial pigmentation. The remainder of the ophthalmic examination OS was within normal limits.

Differential diagnoses for the corneal lesions included neoplasia (squamous cell carcinoma, vascular tumour, mast cell tumour, lymphosarcoma, melanocytic tumour), granulation tissue from a previous injury, infectious keratitis and eosinophilic or other immune-mediated keratitis. Under standing sedation, topical anaesthetic (proparacaine 0.5% ophthalmic solution) was applied to the left cornea, and a shave biopsy of one of the corneal masses was performed using a microsurgical blade (No. 6400 Beaver Mini-Blade). Cytology of impression smears was highly cellular, containing binucleate and multinucleated cells with moderate-to-marked anisokaryosis, anisocytosis and anisonucleolosis. Neoplasia, and particularly squamous cell carcinoma, was suspected due to the numerous criteria for malignancy and lack of inflammatory cells present. Histopathology of the shave biopsy showed focal corneal fibroplasia with no neoplastic cells present. Excisional biopsy with a superficial keratectomy was recommended to obtain a definitive diagnosis. High-frequency ocular ultrasound to determine the depth of the lesions prior to surgery was considered, but was deemed unnecessary. The lesions appeared superficial on examination, and any visible pathological areas would be excised with surgery and followed by adjunctive therapy.

Surgical procedure

One week following initial examination, the horse was anaesthetised and placed in right lateral recumbency. A superficial keratectomy, approximately 20 mm by 15 mm in size and 50% corneal depth, was performed to completely encompass both corneal masses and the surrounding areas of stromal opacity on the dorsal cornea. A second superficial keratectomy, approximately 8 mm in diameter, was performed adjacent to the lateral limbus where stromal haze and corneal surface irregularity were visible upon microscopic examination. This abnormal region was not appreciated on initial examination.

Following the keratectomies, strontium-90 beta irradiation was applied in three separate locations to the dorsal and lateral cornea where the keratectomies were performed as an adjunctive therapy to help prevent recurrence of neoplasia. A radiation dose of 100 Gy was applied to each site.

Two grafts of porcine urinary bladder extracellular matrix (ACell Vet® Corneal Discs)[2] were trimmed to size and sutured over the keratectomy sites using 8-0 polyglactin suture in a simple interrupted pattern. A ventral subpalpebral lavage line was placed for administration of post-operative topical medications. A lateral temporary tarsorrhaphy was placed for protection of the surgical site during initial healing. The horse recovered well from anaesthesia and was discharged the following day. Topical medications OS included neomycin/polymixin B/gramicidin ophthalmic solution six times per day, serum six times per day and atropine 1% ophthalmic solution once daily. The horse was also treated orally with flunixin meglumine at a dose of 1.1 mg/kg twice daily, and sulfamethoxazole and trimethoprim at a dose of 25 mg/kg twice daily.

Histopathology

Histopathological examination of the dorsal corneal biopsy revealed aggregates and clusters of large, round-to-oval neoplastic cells within the surface epithelium, along the stratum basale and within the stroma (**Fig 2**). The cells had large, round-to-oval nuclei, 1-2 nucleoli, an abundant amount of lightly basophilic cytoplasm and distinct cell borders. A few cells contained occasional brown granules interpreted to be melanin (Perl's stain for iron was negative, ruling out the possibility that the pigment was haemosiderin). There was moderate-to-marked anisocytosis and anisokaryosis, and a high mitotic index (25 per 10 – 400 × fields). Additionally, marked infiltrate of lymphocytes and plasma cells, occasional eosinophils, and fibrosis and neovascularisation were observed within the adjacent stroma. Surgical clearance of neoplastic cells appeared complete along the deep and outer margins. The lateral corneal biopsy did not contain neoplastic cells, but had a substantial number of melanophages and other inflammatory cells along the epithelial–stromal interface. Based on these findings, consideration was given to a poorly melanised malignant melanoma or a poorly differentiated squamous cell carcinoma.

Immunohistochemistry (IHC) was performed for four markers: monoclonal mouse anti-vimentin, clone V9; polyclonal rabbit anti-S100 protein; monoclonal mouse anti-human melan-A, clone A103; and monoclonal mouse anti-human cytokeratin, clone MNF116[3]. These markers were chosen to try to differentiate squamous cell carcinoma, a tumour of epithelial origin that would likely express cytokeratin, from a malignant melanoma, a tumour of neuroectodermal origin that would likely express vimentin, S100 and potentially melan-A (Desnoyers *et al.* 1990; Ramos-Vara *et al.* 2000, 2014; de Wit *et al.* 2004). Neoplastic cells had strong cytoplasmic immunoreactivity for vimentin and S100, negative staining for melan-A and weak cytoplasmic immunoreactivity for cytokeratin (**Fig 3**). Poorly melanised malignant melanoma with chronic lymphoplasmacytic keratitis was diagnosed based on the results of IHC, the pattern of neoplastic cells within the cornea, and the presence of a modest amount of brown granular pigment within a few neoplastic cells.

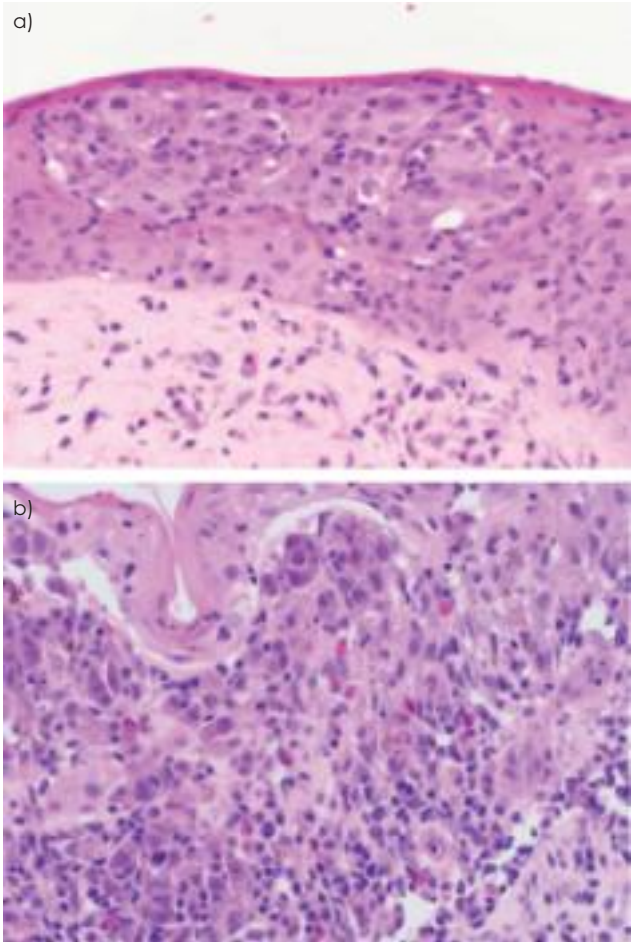

Fig 2: Photomicrographs of the affected cornea. Haematoxylin and eosin staining; 400 × magnification. a) There are neoplastic cells within the epithelium containing occasional brown granules. b) There are clusters of neoplastic cells within the stroma, as well as neovascularisation and infiltrates of plasma cells, lymphocytes and eosinophils.

Follow-up

Initial recheck 5 days post-operatively showed partially epithelialised keratectomy sites with grafts in place. At evaluation 19 days post-operatively, the left cornea was completely epithelialised with negative fluorescein staining; corneal sutures were still present and there were fine blood vessels and mild fibrosis present throughout the keratectomy sites. Treatment with topical mitomycin C 0.04% solution[4] was started on the left eye 25 days following surgery as an adjunctive therapy to help prevent recurrence of neoplasia. The mitomycin C (MMC) was administered four times daily for 7 days, and discontinued for the following 7 days. This 2-week course was repeated for four cycles.

Three weeks after starting MMC therapy, two slightly elevated, 2–3 mm diameter, white lesions were noted on the dorsal left cornea at the keratectomy site. Nonelevated fibrotic tissue with fine blood vessels was also present. The elevated white lesions were thought to be granulation tissue during the healing process, or possibly regrowth of neoplasia. Biopsy was considered, but it was elected to first try treatment with topical neomycin/polymixin B/dexamethasone (NPD) ophthalmic solution three to four times daily. The elevated lesions were resolved by the recheck 4 weeks later (**Fig 4**), consistent with granulation tissue and not regrowth of neoplasia, and the NPD ophthalmic solution was discontinued.

No obvious side effects were noted during the four cycles of MMC treatment. A few days after treatment was completed, the horse began developing depigmentation of the medial upper and lower left eyelids, intermittent epiphora and periocular crusting. The epiphora and periocular crusting resolved quickly with short courses of topical NPD, but recurred several times over the course of 1 month. Depigmentation of the eyelids was progressive during this time.

At the last recheck evaluation, 4 months following surgery, there was no recurrence of corneal neoplasia. Mild fibrosis was present on the dorsal corneal surface, yet no raised lesions were present. The medial periocular skin was depigmented, but no erythema, swelling, or discharge was present. The operated eye was comfortable and visual. Due to distance and travel logistics, the owner elected not to return to ISU-LVMC for additional rechecks. Follow-up phone calls and photographs from the owner 10 months after surgery revealed significant improvement of periocular depigmentation and no evidence of corneal neoplasia recurrence.

Discussion

The corneal masses in our case were originally suspected to be SCC based on appearance, location and initial cytology. However, histopathology of the corneal biopsies did not identify any characteristic features of SCC, such as intercellular bridges, individual cell keratinisation or keratin pearl formation (Grahn *et al.* 2013). Biopsies revealed oval-to-round neoplastic cells with rare cytoplasmic brown granules, and consideration was given to a poorly melanised malignant melanoma. However, a poorly differentiated SCC could not be ruled out based solely on histopathology.

Variable histological and cytological patterns of melanocytic neoplasms can make definitive diagnosis difficult. In veterinary and human medicine, IHC is commonly used to support a diagnosis of melanocytic neoplasia (Ramos-Vara *et al.* 2014). Normal melanocytes are dendritic cells derived from neuroectodermal melanoblasts, so markers of neuroectodermal tissues can assist in diagnosis and differentiation from other types of tumours, such as carcinomas (Koenig *et al.* 2001). Several IHC markers were used in our case, including vimentin, S100, melan-A and cytokeratin.

Neoplastic cells within the cornea had strong immunoreactivity to both vimentin and S100 antibodies. Vimentin is an intermediate filament expressed by mesenchymal and neuroectodermal cells, which has shown high sensitivity but low specificity as a marker for melanocytic neoplasia (Ramos-Vara *et al.* 2000; Ohsie *et al.* 2008). Vimentin consistently stains human melanocytic neoplasms (Nakhleh *et al.* 1990), and expression has been reported in 100% of canine oral melanocytic neoplasms (Ramos-Vara *et al.* 2000). The S100 marker is a calcium-binding protein that is another sensitive but nonspecific marker used for detection of human and animal melanocytic neoplasia (de Wit *et al.*

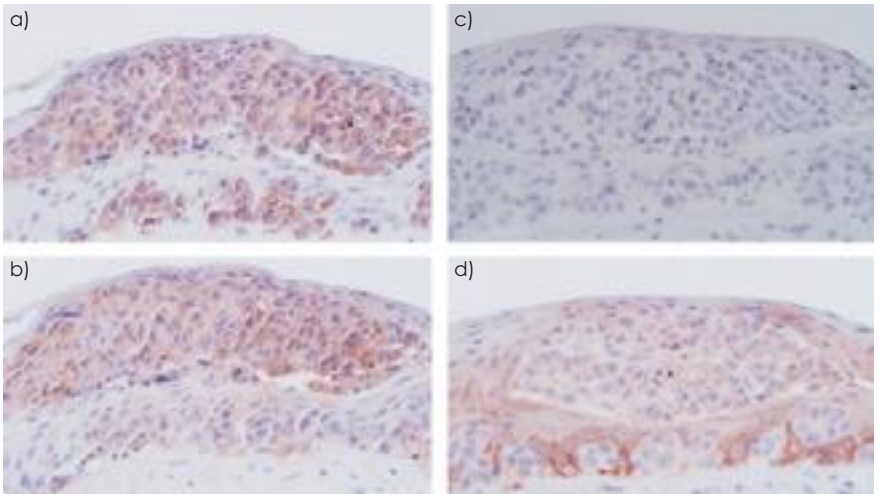

Fig 3: Immunohistochemical staining of neoplastic cells; 400 × magnification. a) Strong cytoplasmic immunoreactivity of neoplastic cells for vimentin. b) Strong cytoplasmic immunoreactivity of neoplastic cells for S-100. c) Negative staining of neoplastic cells for melan-A. d) Weak immunoreactivity of neoplastic cells for cytokeratin, compared to strong immunoreactivity of surrounding normal epithelial cells for cytokeratin.

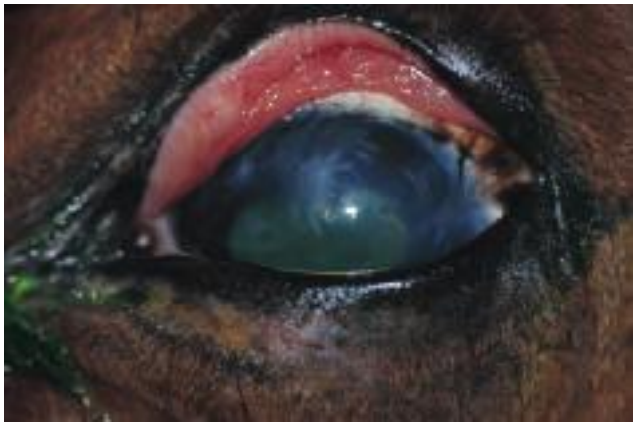

Fig 4: At recheck 11 weeks after surgery and 1 week after finishing mitomycin C therapy, there is mild fibrosis of the dorsal and lateral cornea. The medial periocular skin shows early depigmentation.

2004). Various studies have shown positive S100 immunoreactivity in 87.5% of feline melanocytic neoplasms (Ramos-Vara *et al.* 2002), 76% of canine oral melanocytic neoplasms (Ramos-Vara *et al.* 2000) and 83% of canine amelanotic melanocytic neoplasms (Sandusky *et al.* 1985). Tumours of neuronal and neuroectodermal origin, such as neurofibromas, schwannomas and malignant peripheral nerve sheath tumours, will also stain positive for S100 (de Wit *et al.* 2004). Carcinomas will generally not express vimentin or S100 (Desnoyers *et al.* 1990).

Neoplastic cells were negative for melan-A, a cytoplasmic protein of melanosomal differentiation. While melan-A is generally considered a highly sensitive and specific marker for human and canine pigmented melanocytic neoplasms (Choi and Kusewitt 2003; Smedley *et al.* 2011), it appears to be a less sensitive marker of melanocytic neoplasia in horses. In one study, the monoclonal antibody to melan-A did not react with any of the equine melanocytic tumours evaluated, nor in normal melanocytes of equine skin (Ramos-Vara *et al.* 2014). The neoplastic cells in our biopsy were negative for melan-A, as were the melanocytes at the periphery of the specimen, which served as an internal control.

The last IHC marker evaluated in our case was cytokeratin. Keratins are intermediate filaments found in epithelial cells, and IHC for cytokeratin is commonly used to identify carcinomas (Desnoyers *et al.* 1990). Neoplastic cells in our case had weak cytoplasmic immunoreactivity for cytokeratin, while surrounding normal epithelial cells had strong immunoreactivity. This difference in staining intensity between the neoplastic cells and epithelial cells suggests that the neoplasm was not epithelial in origin, and the weakly positive cytokeratin immunoreactivity could simply be nonspecific staining. Additionally, anomalous expression of keratins and other intermediate filaments is a well-reported phenomenon in some human melanocytic neoplasms (Romano *et al.* 2015), which may also explain the cytokeratin expression. Based on the neoplastic cell distribution and other IHC markers in this case, melanocytic neoplasia was considered the most appropriate diagnosis.

Melanocytes are not normally found in the cornea, thus primary corneal melanocytic neoplasia is an infrequent diagnosis. The lack of melanocytic tumours in other areas of the body in our case (prior or following surgery) makes the possibility that the corneal malignant melanoma was a metastasis from a primary neoplasia less likely, although complete staging was not performed. In humans, corneal melanocytic neoplasms are most commonly a result of contiguous spread from a conjunctival melanocytic neoplasm, but there are rare reports of pigmented and nonpigmented melanocytic tumours isolated to the cornea (Uçakhan-Gündüz *et al.* 2012). In veterinary medicine, corneal melanocytic neoplasms are more commonly seen as extensions from limbal tumours, and this is most frequently diagnosed in dogs (Donaldson, Sansom, Scase, Adams and Mellersh 2006). There is only one report of a dog with a primary corneal melanocytoma, which was completely

excised with a superficial keratectomy and no recurrence was observed for 12 months of follow-up (Bauer *et al.* 2015).

In our case report, chronic keratitis with lymphocytes, plasma cells and eosinophils was diagnosed in addition to the corneal neoplasia, and multifocal pinpoint areas of pigmentation were seen on clinical examination. The marked presence of these inflammatory cells could be a response to the neoplasia or could represent a pre-existing underlying disease process such as immune-mediated keratitis (IMMK). Immune-mediated keratitis is a group of diseases characterised as primary, nonulcerative, noninfectious, chronic corneal inflammation (Matthews and Gilger 2009). In the United States, five types of IMMK have been described in horses based on the depth of the corneal lesion and the type of infiltrate present (epithelial, superficial stromal, midstromal, endothelial, and eosinophilic) (Matthews and Gilger 2009). Clinical signs of IMMK can be unilateral or bilateral and include corneal vascularisation, cellular infiltrate and corneal oedema, with only mild signs of ocular discomfort. In all types except for eosinophilic keratitis, the cellular infiltrate consists primarily of lymphocytes and plasma cells, with variable amounts of macrophages and neutrophils (Gilger *et al.* 2005; Pate *et al.* 2012). With eosinophilic keratitis, cellular infiltrate also contains eosinophils (Yamagata *et al.* 1996; Lassaline-Utter *et al.* 2014), and corneal lesions often appear as white plaques (Brooks 2004). In our case, the presence of eosinophils on histopathology is suggestive of underlying eosinophilic keratitis. Immune-mediated keratitis can be treated with topical anti-inflammatories, although superficial keratectomy to remove the lesion often yields the best response (Yamagata *et al.* 1996; Brooks 2004; Gilger *et al.* 2005). This could help explain why inflammatory lesions did not recur in our case following superficial keratectomy to remove the neoplastic lesions.

If there was an underlying chronic inflammatory disease present, it is possible this led to migration of melanocytes into the superficial cornea as the original source of neoplastic melanocytes. In human patients, chronic inflammation has been shown to predispose an individual to neoplasia, as inflammatory mediators can induce proneoplastic mutations, resistance to apoptosis, and other changes (Shacter and Weitzman 2002). In veterinary medicine, chronic inflammatory conditions affecting the cornea and topical immunosuppressive therapy have been suggested as risk factors for corneal SCC in dogs (Dreyfus *et al.* 2011), and a recent case report discussed a horse that developed suspected neoplastic transformation of immune-mediated keratitis to primary corneal lymphoma (Vallone *et al.* 2016).

This case was treated effectively with a multimodal approach, although the necessity of using all treatment modalities is unknown. Surgical resection was performed to obtain a definitive diagnosis, and surgical clearance appeared to be complete along the outer and deep margins. Beta irradiation using strontium-90 was performed at the surgical sites intraoperatively after resection. This is a noninvasive treatment using a handheld applicator that can apply radiation safely to the ocular surface with minimal tissue penetration (Kirwan *et al.* 2003). This modality was chosen because at the time of surgery, corneal SCC was suspected and beta irradiation has shown to be effective at reducing recurrence of ocular SCC in horses after keratectomy (Walker *et al.* 1986; Plummer *et al.* 2007). This modality has also shown some benefit with melanocytic tumours, so may have been beneficial in our case. A study of canine limbal melanocytic neoplasms treated with surgical resection and adjunctive strontium-90 beta radiotherapy showed good efficacy with a recurrence rate of only one in thirty cases (Donaldson, Sansom and Adams 2006). Strontium-90 beta radiotherapy has also shown good efficacy as an adjunctive therapy for conjunctival melanocytic tumours in humans (Cohen *et al.* 2013).

Topical MMC therapy was also used to help prevent recurrence of neoplastic tissue growth. Mitomycin C is an antineoplastic antibiotic that is used as a chemotherapeutic agent in both human and veterinary medicine. In equine ophthalmology, topical MMC therapy has been reported most commonly for treatment of ocular SCC, and it has shown favourable clinical results when used alone, or as an adjunctive therapy to surgical excision either intraoperatively or post-operatively (Rayner and Van Zyl 2006; Malalana *et al.* 2010; Clode *et al*. 2012). Mitomycin C has also been shown as an effective therapy in people with extensive conjunctival-corneal squamous cell carcinoma (Shields *et al.* 2002), and is reported in people as adjunctive therapy for conjunctival and corneal melanocytic tumours (Kurli and Finger 2005; Russell *et al.* 2010). There are a variety of protocols reported in human and veterinary medicine for administration of MMC. The week-on/week-off regimen has been used relatively safely in horses for up to four cycles, which was the protocol used in our case (Malalana *et al.* 2010; Clode *et al*. 2012). It is thought that this regimen helps reduce ocular toxicity, as it allows time for the slowly dividing epithelial cells and limbal stem cells to repair DNA (Chen *et al.* 2004). Mitomycin C also has antifibrotic effects, as it has been shown to inhibit cell migration and extracellular matrix production (Abraham *et al.* 2006). It is used in human patients to decrease corneal haze following refractive laser eye surgeries (Teus *et al.* 2009), and has shown promise at decreasing corneal scarring in horses and dogs in vitro (Buss *et al.* 2010; Gupta *et al.* 2011). It may have helped decrease scarring in our case post-keratectomy.

Minimal side effects were seen from the ocular treatments in this case. The keratectomy site healed uneventfully within 19 days of surgery and radiation therapy. Raised lesions on the corneal surface were observed after corneal epithelialisation; however, these lesions were presumed to be granulation tissue rather than neoplastic masses based on appearance and the resolution with topical steroid/antibiotic solution. Within a few days of finishing the MMC cycles, the horse developed mild blepharitis and periocular depigmentation, which was presumed to be associated with the MMC therapy. Side effects such as transient conjunctivitis and periocular reactions including ulcerative and nonulcerative blepharitis have been reported in horses secondary to MMC treatment (Malalana *et al.* 2010; Clode *et al*. 2012). More significant MMC complications related to corneal epithelial and stromal defects (e.g. stromal ulcer, descemetocele, bullous keratopathy), which have previously been documented with MMC therapy in horses, were not observed in this case (Clode *et al*. 2012). In this case, MMC therapy was not started until after the keratectomy site was epithelialised, which has been shown to reduce the occurrence of major complications (Clode *et al*. 2012). The timing of complications in our case was unusual, as it did not peak until after the completion of therapy. However, persistent side effects from MMC have been reported in people despite discontinuation of therapy (Kurli and Finger

2005; Russell *et al.* 2010). Fortunately, side effects were mostly controlled in our case with topical steroid/antibiotic solution.

The present case report describes the diagnosis and surgical outcome of a horse with a primary corneal malignant melanoma. This diagnosis should be considered in cases of equine corneal tumours, whether pigmented or not, and special IHC stains may be required to support the diagnosis. In this case, multimodal therapy consisting of surgical excision, strontium-90 beta irradiation, and topical chemotherapy with MMC was performed. This approach has been an effective treatment with minimal side effects and no sign of recurrence after 10 months.

Authors' declaration of interests

No conflicts of interest have been declared.

Ethical animal research

This is a retrospective case report so there were no ethical considerations.

Source of funding

None.

Authorship

All authors contributed equally.

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EQUINE VETERINARY EDUCATION
Equine vet. Educ. (2019) **31** (8) 410-412
doi: 10.1111/eve.12847

Clinical Commentary

Melanocytic ocular and periocular tumours of the horse

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Keywords: horse; melanoma; iris; eyelid; melanocytoma; cancer

Introduction

Reports of ocular melanocytic neoplasms are rare in horses. However, many ocular melanocytic tumours are likely unreported since these lesions often progress slowly or are benign in nature. Therefore, there are limited case reports in the literature, and the biologic behaviour of tissue-specific melanoma as well as the relative effectiveness of specific therapies is not well established. Strauss *et al.* (2019) present the first published case of a primary corneal melanoma in this issue, reminding us of the need for larger, multi-institutional studies to characterise these tumours in the horse.

Ocular melanocytic neoplasms comprise about 9% of all equine ocular neoplasms and have been described in adnexal, epibulbar, intraocular and orbital tissues (Dugan 1992). The most commonly reported ocular melanocytic neoplasms in horses involve the periocular skin (**Figs 1** and **2**). They are more frequently found in older grey or white-coated horses (Fleury *et al.* 2000). Ultraviolet light exposure, chemical exposure, trauma, chronic irritation, concurrent dermal melanomas and genetics have been considered in the pathogenesis of these tumours in humans and animals. No predisposing factors have been identified for the development of corneal and scleral melanocytic proliferation or neoplasia (Gilger 2016a). Intraocular melanocytic neoplasms occur less commonly than periocular tumours and are also more frequently reported in older grey horses. In a retrospective study of 52 horses with intraocular melanocytic neoplasms, 83% had grey coat colour and 67% had concurrent cutaneous melanoma at the time of diagnosis (LaBelle *et al.* 2012).

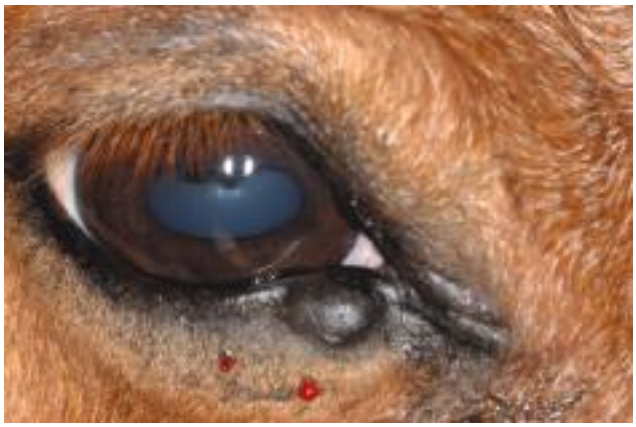

Fig 1: Eyelid melanoma in the medial canthus of a horse. This sessile mass was treated locally with injectable cisplatin resulting in clinical resolution of the neoplasia.

Clinical examination

Periocular melanocytic neoplasms are typically pigmented, partially alopecic, and slowly progressive in nature (Giuliano 2010). Although some masses and adjacent tissues may be heavily pigmented, the tissue may be nonpigmented as in the corneal tumour identified by Strauss *et al.* (2019). For this reason, biopsy is indicated for definitive diagnosis in all cases. Most neoplasms can be identified with direct illumination, but slit lamp biomicroscopy and ocular ultrasound are valuable for determining extent of tissue involvement. In particular, ocular ultrasound or other methods of advanced imaging are helpful in determining the magnitude of tissue involvement; especially with iridociliary tumours. Horses should also be examined for concurrent nonocular cutaneous melanocytic tumours.

Diagnostics

Exfoliative cytology may aid in the diagnosis of eyelid, conjunctival, third eyelid, corneal and scleral tumours, while tissue histopathology remains the gold standard (Dugan 1992; Wang and Kern 2015). Fine needle aspiration of uveal masses is usually nondiagnostic due to limited sample cellularity and may be contraindicated as the uvea is likely to bleed resulting in substantial intraocular sequelae (Singh and Biscotti 2012; Wang and Kern 2015). Unfortunately, biopsy of intraocular tissues is extremely difficult, and for the general practitioner, enucleation is the only method of direct biopsy

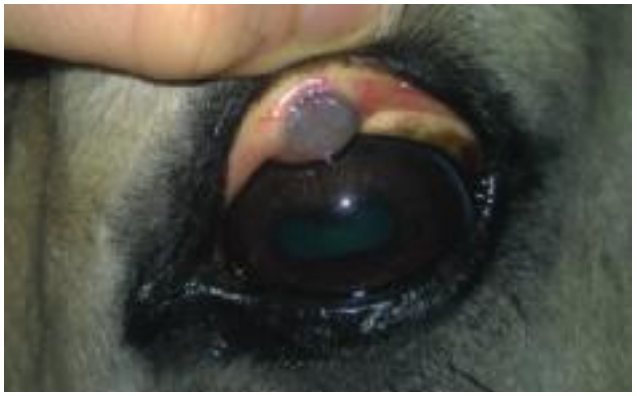

Fig 2: Eyelid melanoma only visible by flipping the lid to examine the conjunctival surface. This neoplasia was not involving the overlying conjunctiva and resolved with local excision rather than full thickness wedge excision. The area was treated with cryotherapy as well and there is no recurrence 5 years post-operatively.

for intraocular tumours. Those who wish to preserve the eye and confirm diagnosis should be referred to an ophthalmologist for diagnostics and potential sector iridectomy or other therapies. Histopathological evaluation with special staining helps confirm diagnosis, but does not consistently define malignant potential in horses. Histological appearance of equine melanocytic tumours is highly variable and should not be used in isolation to predict their clinical behaviour. This adds to the confusion of how to treat these lesions and what to tell clients about prognosis.

Clinical behaviour and treatment

Treatment for periocular melanoma frequently involves surgical excision with or without adjunctive therapy such as cryotherapy, brachytherapy or photodynamic therapy (Giuliano *et al.* 2005). Surgical excision is generally curative for eyelid melanomas in horses, and there are few reports of specific treatments for equine adnexal melanomas; therefore, the success rates of various treatments are unknown. Oral cimetidine has been used to shrink nonocular dermal melanomas in horses, but there have been no published reports on the use of oral cimetidine for the treatment of adnexal melanomas (Goetz *et al.* 1990). There are no known studies on the efficacy of intralesional chemotherapy or immunotherapy for equine adnexal melanomas although the author has had success with intralesional cisplatin.

Epibulbar or limbal melanomas are rarely reported in the horse. Limbal melanomas may be pigmented, fan-shaped, or nodular, and most commonly occur on the dorsal limbus (**Fig 3**). These are predominantly benign growths with the potential for local infiltration (Wang and Kern 2015). Most limbal melanomas can be treated with surgical excision and cryotherapy. A malignant epibulbar melanoma was described in a 6-month-old Hanoverian cross gelding with a rapidly growing pigmented epibulbar mass (McMullen *et al.* 2008). The lesion continued to grow rapidly despite debulking for histopathological evaluation, so enucleation was performed. No recurrence was reported 14 months post-operatively. In contrast to epibulbar melanomas, conjunctival melanomas in humans and domestic animals appear to have a more aggressive clinical nature. Conjunctival melanomas may be variably pigmented and have the potential for metastasis. Unfortunately, there have been few reports of conjunctival melanomas in horses, but uncomplicated cases seem to respond well to surgical excision and cryotherapy (Moore *et al.* 2000; Wang and Kern 2015). Recurrence and malignancy has been reported, such as in the case of a 16-year-old grey Arabian mare with multiple recurrences of a conjunctival melanoma following two surgical excisions and one treatment with cryotherapy. Exenteration was elected and no recurrence was reported 5 years post-operatively (Moore *et al.* 2000).

Melanomas of the anterior uvea are also rare. They are typically nodular, pigmented masses that are benign in nature, but with the potential for rapid local growth. Nodular intraocular neoplasms may cause secondary keratitis, uveitis, cataract and glaucoma. Distortion of the pupil may occur and focal corneal oedema is commonly seen if the mass contacts the cornea (**Fig 4**). Tumours in horses with lightly pigmented irises may appear pink and fleshy. In the case of rapidly growing tumours, or those involving ciliary body, sector iridectomy, enucleation, or exenteration should be considered. There has been high success with these procedures and metastasis has not been reported (Gilger 2016b). Small, uncomplicated tumours may be monitored. Local laser therapy may also be considered to shrink melanocytic neoplasms; however, uveitis and corneal oedema are common adverse effects (Gilger 2016b).

Collectively, there are few reports of ocular melanomas in horses and the effectiveness of specific therapies, so prognosis is difficult to determine. There is a need for a

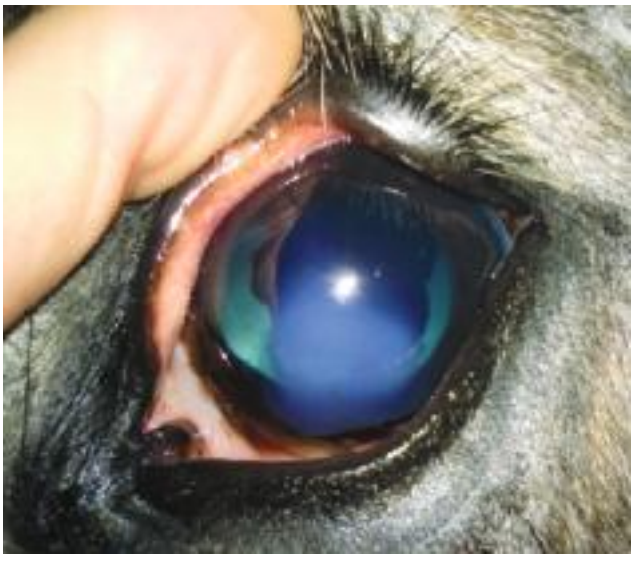

Fig 3: Perilimbal, heavily pigmented mass suspicious for conjunctival or epibulbar melanoma. Histopathology was inconclusive in this case and surgical excision and concurrent cryotherapy resolved the lesion in this case. Any limbal mass should be biopsied in an attempt to confirm diagnosis.

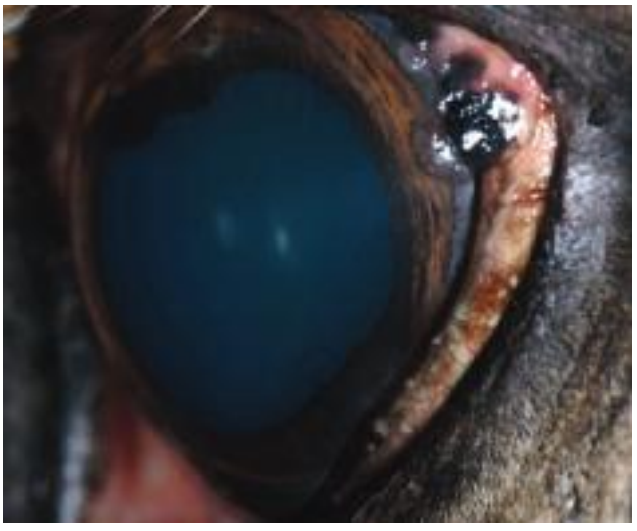

Fig 4: Intraocular melanoma associated with the corpora nigra. This mass was associated with cornea oedema due to tumour touch on the endothelial surface. Additionally, this mass was associated with intermittent haemorrhage and uveitis within the eye. Enucleation was curative in this horse and no evidence of melanoma elsewhere in the horse was found.

generalised consensus on the behaviour and treatment of ocular melanomas in the horse.

Authors' declaration of interests

No conflicts of interest have been declared.

Ethical animal research

Not applicable.

Source of funding

None.

Authorship

C. Sheridan performed a literature review and K. Myrna wrote the commentary.

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EQUINE VETERINARY EDUCATION
Equine vet. Educ. (2019) **31** (8) 413-418
doi: 10.1111/eve.12821

Case Report

PCR for antigen receptor rearrangement (PARR) clonality testing in a horse with a solitary retropharyngeal lymphoma

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Keywords: horse; lymphoma; PCR for antigen receptor rearrangement; antigen receptor rearrangement; clonality

Summary

A 19-year-old Quarter Horse gelding was evaluated for respiratory distress and a rapidly enlarging retropharyngeal mass. Initial evaluation revealed severe respiratory distress, and a large, firm mass, visibly appreciable as 12 × 12 cm, in the left retropharyngeal and perilaryngeal region, with surrounding left and right retropharyngeal swelling. No significant abnormalities were present on complete blood count and serum biochemistry analyses. Endoscopy revealed severe pharyngeal collapse restricting airflow without gross abnormalities of the pharyngeal mucosa other than inflammation and irritation. A multilobular retropharyngeal mass, diffusely heterogeneous in echogenicity, was present adjacent to, but not occluding, the carotid artery as assessed by ultrasonography. Initial needle aspirate suggested lymphoma. Tissue biopsy and histopathology confirmed a round cell tumour. A temporary tracheotomy was performed to provide respiratory relief, and the horse was managed on oral antibiotics and anti-inflammatory medications while awaiting histopathological results. The decision was made to humanely euthanise the horse after biopsy results indicated lymphoma. Definitive diagnosis of T cell rich, large B cell lymphoma was made by combination of cytology, immunohistochemistry and molecular clonality PCR (PARR) testing. Lymphoma should be considered in horses with focal masses of the retropharyngeal region. Although treatment was not pursued, PARR testing was successful in this case and may be helpful for accurate characterisation of lymphoma in horses to more precisely determine prognosis and the most effective treatment plans, as it has been in human patients and small animals.

Introduction

Lymphoma is one of the most common malignant neoplasms of the horse, and the most common neoplasm to affect the haemolymphatic system of the horse (Sellon 2004; Aleman 2009; Munoz *et al.* 2009). Lymphoma can present in nearly any location, cause varying and often nonspecific signs including enlarged lymph nodes, weight loss, ventral and limb oedema, lethargy and fever (Savage 1998; Meyer *et al.* 2006; Munoz *et al.* 2009; Taintor and Schleis 2011; Durham *et al.* 2013), and occur in horses with a wide range of ages (2 months to 30 years). Clinicopathological findings are usually nonspecific and may include anaemia, neutrophilia, hyperfibrinogenaemia, hyperglobulinaemia, hypoalbuminaemia and hypercalcaemia (Savage 1998; Meyer *et al.* 2006; Munoz *et al.* 2009; Taintor and Schleis 2011).

Lymphoma can present diagnostic challenges in animals, particularly in cases with inaccessible masses or cases without a discrete mass (Burba *et al.* 1991; Saulez *et al.* 2004; Meyer *et al.* 2006; Marques *et al.* 2012; Gress *et al.* 2016). The inability to distinguish between populations of lymphocytic cells has frequently led to nebulous diagnoses that waver between neoplastic or reactive in nature (Meyer *et al.* 2006), and PCR-based clonality testing has been helpful in other species (Gress *et al.* 2016). Recently, clonality has been assessed using molecular techniques in horses with leukaemia and concurrent lymphadenopathy (Badial *et al.* 2015). The current report represents one of only few reported cases of a solitary lymphoid tumour in a well-fleshed horse with no evidence of multicentric disease, and the first documented use of polymerase chain reaction for analysis of antigen receptor rearrangement (PARR) in equine solitary extranodal lymphoma.

Case history

A 590-kg (1298 lb) 19-year-old Quarter Horse gelding in good body condition (6/9 body condition score) was referred for evaluation of a rapidly enlarging retropharyngeal mass causing respiratory distress. Ten months prior, the gelding had begun making a loud respiratory noise when exercising that the owners described as a roaring sound. No external swelling was visible. Gradually, the gelding developed mild exercise intolerance and reportedly coughed on occasion, but continued to eat and swallow without issue. One-week prior to referral, the gelding was examined by the referring veterinarian for a left sided retropharyngeal swelling. Blood was obtained on a fine needle aspirate, and endoscopy demonstrated left pharyngeal compression. The horse remained stable until, when attempting to load the horse for transport the morning of referral, the gelding became distressed. The referring veterinarian was called to the farm and noted that the mass/swelling had significantly enlarged. He administered 40 mg of dexamethasone i.m. and monitored the horse until he was stable and could be transported for referral.

Diagnostic investigations and case management

Clinical examination and clinical pathology

The horse presented in severe respiratory distress, which included signs of increased respiratory effort, tachypnoea, nostril flaring and anxious behaviour. Loud respiratory noises were audible on inhalation and exhalation. A large, discrete, approximately 12 × 12 cm retropharyngeal mass (**Fig 1**) was visible on the left side, just caudal to the ramus of the

mandible, centred within Viborg's triangle (McCarthy 1990). On palpation, the mass was firm and extended in a rostral direction, medial to the mandible. It also extended across midline, dorsal to the larynx and could be palpated on the right side. Due to the gelding's severe respiratory distress, a tracheotomy was performed without delay, approximately 20 cm caudal to the larynx. The horse's respiratory distress resolved immediately upon placement of a tracheotomy tube.

After the tracheotomy was performed, the gelding had a normal heart rate of 44 beats/min, respiratory rate of 20 breaths/min and temperature of 37.2°C (99.9°F). Jugular fill was within normal limits bilaterally when the vein was distended. Clear mucoid discharge was present in the right nostril. Except for the aforementioned left retropharyngeal mass and associated swelling, all other physical examination parameters were within normal limits. Packed cell volume was normal at 37%, as was the total solids concentration at 7.2 g/dL (reference range, 6.0–8.5 g/dL). Complete blood count and chemistry were fairly unremarkable, with a very mild neutrophilia (6.1×10^9 neutrophils/L; reference range, $3.0–6.0 \times 10^9$ neutrophils/L) as the only abnormal finding. Total white blood cell count and morphology (8.3×10^9 WBC/L; reference range, $6.0–12.0 \times 10^9$ WBC/L), lymphocytes (1.6×10^9 lymphocytes/L; reference range, $1.5–5.0 \times 10^9$ lymphocytes/L) and fibrinogen (3.0 g/L; reference range, 1.0–4.0 g/L) were within normal limits.

Endoscopy

Upper airway endoscopy revealed normal anatomy from the nostrils to the dorsal pharyngeal recess. Extending caudally from the dorsal pharyngeal recess, the dorsal pharyngeal wall coursed ventrally (complete ventral collapse), obscuring any view of the larynx. Once the endoscope was advanced beyond the ventral pharyngeal collapse, the caudal left and right pharyngeal walls were severely collapsed axially, creating a very small airway to the larynx. The arytenoids were observable through a small opening in the compressed and inflamed pharynx. The arytenoids appeared to have a small amount of movement, but due to the amount of compression and swelling, there was very little room for them to abduct. White, foamy mucus was present in the caudal pharynx and entering the trachea. A small amount of blood, likely from the tracheotomy procedure, and a moderate amount of mucus were present in the trachea. Both guttural pouches were within normal limits.

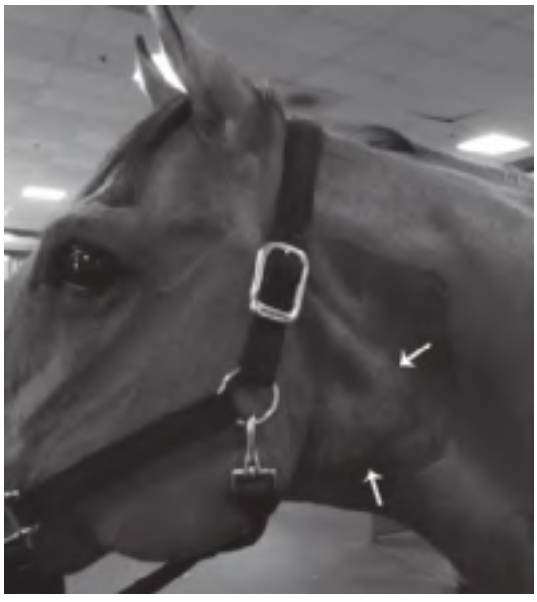

Fig 1: A large, firm soft tissue mass (arrows), externally appreciable as approximately 12 × 12 cm (craniocaudal by dorsoventral), is present in the left retropharyngeal region. Image taken after the placement of a tracheotomy tube.

Ultrasonography

Ultrasonography of the mass and surrounding region was performed. The mass was multilobular with a diffusely heterogeneous echogenicity and indistinct margins. It enveloped the left common carotid artery without occluding it. The adjacent tissues were oedematous.

Aspirate and biopsy

Needle aspiration of the mass was performed to rule out an abscess and collect a sample for cytology, which revealed cellular samples that contained a predominance of intermediate to large, atypical lymphoid cells, with few small, mature lymphocytes, suggestive of lymphoma (**Fig 2**). A tissue biopsy was obtained from the ventral aspect of the mass. The horse was maintained on trimethoprim-sulfamethoxazole[1] (25 mg/kg bwt per os q. 12 h) and flunixin meglumine[2] (1.1 mg/kg bwt per os q. 12 h; Prevail) while awaiting results.

Histopathology demonstrated dense sheets of pleomorphic round cells interspersed by large confluent regions of necrosis (representing about 25% of the sample). The background stroma was fine and contained many small tortuous vessels. Some small mature lymphocytes were present (**Fig 3**). Neoplastic cells were large (often 3× the size of a red blood cell or larger). There were up to 50 mitoses in 10 high-power fields. These preliminary histopathological findings supported the cytological diagnosis of a round cell tumour, likely lymphoma.

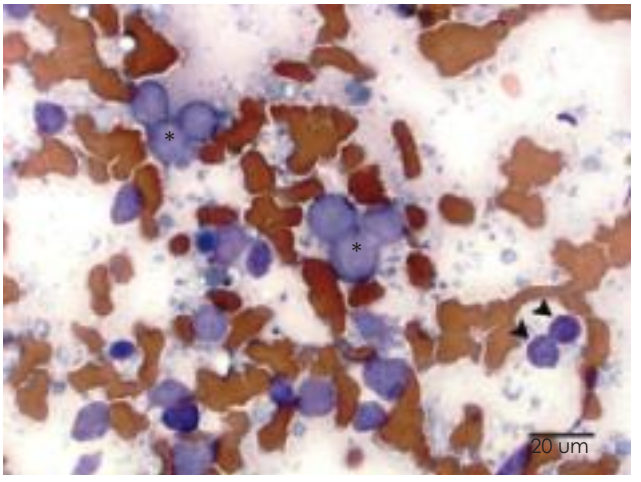

Fig 2: Cytological findings of a malignant round cell neoplasm, interpreted as lymphoma, from a needle aspirate of a mass in the retropharyngeal region of a horse. Large atypical lymphoid cells (asterisks) predominate, with few small, mature lymphocytes (arrow heads) and erythrocytes scattered throughout. (Wright's stain; 20× objective; bar = 20 μm).

After considering the diagnosis of lymphoma with concern for multicentric disease, the potentially poor prognosis, the treatment options and the well-being of the horse, the owners elected for humane euthanasia.

Post-mortem findings

Necropsy revealed a multilobular, soft, pale, tan, fleshy, irregular and infiltrative mass within deep connective tissue that measured approximately 23 × 18 × 18 cm and enveloped significant vasculature, including the common carotid artery and its branches (**Fig 4**). Also present were multifocal zones of necrosis deeper in the mass. The mass was expansile within soft tissue, obliterating normal architecture. The mass compressed the pharyngeal and epiglottic region, but did not involve the pharyngeal mucosa. Histological examination of the pharyngeal wall near the tumour revealed prominent submucosal lymphoid nodules with occasional mitoses, but with a mixture of medium and small sized lymphocytes and plasma cells. Overall, the submucosal lymphocytes were smaller than cells within the mass tissue, and, based on cellular differentiation, were considered to be reactive rather than neoplastic. Adjacent salivary tissue and thyroid lobes were normal in appearance. No enlarged lymph nodes, mediastinal masses or other internal masses were found, save a 3–4 cm sessile lipoma on the surface of the jejunum. The remainder of the necropsy was unremarkable.

Sections of the primary mass underwent immunohistochemical staining for CD20 and CD3. The larger neoplastic cells had marked expression of CD20 (**Fig 3**), consistent with a B cell phenotype. Smaller cells scattered throughout the mass were CD3 positive, consistent with surrounding T cells. Histological and immunophenotypical analyses were consistent with a T cell rich, large B cell lymphoma (TCRLBCL). Stained and unstained aspirates were submitted[3] for clonal assessment by polymerase chain reaction for analysis of antigen receptor rearrangement (PARR testing). Molecular clonality assessment of genomic DNA was performed using capillary zone electrophoresis[4,5]. Clonality analysis of IgH2, IgH3 and KDEi (B cell) genes yielded clonal rearrangements, and analysis of TCRG (T cell) yielded polyclonal gene rearrangements (**Fig 5**). These findings supported the presence of a B cell neoplasm with T cell proliferation consistent with TCRLBCL.

Discussion

The current case represents one of only few reported cases of a solitary lymphoid tumour in a well fleshed horse with no evidence of multicentric disease. With a previously undescribed presentation and a lack of additional signs, this case demonstrates that lymphoma should be on the differential diagnoses list of practitioners investigating a solitary soft tissue mass in the horse.

Lymphoma in animals has been divided into four main categories based upon location: multicentric, intestinal, mediastinal and cutaneous (Meyer *et al.* 2006; Munoz *et al.* 2009). Additionally, in horses, solitary tumours of extranodal

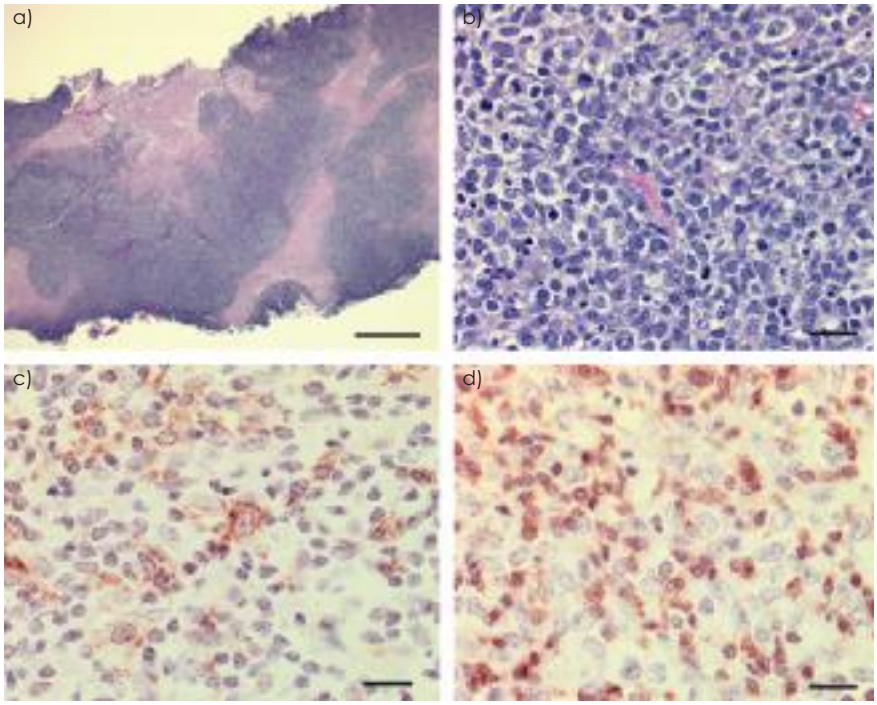

Fig 3: Incisional biopsy of retropharyngeal mass. a) The mass contains a dense population of round cells with large areas of necrosis. H&E stain; 2× objective; bar = 1 mm. b) The mass is composed of a mixed population of round cells. There are large round cells (approximately 2–3 times an erythrocyte) with euchromatic chromatin and irregularly round nuclear membranes and euchromatic chromatin. These are interspersed by many small mature lymphocytes. There are frequent mitotic figures with individual cell death and tingible body macrophages. H&E stain; 60× objective; bar = 20 μm. c) The population of large round cells has intense membranous CD20 immunoreactivity (B cells). CD20 immunohistochemical staining; bar = 20 μm. d) Small round lymphocytes within the mass have intense membranous immunoreactivity (T cells). CD3 immunohistochemical staining; bar = 20 μm.

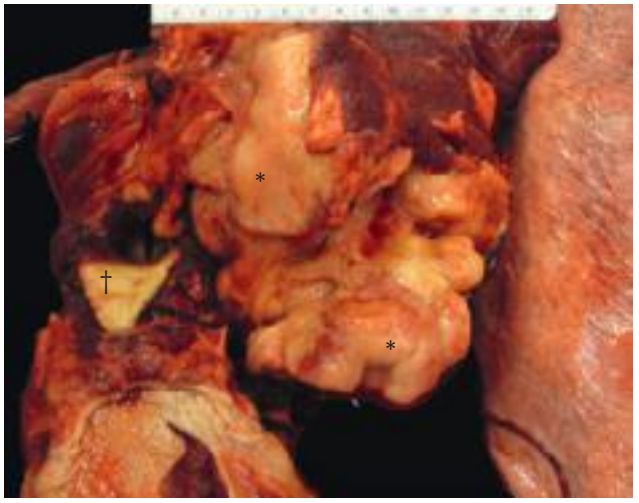

Fig 4: A multilobular, irregular mass (asterisk; lymphoma), which measured approximately 23 × 18 × 18 cm, enveloped significant vasculature, and caused compression of the pharynx, was located (asterisk) lateral and adjacent to the larynx (†: epiglottis) within the left retropharyngeal region.

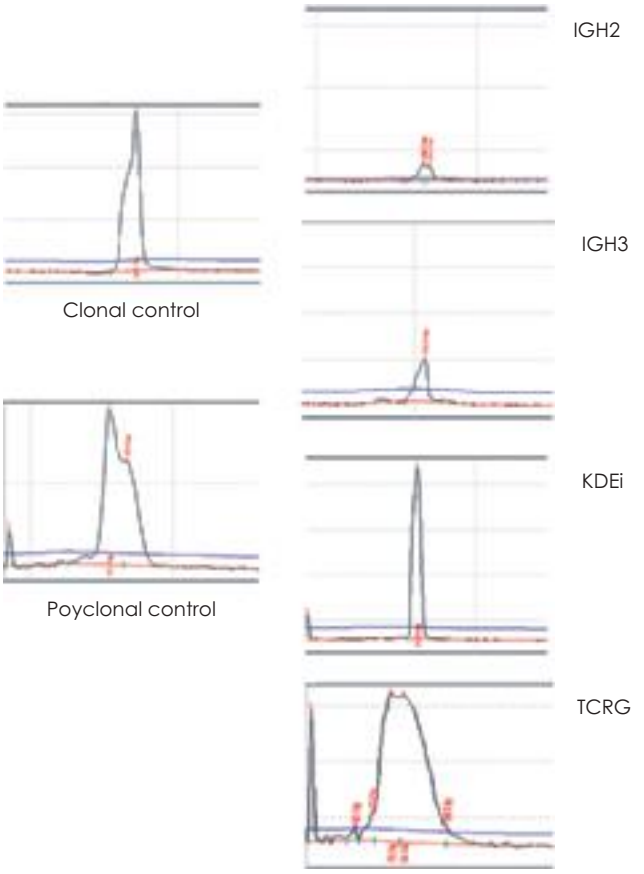

Fig 5: Molecular clonality assessment of genomic DNA from a large retropharyngeal tumour in a horse was performed using capillary electrophoresis and BioCalculator analysis. Clonal and polyclonal controls (left) are shown for comparison. Electropherotograms from the horse's tumour cells (right) for IgH2, IgH 3, KDEi and TCRG primers demonstrate reproducible (done in triplicate) clonal spikes using immunoglobulin heavy chain (IgH) and KDEi (B cell) primers (top three images on right). There is a broad polyclonal result when the neoplastic cells were assessed with T cell receptor gamma (TCRG) primers (bottom image on right). These findings supported the presence of a B cell neoplasm with T cell proliferation consistent with T cell rich, large B cell lymphoma. The presence of the second clonal peak (KDEi and TCGR) is a common artefact seen with capillary electrophoresis of this type (Keller et al. 2016).

sites have also been infrequently reported in regions such as the tongue (Rhind and Dixon 1999), palatine tissues (Lane 1985), urethra (Montgomery *et al.* 2009), bladder and spleen (Tanimoto *et al.* 1994). This report adds an additional case of solitary extranodal lymphoma. Equine lymphoma has also been subtyped into 14 categories based on the World Health Organization (WHO) classification criteria (Durham *et al.* 2013). The current case was diagnosed as TCRLBCL. TCRLBCL is the most common form of lymphoma in the horse, representing up to 87% of equine lymphoma diagnoses (Durham *et al.* 2013), and is frequently multicentric.

Lymphoma can present diagnostic challenges in animals (Burba *et al.* 1991; Saulez *et al.* 2004; Meyer *et al.* 2006; Marques *et al.* 2012; Gress *et al.* 2016), and PCR-based clonality testing has been helpful in other species (Gress *et al.* 2016). The process of clonal assessment by PARR testing has been previously described (Burnett *et al.* 2003; Thalheim *et al.* 2013; Keller *et al.* 2016), and has recently been described for lymphoma in horses (Meichner *et al.* 2017). Briefly, PARR testing assesses DNA from cells to ascertain if they come from the same cell line, termed monoclonal (typically neoplastic), or from different cell lines, termed polyclonal (typically reactive) (Avery 2009). Because PCR testing is an assay in which DNA is amplified, assessment of clonality can be accomplished on small sample size with a high level of sensitivity (Avery 2009). PARR testing is advantageously sensitive, identifying even one neoplastic cell in 100 cells (Avery 2009). PCR primers analyse for the conserved regions of receptors or genes (use the most specific lymphoid receptors) that flank hypervariable regions. PCR products are then separated by capillary gel electrophoresis. A single sized or sequence PCR product indicates clonality, while multiple PCR products indicate polyclonality, or a reactive process. The addition of immunophenotypic, genomic and molecular advancements have allowed for an ever-growing selection of diagnostics to characterise and more definitively diagnose lymphoma, particularly in animals (Strauchen 2004; Meyer *et al.* 2006). PARR testing is the only diagnostic available to veterinarians to verify cellular clonality. Final diagnosis in the current case was eventually made through a combination of cytology, histology, immunohistochemistry and PARR testing. Recently, PARR has been assessed using molecular techniques in horses with a rare condition of monoclonal gammopathy, B cell leukaemia and concurrent lymphadenopathy (lymphoma/leukaemia) (Badial *et al.* 2015). Additionally, PARR has been used to successfully identify diffuse intermediate to large B cell lymphoma with a robust haematogenous phase (TCRLBCL) in both blood and infiltrative tissue from a horse with multicentric disease (Meichner *et al.* 2017). To the authors' knowledge, this is the first documented use of PARR in equine solitary extranodal lymphoma.

In the dog, lymphoma is staged (I–V) for prognostication, and recently, PARR has been used to provide a more sensitive testing for the presence of neoplastic cells in the

blood (Stage V) (Lana *et al.* 2006). As mentioned, PARR testing can assist in differentiating between reactive lymphocytes (polyclonal) and neoplastic lymphoid tissue (monoclonal), and may be helpful in supporting a diagnosis of lymphoma in horses with an unclear diagnosis. Clonality results should, however, be examined alongside cytology and histology, and PARR should not be relied upon to definitively phenotype lymphoma as clonal rearrangement of both B and T cell receptors has been demonstrated in human, canine and feline lymphomas (Weiss and Spagnolo 1993; Strauchen 2004; Wilkerson *et al.* 2005; Avery 2009; Sato *et al.* 2011; Keller *et al.* 2016). In addition, recent advances in dogs using real time PARR testing for clonality (utilising melting curve analysis) demonstrates promise in making this testing more readily accessible for clinical use (Langner *et al.* 2014).

While PARR testing is sensitive, it is not infallible, and false negatives or positives can occur (Keller *et al.* 2016). As mentioned, PARR testing should be examined alongside additional diagnostics, as was performed in the current case. PARR testing was used to verify that the B cell population was indeed the neoplastic population and that the T cell population was the infiltrate, thus verifying the diagnosis as a TCRBCL. As equine lymphoma is frequently a more insidious disease, often poorly exfoliative into body cavity fluids and typically comprised of a heterogeneous population of lymphoid cells, initial diagnosis by cytology can be difficult. PARR testing was successful in this case of extranodal lymphoma, and could be a useful tool for equine practitioners. While this particular tumour was accessible, the enhanced sensitivity of PARR testing could potentially play a role in detecting hard to find tumours within the thoracic cavity, abdominal cavity or blood. This enhanced sensitivity could potentially aid in detection of lymphoma within body cavity fluid when biopsy is contraindicated or difficult to achieve. Additional research is warranted to assess the use of PARR testing in horses with suspected but undiagnosed or inaccessible tumours.

Due to the severe respiratory distress, the unknown and potentially poor prognosis, the possibility of unrecognised multicentric disease, and the owner's concern for the horse's well-being, humane euthanasia was elected in this case. Because of the close proximity of the mass to important neurovascular structures, complete surgical resection with appropriate margins was not considered possible, and necropsy findings confirmed this. Potential treatment plans were discussed, including chemotherapy or radiation to decrease the size of the mass, possibly allowing for adequate surgical excision, which could have been followed by other adjunct therapies. With the exception of cutaneous lymphoma, prognosis and treatment success is generally viewed as poor in equine lymphoma (Weaver *et al.* 1996; Rhind and Dixon 1999; Montgomery *et al.* 2009), but is largely unknown (Taintor and Schleis 2011). In cases of localised disease, most reports describe that, due to poor prognosis, treatment was not attempted (Lane 1985; Lester *et al.* 1992; Rhind and Dixon 1999; Montgomery *et al.* 2009; Marques *et al.* 2012) or was unsuccessful (Burba *et al.* 1991), although surgical excision (Rebhun and Del Piero 1998), radiotherapy (Weaver *et al.* 1996), and systemic chemotherapy (Saulez *et al.* 2004) have had occasional success. An early and better characterised diagnosis, in combination with treatment earlier in the course of disease, may result in improved success with treatment.

Although thoracic and laryngeal lymphoma has been documented to cause respiratory distress (Burba *et al.* 1991; Lawn 2005; Marques *et al.* 2012), to the authors' knowledge, focal, solitary, extranodal retropharyngeal lymphoma causing respiratory distress in a horse has not been reported. Lymphoma should be considered in horses with focal masses of the retropharyngeal region. Although treatment was not pursued, PARR testing was successful in this case and may be helpful for accurate characterisation of lymphoma in horses to more precisely determine prognosis and the most effective treatment plans, as it has been in human species and small animals.

Authors' declaration of interests

No conflicts of interest have been declared.

Ethical animal research

The hospital from which material were derived for this study consistently uses an admission/consent form that includes an option for owners to opt out of research studies. Approval was given by this owner for use of photos, video or tissue samples to be used for research purposes.

Source of funding

None.

Authorship

E. Collar was involved in all aspects of horse care, tissue sampling, data analysis and interpretation, and major preparation of the manuscript. J. Parker was involved in all aspects of horse care, tissue sampling, data analysis and interpretation. E. Gorman assisted with cytology and histology analysis and interpretation, diagnostic recommendations and manuscript preparation. D. Russell assisted with histology analysis and interpretation, and manuscript preparation. B. Valentine performed the post-mortem examination. All authors contributed to critical manuscript review and approved the manuscript.

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[2]VetOne, Norbrook Laboratories Ltd, Newry, Northern Ireland, UK.
[3]Leukocyte Antigen Biology Laboratory, UC Davis, Davis, California, USA.
[4]Qiagen QIAxcel capillary electrophoresis system, Qiagen, Valencia, California, USA.
[5]BioCalculator software, Qiagen, Valencia, California, USA.

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EQUINE VETERINARY EDUCATION
Equine vet. Educ. (2019) **31** (8) 419-420
doi: 10.1111/eve.12857

Clinical Commentary

PCR for antigen receptor rearrangement (PARR) in equine veterinary medicine

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Keywords: horse; lymphoma; lymphosarcoma; malignancy; tumour; cancer

Summary

PCR for antigen receptor rearrangement (PARR) is a technique developed to aid with the assessment of lymphoid malignancies. The technology relies on the assessment of clonality of T or B cells, and there are many pitfalls. One major advantage is that only a small amount of sample is required to detect clonality, and it can be performed on any type of sample, including effusions, formalin-fixed paraffin-embedded tissues and cytological samples. However, the risk of both false negative and false positive results means that PARR should not be used to diagnose a lymphoid malignancy in isolation, but that results should be considered in conjunction with a full clinical and clinicopathological assessment.

Normal lymphoid cells contain unique DNA sequences, which occur as a result of rearrangements of the V, D and J genes in B cells and V and J genes in T cells during their development. During this normal antigen receptor rearrangement, one each of multiple V, D and J genes are joined by removal of intervening DNA, followed by the addition or removal of nucleotides from the ends of these joined genes. This leads to unique, specific antigen-binding receptors on B and T cells and is essential for the normal function of the adaptive immune system (Cooper and Alder 2006). Normally, when responding to an infectious agent, there will be a heterogenous population of lymphocytes with wide variation in the antigen receptor genes. In the case of lymphoid neoplasia, the abnormal lymphocytes are derived from a single cell (or clone) and will therefore have identical V, D and J genes. The presence of a single clonal rearrangement of the V, D and J genes may therefore indicate a lymphoid malignancy, and it is this premise that led to the development of the PCR for antigen receptor rearrangement (PARR) test in human medicine. PARR is an assay that assesses clonality in a population of lymphoid cells by amplification of DNA encoding these variable regions of the T and B cell receptor (Burnett *et al.* 2003; Lana *et al.* 2006). It is important to note that benign clonal expansions are possible and can lead to a false positive result, and false negatives are also found, especially where a polyclonal background is present (Keller *et al.* 2016). Polyclonal backgrounds are often found where there is a mixed cellular infiltrate into a tumour (Keller *et al.* 2016). PARR incorrectly determined the immunophenotype of 33% of B cell lymphomas and 25% of T cell lymphomas in one case series of lymphoma in dogs (Thalheim *et al.* 2013). The overall sensitivity of PARR for the detection of clonal rearrangements of B cell and T cells in this series was 74%, compared with a sensitivity of 98% for flow cytometry (Thalheim *et al.* 2013). The positive predictive value of PARR in the same case series was 92% for T cell lymphomas and 100% for B cell lymphomas, and the negative predictive value was 92% for T cell lymphomas but was only 52% for B cell lymphomas (Thalheim *et al.* 2013). These data are not currently available for the equine patient, but it is clear that results should be interpreted with caution, especially as the most common lymphoid neoplasia in the horse is T cell-rich large B cell lymphoma, where large neoplastic B cells are accompanied by an infiltration of non-neoplastic, mature T cells (Meyer *et al.* 2006; Durham *et al.* 2013; Badial *et al.* 2015). However, PARR has a major advantage over flow cytometry in that PARR can be performed on any cell, dead or alive; it can be performed on cytological samples, effusions and formalin-fixed paraffin-embedded tissue, and has a high specificity so may be an acceptable assay if fresh samples are not available (Thalheim *et al.* 2013). Interestingly, the assay is considered to be less sensitive when lymphoid tissue is examined compared with nonlymphoid tissue (Burnett *et al.* 2003), so it may be more useful to detect clonality in effusions than from lymph node aspirations or biopsies. It can also provide useful information in ambiguous cases, where lymphoid neoplasia is suspected but has not been confirmed, and remains the only test available to assess clonality in lymphoid cells.

In human medicine, PARR testing is usually used for molecular staging and therapeutic monitoring rather than as a diagnostic tool. Its reported use in equine veterinary medicine is limited; the case report in this issue describes its use to help confirm a case of T cell-rich B cell lymphoma (Collar *et al.* 2019), and it has also been used to aid the diagnosis of a diffuse large cell lymphoma (Meichner *et al.* 2017) and for characterisation of B cell neoplasms (Badial *et al.* 2015).

It is clear that PARR clonality testing should not be performed in isolation; it can give useful adjunctive information but should follow clinical, morphological and immunophenotypical assessment, and it is generally considered unnecessary in obvious lymphoid neoplasms (Keller *et al.* 2016). This technology is still in its infancy in veterinary medicine and there is a lack of standard terminology, interpretation and laboratory practices which raise concerns as to the reproducibility of results obtained via these methods (Keller *et al.* 2016). However, with advances in the technology and increasing availability, it may become a useful part of the diagnostic and prognostic work-up of cases of suspected lymphoid neoplasia in the horse.

Author's declaration of interests

No conflicts of interest have been declared.

Source of funding

Anna R. Hollis is generously supported by the Margaret Giffen Charitable Trust.

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EQUINE VETERINARY EDUCATION
Equine vet. Educ. (2019) **31** (8) 421-426
doi: 10.1111/eve.12796

Original Article

Extraction of 22 equine cheek teeth with displaced sagittal fractures using polymethylmethacrylate stabilisation (2011–2016)

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Keywords: horse; dental extraction; equine cheek tooth; polymethylmethacrylate (PMMA); sagittal fracture

Summary

Displaced sagittal cheek tooth fractures are a cause of oral pain, quidding and apical infection. Intraoral extraction is the preferred technique to remove affected teeth, but can be difficult due to displaced and friable fracture fragments. Stabilising fracture fragments via filling of the fracture space with polymethymethacrylate (PMMA) prior to removal may be a useful method to facilitate intraoral extraction. Case details were examined retrospectively. A total of 22 cheek teeth required extraction in 20 horses because of displaced sagittal fractures. Clinical diagnoses were made using oral examination, oral endoscopy, skull radiography and computed tomography. All procedures were performed in standing, sedated horses in stocks. Fracture spaces were cleaned and packed with PMMA and teeth removed using a routine intraoral extraction technique. Digital photographs of extracted teeth were taken and tooth measurements calibrated using digital image software. Intraoperative difficulties, as well as post-operative complications were recorded. A total of 21 maxillary and one mandibular cheek teeth were extracted. All maxillary teeth had advanced infundibular caries. Intraoral extraction was successful in 16 cases; six were unsuccessful and required repulsion due to tooth fragmentation or abnormal dental anatomy. In 11 cases, maxillary or conchofrontal sinus trephination was performed to either treat sinusitis, repulse the tooth, or both. Two horses developed short-term complications following local anaesthesia of the maxillary nerve. The mean ratio of fracture depth to tooth length was 0.59 and mean ratio of fracture width to tooth width 0.53. The limitations of the study are its small sample size, retrospective nature and lack of control group to compare extraction success in PMMA and non-PMMA groups. It was concluded that using PMMA to stabilise displaced sagittal fractures in equine cheek teeth is a simple, effective method of facilitating intraoral extraction and may reduce the need for more invasive procedures.

Introduction

Fractures of equine cheek teeth are a cause of oral pain and risk for apical infection, particularly when fracture planes involve pulp chambers (Dacre *et al.* 2007). They can be of traumatic origin, iatrogenic, or may have no known history of trauma. The latter group have been termed 'idiopathic' (Dixon *et al.* 2000). Idiopathic cheek teeth fractures have a low prevalence (0.4%) in the general horse population and varying clinical signs range from mild halitosis to severe apical infection with associated draining tracts or sinusitis (Taylor and Dixon 2007). Studies of idiopathic maxillary cheek teeth fracture configurations have revealed buccal slab fractures to be the most prevalent followed by sagittal (or midline) cheek teeth fractures (Dixon *et al.* 2000; Dacre *et al.* 2007). Sagittal fractures in maxillary cheek teeth typically course through both infundibulae and have been shown to be the result of coalescence of severely carious infundibulae with the 109 or 209 cheek teeth commonly affected (Dixon *et al.* 2000, 2014; Dacre *et al.* 2007; Taylor and Dixon 2007; Bienert and Bartmann 2008; van den Enden and Dixon 2008). Approximately 70% of sagittal fracture cases may be symptomatic, with clinical signs including quidding, behavioural problems and halitosis (Taylor and Dixon 2007). In more advanced cases significant displacement of the fracture fragments occurs secondary to food packing into the fracture space. Despite the common nature of sagittal fractured cheek teeth, an extraction technique specific to this pathology has not been described.

Standing intraoral dental extraction is the treatment of choice for diseased cheek teeth in horses (Prichard *et al.* 1992; Dixon *et al.* 2005; O'Neill *et al.* 2011; Reichert *et al.* 2014). Intraoral extraction may not always be possible in teeth with pre-existing fractures or dental caries, due to significantly reduced crown surface area and the friable nature of fracture fragments (Tremaine 2004). Additionally, deformed or malformed roots are often present and become fractured when buccal and palatal or lingual fragments are compressed. Alternatives to conventional oral extraction include traditional buccotomy, tooth repulsion and minimally invasive transbuccal intradental screw placement (Prichard *et al.* 1992; O'Neill *et al.* 2011; Langeneckert *et al.* 2015). These techniques can be associated with higher complication rates than conventional oral extraction, involve general anaesthesia, or require specialised equipment.

Polymethymethacrylate consists of polymerised methyl methacrylate monomers and has several biological uses (Cruz *et al.* 2006; Hirvinen *et al.* 2009). It is available in powder form (Technovit powder and liquid)[1] and sets to a hard substance via an exothermic polymerisation process within 2–10 min after mixing with a hardener. The purpose of this report is to describe utilising PMMA to reinforce the clinical crown of sagittally-fractured displaced cheek teeth as an aid in intraoral extraction and secondarily to document the clinical appearance of displaced sagittal cheek teeth fractures. The technique is described, intraoperative difficulties discussed and post-operative

complications, outcomes and limitations of the technique are reported.

Materials and methods

Case details of 22 dental extractions on 20 horses utilising PMMA at the Marion DuPont Scott Equine Medical Center, Leesburg, Virginia from October 2011 to August 2016 were examined retrospectively. Extractions were considered successful if the entire targeted tooth was extracted without requiring repulsion to remove the tooth or residual fragments. The authors performed all procedures. Case information extracted from medical records included signalment, affected tooth (Floyd 1991), clinical signs, diagnostic imaging, technical difficulties, post-operative complications and time to resolution of clinical signs. Each tooth extraction using PMMA was considered a separate procedure, with two horses undergoing two extractions each.

Clinical diagnoses were made using visual oral and dental examination, oral endoscopy, radiography and, more recently, standing computed tomography (Pegaso HD CT)[2]. All procedures were performed with animals sedated and standing in stocks. Sedation was achieved using detomodine hydrochloride (Dormosedan)[3] i.v. at 0.01 mg/kg bwt or xylazine hydrochloride (Rompun)[4] i.v. at 0.4 mg/kg bwt alone, or in combination with butorphanol tartrate (Torbugesic)[5] i.v. at 0.01 mg/kg bwt. Perioperative analgesia was provided with flunixin meglumine (Flunazine 1.1 mg/kg bwt i.v.)[6], or phenylbutazone (ButaJect 2.2 mg/kg bwt i.v.)[7]. Perioperative antimicrobial coverage with ceftiofur crystalline (Excede 6.6 mg/kg bwt i.m.)[8] was administered in cases with pre-existing sinusitis, as broad spectrum antimicrobial coverage is advised in these cases to reduce the risk of bacteraemia (Bartmann *et al.* 2002; Bienert *et al.* 2003). Regional anaesthesia was performed on either the maxillary or mandibular nerves, as described elsewhere (Tremaine 2007). Initially, perineural injection of the maxillary nerve was performed blindly and latterly under ultrasound guidance (O'Neill *et al.* 2014). Following placement of a full mouth speculum, the mouth was rinsed with copious amounts of water and a pressurised stream of dilute betadine and dental pick used to dislodge the feed material.

Polymethymethacrylate powder was then mixed until it formed a thick slurry and continually mixed until it became mouldable. Fracture spaces were packed with PMMA to the level of the occlusal crown and allowed to harden (**Fig 1**). Intraoral dental extraction was then performed as described in detail elsewhere (Tremaine 2004; Dixon *et al.* 2005). Occasionally following cheek tooth spreading, PMMA packing would loosen and fall out of the fracture space. In these instances, new PMMA was prepared and allowed to harden prior to applying extraction forceps. Trephination of the conchofrontal or maxillary sinuses was performed to facilitate sinus lavage, or to enable repulsion, where required (Barakzai and Dixon 2014). Following sinus lavage and/or tooth repulsion, skin incisions were opposed primarily using sterile stainless steel skin staples.

Following extraction, tooth alveoli were lavaged with dilute betadine solution. Dental impression material (Splash! super hydrophilic impression material)[9], impregnated with 1 g crushed metronidazole powder[10], was then mixed together and packed in the empty alveoli and allowed to harden. Metronidazole was chosen due to the reported prevalence of anaerobes in dental related sinusitis cases (Bartmann *et al.* 2002; Bienert *et al.* 2003).

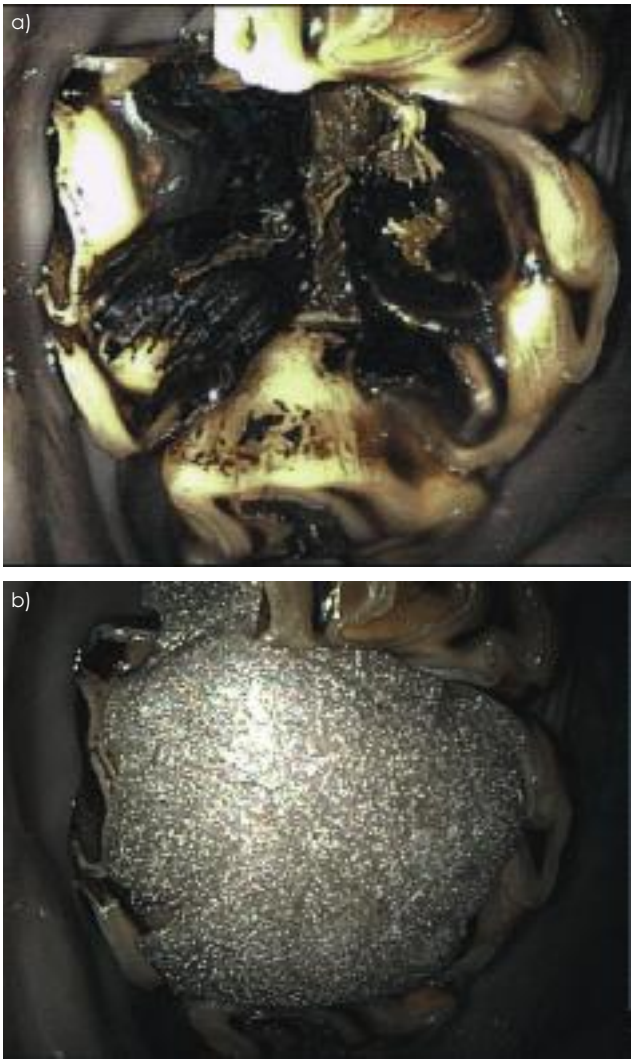

Fig 1: Oral endoscopy images of a sagittal fractured maxillary cheek tooth. a) shows the tooth before and b) after filling with PMMA.

Digital photographs of extracted teeth were taken using a digital camera[11] (**Fig 2**). Tooth measurements were made using digital image software (ImageJ version 1.49)[12] and calibrated against a ruler in the image. Teeth were measured longitudinally from the longest root to the ipsilateral occlusal surface and transversely across the widest point of the occlusal surface. Fracture depth was measured from the most apical extent of the carious lesion to a point in line with the occlusal surface, approximately half way between each fragment and transversely at the widest point.

Follow-up information was obtained from owners at 3–48 months post-operatively via email or telephone conversation. Enquiries were specifically made regarding time to resolution of clinical signs, post-operative complications and return to intended use.

Data analysis

Summary statistics and histograms of tooth measurement data were performed. Tooth measurements are presented as mean ± s.d. Comparison of intraoral extraction success with other categorical variables was not performed as this was not

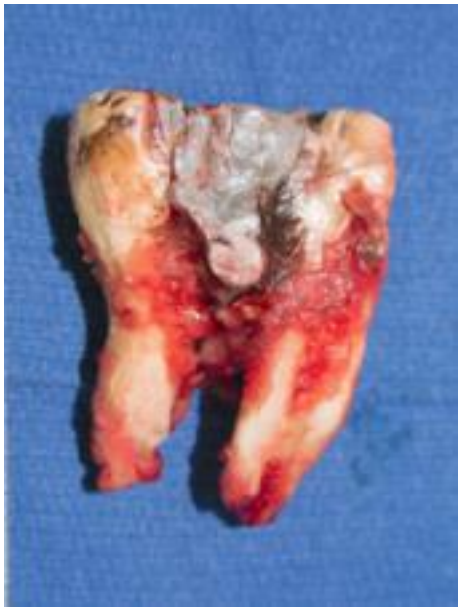

Fig 2: A successfully extracted 109 tooth with PMMA packing the fracture space. Note the fermented feed material that remains lodged at the apex of the fracture.

a primary objective of the study. All statistical analysis was performed using Microsoft Excel[13] (2010).

Results

The median age of horses in the study was 15 years with a range of 9–32 years. There were seven Warmbloods, four ponies, two draught crossbreds, two Quarter Horses, two Arabians and three other breeds. Fourteen were geldings, six were mares. Six did not have symptoms reported by the owner on presentation. Of the 14 horses with symptoms, nine had purulent nasal discharge, three halitosis, two quidding, one with facial swelling and one displayed signs of oral discomfort. Three animals had multiple clinical signs.

A total of 21 maxillary and one mandibular cheek teeth were extracted. In animals with multiple tooth extractions, teeth were extracted in independent procedures separated by one month. The most commonly affected teeth were the Triadan 109 and 209s, forming 50% of all cases (**Fig 3**). This was followed by 110 and 210s, which formed 32%. All maxillary cheek teeth had grade 4 infundibular caries, defined as having the integrity of the tooth affected (Honma *et al.* 1962).

Preoperative radiographs were taken for 17/22 cases. All had a fracture space on dorsoventral projection and seven cases had sinus opacity consistent with sinusitis. In 10 cases, widening of the periodontal ligament space and periapical lucency was present, 10 affected teeth showed periapical sclerosis and in two, a small, apical, circular radiopacity consistent with reactive hypercementosis was present. Standing computed tomography became available and was used for the last case, a 32-year-old Welsh pony. Preoperative standing computed tomography of the skull revealed a complete sagittal fracture with displaced palatal and buccal fragments (**Fig 4**). Loss of alveolar bone and trabecular architecture of the maxilla immediately surrounding the root apices at the fracture space was evident.

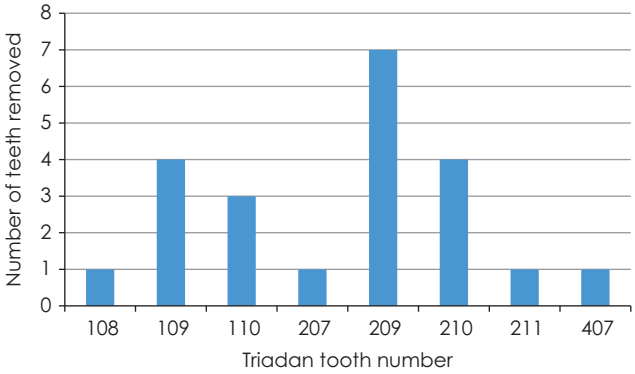

Fig 3: Number of cheek teeth listed by Triadan position.

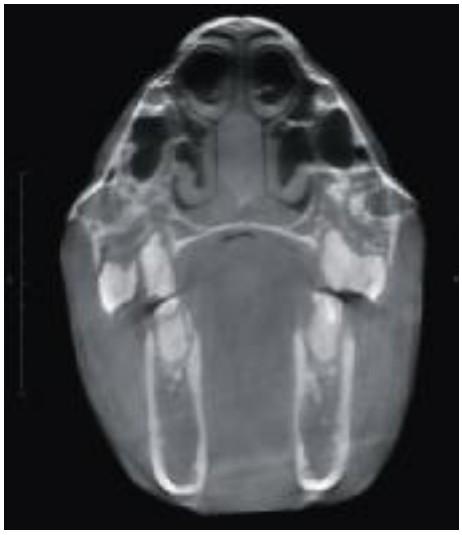

Fig 4: A transverse computed tomography image through a sagittal fractured 209 tooth, showing the fractured, displaced tooth.

Intraoral extraction was successful in 16 cases (73%). Individual case details are listed in **Supplementary Item 1**. Of the six failures, three were due to root fractures, two failed because of fragmentation of the clinical crown and in one case, abnormality in size and shape of the tooth would not allow oral extraction. Eight of the nine cases with preoperative sinusitis had paranasal sinus lavage via a conchofrontal (n = 4), rostral maxillary (n = 2), or caudal maxillary (n = 2) sinus trephine. One case had a significant oroantral fistula used to lavage the rostral maxillary sinus retrograde following successful intraoral extraction. Three cases that did not have preoperative sinusitis required trephination to facilitate repulsion of the tooth via transcortical bone (n = 1), rostral maxillary (n = 1), or caudal maxillary (n = 1) trephines. Two cases with preoperative sinusitis also had persistent post-operative sinusitis, one of which had the tooth repulsed (resolved after 29 days) and one which did not require repulsion (resolved after 50 days). Overall, of nine cases with preoperative sinusitis, seven resolved immediately after sinus lavage and tooth extraction. Two horses that did not have preoperative sinusitis developed post-operative sinusitis which resolved after 28 and 109 days. For all cases, repeat examinations and dental plug changes were scheduled for 14 days post-operatively, then as

TABLE 1: Summary tooth measurement data

	Fx depth (mm)	Tooth length (mm)	Fx width (mm)	Tooth width (mm)	Fx depth/ tooth length	Fx width/ tooth width
IOE Successful mean (n = 15)	30.86	53.07	19.15	36.54	0.60	0.54
IOE failure mean (n = 3)	34.33	61.33	14	31	0.55	0.45
Overall mean* (n = 18)	31.47	54.53	18.47	35.8	0.59	0.53

* For some teeth, not all measurements were available. For individual tooth data, see Supplementary Item 2.
Fx, fracture.

required. The horse with persistent sinusitis for 109 days following extraction did not present for routine recheck at 2 weeks. When examined at 109 days, sequestered right ventral concha bulla bone was removed via the nasal passages and the sinusitis subsequently resolved.

A total of 82% of teeth (18/22) were measured following extraction (**Table 1**). Mean tooth length was 54.53 ± 10.79 mm, mean fracture length 31.47 ± 8.16 mm, mean tooth width 35.80 ± 10.16 mm and mean fracture width 18.47 ± 6.20 mm. The mean ratio of fracture depth to tooth length was 0.59 ± 0.15 and mean ratio of fracture width to tooth width 0.53 ± 0.13. Individual tooth measurement data are listed in **Supplementary Item 2**.

There were two cases with maxillary nerve block complications. One developed a retrobulbar haematoma and periocular swelling which resolved in 24 h. This was treated with topical triple antibiotic ophthalmic ointment (Neomycin and Polymyxin B Sulfates and Bacitracin Zinc Ophthalmic Ointment USP)[14] applied to the cornea and a pressure bandage placed over the eye for 12 h. The other developed ocular pruritis, blepharospasm, conjunctivitis and a small superficial corneal ulcer. Resolution was achieved following 4 days of treatment with a face mask to protect the eye, triple antibiotic ophthalmic ointment every 6 h and flunixin meglumine (1.1 mg/kg bwt i.v. q. 12 h).

Follow-up was available for 18 cases at a median of 20.5 months post-tooth extraction (range 3–48 months).

Discussion

Utilising PMMA to fill large defects in displaced sagittal fractures was successful in 73% of cases and therefore we found it to be an effective method of assisting intraoral extraction of this type of cheek tooth fracture. In our clinical experience, attempting oral extraction of displaced sagittally-fractured cheek teeth without stabilisation usually resulted in iatrogenic fracture of one or both of the fracture fragment clinical crowns, similar to what has been reported (Dixon *et al.* 2005).

The study population consisted of middle-aged horses with a median age of 15 years, similar to a survey based report of sagittal fractured cheek teeth (Taylor and Dixon 2007). It is likely that this reflects the duration of progression of infundibular caries as the majority (21/22) of teeth extracted had an appearance consistent with grade four infundibular caries. The one mandibular cheek tooth had a vertical fracture coursing through pulp chambers one and two (Du Toit *et al.* 2008). No breed predilection was observed throughout the study; the higher number of Warmbloods is likely to reflect the hospital referral population. The majority of cases were geldings (70%) and the reason for this is unknown.

The prevalence of clinical signs (64%) in this case series is consistent with a previous study of idiopathic cheek tooth fractures that reported 70% in maxillary midline fractures (Taylor and Dixon 2007). In that study, quidding, bitting/ behavioural problems and halitosis were the main clinical signs with only one case having purulent nasal discharge. In the current study, 41% of horses had purulent nasal discharge and confirmed sinusitis, indicating a greater propensity for clinical progression of this fracture type in our study population. Sinusitis may be the result of either extension of periodontal infection or pulpar exposure. In a report of 31 idiopathic cheek teeth fractures, 42% of extracted teeth had evidence of pulpar exposure (van den Enden and Dixon 2008). In the present study, it was assumed that pulpar exposure through the common chamber had occurred. Examination of extracted teeth apices often revealed loss of the periodontal ligament as a result of infection caused by feed material packing into the fracture plane.

To our knowledge, displacement and depth of sagittal fractures have not been previously described. The fractures in this report were highly displaced with a mean fracture depth of 31 mm and a mean fracture width of 18 mm. This allows significant feed packing into fracture spaces, leading to fermentation and displacement of the tooth fracture fragments. In the one case in which computed tomography was performed (**Fig 4**), buccal and palatal displacement of the exposed crown was evident indicating remodelling of the distal periodontal ligament and alveolar bone to accommodate the expansion of the fracture space.

All maxillary cheek teeth in the study had grade 4 infundibular caries (Honma *et al.* 1962). The aetiopathogenesis of sagittal fractures in maxillary cheek teeth is thought to be the result of coalescing infundibular caries (Dixon and Dacre 2005). In turn, infundibular caries may result from cemental hypoplasia, a process whereby the central apical portion of infundibular cement is incomplete (Staszyk *et al.* 2015). Triadan maxillary 09 cheek teeth are more prone to hypoplasia and it is thought that this is because of their relatively earlier eruption precluding complete filling of the infundibula with cementum (Staszyk *et al.* 2015; Suske *et al.* 2016a). The hypoplastic infundibula are then potentially vulnerable to carious damage when exposed through attrition (Suske *et al.* 2016b). A higher percentage of Triadan 09 teeth were affected in the present study, consistent with the hypoplastic infundibula hypothesis and previous reports of cheek teeth fracture patterns. The one mandibular cheek tooth in this study had a miscellaneous fracture pattern (Dacre *et al.* 2007). This horse presented with quidding and had radiographic evidence of periapical infection. The exact pathogenesis of the fracture is unknown in this case. In a previous report of a similar fracture type, no underlying reason for the fracture was found histologically (Dacre *et al.* 2007).

The complications associated with the PMMA stabilisation technique reported in this study were minimal. Two cases that developed maxillary nerve block complications resolved with medical management within 4 days. Two cases which did not have preoperative sinusitis developed sinusitis following tooth removal. In one of those, leakage of feed material around the dental alveolar plug following tooth repulsion had occurred, tracking into the sinus and establishing infection. Resolution was obtained with gauze debridement of the orosinus fistula, plug replacement and antibiotic therapy. The second case developed sinusitis following successful oral extraction, and resolved when sequestered right ventral concha bulla bone was removed.

The short- and long-term post-operative complications, following successful intraoral extraction of PMMA stabilised teeth, is in line with the low complication rate reported for oral extraction of diseased cheek teeth and compares favourably with alternative extraction techniques. In a report of standing maxillary and mandibular cheek tooth repulsion, 41% of extractions required follow-up medical or surgical treatment to resolve signs of maxillary sinusitis or persistent mandibular drainage (Coomer *et al.* 2011).

Alternative approaches to intraoral extraction are associated with higher prevalence of intra- and post-operative complications such as oromaxillary fistula formation, alveolar sequestrae formation and tooth fragments remaining in the alveolus. These complications were described in 15/23 (65.2%) cases following failed intraoral extraction (Reichert *et al.* 2014), alternative approaches included minimal invasive buccotomy, classical lateral buccotomy and a combination of repulsion and minimal invasive buccotomy. In the case series by Reichert et al. (2014), the most common reason to use an alternative procedure to oral extraction was complete fracture of the clinical crown. A report of lateral buccotomy in 114 horses described complications in 30% of cases, including partial wound dehiscence and infection, temporary and permanent facial nerve paralysis, myositis, sinusitis and establishment of an oroantral fistula (O'Neill *et al.* 2011).

A minimally invasive transbuccal approach and intradental screw placement for standing cheek teeth extraction has been described (Langeneckert *et al.* 2015). While good overall success (81%) and minimal post-operative complications were reported, the technique was less successful in teeth that were carious and friable.

In 10 cases (45%), the tooth fragmented during molar spreading or extraction and six of those had fragments that required repulsion. In four cases useful loosening of the tooth was achieved prior to fragmentation, which enabled easy fragment extraction with root forceps. It is also conceivable that loosening, facilitated by PMMA stabilisation and molar spreading, is beneficial even when the tooth is ultimately repulsed.

An initial concern in the development of the technique was whether the PMMA would bond to the adjacent mesial and distal teeth. Clinically, cheek tooth spreaders were able to be placed without difficulty in all cases. The exothermic nature of PMMA hardening did not cause any known long-term pathology to the adjacent mesial and distal teeth.

In conclusion, significant fracture displacement was recorded in this study population. Utilising PMMA to stabilise sagittally fractured cheek teeth for intraoral extraction is a useful technique with minimal complications. It is simple to implement, although operators are encouraged to have experience with standing oral extraction as well as adequate facilities, equipment and expertise to undertake repulsion or other minimally invasive extraction techniques should it be required.

Authors' declaration of interests

No conflicts of interest have been declared.

Ethical animal research

Research ethics committee oversight is currently required by this journal for 'some retrospective studies'. In this retrospective study of clinical records, informed owner consent was diligently sought and obtained from 17 out of 20 horses in the study; the remaining three owners were unable to be contacted.

Source of funding

None.

Authorship

Both authors approved the final version of the manuscript and contributed to study design, data collection and study execution, data analysis and interpretation and preparation of the manuscript.

Manufacturers' addresses

[1]Jorgensen Laboratories Inc., Loveland, Colorado, USA.
[2]Epica Medical Innovations, San Clemente, California, USA.
[3]Orion Corporation, Espoo, Finland.
[4]Bayer, Shawnee Mission, Kansas, USA.
[5]Pfizer Inc., New York, New York, USA.
[6]Bimeta Inc., Le Sueur, Minnesota, USA.
[7]Henry Schein Animal Health, Dublin, Ohio, USA.
[8]Zoetis, Kalamazoo, Michigan, USA.
[9]DenMat Holdings, Lompac, California, USA.
[10]Unichem pharmaceuticals (USA) Inc., Rochelle Park, New Jersey, USA.
[11]Nikon J1, Nikon Corporation, Shinagawa, Tokyo, Japan.
[12]U.S. National Institutes of Health, Bethesda, Maryland, USA.
[13]Microsoft Corporation, Redmond, Washington, USA.
[14]Bausch & Lomb Inc., Tampa, Florida, USA.

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Supporting information

Additional Supporting Information may be found in the online version of this article at the publisher's website:

Supplementary Item 1 Summary data for the removal of 22 displaced sagittal fractured cheek teeth.

Supplementary Item 2 Measurements of cheek teeth with sagittal fractures.

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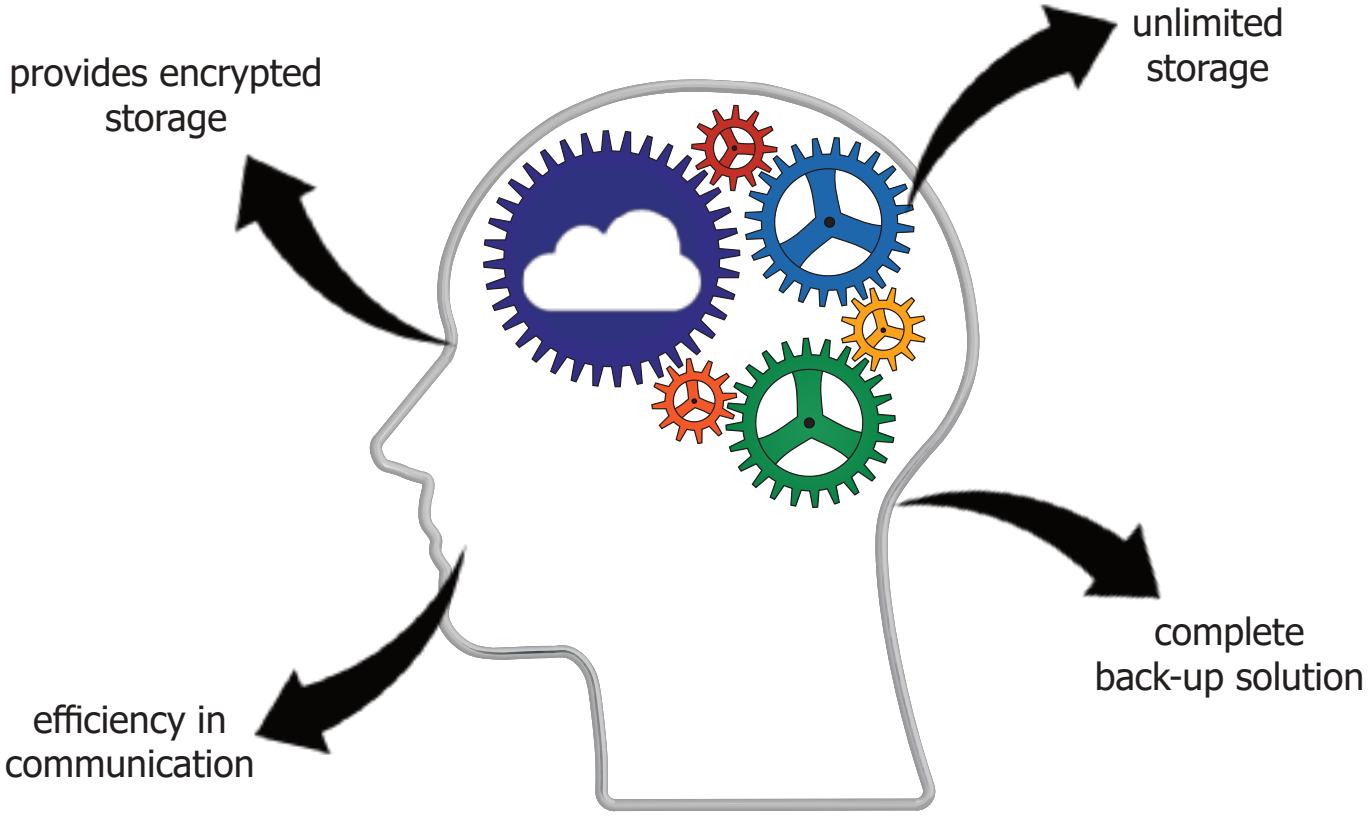

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EQUINE VETERINARY EDUCATION
Equine vet. Educ. (2019) **31** (8) 427-431
doi: 10.1111/eve.12816

Original Article

Comparison of the use of a braided multifilament transfixation suture for field castration with other castration techniques

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Keywords: horse; castration; complications; transfixation; suture

Summary

Complications with castrations occur commonly and are usually not life-threatening, with the exception of evisceration or haemorrhage, which are uncommon. Primary closure castration (closing deeper tissue layers and skin) or use of a transfixation ligature alone to prevent evisceration has been recommended. The goal of this study was to investigate the use of a closed technique with multifilament suture for transfixation of the spermatic cord alone in field castrations. The results of this study support our hypothesis that a closed castration technique with a multifilament, transfixing ligature (No. 2 polyglactin 910) did not result in additional post-operative complications when compared with a closed castration technique without ligature placement or a primary closure castration technique in a hospital setting.

Introduction

Castration is one of the most common surgical procedures performed in male horses. The procedure can be performed with the horses either standing under heavy sedation with local anaesthesia, or in recumbency under general anaesthesia. Open, closed and semiclosed surgical castration techniques are used for horses with or without primary wound closure (Cox 1984; Searle *et al.* 1999). Choice of anaesthetic and surgical technique vary based on the surgeon's preference, practice tradition, owner's desire, behaviour of the horse, descent of the testicles and surgery location (field or operating room) (Pleasant 1999).

Complications that have been reported following castration include scrotal swelling, oedema, haemorrhage, omental herniation, evisceration, penile trauma, bacterial infection of the spermatic cord (scirrhous cord formation or funiculitis), incisional infections, hydrocele formation and peritonitis (Thomas *et al.* 1998; Pleasant 1999; Searle *et al.* 1999). Certain breeds such as Standardbreds and draught horses have been reported to be more prone to omental herniation and evisceration following open and closed unsutured castration techniques (Shoemaker *et al.* 2004). Therefore, ligation of the spermatic cord or primary closure castration has been recommended to possibly reduce the risk of post-operative complications (Moll *et al.* 1995; Schumacher 1996, 2012). Primary closure castration typically requires referral to a surgical facility and is costlier than ligation of the spermatic cord alone (Mason *et al.* 2005). In a recent study, 6–9 months old colts castrated utilising a closed technique and a pretied monofilament suture under field conditions were reported to have low post-operative infection rates (Carmalt *et al.* 2008). Use of a monofilament transfixation suture and emasculators increased parietal tunic tensile strength and may reduce the risk of evisceration (Rijkhenuizen *et al.* 2013; Comino *et al.* 2016). Monofilament suture would be desirable to use under field conditions due to minimal tissue drag and a reduced risk of infection, but increased bending stiffness and memory are associated with poorer handling properties and reduced knot security (Kümmerle 2012), which could outweigh the benefits. Multifilament suture, particularly polyglactin 910, is easier to handle and less likely to break. Along with these properties, it has a greater initial breaking strength than monofilament suture (Kümmerle 2012), making it a more secure suture to use for castrations. Few reports outline surgical characteristics of suture type and post-operative complications in colts for castrations.

Therefore, the goal of this study was to investigate the use of a closed-castration technique with a multifilament suture for transfixation ligation under field conditions. We hypothesised that using multifilament (braided) suture material under field conditions (Group 1) would not result in an increase in post-operative complications compared with a closed field castration technique without suture (Group 2) or a primary closure castration approach in a hospital setting (Group 3).

Material and methods

Animals

A total of 90 horses were placed into three separate surgical castration groups (30 horses each). All colts were deemed healthy based on a physical examination and the scrotum was palpated to ensure both testicles were present prior to surgery.

Group 1 (n = 30) were part of a prospective randomised clinical trial about parallel field anaesthesia and were included in the closed castration with transfixation suture group (Sinclair *et al.* 2013). All procedures were approved by the Animal Care Committee, University of Guelph, and followed the Canadian Council on Animal Care Guidelines with owner consent. Surgeries were performed over a 2-year period in the spring and fall (2012 and 2013).

Other horses were included to compare surgical complications retrospectively as follows: medical records of horses undergoing castration at the Ontario Veterinary College (OVC) over a 3-year period (2012–2015) were

reviewed, and based on the type of castration performed, 30 horses were assigned to Group 2 (field control group) and 30 horses to Group 3 (primary closure castration).

Group 2 ('field' control group) included only horses that underwent routine field castration under injectable general anaesthesia either on a farm or in the OVC arena without any suture with the incisions left open to drain.

Group 3 included 30 horses that underwent primary closure castration in the operating room at the OVC Large Animal Referral Hospital as the 'cleanest environment' control group.

Surgical Procedures

Group 1 – closed castration with transfixation

Horses were placed in lateral recumbency on either straw, grass or sand with the upper leg tied. Liquid soap and water were used to wash the surgical field, then the area was dried with clean lap sponges. Depending on the anaesthesia research, the testicles were injected with lidocaine injection (lidocaine 2% HCl, 10–15 mL/testicle based on size) or an equal volume of saline (Sinclair *et al.* 2013). The testicles were pushed into the scrotum with one hand until the skin was taut. Using a scalpel, an incision was made parallel to the median raphe through the scrotal skin, tunica dartos and scrotal fascia until the parietal tunic was encountered. The testicle, still encapsulated by the parietal tunic, was grasped, and the scrotal fascia was 'stripped' (separated using a sterile gauze) from the parietal tunic until the cremaster muscle and tunic were fully exposed. In horses 2 years of age or older, the cremaster muscle was separated from the parietal tunic encasing the spermatic cord for a length of 3 or 4 cm and Serra emasculators were applied to the cremaster muscle and left in place for 30 s. Kocher forceps were then applied to the edge of the spermatic cord, close to the body wall. Approximately 3 cm distal to the body wall, a transfixation suture using No. 2 polyglactin 910 (coated VICRYL)[1] was applied to the spermatic cord (including the cremaster in horses under 2 years) in the following manner: For the single knot ligature, the suture material was fixed through approximately 2 mm of the vaginal tunic in order to prevent slippage of the suture, and held in place with a simple throw, and then the suture material was looped around the spermatic cord, fixed with a surgeon's knot, and followed by three more throws. Next, Serra emasculators[2] were applied approximately 1.5 cm distal to the transfixation suture and held in place for at least 2 min. In horses with large spermatic cords (either over 650 kg or active breeding stallions), the emasculator was held in place for 3 min and then removed. The surgical site was checked for bleeding, and the other testicle was castrated in the same fashion. All castrations were performed by one of two board certified surgeons.

Group 2 – unsutured castration

Horses were anaesthetised as described above. Horses were placed in lateral or dorsal recumbency based on surgeons' preference on either straw, grass or sand with the upper leg (lateral) or both legs (dorsal) tied. The same preparation and the same approach were used as described above for closed castration, except that no transfixation suture was placed. All castrations were performed by one of two board certified surgeons.

Group 3 – primary closure castration

For the in-clinic castrations, a 14-gauge i.v. jugular catheter (BD Insyte -W)[3] was routinely placed sterilely. All horses were medicated with xylazine (0.8–1 mg/kg bwt i.v.)[4] as required to move into the induction stall. Anaesthesia was induced with ketamine (2 mg/kg bwt i.v.)[5] and diazepam (0.02–0.04 mg/kg bwt i.v.)[6], and horses were orotracheally intubated and maintained on isoflurane (Isoflurane USP)[7] in oxygen via a circle rebreathing system. The surgical field was aseptically prepared, and after draping, scrotal skin incisions were made as described above and a closed castration technique with transfixation suture (absorbable suture material used was based on surgeons' preference) was used. After ensuring that there was no bleeding from the transected cords, the median raphe was resected. Two deep fascia/subcutaneous layers were sutured using a continuous suture pattern to obliterate dead space. Then, an intradermal suture pattern was used. An absorbable suture material used for the three layers was based on the surgeons' preference (Cox 1984). All castrations were performed by one of five board certified surgeons.

The following information was collected: age, breed, surgeon, position in surgery, volume of lidocaine injected into the testicle, medications used, surgery time in minutes, recovery score from anaesthesia and suture used. Follow-up post-operative information was obtained by telephone survey of owners or trainers (2014–2016) and included the following complications: scrotal swelling, excessive haemorrhage, omental herniation, intestinal herniation, incisional infection and septic funiculitis. Scrotal swelling was defined as swelling of the scrotal area/prepuce severe enough to require veterinary attention. Excessive haemorrhage was defined as post-operative bleeding requiring veterinary intervention by either packing of the surgical site or ligation of a vessel to stop the bleeding; incisional infection was defined as scrotal/preputial swelling severe enough to require opening of the incisions and antibiotic treatment by the treating veterinarian; septic funiculitis was defined as infection of the remainder of the spermatic cord requiring referral to a surgical facility and necessitating surgical removal of the stump and debridement (Mason *et al.* 2005; Schumacher 2012).

Statistical analysis

The data were analysed with SAS 9.4[8].

Variables included nominal (surgical technique, breed, surgeon, positioning during surgery (dorsal/lateral), type of suture, medications used) and discrete variables (duration of surgery in minutes, age in years, dose of lidocaine, number of complications). The recovery score (1–4) and most discrete variables were initially handled as continuous variables, but on occasion (e.g. age) grouped as categorical variables (e.g. age 1, 2, 3 +) for further analysis. Complications (scrotal swelling, haemorrhage, omental or intestingal herniation, incisional infection or scirrhous cord) were recorded in dichotomous fashion (not/observed) and summarised as number of complications and dichotomous complications (yes/no). Categorical observations were summarised with frequency statistics (PROC FREQ) or summary statistics for continuous variables (PROC MEANS). Fisher-exact tests for categorical variables (e.g. age group (1, 2, 3 +) and surgical approach) or ANOVA for continuous variables (e.g. surgical approach and duration of surgery). Alpha was set at ≤ 0.05.

Results

Post-operative complications are summarised in **Table 1** for all three groups.

Group 1 – closed castration with transfixation

Thirty horses of the following breeds were included in this group as follows; 11 Thoroughbreds, 9 Warmbloods, 3 draught horses, 3 ponies, 2 Quarter Horses and 2 Standardbreds. They were between 1 and 5 years of age (Mean age ± s.d.: 1.8 ± 1 years) and weighed between 250 and 650 kg (Mean kg ± s.d.: 395.3 ± 106.6 kg). All castrations were performed by two board certified surgeons. Eleven horses were positioned in right lateral recumbency and 19 in left lateral. Lidocaine was injected into the testicles of 21 horses with a volume range from 5 to 20 mL based on size; the remaining horses received saline (n = 9). All horses were injected with penicillin (Procaine Penicillin G; 20000 IU/kg bwt i.m.)[7] and phenylbutazone (Phenylbutazone 20% INJ; 4.4 mg/kg bwt i.v.)[9]. The duration of surgery ranged from 9 to 22 min, with a mean of 13.4 min. The transfixation suture was No. 2 polyglactin 910 in all horses. Most horses (n = 25) had a recovery score of 1 (excellent), while five horses had a recovery score of 2 (good). It was recommended to continue oral phenylbutazone treatment at home for 3–5 days, but owner compliance was not recorded.

Four out of 30 horses had a total of nine post-operative complications (one or more than one complication/horse; **Table 1**; 13.3%). In the three horses with post-operative signs of infection, two of them resolved with antibiotic treatment, and one continued to have issues necessitating surgical removal of the infected spermatic cord (septic funiculitis).

Group 2 – unsutured castration

Thirty horses of the following breeds were included in this group as follows: 8 Quarter horses, 8 Thoroughbreds, 5 Warmbloods, 5 ponies and 4 Standardbreds were included. They ranged in age from under 1 year to 6 years of age (mean age ± s.d.: 2.6 ± 1.6 years) and weighed between 220 and 550 kg (mean kg ± s.d.: 448 ± 116.3 kg). Right lateral positioning was used in 14 horses, and dorsal and left lateral in eight horses each. All horses except for two had lidocaine injected into the testicles, and volume ranged from 5 to 30 mL. All horses were given phenylbutazone (4.4 mg/kg bwt i.v.) except one, which received flunixin meglumine (Banamine; 1.1 mg/kg bwt i.v.)[10]. Horses received either i.m. penicillin (n = 22), i.v. trimethoprim sulfa (n = 4; 24 mg/kg bwt i.v.), i.v. gentamicin (n = 2; Gentocin; 6.6 mg/kg bwt i.v.)[11] or no prophylactic antibiotics (n = 2). Surgery time ranged from 7 to 21 min with a mean of 12.6 min. A recovery score of 1 (n = 18), 2 (n = 10), 3 (n = 1) and 4 (n = 1) was noted. It was recommended to continue oral phenylbutazone treatment at home for 3–5 days, but owner compliance was not recorded.

Three out of 30 horses had a total of eight post-operative complications (one or more than one complication/horse; **Table 1**; 10%).

Group 3 – primary closure castration

The majority of cases in this group were Standardbreds (with exception of one draught horse). They were between 1 and 6 years of age (mean ± s.d.: 2.3 ± 0.9 years) and weighed between 250 and 575 kg (Mean kg ± s.d.: 411.3 ± 82.8 kg). All horses were positioned in dorsal recumbency on a surgery table. Lidocaine was injected into the testicles in 12 of the horses, with a volume ranging from 8 to 40 mL. Flunixin meglumine was administered to 21 horses, and nine received phenylbutazone. All horses received i.v. penicillin (Penicillin G Sodium; 20 000 IU/kg bwt)[7], and seven horses additionally received gentamicin. Surgery time ranged from 40 to 105 min (mean ± s.d.: 71 ± min). The transfixation suture of the spermatic cord was done with braided suture material in 18 horses and monofilament in 10 horses (see **Table 2**). Transfixation suture type was not recorded in two horses. For the two fascia/subcutaneous layers and the intradermal sutures, monofilament was used in 28 horses and braided suture in two (see **Table 2**). Recovery scores of 1 were recorded in 21 horses and of 2 in nine horses. It was recommended to continue oral phenylbutazone treatment at home for 3–5 days, but owner compliance was not recorded.

Seven out of 30 horses had a total of 14 post-operative complications (one or more than one complication/horse; **Table 1**; 23.3%). In **Table 1**, five horses are listed with post-operative infection, three resolved with medical therapy, but two needed surgical removal of the infected cord.

Group comparisons

More 2-year-old Standardbred horses underwent primary closure castrations in the hospital (Group 3) than any other age group ($P<0.01$) or breed ($P<0.01$). In addition, significantly more horses were placed in dorsal recumbency (n = 38) than in left (n = 27) or right (n = 25) lateral recumbency, with all the primary closure cases being placed in dorsal recumbency ($P<0.01$). Primary closure castrations took longer (71 ± 16.9 min) than transfixed closed (13.4 ± 2.7 min) or unsutured closed (12.6 ± 4.3 min) castration ($P<0.01$). However, no significant difference was noted between groups for lidocaine volume injected into the testicle, surgeon, medications used or recovery score from anaesthesia.

The proportion of post-operative complications was 3.3% of the overall study population, and did not differ statistically between the three groups ($P = 0.44$). A recovery score of ≥3 was only observed in two horses but was significantly associated with having post-operative complications ($P<0.01$). Regardless of the group, horses experiencing haemorrhage were 13 times more likely to develop scrotal swelling then those without (OR 13.8; 95% CI: 1.2–165.4; $P = 0.053$). The type

TABLE 1: Post-operative castration complications encountered for each surgery group; Group 1: Closed castration with transfixation; Group 2: Unsutured castration; Group 3; Primary closure castration

Complications	Swelling	Haemorrhage	Omental herniation	Intestinal herniation	Incisional infection	Septic funiculitis	Group total
Group 1	4	1	0	0	3	1	9
Group 2	2	2	1	0	2	1	8
Group 3	7	0	0	0	5	2	14
Total numbers	13	3	1	0	10	4	31

TABLE 2: Detailed description of type of suture used for primary closure castration

Suture type Suture size	Transfixation suture No. 1 or No. 2	Deep tissue layers No. 2.0 or No. 3.0	Intradermal No. 2.0 or No. 3.0
Lactomer 9-1/ Polyglactin (Polysorb®)[12]	18	2	2
Glycomer 631 (Biosyn®)[12]	3	26	24
Polyglyton 6211 (Caprosyn®)[12]	2	2	4
Polydioxanone (PDS®)[13]	5	0	0

of suture used (braided, monofilament or no suture) did not influence the occurrence of post-operative complications in this study.

Discussion

This is the largest study to date to compare the occurrence of post-operative complications of three different castration techniques. The results of our study support our hypothesis that using a closed castration technique with transfixation using a multifilament suture material (No. 2 polyglactin 910) under field conditions did not result in more post-operative complications than a closed field castration technique without suture or a primary closure castration in a hospital setting. In fact, almost twice the number of horses in the primary closure castration group (Group 3) had post-operative complications (n = 7 in comparison to the field castrations Group 1; n = 3 and Group 2; n = 4, respectively), particularly swelling of the surgical site. Unfortunately, the statistical power of our study was too low to detect a significant difference between complications in the three groups as a power analysis indicates that 200 horses per group would be necessary to detect this 11–13% risk difference in post-operative complications. Despite the overall lack of significance in our study for specific complications, application of a transfixation suture did not prolong surgery or anaesthesia time, and anaesthetic recovery scores were comparable between Group 1 and Group 2 with injectable anaesthesia. Considering this, transfixation is a viable option for equine practitioners under field conditions as a strategy recommended to reduce the risk of serious post-operative complications such as evisceration (Moll *et al.* 1995; Schumacher 1996, 2012). However, this recommendation is only applicable to horses being anaesthetised under field condition. An evaluation of the transfixation technique in castrations performed under standing sedation, would provide additional useful information.

It is important to consider the option of transfixation for field castrations, especially for draught and Standardbred horses which reportedly have higher rates of evisceration (Moll *et al.* 1995; Schumacher 1996), as there are disadvantages to primary closure castrations. A primary closure castration is significantly more expensive and takes a significantly longer time to perform than either transfixed closed or unsutured closed castration. With an increased length of surgery time over 60 min, supplemental oxygen or general inhalant anaesthesia is recommended to support hypoventilation and the development of hypoxaemia reported in horses with prolonged injectable techniques on room air (McCarty *et al.* 1990; Mama *et al.* 2005). Consequently, an increase in anaesthetic complications with inhalational anaesthesia compared to injectable anaesthesia may arise (Johnston *et al.* 2002). Also, a longer surgery time may increase the risk of infection and provide an explanation why post-operative infection rate in Group 3 was so much higher.

In Group 1, the closed castration with transfixation had three horses with post-operative signs of infection. Two of them resolved with antibiotic treatment, and one continued to have issues necessitating surgical removal of the infected spermatic cord (funiculitis). Interestingly, even though statistically not significant, in the primary closure castrations (Group 3) performed in the operating room, were five horses with post-operative infection, and three resolved with medical therapy, but two needed surgical removal of the infected cord. Post-operative infection rates were reported to range from 2.1% in castrations performed in an operating theatre under sterile conditions to 20.7% in standing castrations (Mason *et al.* 2005). There are only minor differences in technique that do not explain why the post-operative infection rate in Group 3 was so much higher than reported by described by Mason *et al.* 2005. In the former study cat gut was used instead of Vicryl to transfix the spermatic cord, but the other tissue layers in our study were sutured with similar material and suture pattern, except for the skin in Masons report, which was only sutured with three interrupted sutures instead of a continuous pattern. In one report where researchers used a questionnaire to obtain details about castration technique and post-operative complications in 23,229 horses, respondents felt that there was a significant rate of infection (13.5%) in using a ligature versus no ligature (2.8%) (Moll *et al.* 1995). This was only the impression of the 31 respondents that routinely used a suture around the spermatic cord and it is unclear what surgical castration technique was used in these cases and if this was performed standing or recumbent.

Standardbred horses (except for one draught colt) were the main breed in our primary closure castration Group 3, which is not surprising as Standardbred or draught horses are the breeds most commonly referred for primary closure castration at our clinic. In Standardbreds, an excessive size of the inguinal rings was perceived to be responsible for a higher rate of evisceration but was reported to only occur in less than 1% of horses (Moll *et al.* 1995). Furthermore, a recent study in draught foals reported a much higher rate of evisceration (4.8%) independent of open or closed castration technique (Shoemaker *et al.* 2004). A recent ex vivo study showed that use of a transfixation technique in closed castration resulted in significantly higher parietal tunic tensile strength and should be used to reduce the risk of evisceration (Comino *et al.* 2016). Similarly, a technique using a pretied loop (4S modified Roeder knot) of 1 polyglyconate (Maxon) placed over the emasculator and tightened around the common vaginal tunic and attached cremaster muscle (Carmalt *et al.* 2008), reduced the incidence of post-castration evisceration in draught foals (Carmalt *et al.* 2008).

Another controversy exists about whether the placement of a transfixation suture can reduce the risk of post-operative haemorrhage. A transfixation knot (Rijkhenuizen *et al.* 2013) or modified Roeder knot (Carmalt *et al.* 2008) has been recommended as the optimal method to use in castration.

For example, it has been reported that the blood vessels of the spermatic cord of donkeys are larger than in horses and a ligature around the spermatic cord could prevent haemorrhage (Sprayson and Thielmann 2007). Similar to previous reports, the frequency of post-operative haemorrhage was reported as 3.3% in this study. In our study, three out of 90 horses experienced haemorrhage. None of the horses in the primary closure castration group haemorrhaged, while one horse in the transfixed group and two in the closed group experienced this complication. Unsurprisingly, horses with haemorrhage had 13 times the odds of developing scrotal swelling than those without.

Higher infection rates are reported when ligatures around the spermatic cord were used (Moll *et al.* 1995). Therefore, monofilament suture material has been recommended for use under field conditions due to the decreased risk of infection and reduced tissue drag (Carmalt *et al.* 2008; Kümmerle 2012; Rijkhenuizen *et al.* 2013), but the disadvantage of monofilament suture is multifilament suture is easier to handle and less likely to break, particularly polyglactin 910 has a greater breaking strength (Carpenter *et al.* 2006; Kümmerle 2012). Considering this, we chose No. 2 polyglactin 910 for transfixation of the spermatic cord in our methods.

In conclusion, the use of a transfixation suture with No. 2 polyglactin 910 is a viable option for field castrations under general anaesthesia to minimise risk of evisceration without evidence of a higher infection rate after castration. While infection rates were low in our study and did not differ significantly between groups, examination of additional cases would help confirm the finding that use of a transfixation ligature does not significantly increase the risk.

Authors' declaration of interests

No conflicts of interest have been declared.

Ethical animal research

The procedures (anaesthesia and castration) of horses used in Group 1 followed the standards and were approved by the Animal Care Committee of the University of Guelph.

Source of funding

None.

Authorship

J. Koenig and M. Sinclair contributed to study design, study execution, data analysis and interpretation, and preparation and final approval of the manuscript. U. Sorge contributed to data analysis and interpretation, and preparation and final approval of the manuscript.

Manufacturers' addresses

[1]Ethicon, Somerville, New Jersey, USA.
[2]Jorgenson Laboratories, Loveland, Colorado, USA.
[3]BD Vialon, Mississauga, Ontario, Canada.
[4]Bimeda Canada, Cambridge, Ontario, Canada.
[5]Vetoquinol N.A. Inc., Georges Lavaltrie, Quebec, Canada.
[6]Sandoz Canada Inc. (Novartis Company), Boucherville, Quebec, Canada.
[7]Fresenius Kabi Canada Ltd., Richmond Hill, Ontario, Canada.
[8]SAS Institute, Cary, North Carolina, USA.
[9]Rafter 8 Products Inc, Calgary, Alberta, Canada.
[10]Pfizer, Kirkland, Quebec, Canada.
[11]Merck Intervet Canada Corp, Kirkland, Quebec, Canada.
[12]Covidien Canada 8455 Trans-Canada Highway Saint-Laurent, Québec, Canada.
[13]Johnson & Johnson Medical Company (Ethicon), Mississauga, Ontario, Canada.

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EQUINE VETERINARY EDUCATION
Equine vet. Educ. (2019) **31** (8) 432-440
doi: 10.1111/eve.12757

Review Article

Local anaesthetic techniques for the equine head, towards guided techniques and new applications

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Keywords: horse; anaesthetic; head; perineural nerve blocks; ultrasound

Summary

Perineural nerve blocks are often used in equine practice, especially since the use of diagnostic and surgical procedures in the standing sedated horse have expanded over recent decades. The purpose of this review is to discuss the different perineural nerve blocks for the equine head. The review starts with the currently most used blind approaches as described in textbooks and scientific studies. In human medicine, the role of guided techniques, such as ultrasound guidance, advanced imaging guidance and nerve stimulator guided techniques, is very extensively described. These techniques are promising to use in equine medicine as well. The first studies that describe these techniques in equine cases are also discussed in this review, as well as the possibilities for neuromodulation in equine pain syndromes like equine trigeminus-mediated headshaking and the role of perineural nerve blocks in diagnosing this syndrome.

Introduction

In modern equine veterinary practice, surgical and diagnostic procedures in the standing sedated horse are expanding, especially with the growing interest in minimally invasive surgery (Dixon *et al.* 2005; Coomer *et al.* 2011; De Linde Henriksen and Brooks 2014; Menzies and Easley 2014). Reliable and stable sedative planes are very important and various studies have been performed to assess the effects of sedative and analgesic pharmaca (Ringer *et al.* 2013; Marly *et al.* 2014). Both in the sedated horse and in the horse under general anaesthesia, the beneficial effects of locoregional techniques (diminished levels of sedation or anaesthesia, prevention of harmful reflexes and pre-emptive analgesic effects) are clearly described (Ong *et al.* 2005; Oel *et al.* 2014). Furthermore, in the standing equine patient it is especially important to provide a reliable local anaesthetic block regarding the safety of the horse, the veterinary surgeon and animal handlers.

Previous reviews have very extensively described the use and anatomical landmarks for all relevant local anaesthetic techniques for the equine head (Tremaine 2007; Labelle and Clark-Price 2013). Experience of the performer is very important in the success rates of local anaesthetic techniques, as was shown by Wilmink *et al.* (2015) for the perineural block of the maxillary nerve. Although blind techniques used to be the gold standard, nowadays ultrasound-guided techniques are quickly being adopted. They are broadly used in human medicine where there has been a rapid development in different ultrasound-guided approaches in recent years (Helayel *et al.* 2007). Ultrasound-guided local anaesthetic techniques enhance both the quality and duration of peripheral-nerve blockade and reduce the incidence of complications. Meta-analyses comparing ultrasound-guided local anaesthetic techniques to blind techniques in man have shown that ultrasound guidance reduces complication rates and improves quality of the block (both for sensory and motor blocks); the technique further reduces performance time and results in quicker onset of the block in peripheral nerve blockade in adult humans (Walker *et al.* 2009; Lewis *et al.* 2015). Furthermore, ultrasound guidance reduces the incidence of local anaesthetic systemic toxicity (neuro- and cardiotoxicity consequent to unintended intravascular injection or delayed tissue uptake; El-Boghdadly and Chin 2016; Neal 2016) and unintended paresthesias (Soeding *et al.* 2005). For infants, a recent systematic review by Guay *et al.* (2016) showed that ultrasound guidance improved the success rate and duration of perioperative neuraxial and peripheral local blocks. Additional data are required to assess the potential effect of ultrasound guidance on reducing the rate of inadvertent puncture of blood vessels. Besides these advantages, ultrasound guidance was found to improve the learning curve of clinicians and it has been stated that the technique should have a role in future training (Marhofer *et al.* 2005). To date, the role of ultrasound-guided techniques and advanced imaging is still limited in the performance of local nerve blocks in the equine patient.

This review discusses the perineural nerve blocks for the equine head and describes blind approaches as well as those guided by ultrasound, nerve stimulation, or advanced imaging techniques and compares human and equine literature. As perineural techniques can also have diagnostic and therapeutic value, the possibilities for neuromodulation in equine pain syndromes such as equine trigeminus-mediated headshaking and the role of perineural nerve blocks in diagnosing this syndrome are also discussed.

Perineural nerve blocks for the equine head: classic approach

The most commonly used local anaesthetic techniques for the equine head are well described in various review articles (Tremaine 2007; Labelle and Clark-Price 2013) and veterinary textbooks (Easley *et al.* 2011; Gilger 2011). Perineural nerve blocks are often used in equine dentistry and equine ophthalmology. **Figure 1** shows the four anatomical locations for the techniques most often used in equine dentistry: the

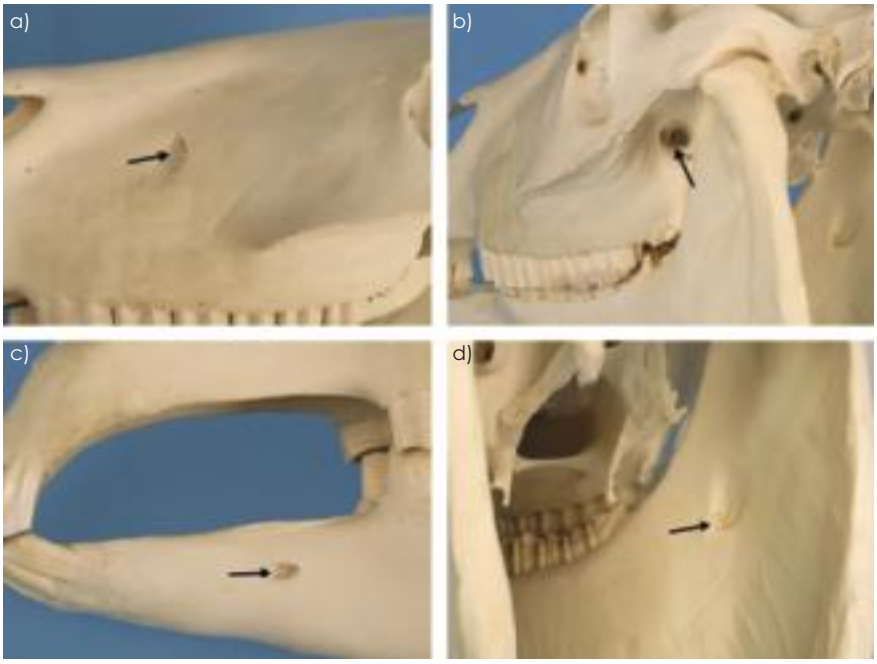

Fig 1: Most important anatomical structures for nerve blocks used in equine dentistry. a) Infraorbital foramen for infraorbital nerve block, b) caudal entrance of infraorbital canal for maxillary nerve block, c) mental foramen for mental nerve block and d) mandibular foramen for inferior alveolar nerve block.

maxillary, mandibular, infraorbital and mental nerve blocks with their relevant anatomical foramina. The infraorbital and mental nerves are desensitised outside the infraorbital and mental foramina for procedures involving the mandibular and maxillary soft tissues, such as treatment of soft tissue trauma in these areas. For painful procedures involving the incisor teeth (such as extraction in equine odontoclastic tooth resorption and hypercementosis), mandibular or maxillary fractures of the incisive (premaxillary) bone or extraction of the first cheek teeth, the infraorbital or mental nerve should be desensitised inside the respective foramina because of branching nerves to these structures. **Figure 2a** shows the localisation of the infraorbital foramen, relative to the surrounding structures (rostral edge of the facial crest and nasoincisive notch).

Maxillary nerve block

Several techniques for the maxillary block have been described in various studies: Bardell *et al.* (2010) described two approaches for the maxillary nerve block in equine cadavers with different orientations of the needle (perpendicular vs. angled needle placement in relation to the skin). Staszyk *et al.* (2008) compared a superficial approach (superficial advancement of the needle into the extraperiorbital fat cushion underneath the masseter muscle) to a deep approach (deep advancement of the needle into the pterygopalatine fossa of the palatine bone). The advantage of the superficial technique is the decreased risk of haemorrhage, because the major vascular structures (infraorbital artery, deep facial vein and descending palatine artery) are located close to the palatine bone.

Nannarone *et al.* (2016) described a retrograde maxillary nerve perineural injection within the infraorbital canal towards the maxillary foramen using a Tuohy needle, thereby avoiding the periocular region and possibly the described complications (Tremaine 2007; Staszyk *et al.* 2008).

For surgical procedures involving the paranasal sinuses and the nasal cavity, a maxillary nerve block will in some instances not be sufficient due to sensory innervation of these structures by the ophthalmic branch of the trigeminal nerve

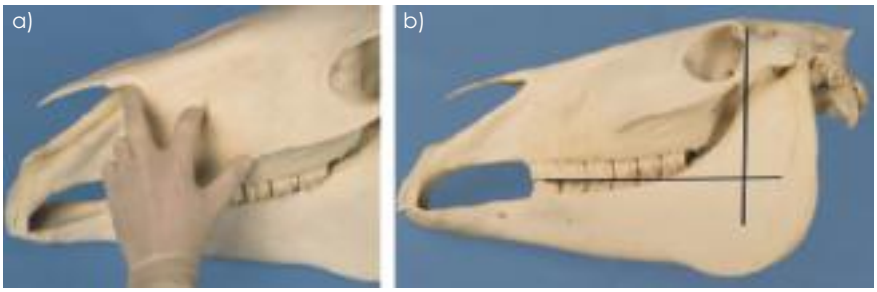

Fig 2: Anatomical localisation of the infraorbital and mandibular foramen. a) Anatomical structures (rostral edge of the facial crest and nasoincisive notch) and handsetting to determine the infraorbital foramen. b) Determination of position of the mandibular foramen using perpendicular lines through occlusion of the maxillary and mandibular cheek teeth and the lateral canthus of the eye.

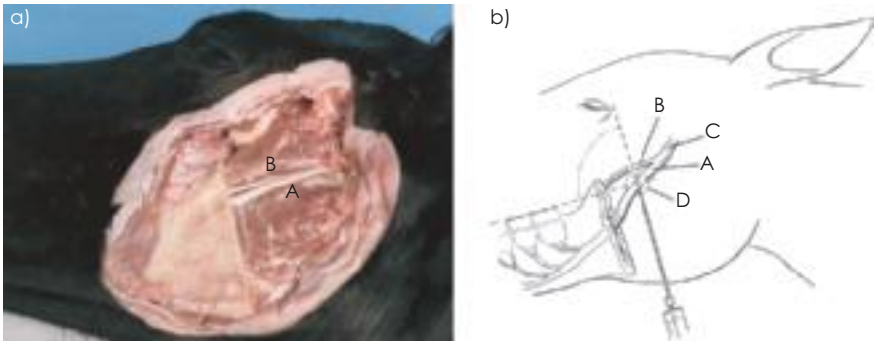

Fig 3: Localisation of the lingual and inferior alveolar nerves. a) In-situ anatomical orientation of inferior alveolar nerve (A) and lingual nerve (B). The latter branches off the mandibular nerve (C) before it enters the mandibular foramen (D) as the inferior alveolar nerve. b) Schematic orientation of both nerves. Figure 3b reproduced with permission by Caldwell and Easley (2012).

and an additional block of the nerve in addition to the maxillary nerve block can be beneficial in these cases. Caruso III *et al.* (2016) described a technique to desensitise the ethmoidal nerve. The ethmoidal nerve branches off the nasociliary nerve, which proceeds as the infratrochlear nerve. The ethmoidal nerve is blocked at the rostromedial aspect of the supraorbital fossa and the technique has proven to be reliable and simple.

Inferior alveolar nerve block

The mandibular or inferior alveolar nerve block has been described in various studies. Harding *et al.* (2012) described two extraoral approaches (vertical vs. angled technique), while Henry *et al.* (2014) described an intraoral technique. In the latter, a custom-made device inserted into the mouth is used to anaesthetise the inferior alveolar nerve with a relatively small volume of local anaesthetic (5 mL). This alternative technique could decrease the risks of side-effects of this block such as self-inflicted trauma of the tongue due to accidental desensitisation of the lingual nerve that branches off the mandibular nerve very close to the mandibular foramen (**Fig 3**). Several cases of this self-inflicted tongue trauma after bi- and unilateral inferior nerve blocks have been described by Caldwell and Easley (2012). Localisation of the mandibular foramen on the medial side of the mandible can be realised using the perpendicular lines passing through the occlusal surface of the maxillary and mandibular cheek teeth and the lateral canthus of the eye (Tremaine 2007; **Fig 2b**). Harding *et al.* (2012) determined the accuracy of this technique of localising the mandibular foramen using radiography, revealing that the mandibular foramen was consistently located in close proximity to the intersection of these perpendicular lines. The described topographical landmarks were found to be accurate in locating the mandibular foramen (Harding *et al.* 2012). The relative position of the lingual and the inferior alveolar nerves is shown in **Figure 3**.

Ophthalmic nerve blocks

For ophthalmic procedures, various blocks can be used to enable minor and major surgical procedures in the standing horse or in the horse under general anaesthesia. The retrobulbar block (Tremaine 2007; Labelle and Clark-Price 2013) can effectively be used in surgical procedures such as enucleation and for minor procedures such as intraocular injection of tissue plasminogen activator in cases with recurrent uveitis with fibrin formation in the anterior chamber or for episcleral placement of cyclosporin implants. With the retrobulbar block, the oculomotor, trochlear and abducens nerves are desensitised, resulting in paralysis of all the straight and oblique ocular muscles, and the retractor bulbi muscles, which leads to a stable forward-positioned eye. Additionally, the ophthalmic and maxillary branches of the trigeminal nerve and the optic nerve are blocked, providing desensitisation of the eye and ocular adnexa. **Figure 4** shows the 'diamond block', which comprises the supraorbital, lacrimal, infratrochlear and zygomaticofacial nerves. This block results in desensitisation of respectively the medial two-thirds of the superior eyelid, the temporal canthus of the eye, nasal eye canthus and the temporal 75% of the inferior eyelid. The block is useful for surgical repair of eyelid lacerations or several diagnostic procedures of the eye.

Ultrasound-guided local anaesthetic techniques for the equine head

In modern human and veterinary clinical anaesthesiological practice, ultrasonography is becoming a more important technique that improves accuracy and safety of local anaesthetic techniques. Ultrasound can help to determine the exact location of the needle placement relative to the anatomical landmarks and the peripheral nerve that is aimed for. By means of ultrasound guidance, the amount of local anaesthetic needed to desensitise a nerve can be minimised due to the close proximity of the needle in relation to the peripheral nerve. The resultant influence on volume and concentration of the local anaesthetic at the level of the nerve improves the anaesthetic block quality in terms of time of onset and duration of effect. Ultrasound can also help to determine anatomical structures such as adjacent blood vessels, hence reducing the risk of side-effects such as haemorrhage from puncturing a vessel or inadvertent injection of the nerve. On ultrasound, nerves appear as single or multiple round or oval hypoechoic areas surrounded by a relatively hyperechoic area in the transverse scanning orientation (Alexander and Dobson 2003). In the longitudinal view, the nerve presents as a hyperechoic band characterised by multiple discontinuous hypoechoic stripes separated by hyperechoic lines, creating a fascicle pattern

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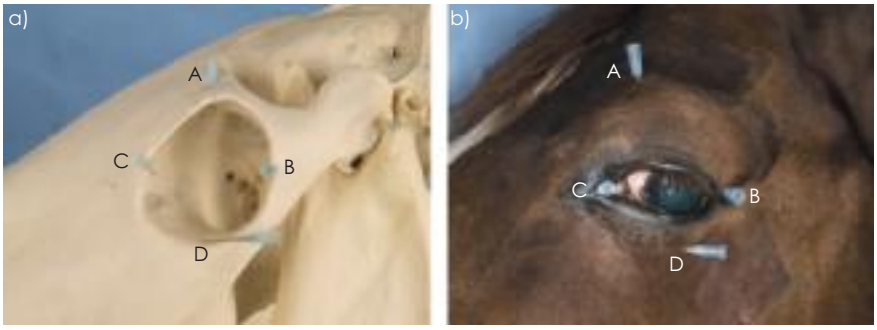

Fig 4: Diamond block. Needle setting on a) skull and b) head for diamond block (A, supraorbital nerve; B, lacrimal nerve; C, infratrochlear nerve; D, zygomatofacial nerve).

(Beukers *et al.* 2016). The hyperechoic structures are the fascicles of the nerves; the hypoechoic background reflects the connective tissue between neuronal structures. The nerve's image is sensitive to the angle of insonation because of the presence of fat (Re *et al.* 2016).

Maxillary nerve block

Ultrasound guidance for the maxillary nerve block has been described both in man (Bouzinac *et al.* 2014) and in horses (O'Neill *et al.* 2014). In the study by Bouzinac *et al.* (2014) efficacy of the block was not tested by pinpricks since the patients were under general anaesthesia, but ultrasound guidance allowed for accurate placement of the needle by visible spreading of the local anaesthetic into the pterygopalatine fossa. O'Neill *et al.* (2014) described the technique both in equine cadavers and in a number of clinical cases in a standing procedure. Ultrasound permits identification of the bony landmarks of the pterygopalatine fossa that can guide the needle towards the maxillary nerve and determination of the position of the needle relative to the greater vascular structures accompanying the maxillary nerve (deep facial vein, infraorbital artery, descending palatine artery). Puncturing of these vascular structures can cause serious side-effects such as temporary blindness, haemorrhage and severe swelling. Ultrasonography can be very helpful in improving the safety and efficacy of maxillary nerve blocks (**Fig 5**) and has proven to be an accurate and precise technique without any complications in clinical cases (O'Neill *et al.* 2014), compared to the blind technique (Bardell *et al.* 2010). Colour flow Doppler can be very useful

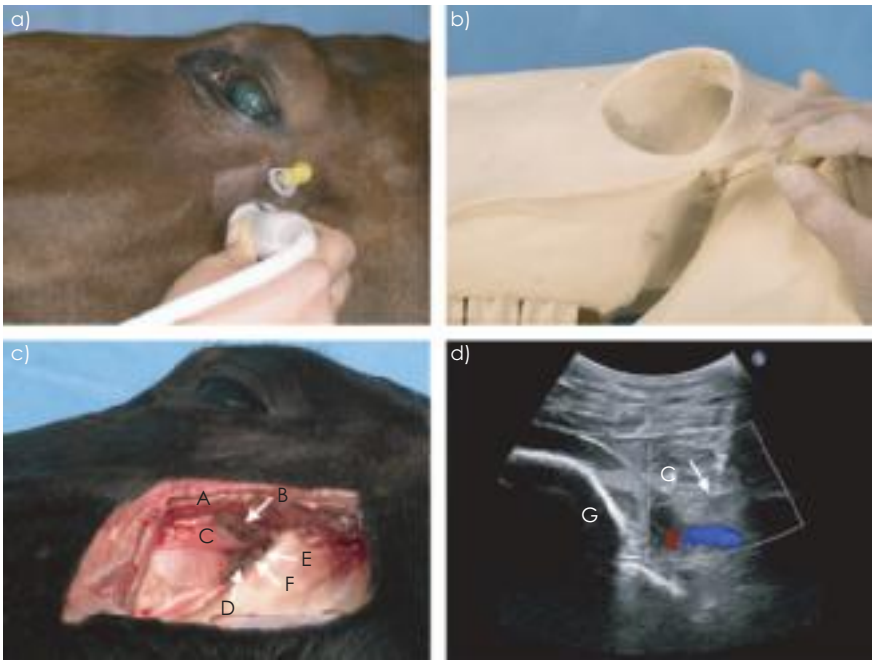

Fig 5: a) and b) relative positioning of the ultrasound probe and the needle for ultrasound-guided maxillary nerve block. c) masseter muscle (A) and underlying extraperiorbital fat tissue (B) with the deep facial vein (C) embedded in the extraperiorbital fat tissue and the maxillary nerve situated deeper (D), in close proximity of the infraorbital artery (E) and the descending palatine artery (F). d) Ultrasound with colour Doppler image showing the maxillary nerve at the needle tip (white arrow) and associated vascular structures (C, deep facial vein, red and blue Doppler traces are infraorbital artery and the descending palatine artery; G, tuberosity of the maxillary bone).

as well to identify vascular structures and is often used in human patients (Marhofer *et al.* 2005), although blood flow or pulsation can often easily be identified in horses without the use of colour flow Doppler (O'Neill *et al.* 2014).

Inferior alveolar nerve block

In man, the use of ultrasound guidance has been described for the desensitisation of the inferior alveolar nerve block (Hannan *et al.* 1999; Chanpong *et al.* 2013). In order to visualise the inferior alveolar nerve that runs medial of the mandibular ramus, the ultrasound probe is placed intraorally medial to the mandibular ramus (Hannan *et al.* 1999; Chanpong *et al.* 2013). Introduction of the technique has substantially improved outcome compared to the formerly used blind technique (Chanpong *et al.* 2013), of which failure rates were as high as 60% (Montagnese *et al.* 1984) and this position of the ultrasound probe helps to determine the inferior alveolar nerve. In literature, failure rates of up to 62% for the inferior alveolar block have been described in older human studies (Montagnese *et al.* 1984), greatly caused by anatomical variation and blind needle placement. Ultrasound guidance may improve the outcome of the inferior alveolar nerve blocks (Chanpong *et al.* 2013) and, at the same time, it could decrease the risk of potential side effects such as vascular punctures. This technique seems rather impractical in the equine head because of the long mouth, but, alternatively, ultrasound guidance could potentially be applicable in the equine head as well with a modified approach from the ventromedial aspect of the mandibular ramus. This technique has not yet been described in the horse and may prove impractical due to the deep location of the nerve.

Retrobulbar block

In man, ultrasound-guided retrobulbar blocks have been described by Luyet *et al.* (2008). They used a cranial approach and ultrasound guidance allowed the needle tip to be advanced up to 2 mm from the optic nerve. No side effects such as contrast injection into the eyeball or into the optic nerve were seen. For ultrasonography of the retrobulbar space by the transbulbar approach, curvilinear array transducers are most suitable at low to intermediate frequencies of 3–8 MHz. Phased array or linear array transducers can be used, but produce a less optimal image for needle guidance. Two indices, thermal index (TI) and mechanical index (MI), are denotive of heat and mechanical agitation that are generated by every ultrasonographic transducer. Low TI- and MI-values are preferred for ultrasonography of the eye (Morath *et al.* 2013). For the human eye, the maximally allowed TI is 1.0 and the maximally allowed MI is 0.23 (these values are much lower for the eye compared to other tissues).

In the horse, the technique of the ultrasound-guided retrobulbar block has been explored in a cadaver study by Morath *et al.* (2013). The effect of the volume that was injected was assessed, as was desensitisation of the orbital fissure after both intra- and extraconal injection of contrast medium. The study used a caudal (supraorbital) approach (comparable to the technique shown in **Fig 6**) and the spread of contrast medium was evaluated by computed tomography (CT). Needle placement within the cone formed by the retractor bulbi muscles was found to lead to a more effective spread of the injected fluid towards the orbital fissure and the subjective evaluation of ultrasound performance appeared to correlate well with the results of

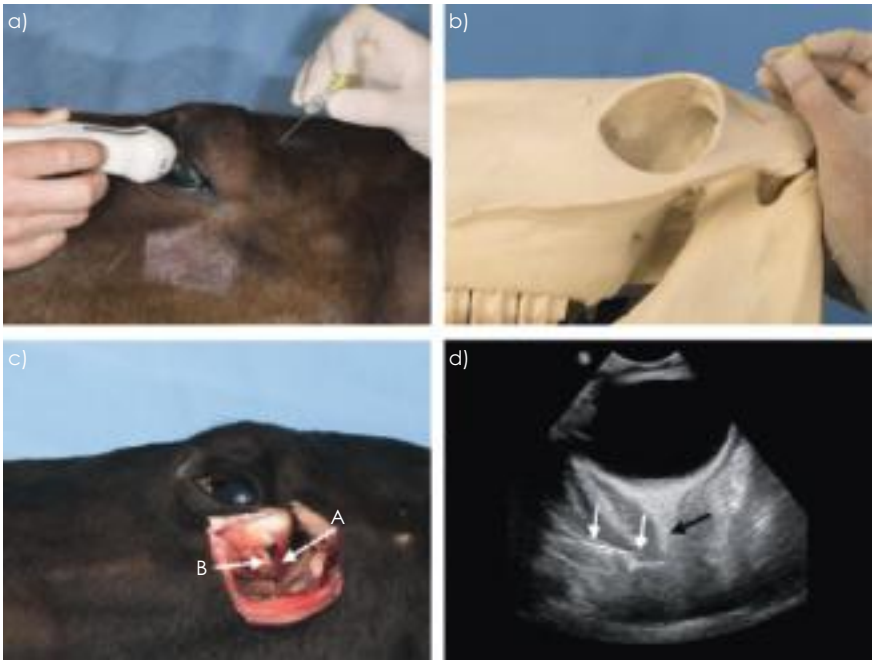

Fig 6: Ultrasound-guided retrobulbar nerve block. a) Positioning of the ultrasound probe and the needle on the head. b) Needle placement with respect to the bony landmarks. c) Extrinsic straight eye muscles (A) within the covering fascia (cone) and the optic nerve in the centre of the straight muscles (B). d) Ultrasound image of retrobulbar needle placement and associated structures (white arrows show needle placement with tip at the height of the right arrow, black arrow shows the optic nerve).

the CT images. Toth and Hollerrieder (2013) also have described an ultrasound-guided retrobulbar block in horses with a similar approach (**Fig 6**), followed by CT assessment of local anaesthetic spread. They describe the use of Tuohy needles to advance a catheter into the retrobulbar intraconal location for repeated injections.

Internal auricular nerve block

To enable otoscopic examination of the equine external ear canal including the tympanic membrane in sedated standing horses, the internal auricular nerve block can be performed. Sommerauer *et al.* (2012) provided a detailed anatomical dissection of the equine external ear canal and its nerve supply and described anaesthesia of the equine external ear canal by desensitising the internal auricular nerve. Ultrasound-guided localisation of the styloid process of the auricular cartilage was described as a reliable landmark for desensitisation of the internal auricular nerve, which is a branch of the facial nerve that provides most of the sensory innervation to the equine external ear canal.

Advanced imaging guided procedures

The role of modern imaging techniques, such as CT and magnetic resonance imaging in equine medicine, has expanded in the last decades and these techniques are becoming more widely available for clinical use. For visualisation of the equine skull and the related perineural anaesthetic techniques, CT imaging can especially be very helpful as has been stressed in various review studies (Porter and Werpy 2014; Manso-Díaz *et al.* 2015). The introduction of sliding gantry CT scanners that allow for standing procedures in sedated horses without exposure of personnel, has further increased the possibilities to use this technique without significant risks for the patient (such as complications related to general anaesthesia). These imaging techniques are very useful for assisting with locoregional techniques as well. This clinical application is still under development and should be explored further.

Tomaszewska *et al.* (2014), used a CT scan to determine anatomical landmarks for localisation of the greater palatine foramen in man. CT scans have been used to compare two different techniques for maxillary nerve blocks in horses and yielded very useful information on anatomical landmarks for these techniques (Staszyk *et al.* 2008). These authors assessed a modified superficial (extra periorbital fat body) technique, in which the needle is not inserted up to the palatine bone (where the maxillary nerve is located) and showed it to be effective and safer than the more conventional palatine bone technique. The cadaveric and CT images from this study provide invaluable information about the regional anatomy of the maxillary nerve in relation to accompanying structures such as blood vessels (**Fig 7**). CT has also been used to aid maxillary nerve block placement in a human

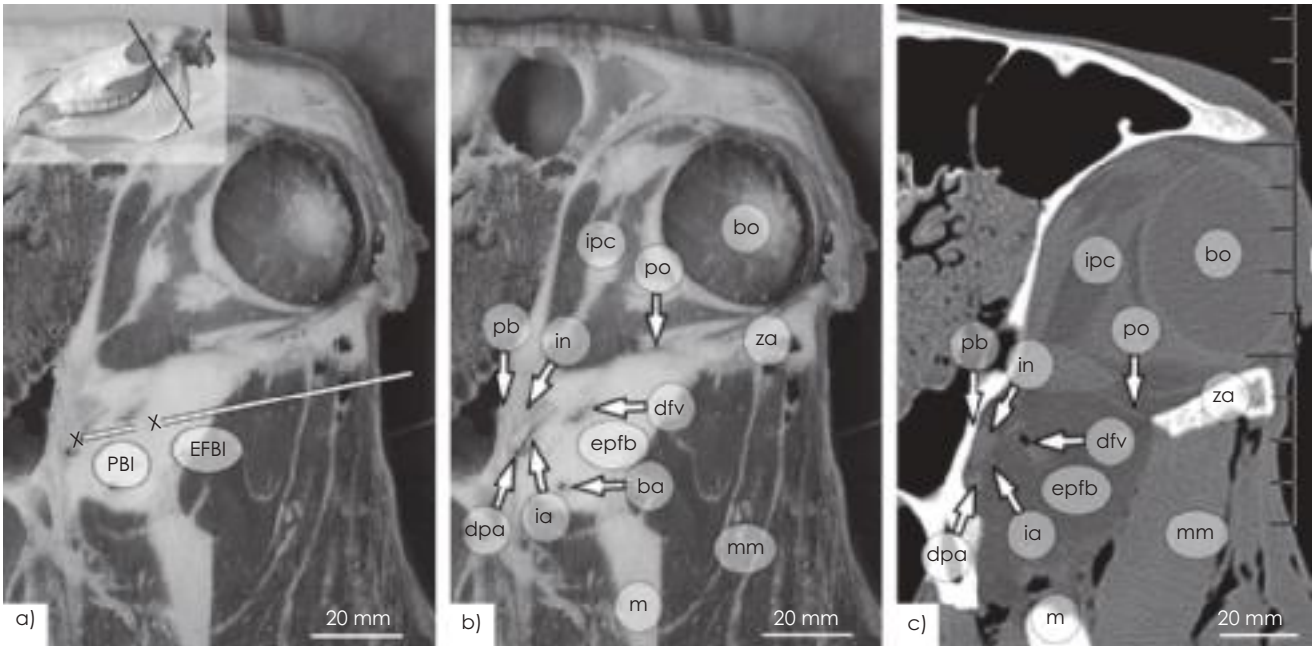

Fig 7: Regional anatomy of the maxillary nerve in cadaveric specimens and matching CT scans. a) Transverse section through the left pterygopalatine fossa of a deep frozen specimen, rostral view. The inset indicates the section plane at the level of the caudal third of the eyeball. The lines indicate the position of the tip of the needle for performing a maxillary block (x). The needle was inserted either until its tip touched the palatine bone (PBI: Palatine Bone Insertion) or the needle was inserted only for 15–20 mm into the extraperiorbital fat body (EFBI: Extraorbital Fat Body Insertion). b) The same picture as in (a) to demonstrate selected anatomical landmarks. The transition from the masseter muscle (mm) to the extraperiorbital fat body (epfb) is clearly visible. c) CT image corresponding to (a) and (b). The periorbita (po) separates the extraperiorbital fat body (epfb) from the intraperiorbital compartment (ipc). Note that the infraorbital nerve (in) is located directly next to the palatine bone (pb). The infraorbital nerve is accompanied by the infraorbital artery (ia) laterally and by the descending palatine artery (dpa) ventrally. The deep facial vein (dfv) is embedded in the extraperiorbital fat body (epfb). ba – buccal artery; pb – perpendicular plate of the palatine bone; za – zygomatic arch; m – mandible; bo – bulbus oculi. Reproduced with permission by Staszyk et al. (2008).

patient with trigeminal neuralgia, in which the classical approach using anatomical landmarks was confounded because of anatomical variations (Okuda *et al.* 2000). A similar approach could be used in equine cases in which anatomical variations impede the use of anatomical landmarks. In cows, magnetic resonance imaging scans were used to compare two different techniques for retrobulbar blocks using contrast medium in cadaveric heads (Pearce *et al.* 2003).

Nerve stimulator guided local techniques for the equine head

Since the inferior alveolar nerve is a sensory nerve with certain motor components as well, it is a very suitable nerve for nerve stimulator guidance when it needs to be desensitised. In various studies, the use of nerve stimulator guidance of the inferior alveolar block in man has been described (Simon *et al.* 2010; Espitalier *et al.* 2012; Kumar *et al.* 2012). Stimulation of the nerve using a lateral extraoral approach results in a motor response of the temporal and masseter muscles, apparent as a jaw jerk.

Cheetham *et al.* (2009) used a peripheral nerve locator to perform a bilateral block of the common trunk of the hypoglossal nerve in 10 horses to determine the role of the hypoglossal nerve in equine nasopharyngeal stability. During this study, no complications were associated with the technique.

In literature, use of a nerve stimulator to assist locoregional techniques in the equine head has not been described, but the technique should be feasible and merits further investigation.

Neuromodulation of trigeminal nerve branches in the equine head

Desensitisation of peripheral nerves of the equine head is very important for surgical procedures; however, perineural techniques are used as well in diagnosing peripheral neuropathies in horses (such as headshaking) and neuromodulation of these peripheral nerves can also be of therapeutic value in these cases. Various studies on the technique and reliability of the maxillary nerve block for diagnosing headshakers have been published (Newton *et al.* 2000; Roberts *et al.* 2013; Wilmink *et al.* 2015). Where few horses with idiopathic headshaking showed improvement on anaesthesia of the infraorbital nerve, the majority (13 out of 16 = 81%) showed complete or partial improvement after anaesthesia of the posterior ethmoidal branch of the maxillary nerve (Newton *et al.* 2000). This block is called caudal anaesthesia of the infraorbital nerve by Roberts *et al.* (2013) and caudal nasal nerve block by Dyce *et al.* (2002). This latter nerve branches off the maxillary nerve just proximal to the maxillary foramen and enters the caudal nasal foramen before running towards the dorsal meatus of the nasal cavity to innervate the nasal mucosa.

A recent study by Roberts *et al.* (2016) describes the first results of neuromodulation of the maxillary nerve in equine headshakers, by use of a percutaneous electrical stimulation protocol featuring alternating high (100 Hz) and low (2 Hz) frequency stimulations. The infraorbital nerve was located by ultrasound guidance and the nerve was stimulated for 25 min. Preliminary results show partial or complete remission for a prolonged period in five out of seven cases after several treatments (up to 12–28 weeks after four treatment sessions).

Neurostimulation for the treatment of humans with neuropathic pain has been well described (Papúc and Rejdak 2013; Shaparin *et al.* 2015; Maniam *et al.* 2016). Peripheral stimulation is categorised into transcutaneous electrical nerve stimulation (TENS), peripheral nerve stimulation (PNS) and nerve root stimulation. With TENS, surface electrodes are used while electrodes are percutaneously implanted to directly contact the nerve in PNS. In the study by Roberts *et al.* (2016) in horses, stimulation is realised through needle electrodes that are inserted in close proximity to the nerve, placing this technique more or less between TENS and PNS. After stimulation, the needles are removed in contrast to the practice with PNS in man (in which the electrodes stay in contact).

There are various hypotheses about the working mechanism of peripheral nerve stimulation. Stimulation is thought to produce paraesthesia that spreads along the territory innervated by the stimulated nerve (Abejón and Krames 2009). Exogenous electrical stimulation might also lead to signal modification of intrinsic electrical impulses (Shaparin *et al.* 2015), or stimulation may inhibit central nociceptive transmission or lead to partial sympathetic blockade and local blood flow alterations (Shaparin *et al*. 2015). Papúc and Rejdak (2013) proposed that high frequency stimulation would lead to inhibition exerted by large-size afferents on spinothalamic pathways. Low-frequency stimulation is thought to activate the antinociceptive systems, mediated in part by the opioid system. The gate-control theory by Melzack and Wall (1965), which states that competing nociceptive and innocuous signals influence second-order neurons to transmit pain signals for higher processing, may also be useful for understanding the effect of peripheral nerve stimulation.

Conclusions

Local anaesthetic techniques have been used in equine practice for a very long time, but the use of ultrasound (or nerve stimulator) guided techniques is new and still limited to date in equine cases. Nevertheless, these techniques are very promising and can possibly be of similar benefit as they have been shown to be in human medicine. Ultrasound-guided techniques can also become very prominent in training young professionals in performing various local blocks. As the immediate real-time feedback results in steeper learning curves, the quality and accuracy of the blocks is most likely to improve considerably, as in human medicine. So far, most studies have been performed on equine cadavers with the main aim of describing the technique. Efficacy and safety studies have not been performed in horses yet. For the further development of the techniques and their implementation in practice, clinical studies such as those that are described in the various systematic reviews in humans are needed.

This review shows that guided techniques (both by ultrasound and nerve stimulator) and modern imaging techniques are becoming more important in veterinary practice and have huge potential. Furthermore, the implementation of peripheral nerve stimulation protocols such as described for equine trigeminus-mediated headshaking is a promising development. It is not unlikely that these techniques will become as important in equine pain management as they are in human pain management.

Authors' declaration of interests

No conflicts of interest have been declared.

Authorship

All authors contributed to study execution and preparation of this manuscript and approved the final version of the manuscript.

Acknowledgements

We would like to thank Joop Fama for his help with photography, Jacobine Schouten for her help with dissection of anatomical structures and René van Weeren for critical reading of the manuscript.

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EQUINE VETERINARY EDUCATION
Equine vet. Educ. (2019) **31** (8) 441-446
doi: 10.1111/eve.12894

Hypothesis Article

Courses for horses: Rethinking the use of proton pump inhibitors in the treatment of equine gastric ulcer syndrome

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Keywords: horse; stomach; ulcer; treatment; omeprazole; esomeprazole

Summary

Oral omeprazole has been the cornerstone of equine gastric ulcer syndrome (EGUS) treatment for nearly 20 years. However, approximately 15–30% of equine squamous gastric disease (ESGD) cases and 75% of equine glandular gastric disease (EGGD) cases fail to heal within current treatment guidelines. Recently, a number of factors that may affect the efficacy of oral omeprazole have been highlighted and the pharmacodynamics of a number of novel proton pump inhibitors (PPIs) have been described in the horse. The purpose of this article is to review the factors that affect oral omeprazole efficacy, with the goal of maximising therapeutic response, and the novel PPIs recently described.

Introduction

It has been reported that the prevalence of squamous and glandular gastric disease are unrelated to each other (Murray *et al.* 2001; Luthersson *et al.* 2009a) and it has been shown that the risk factors for glandular disease are different from those for squamous disease (Habershon-Butcher *et al.* 2012). This suggests that they are separate, distinct disease entities and the European College of Equine Internal Medicine (ECEIM) consensus statement recently sought to clarify the terminology recognising that within the umbrella term of equine gastric ulcer syndrome (EGUS) numerous disease entities exist, with equine squamous gastric disease (ESGD) and equine glandular gastric disease (EGGD) the most common conditions in the adult horse (Sykes *et al.* 2015a).

Regardless of the cause of disease, acid suppressive therapy is considered a cornerstone of treatment. The current ECEIM consensus statement recommendations reflect this, and oral omeprazole is currently considered the drug of choice (Sykes *et al.* 2015a). However, despite its widespread use, until recently little attention has been given to the factors that affect efficacy of oral omeprazole, or the management of refractory cases. The purpose of this article is to review these factors with specific focus on the role that diet and individual dose responsiveness play. Further, the author proposes that the use of individually tailored treatment plans that take into account the individual's dietary conditions and individual dose responsiveness should be considered in lieu of the current blanket dosing recommendations for treatment and prevention of EGUS.

Disclaimer: Unregistered medications and off-label dosing discussed in the current manuscript should be used in accordance with local regulatory frameworks and best practice guidelines.

Equine squamous gastric disease (ESGD)

The pathogenesis of ESGD is well described with acid injury to the squamous mucosa, which has limited defence mechanisms and is not normally exposed to a pH of <4, considered the primary factor in disease development (Sykes *et al.* 2015a). Hydrochloric acid is the dominant factor, but volatile fatty acids produced locally in the stomach associated with grain feeding are also considered important contributors to disease (Vatistas *et al.* 1999; Frank *et al.* 2005; Luthersson *et al.* 2009b). Exercise, and the associated increase in intra-abdominal pressure that results in disruption of gastric pH stratification and 'splashing' of highly acidic fluid from the ventral stomach onto the squamous mucosa, is also considered a key factor (Lorenzo-Figueras and Merritt 2002).

Inhibition of acid production, and the consequent increase in intragastric pH, results in healing of ESGD in the majority of patients with healing rates of 70–85% over a 4-week treatment duration consistently reported in the literature with once daily administration of oral omeprazole (Andrews *et al.* 1999; MacAllister *et al.* 1999; Lester *et al.* 2005; Sykes *et al.* 2015b). However, to date, little attention has been paid to the 15–30% of ESGD cases that fail to heal completely within this time period. Whether these cases represent the failure to address risk factors such as diet and exercise in clinical studies, or sub-therapeutic responses to oral omeprazole is unclear. In an early study, a 100% healing rate for ESGD was reported over a 3-week period in clinical cases, without dietary management (Murray *et al.* 1999), suggesting that if adequate acid suppression is achieved healing will occur regardless of the ongoing presence of dietary and exercise risk factors. This is further supported by the 100% healing observed over a 2-week period following the administration of a novel, long-acting, injectable omeprazole formulation that has been shown to be a potent inhibitor of acid production (Sykes *et al.* 2017a). Considering this, it is the author's opinion that the persistence of ESGD lesions after 3 weeks of oral omeprazole is an indicator of sub-therapeutic acid suppression, rather than the contribution of other co-founding risk factors such as diet, exercise and changes in bacterial populations that have been proposed as contributory factors (Vatistas *et al.* 1999; Lorenzo-Figueras and Merritt 2002; Frank *et al.* 2005; Al Jassim *et al.* 2008; Luthersson *et al.* 2009b). This is consistent with the 'no acid, no ulcer' mantra (Malfertheiner *et al.* 2009) in human medicine.

Equine glandular gastric disease (EGGD)

The pathogenesis of EGGD is poorly described and the risk factors that contribute to disease are yet to be fully elucidated. In contrast to ESGD, which occurs because of increased acid exposure in a region with limited defence mechanisms, EGGD is believed to result from a breakdown of the normal defence mechanisms and consequent exposure of sensitive tissues to acid (Sykes *et al.* 2015a). However, the response to oral omeprazole monotherapy is poor with EGGD healing responses of only 9–32% with 28–35 days of omeprazole therapy at 4.0 mg/kg by mouth once daily reported (Sykes *et al.* 2014a,b, 2015b).

The factors that contribute to the poor response rate are not fully elucidated. The author proposes four mechanisms that likely contribute and that warrant review. Namely;

- Is adequate intraday acid suppression being achieved?
- Are adequate durations of treatment being used?
- Is adjunctive therapy such as sucralfate or misoprostol required?
- Is acid suppression the primary mechanism required for EGGD healing?

Intraday acid suppression

In humans, good healing rates for gastroesophageal reflux disease, which is analogous to ESGD, are achieved when the percentage of time that pH exceeds 4 (%tpH >4) exceeds 66% (Bell *et al.* 1992), while a %tpH >3 of greater than 66% is required for healing of glandular disease (Bell *et al.* 1992). In a study measuring intragastric pH over 24 h periods using pH probes inserted retrograde into the indwelling gastric cannula in six horses fed ad libitum hay supplemented with a small grain meal twice daily a pH exceeding 4 was achieved, on average, for only 14 and 11 h on Days 2 and 7 of treatment, respectively, following the administration of a commercial, buffered, paste formulation of omeprazole at 4 mg/kg bwt by mouth once daily (Merritt *et al.* 2003). Similarly, it has recently been reported that following the administration of the same commercial, buffered, paste formulation of omeprazole the average %tpH >4 within the ventral stomach on Day 5 may be as little as 30–40% in horses consuming high roughage diets at the registered treatment dose of 4 mg/kg bwt by mouth once daily (Sykes *et al.* 2017b). This is below the threshold for healing reported in humans (Bell *et al.* 1992) and provides a potential explanation for the poor EGGD healing rates reported, especially in animals receiving ad libitum hay which is widely recommended for the management and prevention of EGUS (Sykes *et al.* 2015a).

Duration of treatment

The duration of treatment required for the resolution of EGGD has not been well studied. Current treatment recommendations of a minimum of 4 weeks duration (Sykes *et al.* 2015a) are based primarily on existing recommendations for ESGD and clinical experience. Given the differences in pathophysiology between ESGD and EGGD, it is possible that, simply, a longer duration of treatment may be required for EGGD healing to consistently occur. In humans, the duration of treatment required for healing of NSAID induced ulceration was 8 and 12 weeks for 84% and 100% healing, respectively, in one study (Lancaster-Smith *et al.* 1991). If a similar effect was present in the horse, then longer treatment durations may be required. However, a recent study investigating the efficacy of a novel, long-acting, injectable omeprazole formulation reported an EGGD healing rate of 75% with 2 weeks of treatment (Sykes *et al.* 2017a). This suggests that EGGD healing is likely dependent on acid suppression, and that EGGD healing does occur within a short period of time if the magnitude and duration of acid suppression is adequate.

Adjunctive therapy

The use of mucosal barrier protectants is logical given the proposed failure of mucosal defence mechanisms in the pathogenesis of EGGD. The use of sucralfate at a dose of 12 mg/kg bwt by mouth twice daily in addition to omeprazole at 4 mg/kg bwt by mouth once daily has been described in a UK sport and leisure horse population with a healing response rate of 63.2% reported (Hepburn and Proudman 2014). However, more recently, a study in a similar population reported a healing rate of only 22% over a 28 ± 5 day period using omeprazole (4 mg/kg bwt by mouth once daily) and sucralfate (10 mg/kg bwt by mouth twice daily) (Varley *et al.* 2016) suggesting that the benefits of the addition of sucralfate to omeprazole monotherapy may be limited. Misoprostol has been proposed as an alternative, or adjunctive, treatment and a 73% response rate over a 28 ± 5 day treatment period at a dose of 5 μg/kg bwt misoprostol by mouth twice daily has been reported (Varley *et al.* 2016). As such, misoprostol appears to warrant further consideration but detailed discussion is beyond the scope of this paper.

The role of bacteria in the pathogenesis of EGGD is controversial. However, no direct evidence supports the use of antimicrobials, and the addition of trimethoprim-sulfdimidine at 30 mg/kg bwt by mouth once daily to omeprazole at 4.0 mg/kg bwt by mouth once daily failed to improve the treatment response over omeprazole monotherapy (Sykes *et al.* 2014c). As such, and in line with the principles of responsible use of antimicrobials, the current recommendation is that their use in the routine treatment of EGGD is not justified (Sykes *et al.* 2015a).

The role of acid suppression

Lastly, the role of acid suppression in the treatment of EGGD has not been fully validated. As such, it could reasonably be argued that acid suppression may not be, or at least has not been demonstrated to be, a critical factor in EGGD healing. However, as discussed above, a recent study investigating the efficacy of a novel, long-acting, injectable omeprazole formulation reported an EGGD healing rate of 75% over 2 weeks of treatment (Sykes *et al.* 2017a), suggesting that EGGD healing does occur if the magnitude and duration of acid suppression is adequate.

Factors affecting oral omeprazole efficacy

Consistently good rates of healing are observed for ESGD when appropriate magnitude and durations of acid suppression are achieved (Andrews *et al.* 1999; MacAllister *et al.* 1999; Lester *et al.* 2005; Sykes *et al.* 2015b). Similarly, preliminary data from a pilot study, in a small number of horses, suggest that good rates of EGGD healing can be achieved when prolonged acid suppression is achieved (Sykes *et al.* 2017a). As such, a key goal in the

pharmacological management of EGUS should be the optimisation of acid suppression therapy. Oral omeprazole remains the most commonly used medication and an understanding of the factors that affect its efficacy is important in tailoring treatment plans for affected individuals.

Diet

Until recently the role of diet in the efficacy of oral omeprazole has been understated and the potential for feeding recommendations to potentially interact with drug efficacy largely ignored. Current recommendations include the provision of ad libitum roughage (Sykes *et al.* 2015a), which is logical given the dietary risk factors that have been identified for ESGD, but do not distinguish between recommendations during the therapeutic and preventative stages of disease management. In the author's opinion, this is an error in clinical reasoning, and inconsistent with current evidence regarding the significant impact that feeding has on omeprazole absorption and efficacy.

Ad libitum feeding, when compared with horses that have had feed withheld overnight, reduces bioavailability, as measured by area-under-the-curve (AUC) of buffered formulations of omeprazole by approximately 50–66% (Daurio *et al.* 1999; Sykes *et al.* 2015c, 2017c). The primary determinant of efficacy of omeprazole in humans (Lind *et al.* 1983) and dogs (Abelö *et al.* 2000) is AUC, and, as such, conditions that decrease AUC likely decrease the efficacy of omeprazole. Consistent with this, it has been shown that the magnitude and duration of acid suppression achieved in horses receiving an ad libitum hay diet is less than horses receiving a high grain/low fibre diet with an overnight fast and omeprazole administered 2 h prior to morning feeding (Sykes *et al.* 2017b). Equally important is that in the group receiving ad libitum hay minimal, if any, acid suppression was observed over a 5-day period in 3/6 animals even at a dose of 4 mg/kg bwt by mouth once daily (Sykes *et al.* 2017b). This suggests that under such conditions the current recommended doses are likely to be ineffective in some, if not many, animals when omeprazole is administered concurrently with ad libitum feeding.

Considering this, the author suggests that current recommendations that do not distinguish between feeding management during treatment and prevention are inappropriate. Instead, the author proposes that the recommendations should be updated to include that, where possible, omeprazole be administered after an overnight fast. This small management change has significant potential to increase the efficacy of oral omeprazole in many patients. Once omeprazole treatment is completed, the current recommendation for ad libitum roughage as part of prevention management is appropriate as long as concurrent oral omeprazole therapy is not required for prevention.

Timing of feeding

To date, little attention has been paid to the timing of feeding in regard to omeprazole administration and the impact of different meal feeding schedules has not been specifically studied. However, it is likely that the timing of feeding is important to the drug's efficacy. Although horses are considered to be constant acid secretors, and the consistently low pH in the ventral stomach reflects this (Husted *et al.* 2008; Sykes *et al.* 2017b), there is also a significant prandial effect and meal feeding increases plasma gastrin concentrations (Sandin *et al.* 1998). Proton pump inhibitors are prodrugs and require gastric acid secretion to be converted to their active form, and thus to inhibit acid secretion (in effect the proton pumps need to be turned on and producing acid to be inactivated and stop producing acid), and it is important the maximal stimulation of pumps occurs while drug concentrations are present (Shin and Sachs 2008). In the horse, the half-life of omeprazole is only approximately 30 min (Jenkins *et al.* 1992; Sykes *et al.* 2015d). Maximal serum concentration occurs at around 45–90 min (Sykes *et al.* 2015c,d, 2016, 2017c) and it is important that maximal stimulation occurs within this period. Further, the type of meal may be important as gastric distention appears to play a role in gastrin release in the horse. Larger amounts of gastrin are released more rapidly in response to voluminous, roughage-based meals when compared with smaller grain meals (Sandin *et al.* 1998). Considering this, the author recommends withholding of feed overnight, followed by the feeding of a large, roughage-based meal 60–90 min after administration of oral omeprazole, then any required grain/supplement feeding.

Dose and individual variation

Current dosing recommendations include blanket dose rates for treatment and prevention; however, these fail to take into account the wide range of individual dose responses that have been reported in the horse. When feed was withheld overnight, the bioavailability of an enteric-coated omeprazole formulation varied between approximately 5% and 50% in one study (Sykes *et al.* 2015d), and similar magnitudes of variation have been reported for buffered (Daurio *et al.* 1999; Sykes *et al.* 2016, 2017c) and plain (Sykes *et al.* 2015d) formulations. Given this wide variation, the use of a dose determined on average response means that some animals will be undertreated (potentially explaining the 15–30% of ESGD nonresponders) and that some animals will be overtreated (wherein, a lower dose may improve compliance through increased affordability of the medication).

In studies monitoring intragastric pH, the author has consistently observed individual animals which fall into good, average and poor responder groups, regardless of the dose and conditions studied. To the author's knowledge, there is no evidence in the horse that the magnitude and duration of acid suppression required for treatment differs from that required for prevention. Further, in monitoring intragastric pH levels, the author has repeatedly observed a threshold effect wherein a 50% reduction in dose does not equate to a 50% reduction in acid suppression. Instead, a threshold effect appears to be apparent where once a minimally effective dose is achieved for a specific individual under specific dietary conditions the effect goes from minimal to adequate acid suppression. Considering this, the author believes that individual responsiveness is likely a more important factor in tailoring the management of an individual animal, rather than blanket recommendations for treatment and prevention doses, as currently recommended (Sykes *et al.* 2015a).

As such the author believes that dosing should be considered on an individual level with the goal of finding the minimally effective dose for each individual animal under specific dietary conditions, rather than the current focus on treatment and prevention doses. In practice, the author uses

a dose escalating strategy to achieve this outcome. After assessment of the diet, ideally including an overnight fast to maximise drug absorption, an initial dose is selected, typically 1 or 2 mg/kg bwt by mouth once per day for an enteric coated or buffered formulation, respectively, treatment is initiated and the response to treatment followed. For horses with clinical signs, a change is expected within 7 days while animals with gastroscopically diagnosed disease should be reassessed at 3 weeks. If clinical signs persist beyond 7 days, or ESGD lesions are still present at 3 weeks, then the dose is doubled and the monitoring continued.

Drug interactions

The potential for drug interactions, specifically sucralfate, to affect the absorption of omeprazole in the horse has previously been highlighted (Sykes *et al.* 2015a). As discussed above, the role of sucralfate in the management of EGGD is likely less important than previously believed as it appears, based on pilot data, that good rates of EGGD healing can be achieved with acid suppression alone when an appropriate magnitude and duration of suppression is achieved (Sykes *et al.* 2017a). However, anecdotally, sucralfate may still be advantageous in the management of clinical signs, especially in the early stages of treatment, and insufficient evidence is presently available to completely discount its potential utility as an adjunctive agent in treatment. If it is to be administered, it should not be administered concurrently with omeprazole. Instead, a minimum of 60–90 min following omeprazole administration should be allowed before sucralfate administration to ensure absorption of the majority of the oral omeprazole dose before any potential interaction may occur. In practice, the author recommends administration of sucralfate at the same time as morning and evening feeding.

Novel alternatives to oral omeprazole

Oral omeprazole has been a cornerstone of EGUS treatment for nearly 20 years. However, as discussed above, some patients demonstrate an inadequate response to current treatment regimens, in particular patients with EGGD. Equally, the use of an oral medication is not possible in some patients such as refluxing or dysphagic patients that cannot tolerate oral medications, or animals with delayed gastric emptying, where the increased dwell time of omeprazole in the stomach is likely to result in increased acid degradation and thus reduced efficacy. Given these factors, recent research has focused on alternative PPI therapy for the horse. Specifically, esomeprazole (both oral and i.v.) and a novel, long-acting, injectable formulation of omeprazole have recently been reported and warrant discussion for the management of cases where traditional oral omeprazole therapy is ineffective or inappropriate.

Esomeprazole

In humans, esomeprazole, the S-enantiomer of omeprazole, is considered the PPI of choice with two meta-analysis studies demonstrating its superiority over other PPIs in treating clinical disease (Edwards *et al.* 2006; Gralnek *et al.* 2006). When compared with omeprazole in humans, it has a superior pharmacokinetic profile attaining a higher area-under-the curve (AUC), longer duration of action and higher intragastric pH with less interindividual variability observed (Johnson 2003; Shin and Sachs 2008). Although the pharmacokinetics of oral esomeprazole have not been described in the horse, the pharmacodynamics of a novel, enteric coated formulation of esomeprazole has recently been described (Sykes *et al.* 2017d). When administered to horses following the withholding of feed overnight, the magnitude and duration of acid suppression observed following the administration of 0.5 and 2.0 mg/kg bwt by mouth once daily was comparable to that achieved in the same horses receiving 1.0 and 4.0 mg/kg bwt by mouth once daily, respectively, of commercial, buffered omeprazole paste (Sykes *et al.* 2017b, d). However, when administered to horses consuming ad libitum roughage at a dose a 2.0 mg/kg bwt by mouth once daily a more pronounced and consistent acid suppressive response was observed with esomeprazole when compared with the administration of 4.0 mg/kg bwt by mouth once daily of commercial, buffered omeprazole paste in the same horses under the same conditions (Sykes *et al.* 2017b,d). Specifically, on Day 5 the therapeutic threshold for healing, as reported in other species, was exceeded in 5/6 horses on esomeprazole at 2.0 mg/kg bwt by mouth once daily paste (Sykes *et al.* 2017d) compared with 3/6 horses on omeprazole at 4.0 mg/kg bwt by mouth once daily (Sykes *et al.* 2017b). These findings suggest that oral, enteric coated esomeprazole may be advantageous in some horses consuming ad libitum roughage diets.

The pharmacodynamics of i.v. esomeprazole has also been described following the administration of 0.5 mg/kg bwt i.v. once daily over a 14-day period (Videla *et al.* 2011). On Days 5 and 14, gastric juice pH was higher in esomeprazole treated horses than saline treated controls. The authors concluded that i.v. esomeprazole shows promise for treatment of horses with conditions that restrict oral intake of omeprazole paste (Videla *et al.* 2011).

Parenteral administration of omeprazole

The use of i.m. omeprazole, using a traditional formulation, has previously been described and i.m. dosing was more effective than oral administration at the equimolar dose (Sandin *et al.* 1999). However, traditional formulations of omeprazole are unstable once reconstituted, while their highly alkaline nature is potentially irritant (Jenkins *et al.* 1992), which reduces their clinical usefulness. Intravenous administration of a traditional formulation of omeprazole has also been described (Andrews *et al.* 2006). Although a pronounced and consistent acid suppressive effect was observed one hour after administration on Day 5, the magnitude of acid suppression measured one hour after administration on Day 1 was marginal, and the effect observed at this time point was inconsistent and highly variable (Andrews *et al.* 2006). Similarly, although the mean magnitude of acid suppression following the 4th dose, as measured one hour prior to administration of the 5th dose on Day 5, was above the therapeutic threshold, the duration of acid suppression also appeared highly variable and inconsistent (Andrews *et al.* 2006). A potential explanation for the delayed, and highly variable, response observed following i.v. administration of traditional formulations is that omeprazole has a very short half-life following i.v. administration in the horse (approximately 30 min) (Jenkins *et al.* 1992; Sykes *et al.* 2015d). This makes traditional formulations pharmacokinetically unsuitable for parenteral administration as serum concentrations are only present for a limited duration within each 24-h treatment interval.

Most recently a novel, long-acting, injectable formulation of omeprazole, which is formulated for sustained release, to overcome the short half-life observed following the parenteral administration of traditional formulations, is stable at room temperature for extended durations, and appears well tolerated following i.m. injection, has been described (Sykes *et al.* 2017a). Following the administration of a total body dose of 2.0 g (equivalent to a dose of 4 mg/kg once per week for a 500 kg horse), marked acid suppression was observed within hours of administration. Pronounced acid suppression (%tpH >4 exceeding 66%) persisted for 4 days in all (6/6) animals, and for at least 7 days in 4/6 animals. Although the pharmacokinetics of the formulation has not been described, the use of a slow release, long-acting formulation overcomes the primary limitation of oral omeprazole, being a very short half-life, which conflicts with the need for the drug to be present when proton pumps are activated throughout the intertreatment interval. Equally, it can reasonably be assumed that the wide degree of variation observed in bioavailability following oral administration is less likely to be present with an i.m. formulation, and this is evidenced by the consistent acid suppression achieved over the 4–7 day period.

A pilot clinical investigation of the formulation consisting of 24 horses treated twice at weekly intervals (on Days 0 and 7) and re-examined at Day 14 yielded favourable results (Sykes *et al.* 2017a). In 22 horses affected with ESGD, healing was observed in 100% of animals. This compares favourably with previous reports of once daily administration of oral omeprazole with a 70–85% healing rate over a 4-week treatment duration consistently reported in the literature (Andrews *et al.* 1999; MacAllister *et al.* 1999; Lester *et al.* 2005; Sykes *et al.* 2015b). Similarly, in 12 horses affected with EGGD, healing was observed in 75% of animals. This compares favourably with the EGGD healing response rates of only 9–35% with 28–35 days of oral omeprazole therapy at 4.0 mg/kg once daily that have been reported in a similar population of horses (Sykes *et al.* 2014a,b, 2015b). Although only a small number of animals were studied in the pilot study, the results of the study suggest that the formulation may be advantageous in the management of ESGD or EGGD cases refractory to oral omeprazole therapy, or where the diet of the individual animal is expected to interfere with the therapeutic response to oral therapy. Further, the use of parenterally administered omeprazole, in lieu of oral medications, as a frontline therapy warrants consideration in animals with overt clinical signs on welfare grounds, due to the rapid and predictable nature of acid suppression achieved with parenteral administration.

Conclusion

Oral omeprazole is currently the cornerstone of the treatment of EGUS. However, greater attention should be given to the impact of feeding on drug absorption and the role of individual dose responsiveness when tailoring treatment plans for individual animals. Several new generation PPIs have been described that likely have a role in the management of EGUS where oral therapy is not possible, or where an inadequate response has been observed with oral omeprazole treatment. It should be emphasised that the use of unregistered medications and off-label dosing should be done in accordance with local regulatory frameworks and best practice guidelines.

Author's declaration of interests

Two studies reviewed in the current manuscript were funded by Luoda Pharma and Bova Compounding. In addition to a part-time post as an Adjunct Associate Professor with The University of Queensland, B.W. Sykes is employed by Bova Australia, acts as an advisor to Bova UK and is a paid consultant to Luoda Pharma Pty Ltd.

Ethical animal research

Not applicable to this article.

Source of funding

None.

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EQUINE VETERINARY EDUCATION
Equine vet. Educ. (2019) **31** (8) 447-448
doi: 10.1111/eve.12901

Critically Appraised Topic

What is the risk that corticosteroid treatment will cause laminitis?

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Background

The association between corticosteroids and laminitis has been the subject of controversy for many years. The topic has been thoroughly appraised in two recent publications (Menzies-Gow 2015; McGowan *et al.* 2016). Studies published since these reviews may provide further information.

Question

In a mature horse or pony in which corticosteroids are indicated for therapeutic use, does corticosteroid treatment increase the risk of developing laminitis compared with no corticosteroid treatment?

Search strategy

Literature searches were conducted for articles published in English on the Medline database (inception to April 2017), the CAB Abstracts database (1973 to January 2017) and using Google Scholar. Search terms included 'equine', 'laminitis' and 'corticosteroid'. Recent conference proceedings were also checked. Articles were included if they reported systemic corticosteroid treatment of horses and evidence of subsequent

TABLE 1: Relevant papers

First author, year	Patient group	Study type	Outcomes	Key results	Study weaknesses
Welsh et al. (2017)	7 UK first-opinion practices. Data mining of 70,481 records. Effect of triamcinolone, prednisolone, dexamethasone, methylprednisolone considered	Retrospective cohort. Text mining clinical records	a) Initial episode of laminitis b) Laminitis recurrence	a) No association between corticosteroid use and initial episode b) Prednisolone associated with laminitis recurrence. Hazard ratio 5.23 (CI 2.59–10.63)	Retrospective, record accuracy. Potential prescribing and diagnosis biases
Jordan *et al.* (2016)	416 horses treated with oral prednisolone (P) + 814 time matched controls (C) Ambulatory practice UK	Retrospective cohort. Review of clinical records	Laminitis a) during treatment b) over study period	Laminitis incidence/100 horse years (95% CI) a) during treatment: P: 20.84 (95% CI 8.35–42.95) C: 5.66 (95% CI 1.14–16.54) b) over study period P: 2.60 (95% CI 1.49–4.22) C: 3.46 (95% CI 2.54–4.62) No significant differences between groups	Retrospective, record accuracy. Potential prescribing + diagnosis biases. Fairly small sample (wide CIs) during treatment
Potter *et al.* (2016)	889 adult horses treated with corticosteroids 2 UK sites	Prospective cohort/ follow-up	Veterinary awareness of laminitis within 2 weeks of treatment	Laminitis in 5 (0.6%) cases. Associations between laminitis and pre-existing risk factor e.g. previous laminitis, endocrine disease	No control group. Potential prescribing and reporting biases
Coleman *et al.* (2016)	199 laminitis cases. 198 healthy controls, 153 lameness controls. Multicentre, USA	Case–control	Evaluation of horse, medical and management factors in 30 days prior to onset of pasture or endocrine-associated laminitis	Corticosteroid use in previous 30 days associated with increased risk of laminitis	Conference proceedings – detail limited but full publication expected. Recall bias?

laminitis. Articles describing single cases, only intra-articular or topical corticosteroid use, ex vivo data or those that did not contain original data (i.e. expert opinion) were excluded. Articles were appraised for inclusion using guidelines established in human medicine (Centre for Evidence-Based Medicine: OCEBM-Levels-of-Evidence-Working-Group 2011; Higgins and Green 2011).

Search outcome

Many of the papers included in the recent appraisals (Menzies-Gow 2015; McGowan *et al.* 2016) were retrieved and are not re-appraised in this summary. The highest quality evidence retrieved was considered to be level 3 and 4 evidence. The most relevant recent papers and the strongest clinical evidence are summarised in **Table 1**.

Discussion

Previous appraisals concluded that there was little or no evidence to indicate a significant risk of laminitis in a healthy adult horse or pony due to corticosteroid treatment but that there was weak evidence for increased risk in animals with pre-existing risk factors (Menzies-Gow 2015; McGowan *et al.* 2016). Recent UK evidence supports this conclusion assuming the healthy horse has no history of previous laminitis. Numerous episodes of corticosteroid use without adverse effects are clearly documented in recent publications (Jordan *et al.* 2016; Potter *et al.* 2016; Welsh *et al.* 2017). However, this evidence relates to the prescribing patterns of the veterinary surgeons involved and the population treated. Other prescribing patterns, for example higher doses, longer courses or indiscriminate use may have other consequences.

Data from a case–control study in the USA identified corticosteroid use in the previous 30 days as a risk factor for laminitis. Full details of these data are not yet published and further evaluation is required. A UK-based case–control study (Wylie *et al.* 2013) did not find an association with recent corticosteroid use but evaluated medication use in the 7 days prior to the onset of laminitis. Population or prescribing differences could also account for these differences. Although case–control studies can highlight relative risks they cannot quantify absolute risk. In circumstances in which the baseline risk is low then a fairly large increase in risk may be of very little significance. Equally if baseline risk is high, for example in an animal with several existing risk factors then a small increase in relative risk may have significant clinical consequences.

Further research is required to determine the risk profiles for different corticosteroid preparations, doses and routes of medication and to investigate potential interactions with other risk factors.

Clinical bottom line

In most healthy adult horses, current evidence does not indicate a significant risk of laminitis with systemic corticosteroid treatment under normal prescribing patterns at several UK practices. Some evidence indicates that corticosteroids are associated with subsequent laminitis development under some circumstances. This is likely to be most apparent in animals with an increased baseline risk, in particular those with a previous history of laminitis. In these cases a more cautious approach to prescribing may be warranted. Corticosteroids are clinically useful and the potential risks of treatment must be weighed against the welfare costs of not treating conditions for which they are indicated.

Author's declaration of interests

No conflicts of interest have been declared.

Ethical animal research

Not applicable.

Source of funding

None.

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