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VOLUME 36 NUMBER 2

FEBRUARY 2024



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

IN THIS ISSUE:

Antimicrobial stewardship: An ethical imperative

Effects of individualised rehabilitation for horses with equine herpesvirus myeloencephalopathy

Static, dynamic and non-weightbearing ultrasound evaluation of the digital flexor tendon sheath improves sensitivity and specificity of manica flexoria tears diagnosis in cobs and ponies

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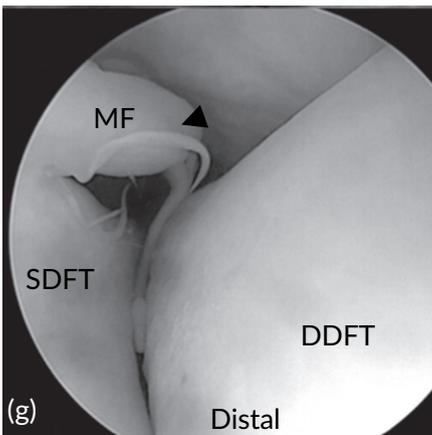
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Antimicrobial stewardship: An ethical imperative

By Barb Crabbe, DVM, MA Bioethics



Dr. Barb Crabbe

We all have heard about antimicrobial stewardship, but are we aware of how big of a problem antimicrobial resistance (AMR) really is? Perhaps most important, have we recognized our role in contributing to this global crisis and accepted our responsibility to be part of the solution? It's important to recognize that how we use antimicrobials in our daily practice has impacts well beyond our own professional bubble.

According to the World Health Organization, AMR is one of the biggest threats to global health. Recent estimates tell us that AMR is directly responsible for over 1 million deaths each year, and it has played a contributing role in more than 4 million deaths worldwide. While these impacts are felt across all geographic and socioeconomic groups, they are borne most acutely by those in poverty-stricken areas—and they are expanding rapidly (Antimicrobial Resistance Collaborators 2022).

Inappropriate or unnecessary use of antibiotics, often associated with inadequate diagnostics, is among the most cited drivers of AMR. While it may feel like antimicrobial resistance is more of a distant problem, local actions have influence. In fact, three of the antibiotic classes that we as equine veterinarians use most frequently—third-generation cephalosporins, aminoglycosides and sulfonamides—are prominent on the lists of antibiotics of high, or even critical, importance to human health. That means we play an important role in helping to solve this problem through responsible use of antibiotics in our daily practice and by working with our clients to help them understand why our commitment to promoting judicious use of antimicrobials is so important.

As a first step, we need to follow the science and pay close attention to rapidly evolving recommendations about antibiotic use. Case in point: Including antibiotics for prophylaxis in a routine joint injection has historically been standard practice for many equine practitioners—with good reason! No one wants to experience a potentially fatal septic joint. Yet recent studies report not only that the incidence of septic arthritis following joint injections is extremely low (<0.1%), but that rates of infection aren't significantly impacted by including antibiotics (Pezzanite 2022). In fact, many of the antibiotics most commonly included in a joint injection can be cytotoxic to cartilage. We can conclude that not only is including antibiotics in

most routine joint injections unnecessary, it could even be harmful while also contributing to the antimicrobial resistance problem.

Another common scenario in equine practice is the client or farm manager who calls requesting antibiotics for a horse with a fever, swollen leg or snotty nose; or the client who requests an examination because their horse's condition isn't responding to the antibiotics they "had on hand." While dispensing bottles of antibiotics for farm use may have been common in years past, times have changed. With the emergence of antimicrobial resistance as a significant threat to both human and animal health, it's especially important that antibiotic prescriptions be limited to cases where they're really needed.

The Centers for Disease Control and Prevention's National Action Plan for combating antimicrobial resistance urges us to "use the right antibiotic, at the right time, for the right duration" (Centers for Disease Control and Prevention 2023). As veterinarians, we're the ones who have the education necessary to formulate those treatment plans and to educate our clients about why it's so important. While it can be difficult to change client expectations, most of our clients mean well. If we take the time to explain that antibiotics not only aren't likely to be needed but can even be harmful, owners will be more willing to accept our recommendations. Older, seasoned practitioners can lead the charge by embracing these changes.

By keeping current with evolving research, demonstrating a willingness to change our treatment strategies and committing ourselves to educating others, we can make a difference in the fight to overcome the devastating impacts of antimicrobial resistance. It's an ethical imperative.

For additional information, refer to the AAEP's guidelines for Judicious Use of Antimicrobials at aaep.org/judicious-use-antimicrobials-2020.

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ETHICAL PRACTICE
Every Day-Every Time

Dr. Crabbe was an equine practitioner and owner of a general practice in the Portland, Ore.-area for over 30 years. She has a Masters in Bioethics from the Neiswanger Institute of Bioethics and serves on the AVMA's Council on Veterinary Services and the AAEP's Professional Conduct and Ethics Committee.

5 things to know about AAEP this month

1. Legislation containing key components of the AVMA/AAEP-endorsed Combating Illicit Xylazine Act passed the U.S. House on Dec. 12 and has moved to the U.S. Senate.
2. Help combat antimicrobial resistance by downloading AAEP's guidelines for Judicious Use of Antimicrobials at aaep.org/judicious-use-antimicrobials-2020.
3. Access free and confidential support for personal or work-related stressors through the AAEP Member Assistance Program. Seek assistance simply by calling (800) 633-3353.
4. U.S. members: Administer compassionate service to horses without incurring financial stress by signing up for Vet Direct Safety Net at aaep.org/vet-direct-safety-net.
5. The Foundation awarded Young Investigators Research Grants totaling \$59,987 in support of three research projects by AAEP-member graduate students, residents or postdoctoral fellows.

Practice Life podcast provides tips for client communication



Communication with clients can be a difficult part of the equine practitioner's job, but it is essential to success and sustainability. During the December episode of the AAEP's Practice Life podcast, hosts Dr. Mike Pownall and Dr. Jessica Dunbar gathered the thoughts of 2023 AAEP

Convention attendees concerning how to talk to clients about costs and expensive procedures as well as how to address challenging clients.

Transparency and treatment options were common themes among respondents in terms of communicating about expensive procedures.

"I like to give clients options and so I break it down as gold standard, silver standard and bronze standard and give them the benefits and risks of going with any of those options and, ultimately, let them make their own decision

so they feel accountable for the decision they make," said Dr. Danielle Price of Columbia Equine Hospital in Gresham, Ore.

"What has evolved for me over time is to present all of the options for treatment with no judgment of the client," said Dr. Becky Ruemmler, owner of Commonwealth Equine in Swansea, Mass. "We need to get from Point A to Point B in your horse's care. I can get there in a Rolls Royce but sometimes I can get there in a Miata and it's OK."

For more of your colleagues' tips on client communication, download or listen to the 26-minute episode on iTunes or at podcast.aaep.org.

THE ART OF HORSE



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Once in the Wiley Online Library, scroll down and click the appropriate issue. In the subsequent Table of Contents, simply select the Case Report you'd like to read in its entirety. You can choose to display the article as a PDF or in html format.



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

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

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Success Story: New Jersey practices join forces through emergency cooperative



Dr. Jessica Martin

“I went from being on call 50% of my life to 16–22% on call per quarter,” said Dr. Jessica Martin of the emergency cooperative established in early 2022 between Mountain Pointe Equine Veterinary Services in Hackettstown, N.J., and nearby B.W. Furlong & Associates.

The catalyst behind its establishment, according to Dr. Martin, an associate with Mountain Pointe Equine, was a mutual interest in improving quality of life for doctors while maintaining a high level of around-the-clock care for the practices’ equine patients in northwestern New Jersey and eastern Pennsylvania.

Getting started

Doctors from both practices initially met informally over dinner to discuss the feasibility of an emergency cooperative. In the ensuing weeks, participants hashed out the logistics more formally via a Google Doc, including client communication and rollout procedures.

Client communication is key

According to Dr. Martin, Mountain Pointe Equine created a list of clients it considered potentially apprehensive about the change in emergency services and called each personally prior to the announcement to discuss the shortage of equine veterinarians, introduce the idea of a cooperative and address any concerns.

“Many of these phone calls were surprisingly well-received, and there was a lot of support from our clients for improving our quality of life,” she said.

Almost four months after the initial group dinner, the practices introduced the emergency cooperative to clients in a joint letter and held a joint Zoom meeting with interested clients to answer questions and allay any concerns.

How it works

Depending on the time of year and availability, between four and eight practitioners participate in the rotation. The emergency coverage radius is approximately one hour in all directions. There is no backup emergency DVM. If the on-call veterinarian is slammed or is tending to an emergency in the opposite direction of a horse that needs to be seen promptly, clients have been provided a list of commercial shippers that could be available to haul their horse into the clinic. In addition, several referral centers within a two-hour radius are available to take emergencies.

During the initial conversation with the on-call veterinarian, the client is informed that payment is required within



24 hours of service and that an Emergency Client Relations Form, including credit card information, must be completed prior to arrival or at the farm as services are rendered. Clients must also enroll in the practice’s EZ Pay service that streamlines the payment process by authorizing the credit card to be charged within 24 hours of service.

Each case is transferred back to the primary practitioner the next business morning via phone call or text message with notes on the case and whether further attention is warranted. Medical records are forwarded to the primary veterinarian’s practice by 9:00 a.m.

“The biggest growing pain was adjusting to a busier on call schedule with clients you may not be familiar with,” said Dr. Martin. “In addition, both of our practices have moved to time-of-service payment, and collecting information from the owners or trainers can sometimes be challenging in the heat of the moment. Most of the clients have come to expect payment at time of service, and this has been extremely helpful in the long run.”

Building trust between the groups

The collegiality established among the emergency cooperative veterinarians continues through a monthly virtual meeting during which they hold journal club discussions and address any concerns from recent cases.

The formation of the cooperative has also created an organic support system for the veterinarians. “We have no hesitation calling one another for case input or advice, as each veterinarian has their own specialties and interests,” said Dr. Martin.

Dr. Martin credits the success of New Jersey’s first emergency cooperative to the comprehensive effort that went into setting it up and communicating the roll out to clients. Within several months of its formation, three additional cooperatives were formed by practices in the state. Her message to other practices that may be thinking of forming a cooperative:

“Find another practice that is the right fit for you and make sure you effectively communicate all expectations with your clients. And don’t be afraid of what clients may think—they may surprise you and be incredibly supportive of your efforts to improve your quality of life and mental health!”

Acquire additional strategies for after-hours care by downloading the AAEP’s Emergency Coverage 2.0 toolkit at <https://tinyurl.com/4764m9px>.

Time running out to submit papers for 2024 convention in Orlando

Papers due March 15 at 3:00 p.m. ET

Secure your spot as a speaker at the AAEP's 70th Annual Convention in Orlando, Fla., Dec. 7–11, by submitting a paper for consideration for presentation. The presenting author of selected papers will receive complimentary registration and an honorarium.

Eligible for consideration are scientific papers, “how-to” papers, review papers, abstracts, and business and practice life papers. All paper presentations are limited to 15 minutes with an additional 5 minutes for Q&A.

Submit papers by March 15 at <https://tinyurl.com/jes67nkh>. Authors should visit the site in advance to set up a profile and provide paper and author information before uploading the paper when it is finished. Complete consid-



erations and ethical guidelines are available in the General Instructions area of the site.

Contact Carey Ross, scientific publications coordinator, at cross@aaep.org with questions concerning educational paper submission.

FOUNDATION

The Foundation invests in clinical advances of emerging researchers

The Foundation for the Horse has awarded \$59,987 for three innovative equine research projects conducted by AAEP-member graduate students, residents or postdoctoral fellows. Since its inception in 2019, The Foundation's Young Investigators Research Grant program has awarded \$496,662 in support of 26 impactful research projects by up-and-coming investigators.

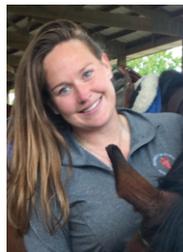
For the fourth consecutive year, The Foundation was joined by the Thoroughbred Education and Research Foundation in helping make these research projects possible. TERF, whose mission is to make racing safer through research, education and scholarships, provided funding and collaborative support for the project by Dr. Kallie Hobbs, presented below. Since 2020, TERF has partnered with The Foundation on six projects for \$106,389.

The supported projects with investigator names, research institutions and brief summaries follow:



Effects of short-term nonstructural carbohydrate supplementation on metabolic function of the oocyte and follicular cells of old mares
Dr. Giovana Di Donato Catandi
Colorado State University

Investigate the extent to which different dietary supplements can reach and alter the follicular environment and, ultimately, the oocyte in aged mares.



Validation of a novel cytokine adsorption device for use in equine patients (TERF sponsored)
Dr. Kallie Hobbs
North Carolina State University

Obtain proof-of-concept data for a clinically relevant approach to treating large animal species with a novel blood purification cartridge (VetResQ®) to improve outcomes for patients with sepsis.



Hepatic gene expression in equine neuroaxonal dystrophy

Dr. Stephanie Ryan
University of California, Davis

Acquire novel insight into the underlying mechanism of eNAD/EDM using RNA-sequencing of liver tissue from 50 eNAD/EDM affected horses and 40 non-eNAD/EDM horses to determine which hepatic genes and pathways are dysregulated with eNAD/EDM.

Equine research is one of three pillars of impact—along with education and horses at risk—supported by The Foundation. The 2024 application window for this research grant program will open in the spring. To learn more, visit foundationforthehorse.org/grants.

The
Foundation
For The HORSE 

Members in the News

Dr. John Pascoe honored in Australia



Dr. John Pascoe

Dr. John Pascoe, who retired in 2023 as associate executive dean at the University of California, Davis School of Veterinary Medicine after 40 years on faculty, received the Vice Chancellor's Alumni Excellence Award from the University of Queensland.

A 1975 veterinary graduate of the University of Queensland, Dr. Pascoe trained 34 surgical residents, five

doctoral candidates and four postdoctoral fellows, and he guided the surgical training of thousands of veterinary students during his time at UC, Davis. Among his many other accomplishments, Dr. Pascoe collaborated with local animal shelters to provide spay, neuter and other surgical procedures to increase adoption rates and support the development of community-based surgical rotations for veterinary students.

Dr. David Levine elected ACVS regent

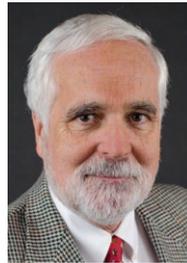


Dr. David Levine

Dr. David Levine, associate professor of clinical large animal surgery and the program director of the large animal residency program at the University of Pennsylvania School of Veterinary Medicine, was elected to the American College of Veterinary Surgeons board of regents.

Dr. Levine, who received his veterinary degree in 2004 from Tufts University, has served as chair of the ACVS Research Committee and been a member of the Resident Credentialing Committee and the Nominating Committee. In addition, he previously served on AAEP's Educational Programs Committee.

Drs. Jack Easley, Eric Mueller receive ACVS awards



Dr. Jack Easley

During its annual surgery summit in mid-October, the American College of Veterinary Surgeons honored a pair of AAEP members for their contributions to veterinary surgery and/or the ACVS.

Dr. Jack Easley, who owns Easley Equine Dentistry in Shelbyville, Ky., received the ACVS Merit Award, given to a non-ACVS diplomate who has made major contributions to veterinary surgery through development of methods, techniques, devices and educational aspects of veterinary surgery.



Dr. Eric Mueller

A 1976 veterinary graduate of Tuskegee University, Dr. Easley previously served on the AAEP board of directors; as chair of the Hospital Planning Committee and Student

Short Courses; and as a member of Professional Conduct and Ethics, Dentistry and other committees.

Meanwhile, Dr. Eric Mueller, a past president of ACVS and professor emeritus of equine surgery at the University of Georgia, received the Al and Carolyn Schiller Distinguished Service Award for exceptional contributions to ACVS.

Dr. Mueller received his veterinary degree from Michigan State University in 1989 and served on the editorial review board of the journal *Veterinary Surgery*. He serves on the AAEP board of directors and is a former chair of the Educational Programs Committee.

Early-career practitioners: Apply for full MentorVet scholarships

AAEP Educational Partner CareCredit is offering 30 full scholarships to early-career equine veterinarians in the U.S. and Canada for the MentorVet Leap program in 2024.

Designed to help early-career veterinarians thrive, MentorVet Leap is an evidence-based program that delivers the peer support, coaching, and mentorship needed to ease into the veterinary profession. This five-month virtual mentorship and professional development program aims to promote wellbeing in the transition to practice by providing a combination of training in professional skills, financial and mental health coaching, and mentorship.

The spring program will extend from Feb. 11–



July 31, 2024. The fall program will extend from Aug. 11, 2024–Jan. 31, 2025. Applicants must have graduated between 2018–2023; new graduates from the class of 2024 will be eligible to participate in the fall program. Applicants must be able to dedicate three-to-four hours per month for five months.

Scholarships are awarded on a first-come basis; apply at mentorvet.net/equinescholarship. For more information about MentorVet, visit mentorvet.net.



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Services provided by



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

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

Dr. Denys Frappier, philanthropic Canadian veterinarian, dies at 84



Dr. Denys Frappier

Dr. Denys Frappier, who retired in 2010 following 16 years of service in Africa at the charitable American Fondouk hospital providing free medical service to the animals of Morocco, passed away Nov. 27 in Morocco at the age of 84.

Dr. Frappier received his veterinary degree in 1970 from the University of Montreal and spent many years as the veterinarian for the Canadian Olympic Equestrian Team before moving to Morocco. Dr. Frappier was a longtime member and former chair of the AAEP's International Committee. He also served on the Horse Show, Membership and Equine Welfare committees.

Canadian sport horse practitioner Dr. Karen Nyrop passes

Dr. Karen Nyrop, who served on the AAEP board of directors from 2012–2014, passed away Jan. 1. She was 69.

Dr. Nyrop focused on English sport horse care and was part of the volunteer veterinary staff for eventing competitors in the 1996 Olympic Games, 2010 FEI Alltech World Equestrian Games and numerous editions of the Rolex Kentucky Three-Day Event.

After receiving her veterinary degree from the University of Minnesota in 1981, Dr. Nyrop completed a large animal surgery residency at Kansas State University. She practiced at a referral hospital in Arizona before establishing her own practice. Dr. Nyrop joined Equine Services, Ltd. in Calgary, Alberta, in 2001.



Dr. Karen Nyrop

Besides her board service, Dr. Nyrop served on eight different AAEP committees, including Educational Programs, Professional Conduct and Ethics, Sports Medicine, and Student Relations. She also served on the Equine Safety and Welfare Committee for the United States Eventing Association, which on Dec. 10 honored Dr. Nyrop with the Ironmaster Trophy for fortitude and courage.

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Whether coping with professional stress, personal grief, a legal matter, or a separate challenge or concern, AAEP members and their immediate family across the U.S. and Canada have been taking advantage of confidential, short-term professional counseling or consultation through the AAEP Healthy Practice Member Assistance Program since September 2022. Program participation is covered as a benefit of AAEP membership.

Now, AAEP members can offer the program's counseling and consultation services to all practice employees at the greatly reduced AAEP program rate of just \$0.22 per month per employee. That's an investment in practice culture and your team's well-being for only \$2.64 per year for each non-AAEP-member employee and serves as a natural complement to the variety of resources



published in recent months by the Practice Culture Subcommittee at <http://tinyurl.com/27eahnd2>.

You can learn more about the array of services provided by visiting aaep.org/healthy-practice-member-assistance-program. If you'd like to explore offering the program to all practice staff, contact Sally Baker at sbaker@aaep.org or (859) 705-0434 and she will walk you through the process.

AAEP Educational Partner Profile: **CareCredit**

CareCredit, a part of Synchrony’s Health & Wellness platform, provides a veterinary financing solution that keeps equine veterinarians at the heart of care by empowering clients with a flexible way to manage the cost of a *lifetime of care* for their horses and pets. The CareCredit health and wellness credit card with budget-friendly financing options helps clients pay at the time of service and the practice gets paid quickly.



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To help veterinarians and clients be prepared for the cost of horse ownership, Synchrony conducted a comprehensive study. Visit equinelifetimeofcare.com to see the findings.

To learn more about CareCredit, visit carecredit.com/equineinsights or call (844) 812-8111.

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¹ Tnibar, A., Schougaard, H., Camitz, L., Rasmussen, J., Koene, M., Jahn, W., Markussen, B., An international multi-centre prospective study on the efficacy of an intrarticular polyacrylamide hydrogel in horses with osteoarthritis: a 24 month follow up. *Acta Vet Scand.* 2015; 57: 20-27.

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RESEARCH HIGHLIGHTS

Highlights of recent clinically relevant papers

COMPARISON OF ESGD TREATMENTS

In this trial, Tania Sundra and co-workers in Australia and the UK compared the efficacy and safety of oral esomeprazole and omeprazole pastes in the treatment of equine squamous gastric disease (ESGD) and, where present, concurrent equine glandular gastric disease (EGGD).

Horses presenting with grade ≥ 2 ESGD lesions were randomly allocated to receive 4 mg/kg of either a buffered esomeprazole or omeprazole paste orally once daily for 28 days before gastroscopy being repeated within a further 3 days. Videos and images were anonymised and subsequently graded blind by one researcher. The severity of ESGD (and EGGD) lesions before and after treatment, and thereby treatment responses, were compared using univariable logistic regression.

Horses were mostly in either low-level or no work, with a mix of breeds represented. A higher proportion of horses had ESGD healing in response to esomeprazole treatment (63/74, 85%) than with omeprazole treatment (43/73, 59%) (odds ratio [OR]: 4.00, 95% confidence interval [CI]: 1.81, 8.82, $p=0.001$). In a subset of horses that had concurrent EGGD, a greater proportion of the horses treated with esomeprazole had lesions \leq grade 1 compared with the omeprazole-treated group (esomeprazole 28/51, 55%; omeprazole 6/24, 25%; OR: 3.65, 95% CI: 1.25, 10.71, $p=0.02$). Using grade 0 as the benchmark for EGGD healing, the difference remained significant (OR: 4.44, 95% CI: 1.33, 14.85, $p=0.02$).

Oral-buffered esomeprazole was a more effective treatment for ESGD (and concurrent EGGD) than oral-buffered omeprazole in this population of horses.

DETECTION OF TAYLORELLA EQUIGENITALIS

In this study, UK-based Ian Mawhinney and Anne Bollard investigated enhanced detection of Taylorella equigenitalis, the contagious equine metritis organism (CEMO), by qPCR using 'Dry' swabs.

The use of swabs without transport medium (Dry swabs) for CEMO PCR was compared with swabs in Amies charcoal transport medium (TM). The experiment was a factorial design using swab type and dilution of organism in culture suspensions, performed in two parts. Simulated genital swabs were prepared in the laboratory by dipping in pairs into culture suspensions containing *T. equigenitalis* with or without other organisms and then inserting them into a sleeve either with or without transport medium. In study 1, the difference in Ct value for the two swab types was compared. In study 2, genital swab material was then also added to culture suspensions

and the swab types were again compared. The swabs were tested by a validated quantitative PCR method. The Ct value of the PCR test was used as the measure for comparison, and the effect of variables was assessed with linear regression.

There was an 7.7% (6.5–8.9) higher mean Ct value of TM versus Dry swabs overall. The Ct difference was more marked at higher dilutions. Addition of genital swab material had no effect on the Ct value. Dry swabs perform at least as well for PCR as swabs in Amies charcoal transport medium, especially when relatively low numbers of organism are present, and are advantageous for routine sampling when culture is not being used.

EFFECT OF TRIAMCINOLONE ACETATE ON INSULIN LEVELS

This study by Brooke Boger and co-workers in the United States aimed to determine the effect of a single intra-articular (IA) dose of triamcinolone acetate (TA) on blood insulin and glucose concentrations.

Ten horses with normal insulin sensitivity as assessed by an oral sugar test received 18 mg of TA into one middle carpal joint. All horses were fed only grass hay during the study period. Insulin and glucose concentrations were evaluated at baseline and 4, 6, 8, 24, 48 and 72 h following IA corticosteroid injection.

Mean \pm SD blood insulin concentration post-IA TA injection was increased at 6 h ($15.8 \pm 3.1 \mu\text{IU/mL}$), 24 h ($23 \pm 5.8 \mu\text{IU/mL}$) and 48 h ($29 \pm 13 \mu\text{IU/mL}$) compared with baseline ($10 \pm 12.3 \mu\text{IU/mL}$), with the peak at 48 h. Median \pm 95% CI blood glucose concentration post-IA TA injection was increased at 6 h ($112.7 \pm 20.3 \text{ mg/dL}$), 8 h ($112.9 \pm 21.4 \text{ mg/dL}$), 24 h (122.6 ± 14.6) and 48 h ($123.5 \pm 15.4 \text{ mg/dL}$) compared with baseline ($89.2 \pm 6.6 \text{ mg/dL}$), with the peak at 48 h.

Blood insulin and glucose concentrations modestly increased for 48 h following IA TA in horses with normal insulin sensitivity.

GRAM-NEGATIVE ORAL MICROBIOTA

This study by José Pimenta and co-workers in Portugal aimed to characterise the oral Gram-negative microbiota of healthy horses and evaluate their antimicrobial susceptibility profile in a One Health approach.

Samples were collected from the gingival margin of healthy horses, free of antimicrobial therapy, cultured in selective media, identified and tested for antimicrobial susceptibility. Fifty-five Gram-negative isolates were identified, with 89.5% being zoonotic and

62% affecting humans, which were also found commonly in the environment. Forty-eight isolates (96%) were multidrug-resistant. The phenotypic resistance presented as higher to macrolides (81.8%), β -lactams (55.4%) and quinolones (50%), and lower to sulfonamides (27.3%), tetracyclines and amphenicols (both with 30.9%). In total, 51.5% of the isolates presented resistance to carbapenems.

In addition to being the first report on the commensal oral microbiota of horses and respective susceptibility profile, this study highlights the horse as a valuable sentinel that can control the evolution and transmission of multidrug-resistant bacteria between the 'One Health triad' since it is in contact with humans, other animals and the environment, in different geographic locations.

ILEAL IMPACTION SURGERY COMPLICATIONS

In this study, Jennifer Ruff and co-workers in the United States compared the occurrence of postoperative complications and survival to discharge in horses with ileal impactions resolved by manual decompression compared with jejunal enterotomy.

Data from the medical records of 121 client-owned horses undergoing surgical correction of an ileal impaction were retrospectively collected. Postoperative complications, survival to discharge or postoperative reflux present were evaluated as dependent variables, and pre-operative PCV, surgery duration, pre-operative reflux and type of surgery were evaluated as independent variables. Type of surgery was divided into manual decompression ($n=88$) and jejunal enterotomy ($n=33$).

There were no significant differences in the development of minor or major complications, presence or amount of postoperative reflux and survival to discharge in horses undergoing distal jejunal enterotomy versus manual decompression for correction of ileal impaction. Pre-operative PCV and duration of surgery were found to be the only predictive factors of survival to discharge. Based on these findings, distal jejunal enterotomy should be considered earlier in horses with moderate-to-severe ileal impactions identified at surgery.

ATYPICAL GUTTURAL POUCH EMPYEMA

This case-control study by Nicole van der Vossen and co-workers in Qatar and the United States documented a previously unreported presentation of guttural pouch empyema (GPE) in a family of juvenile Arabian foals. The study also aimed to document the cytological and microbial composition of the empyema; to identify clinical signs significantly correlated with the presence of GPE, as predictors for the need for guttural pouch (GP) endoscopy; and to demonstrate successful resolution of the identified syndrome with mechanical GP lavage and evidence-based antimicrobial use, improving antibiotic stewardship and the One Health approach to respiratory disease in this demographic of foals.

Upper respiratory disease was reported over many seasons in Arabian foals on a single stud farm in the Middle East. Affected foals were noted to have mucopurulent nasal discharge, cough, fever and tachypnoea. All affected foals had been empirically treated with a macrolide and rifampicin by the referring veterinarian without improvement. On endoscopic examination, all affected foals had significant GPE.

Evaluation and scoring of clinical signs, upper airway endoscopy and thoracic ultrasound were performed in 14 affected foals and 10 age-matched controls, followed by comparative tracheal and guttural pouch sputum culture and cytological evaluation. Therapeutic GP lavage was performed and response to therapy monitored.

GPE cranioventrally distributed ultrasonographic lesions, and opportunistic pathogen infection suggested a primary lesion of GPE with aspiration of GP discharge into the lungs. GP lavage resolved the empyema and associated clinical signs in all cases.

Cytological examination of tracheal and guttural pouch aspirates revealed a neutrophilic exudate with lipid-laden phagocytes, suggestive of milk accumulation. Bacteriology revealed a high prevalence of *Streptococcus equi* ssp. *zooepidemicus* admixed with other opportunistic pathogens. *Streptococcus equi* ssp. *equi* was not isolated in any case.

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CASE REPORT

Effects of individualised rehabilitation for horses with equine herpesvirus myeloencephalopathy

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SUMMARY

At an EHV-1 (equine herpes virus type 1) outbreak in 2019 in Sweden, 19 of the 39 EHV-1-affected horses developed EHM (equine herpes myeloencephalopathy) with varying classical neurological signs. EHV-1/EHM outbreaks have become more common and can result in large economic losses for the horse industry in addition to clear welfare implications. However, there is limited information about estimated time for total recovery from EHM and there is also limited available information regarding adapted rehabilitation programmes following neurological diseases in horses. In this study, we described and evaluated the potential benefit of implementing individual rehabilitation plans for horses recovering from EHM. Four privately owned horses diagnosed with EHV-1/EHM and with similar clinical signs of neurological dysfunction were selected based on owner participation. All horses were initially hospitalised at an intensive care unit for 4–9 days during the first phase of the disease. Approximately 2–3 months after the outbreak, the horses were considered to have recovered sufficiently to start a rehabilitation programme. Throughout the first year, neurological

examination was performed six times and on those occasions, the individual rehabilitation programmes were modified accordingly. The individually designed programmes focused on regaining functional movement, proprioception, core stability and strength training (Figure 1). The owners considered the horses “normal”, both mentally and physically, 6–8 months after the outbreak. All horses were back to their previous level of performance after 8–11 months. Horses suffering from severe EHM may return to an acceptable athletic function given enough time and an individually adapted rehabilitation programme. This study gives us an indication of a timeline that there has to be patience for at least 12 months before a decision is made regarding what level of performance the horse can return to. It is important with an individual rehabilitation plan, close continuous observation and provision of clear guidelines and support to the owners.

KEYWORDS

EHM, EHV-1, horse, neurology, physiotherapy, rehabilitation



FIGURE 1 Foam pad is used to stimulate and improve neuromuscular function.

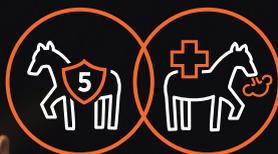
Key points

- Horses affected by EHV-1/EHM assimilates individual rehabilitation in a good way and may return to previous level of performance given time and a proper and individualised rehabilitation plan with a focus on regaining functional movement, training of proprioception, coordination, strength and balance.
- It is of great importance with close, continuous observation and provision of clear guidelines and support for the owners.
- Patience is needed, the timeline is up to 12 months and the horses need a lot of tenderness, love and care.

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CASE REPORT

Colon resection and anastomosis as treatment of an idiopathic colo-colic intussusception in an adult horse

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SUMMARY

An 18-year-old Thoroughbred gelding was referred for treatment of acute colic, unresponsive to initial treatment. The gelding had no previous history of colic or other medical problems. The horse was up to date with routine preventative healthcare including anthelmintic treatment, dental examination/rasping, and vaccinations and was not receiving any regular medication. Rectal palpation revealed a gas-distended, large viscus, on the right of the abdomen with a tight taenial band tracking horizontally across the abdomen. Ultrasonography was unremarkable apart from a gas-distended viscus on the right side of the abdomen. Aseptic abdominocentesis yielded mildly turbid fluid with a total protein of 40g/L and lactate of 1.3mmol/L. Due to incomplete resolution of abdominal pain, suspected large colon distension and elevated peritoneal total protein, an exploratory celiotomy was undertaken. Exploratory celiotomy revealed an intussusception affecting the left dorsal colon (LDC) with the orad LDC (intussusceptum) within the aborad LDC

(intussusciens). The intussusception, approximately 20cm in length, was obstructing the colon. Oral to the obstruction, the colon was markedly distended with fluid ingesta. Exteriorisation and reduction of the intussusception were performed, but the colon wall was markedly thickened and the lumen remained significantly obstructed. Due to the risk of ongoing obstruction, it was elected to perform a partial colon resection of the left dorsal and ventral colon and side-to-side anastomosis. The resected colon was submitted for histopathology. The evaluation showed features representing marked submucosal oedema with dilated submucosal lymphatics and areas of haemorrhages and mild inflammatory infiltrates. There was no evidence of neoplasia or another aetiological agent, nor any other apparent cause. The gelding recovered well from general anaesthesia and progressed well post-operatively. Follow-up (11 months post-operatively) confirmed complete recovery and the horse had returned to the previous level of light exercise.

KEYWORDS

horse, colo-colic, intussusception, large colon resection

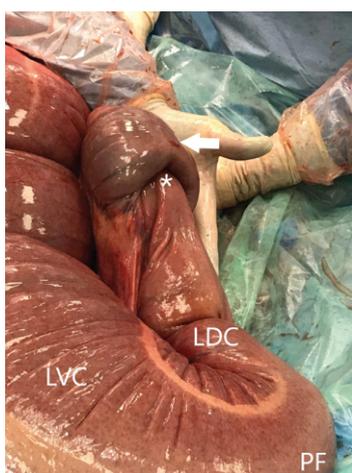


FIGURE 1 Colo-colic intussusception (asterisk [intussusceptum]; arrow [intussusciens]) prior to correction. LDC, left dorsal colon; LVC, left ventral colon; PF, pelvic flexure.

Key points

- Colo-colic intussusception is rare in horses and should be considered in all age groups with evidence of large colon obstruction.
- The prognosis for surgical treatment is good based on this case and four other cases in the literature but may require resection and anastomosis of the colon.
- Even with histopathology in this case, the aetiopathogenesis remained unknown but appeared to be unrelated to intestinal parasitism, neoplasia or dietary changes.

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CASE REPORT

Surgical correction of chronic penile retroversion through a castration incision in a horse

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SUMMARY

A 2-year-old draught horse cross gelding was presented with a 4-month history of his penis exiting through an opening caudal to his prepuce. The owner first noticed the gelding's glans penis exiting through the castration incision 2 months after obtaining the horse from an auction. The horse was able to drop the penis through the scrotum and urinate in a normal stream, which caused occasional apparent discomfort. Manual reduction of the penis in the correct anatomic position was attempted but unsuccessful. Surgery was attempted to correct the chronic penile retroversion. The penis was observed to be exiting through a fistula caudal to the prepuce (Figure 1). An elliptical incision around the fistula present caudal to the prepuce and an incision cranial to the fistula were made to enhance visualisation for further dissection. Multiple fibrous adhesions were found

between the body of the penis to the subcutaneous tissues, which were restricting movement. During dissection of the adhesions, the fistula was found to extend from the scrotum to the right side of the inner lamina of the preputial fold, which allowed retroversion of the distal penis through the scrotal portion of the fistula. Dissection was continued until the penis could be extended normograde through the prepuce, then the incisions were closed. Post-operatively, the horse was immediately able to partially drop his penis through his prepuce and urinate normally and without appreciable pain. The authors hypothesised that the penile body was mistaken for a testicle during castration and dissected free from the subcutaneous tissue resulting in adhesions and the subsequent retroversion. Four years post-operatively, the owner reported the gelding was able to completely retract his penis into the prepuce and partially drop the penis to void urine. This case report highlights a complication of castration that has not been previously reported.

KEYWORDS

horse, castration complications, penile retroversion



FIGURE 1 Pre-operative photograph of the penis emerging through the scrotal fistula from the old castration site.

Key points

- Complications following castration in horses are common and vary in severity.
- Inadvertent dissection of the shaft of the penis during castration can result in significant trauma to the penis and surrounding tissues.
- Surgical correction of a chronic penile retroversion through a previous scrotal incision can be attempted if manual reduction of the penis into the prepuce fails.

CASE REPORT

Diagnosis and surgical reduction of atlanto-axial luxation in a 10-year-old Irish Draught horse

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SUMMARY

A 10-year-old Irish Draught mare was initially admitted to our referral hospital due to dullness and colic signs, which later progressed with worsening dullness, discomfort upon cervical palpation and reduced range of movement. Upon clinical examination, it was noted that the horse could not freely flex its neck to access food from the ground. Further diagnostic investigations, including radiographs and computed tomography (CT), revealed a complete luxation of the atlanto-axial articulation, leading to instability and potential spinal cord compression. This condition was managed surgically under general anaesthesia, involving removal of the dens of C2, realignment of C1 and C2 and the placement of surgical implants to stabilise the vertebral segments.

The surgical procedure was successful, with a duration of 240 min under anaesthesia. The horse's post-operative recovery was aided with multimodal analgesia, and she showed gradual improvement in demeanour and neck mobility. Post-operative monitoring included pain scoring and radiography, which confirmed appropriate vertebral canal alignment and implant positioning.

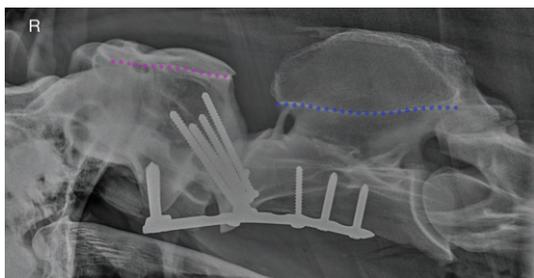


FIGURE 1 A latero-lateral radiograph 48 h post-operatively showing an appropriate alignment between the dorsal lamina of C1 (outlined in pink) and C2 (outlined in blue). A T-plate is contoured to the ventral margins of C1 and C2 with five screws in situ and four trans-articular screws are inserted in a caudoventral-craniodorsal orientation from C2 to C1.

This case report is significant as it describes the successful surgical intervention for presumed traumatic atlanto-axial luxation in an adult horse. We emphasise the importance of thorough clinical examinations in identifying early signs of cervical discomfort, even in cases without apparent trauma. Additionally, we highlight the value of CT in diagnosing and characterising the extent of soft tissue and bone damage, aiding in surgical planning.

In conclusion, this report underscores the rarity of atlanto-axial luxation in adult horses and demonstrates that surgical treatment, involving the removal of the dens, realignment and stabilisation, can lead to a successful outcome. The case serves as a valuable reference for veterinarians encountering similar conditions in equine patients, emphasising the need for a comprehensive approach to diagnosis and treatment.

KEYWORDS

horse, computed tomography, spinal cord, trauma, vertebral



Key points

- The noncongenital atlanto-axial luxation was initially interpreted as signs of colic; however, in-depth monitoring revealed persistent neck discomfort, underlying the importance of performing a thorough examination and considering a broad differential diagnosis list.
- Computed tomography (CT) played a crucial role in characterising the extent of soft tissue and bone damage and aided surgical planning.
- The surgical approach for atlanto-axial luxation reduction involves osteotomy (removing a segment of the dens), reduction of the luxation and stabilisation of the atlanto-axial junctions using surgical implants. This approach provided stability and was considered the most viable option for this adult horse's condition.

Ultrasound-guided perineural injection of the tibial nerve in the horse versus a 'blind' technique

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Summary

Background: Tibial perineural analgesia has often been reported to fail to achieve nerve desensitisation in horses. Ultrasound-guided (US-guided) techniques have recently been described to improve tibial perineural desensitisation.

Objectives: To compare US-guided and 'blind' tibial perineural analgesia techniques in lameness investigation.

Study design: Randomised clinical trial.

Methods: Horses presenting for lameness investigation, which required tibial perineural analgesia, were randomly assigned either to a US-guided or blind injection group. The efficacy of perineural analgesia was assessed by testing the loss of skin sensation at the medial and lateral heel bulbs. Skin sensation was assessed, prior to injection and then at four intervals post-injection (10–15, 20–25, 30–35 and 40–45 min) using a hand-held digital algometer with a 1 mm diameter pin; a value of 25 N was defined as indicative of skin desensitisation. The time taken to perform each injection technique and any adverse reactions were recorded. Summary statistics were performed to examine differences between groups. The frequency of skin desensitisation was compared between groups using a Fisher's exact test and the length of time taken to perform injections was compared using a Mann–Whitney *U* test.

Results: Sixteen US-guided and 11 blind injections were included in the study. All cases undergoing US-guided injection lost skin sensation, whereas this occurred in only one case receiving the blind injection. The US-guided group had a significantly higher probability of skin sensation loss ($p < 0.001$), although the injection technique took significantly longer to complete compared to the blind group ($p < 0.001$). No adverse reactions were noted with either perineural injection technique.

Main limitations: Limited number of cases for each injection group.

Conclusions: These findings suggest that US-guided tibial perineural injection is more likely to result in adequate and prompt tibial perineural analgesia compared to the blind injection technique, although it takes longer to complete.

KEY WORDS

horse, lameness investigation, loss of skin sensation, tibial perineural analgesia, ultrasound-guided injection

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INTRODUCTION

Tibial perineural analgesia is a valuable aid in the diagnosis of musculoskeletal pathology of the hindlimb during lameness examination of the horse, allowing the clinician to identify sources of lameness originating from the distal crus, plantar tarsus or the more distal limb (Bassage & Ross, 2010; Kawcak et al., 2020).

Perineural analgesia of the tibial nerve is achieved by injecting a local anaesthetic agent into the caudomedial aspect of the distal crus, approximately 10cm proximal to the calcaneus between the common calcaneal tendon and the lateral digital flexor muscle (Bassage & Ross, 2010; Dyson, 1984; Moyer et al., 2011).

At this level the tibial nerve is located within the superficial caudal crural compartment (delimited by the superficial and deep caudal crural fasciae), caudomedial to the lateral digital flexor muscle and cranial to the common calcaneal tendon (Denoix et al., 2020) (Figure 1).

Assessment of the response to tibial perineural analgesia has been recommended between 10min and 1h following injection (Bassage & Ross, 2010; Denoix et al., 2020). The potentially prolonged time required for adequate tibial perineural analgesia has been attributed anecdotally to the topography and large size of this nerve which may require a longer period of time for diffusion of the local anaesthetic agent (Denoix et al., 2020; Kawcak et al., 2020). Tibial perineural analgesia can fail because of erroneous subcutaneous injection without penetration of the superficial crural fascia, erroneous intramuscular injection of the lateral digital flexor muscle or intravascular injection of the caudal root of the saphenous or caudal femoral veins (also contained in the superficial caudal crural compartment), requiring the clinician to repeat the perineural injection (Denoix et al., 2020; Pilsworth & Dyson, 2015; Schumacher & Schramme, 2019). Inadvertent contact with the tibial nerve during placement of the needle can result in a violent reaction of the horse

(e.g. kicking out, bucking, etc.) and therefore places the clinician at risk of injury (Moyer et al., 2011; Schumacher & Schramme, 2019).

Ultrasound (US)-guided technique is the accepted gold standard for perineural analgesia in human medicine (Kruisselbrink & Chin, 2015) and is increasingly used in veterinary medicine (Beaumont et al., 2020; Denoix et al., 2020; Portela et al., 2018a, 2018b). Injection under US guidance is reported to increase the accuracy of needle placement compared to 'blind' injection techniques, potentially reducing complications associated with inaccurate deposition of injectate or inadvertent damage to surrounding structures (Jarosinski et al., 2020; Schneeweiss et al., 2012). Therefore, the use of US guidance for perineural analgesia in lameness investigation of the horse could result in an increased success rate of injection, the more prompt onset of analgesia and increased operator and patient safety (Beaumont et al., 2020; Denoix et al., 2020; Kruisselbrink & Chin, 2015).

More recently, US-guided techniques for tibial perineural analgesia have been described and evaluated in cadaver studies (Denoix et al., 2020; van der Laan et al., 2021), but *in vivo* studies supporting the use of US-guided tibial perineural analgesia in lameness investigation are still lacking.

Subjective evaluation of skin sensation by applying firm pressure with a blunt object (e.g. ballpoint pen) is often used to assess if perineural analgesia has been adequately performed (Bassage & Ross, 2010; Schumacher & Schramme, 2019). More recently, algometers, instruments that allow measurement of the pressure applied, have been used to test skin sensation in the research setting (Gozalo-Marcilla et al., 2020; Hinnigan et al., 2014; Hoerdemann et al., 2017; Jordana et al., 2014).

The principal aim of this study was to compare a US-guided tibial perineural analgesia technique with a blind technique in lameness investigation in the horse by assessing the onset of loss of skin sensation in the tibial nerve's autonomous zones using an algometer. A

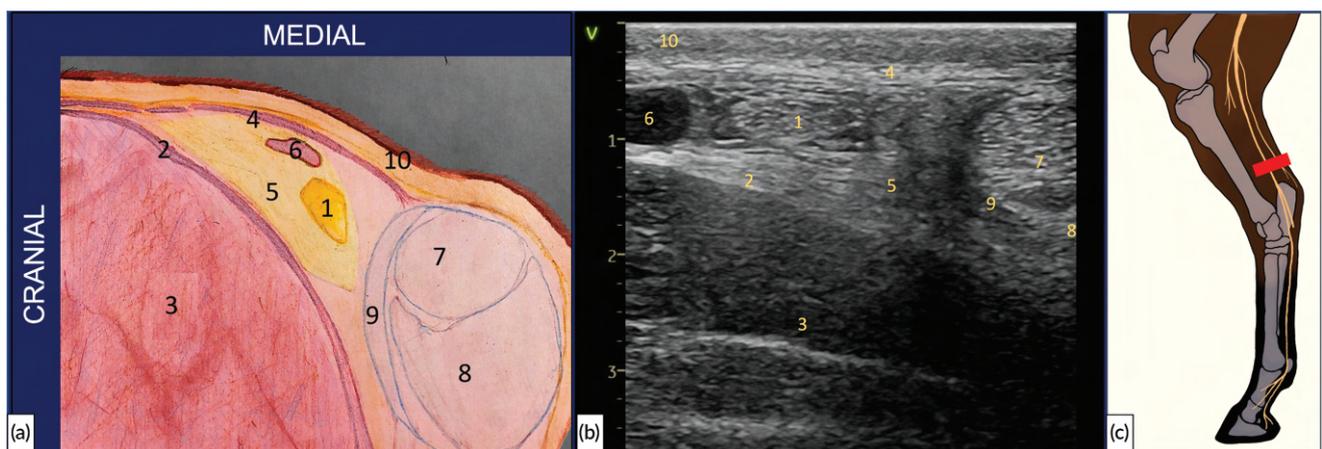


FIGURE 1 (a,b) Drawing of a transverse anatomical section of the caudomedial part of the crus and ultrasonographic image obtained at the injection site for tibial perineural analgesia. 1=Tibial nerve; 2=Deep caudal crural fascia; 3=Lateral digital flexor muscle body; 4=Superficial caudal crural fascia; 5=Fat of the caudal crural compartment; 6=Caudal root of the saphenous vein and caudal femoral vein; 7=Superficial digital flexor tendon; 8=Gastrocnemius tendon; 9=Tendon of the caudal femoral muscles; 10=Skin. (c) Drawing shows the site of a transverse anatomical section and transverse ultrasonographic image with the broad red line indicating the positioning of the ultrasound transducer.

further aim of this study was to compare horses' tolerance of the procedure and operator safety between US-guided and blind tibial perineural analgesia.

We hypothesised that the US-guided technique would result in a quicker and more consistent onset of loss of skin sensation of the distal limb compared to the blind technique. Also, we hypothesised that the US-guided technique would take longer to complete but be better tolerated by the horse compared to the blind technique.

MATERIALS AND METHODS

Animals

Horses were recruited from clinical cases presented for lameness examination to two equine referral hospitals over an 18-month period (2020–2022). All horses included in the study required tibial perineural analgesia for diagnostic purposes as part of a lameness investigation. Ethical approval for the study was granted by the lead institution (School Research Ethics Committee, School of Veterinary Medicine, University of Glasgow, Ref EA28/20) and horse owners gave written consent for participation. Horses were included in the study if no diagnostic analgesia procedures were performed within 6 h preceding tibial perineural analgesia on the limb being investigated, except for perineural analgesia of the superficial and deep peroneal nerves. None of the horses in the study received any sedatives or tranquilisers prior to or during tibial perineural injection.

Study design

It was estimated that 10 cases of US-guided and 10 cases of blind tibial perineural injection would be sufficient to investigate the difference in the time required for loss of skin sensation at the heel bulbs. Sample size calculations were not performed as no pre-existing data were available.

Recruitment of 20 clinical cases was anticipated and these were randomly pre-assigned to either the US-guided or blind tibial perineural injection groups using a random-number generator (Excel, Microsoft Corporation) with an allocation ratio of 1:1. Cases were assigned based on chronological presentation (e.g. case number one was pre-assigned to the blind injection group). After completion of 20 cases, additional cases were sequentially randomised using a web-based programme (random.org Randomness and Integrity Services Ltd).

Skin sensation was assessed prior to performing tibial perineural analgesia and at four subsequent time points following injection: 10–15, 20–25, 30–35, and 40–45 min. One investigator assessed skin sensation in all cases, while four operators, with a similar level of experience, performed the tibial perineural injections (three ECVS-certified surgeons and one surgical resident).

The time taken to complete the tibial perineural injection procedure, whether by US-guided or blind technique, was recorded for

each clinical case, as well as any complications that arose from the procedure, including reactions from the horse at the time of perineural injection that might endanger operator safety.

The effect of tibial perineural analgesia on lameness was purposely not reported as this was beyond the scope of this study.

Tibial perineural analgesia injection techniques

The anatomic site for tibial perineural injection was prepared by clipping the hair using a No. 40 clipper blade, followed by cleaning using a dilute chlorhexidine solution and then alcoholic spirit (95% ethanol and 5% methanol). In all cases, in both groups, 2 mL mepivacaine hydrochloride 2% (w/v) (Intra-Epicaine, Dechra Veterinary Products) was deposited subcutaneously using a 25 gauge 5/8-inch needle prior to performing the tibial perineural injection. Tibial perineural analgesia was performed by injecting 20 mL of mepivacaine hydrochloride 2% (w/v) into the caudomedial aspect of the distal crus, with the limb weightbearing and in a slightly retracted position, 10 cm proximal to the tuber calcanei, between the common calcaneal tendon and the lateral digital flexor muscle. The syringes containing the local anaesthetic agent were connected to the needle via a 200 cm long, 2 mm diameter extension line (Lectrocath, Vygon) in all cases.

US-guided perineural injections were performed using an 8–12 MHz linear transducer (Vivid S60N, GE Healthcare) and a 21 gauge, 1.5-inch needle. The transducer was placed in a transverse plane at the level of the injection site allowing identification of the tibial nerve. The sonographic appearance of the tibial nerve has previously been described by others (Denoix et al., 2020). Briefly, the tibial nerve is oval in outline and echogenic and lies superficial to the deep caudal crural fascia, caudal to the saphenous and femoral veins and cranial to the common calcaneal tendon (Figure 1). Following identification of the nerve, the transducer was moved cranially to create space for needle insertion caudal to the nerve (i.e. a caudal approach was used).

The needle penetrated the limb at a 20–30° angle to the skin on the caudomedial aspect of the distal crus and was visualised along the long axis of the transducer. Following the penetration of the superficial crural fascia, the needle was advanced in the caudal crural compartment until its tip was immediately adjacent to the tibial nerve. The local anaesthetic solution was first injected around the caudal aspect (10 mL) of the nerve and then the needle was redirected, under ultrasound guidance, at a 15–20° angle and advanced superficially in a cranial direction, to enable distribution of the local anaesthetic agent around the cranial aspect of the nerve (10 mL).

Alcoholic spirit (95% ethanol and 5% methanol) was used to provide contact between the ultrasound transducer and the skin.

Two operators were required for the ultrasound-guided technique; one held the transducer in one hand and the needle in the other (Operator A), while the second (operator B) held the syringe (extension set connecting syringe and needle) and injected under the



FIGURE 2 Images showing the US-guided technique being performed. (a) Set-up and positioning of operators when performing perineural injection using the US-guided technique; the transducer is placed on the caudomedial aspect of the distal crus. (b) The image shows the operator handling the linear transducer and the needle attached to the extension line simultaneously; the tip of the needle penetrates the skin on the caudomedial aspect of the distal crus, just caudal to the transducer. (c–e) Sequence of ultrasonographic images showing US-guided perineural injection (caudal is to the right). (c) The tibial nerve is identified in a transverse plane just cranial to the superficial digital flexor tendon. (d) The tip of the needle is then inserted adjacent to the caudal margin of the tibial nerve. (e) Following injection of local anaesthetic around the caudal margin of the nerve the tip of the needle is redirected at the superficial (medial) margin of the nerve to allow further advancement and injection of local anaesthetic around the cranial margin of the nerve.

instruction of operator A (Figure 2). Operator B was also responsible for maintaining the safety of the transducer cable and for moving the ultrasound machine away from the horse if needed.

Operator A stood lateral to the limb being injected. Operator B and the ultrasound machine were positioned on the contralateral side of the horse, such that Operator A had a good view of the ultrasound machine monitor (Figure 2).

Blind perineural injections were performed using a 23 gauge, 1-inch needle. The nerve was first identified by palpation (firm cord-like structure) caudal to the lateral digital flexor muscle and cranial to the common calcaneal tendon with the limb in a flexed position. Then, with the limb in a weightbearing position, the needle was inserted up to the hub over the caudal surface of the lateral digital flexor muscle to position its tip close to the nerve. The needle was then redirected four times in a fan shape (45°, 75°, 105° and 135° angle to the skin) with a 5 mL local anaesthetic agent deposited in each plane to allow distribution around the nerve. The operator performing the injection stood lateral to the limb being injected.

In addition to operator/s involved in the perineural injection, one person was required to restrain the horse.

Skin sensation testing

Skin sensation was assessed by the maximum force that could be applied to the skin prior to inducing a horse's reaction. Application and measurement of force were by a hand-held digital algometer (Prod, TopCat Metrology) attached to a long custom-made handle (Figure 3). An increase in the force, measured in newton (N), reflects an increased mechanical nociceptive threshold (MNT) and a reduction in skin sensation.

The algometer features a silent, pneumatic actuator with a 1 mm diameter flat-ended pin (Figure 3) and was manually applied against the limb's skin. The horses' eyes were covered by the operator holding the horse or by blinkers. The force applied to the skin was progressively increased at a rate of 1–2 N/s. The force rate increase was monitored using LEDs on the algometer, which guided the operator when testing skin sensation: green too slow, red too fast; LEDs are not illuminated when the rate is correct. The algometer was removed as soon as the horse reacted (limb lift or stamp, shoulder muscle contraction, shifting weight to the non-tested limb), with the MNT displayed being recorded, or applied until a value ≥ 25 N was reached.

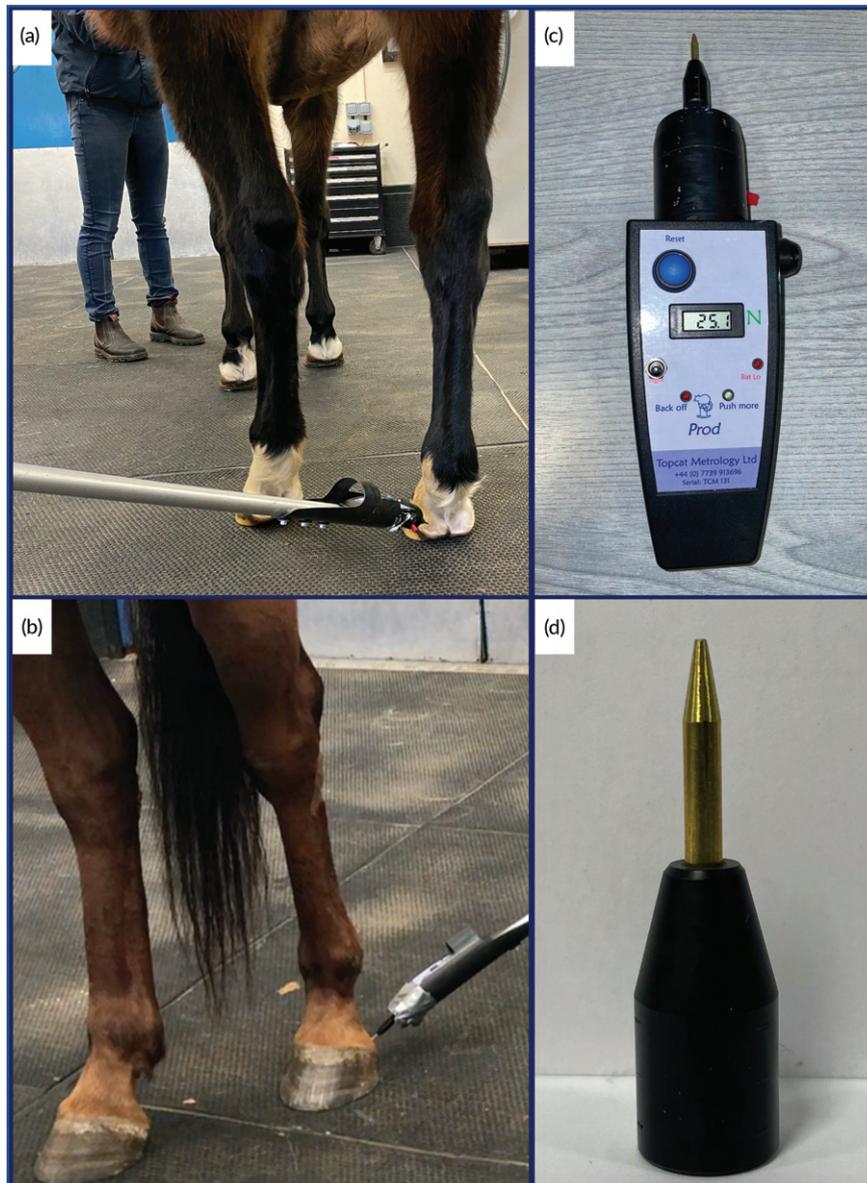


FIGURE 3 Images show skin sensation testing and the digital algometer. (a,b) images show the medial and lateral heel bulbs being tested using the digital algometer attached to a custom-made handle. (c) Digital algometer. (d) Close-up of the 1 mm diameter tip that was used for testing skin sensation.

The MNT value of 25 N achieved using a 1 mm diameter pin indicated a complete loss of skin sensation (Schambourg & Taylor, 2020). Skin sensation measurements were performed prior to performing tibial perineural analgesia and at four time points following injection (10–15, 20–25, 30–35 and 40–45 min after injection). A 5-min window was allowed for each testing time point to allow the operator to complete the task.

Three measurements at a minimum of 30-s intervals were carried out at each time point to ensure the reliability of the readings. If a horse reacted or moved for reasons unrelated to the test, that measurement was discarded and then repeated. The readings displayed were recorded for data analysis.

When a value ≥ 25 N was recorded at a skin location, no further measurements were made at that location at the remaining time points.

The locations used for testing were the lateral and medial heel bulbs (1–2 cm above the coronary band; Figure 3), with measurements completed at the lateral heel bulb at each time point before proceeding to the medial. The heel bulbs were selected following a review of the available literature (Carpenter & Byron, 2017; Labens et al., 2012; Moyer et al., 2011; Prange, 2019; Skarda et al., 2009).

Time required to complete injections

Time required to complete each tibial perineural injection was recorded in seconds using a stopwatch. The time for subcutaneous placement of 2 mL local anaesthetic was not recorded for either technique. For US-guided perineural injections, the stopwatch was started as soon as the transducer contacted the skin. For blind

injections, the stopwatch was started at the time of palpation of the nerve with the limb in a flexed position. The stopwatch was stopped for both injection techniques when injection of the total volume was completed.

Complications and adverse reactions to perineural injections

Any complications of the injection techniques were recorded as well as any adverse reaction of the horse with implications for horse or operator safety; these included: horses kicking out at the time of injection, sudden foot stamping of the horse, horse moving abruptly, injury to the operators and/or horses.

Statistical analysis

Summary statistics were performed to examine differences between groups (US-guided and blind).

The dichotomous outcome 'desensitisation at 40–45 min' 'yes' or 'no' was defined as loss of skin sensation (no response to ≥ 25 N pressure) at the medial and lateral heel bulbs. The frequency of this outcome was compared between US-guided and blind groups using a Fisher's exact test.

The speed of onset of medial and lateral heel bulb desensitisation (≥ 25 N) was evaluated between US-guided and blind groups graphically.

The lengths of time taken to complete the nerve blocks were compared between groups US-guided and blind groups using a Mann–Whitney *U* test.

RESULTS

A total of 27 cases were collected in this study, with 27 tibial perineural injections being performed on 22 horses (8 mares, 14 geldings); breeds included 10 Cob-type horses, 9 Warmblood crossbreed horses and 3 Thoroughbred crossbreed horses. The horses ranged in age from 5 to 20 years of age (mean \pm SD, 10 ± 4 years). Sixteen cases were assigned to the US-guided group and 11 cases were assigned to the blind group.

One horse underwent three blind tibial perineural injections at different times (two left hindlimb and one right hindlimb), one horse underwent one US-guided injection and one blind injection (both right hindlimb) and one horse underwent two blind injections (one left hindlimb and one right hindlimb).

Nine out of 16 cases that underwent US-guided injection were cob types, six were Warmblood crossbreeds and one was a Thoroughbred crossbreed. Five out of the 11 cases that underwent blind injections were Warmblood crossbreeds, four were Thoroughbred crossbreeds and two were cob types.

Four operators performed the tibial perineural injections [three boarded surgeons (JW, MM and CB) and one surgical resident (NB)]. NB performed six out of 11 blind injections and 10 out of 16 US-guided injections. The boarded surgeons performed the remainder: JW one blind injection and six US-guided injections, MM two blind injections and CB two blind injections.

Eleven cases had superficial and deep peroneal perineural analgesia performed at the time of tibial perineural analgesia (6 out of 16 US-guided cases and 5 out of 11 blind cases).

Desensitisation at heel bulbs

There was no difference in timing of desensitisation between lateral and medial heel bulbs. All 16 US-guided injection cases lost skin sensation at the heel bulbs by 30–35 min post-injection. One out of the 11 blind injection cases lost skin sensation (this occurred by 10 min post-injection). Timing of desensitisation for the groups is shown in Figure 4a. Significantly more ($p < 0.001$) cases had lost skin sensation at medial and lateral heel bulbs at 40–45 min post-injection in the US-guided group than the blind group as shown in Figure 4b.

The mechanical nociceptive threshold values recorded for both groups are shown in Figure 5.

Time to complete perineural injections

The mean injection time for the US-guided group (275.5 s, range: 90–485) was significantly longer than for the blind group (115.7 s, range: 40–310), $Z = -3.53$, $p < 0.001$, as shown in Figure 6.

Complications and adverse reactions to perineural injections

The only complication reported was an inadvertent intravenous puncture in one case in the blind injection group. No adverse reactions to perineural injection were observed with either injection technique.

DISCUSSION

This study demonstrated that a US-guided tibial perineural analgesia technique resulted in a greatly increased probability of achieving loss of skin sensation at the heel bulbs compared to an equivalent blind technique.

Skin sensation, which was measured prior to and after performing tibial perineural analgesia, was used to determine the onset of nerve blockade following injection of a local anaesthetic agent. As well as being used clinically, loss of skin sensation has been used

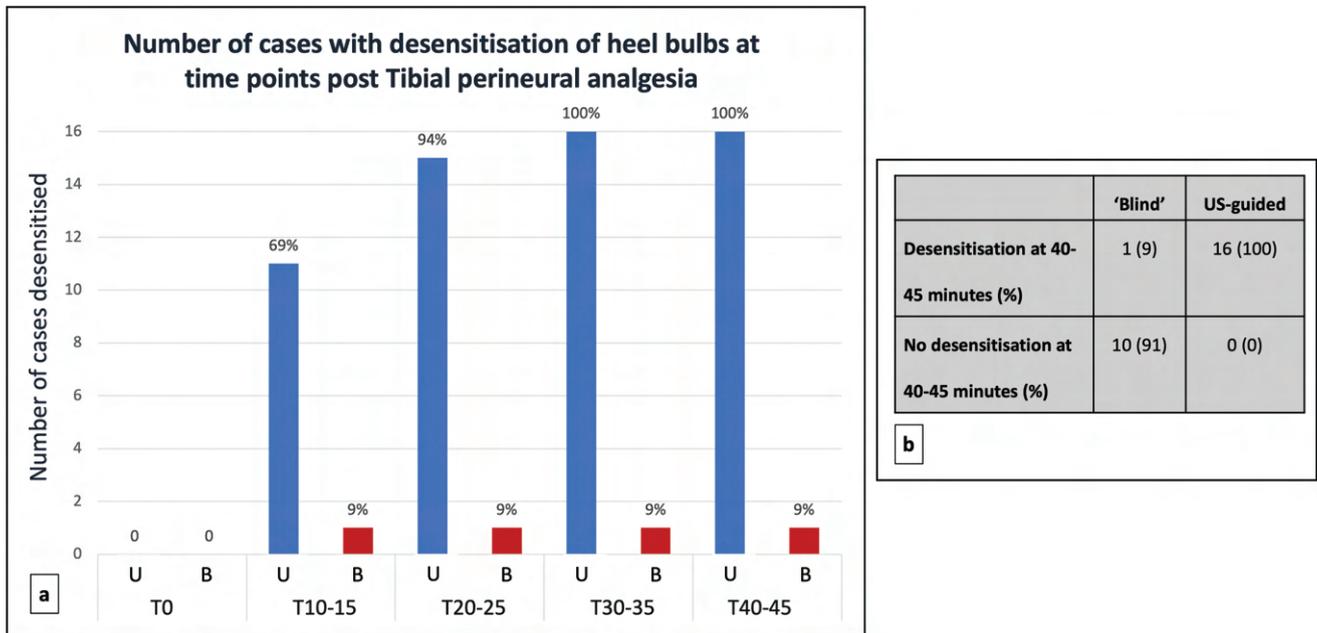


FIGURE 4 (a) Histogram shows the number (and percentage) of cases with desensitisation (no response to ≥ 25 N pressure) of the heel bulbs at time points post (T [min]) tibial perineural analgesia using a 'Blind' (B, columns in red) or a US-guided (U, columns in blue) technique. (b) Table shows the number (and percentage) of cases that had desensitisation (loss of skin sensation [no response to ≥ 25 N pressure] at medial and lateral heel bulbs) or no desensitisation at 40–45 min post-injection, subdivided between injection technique ('Blind' or US-guided).

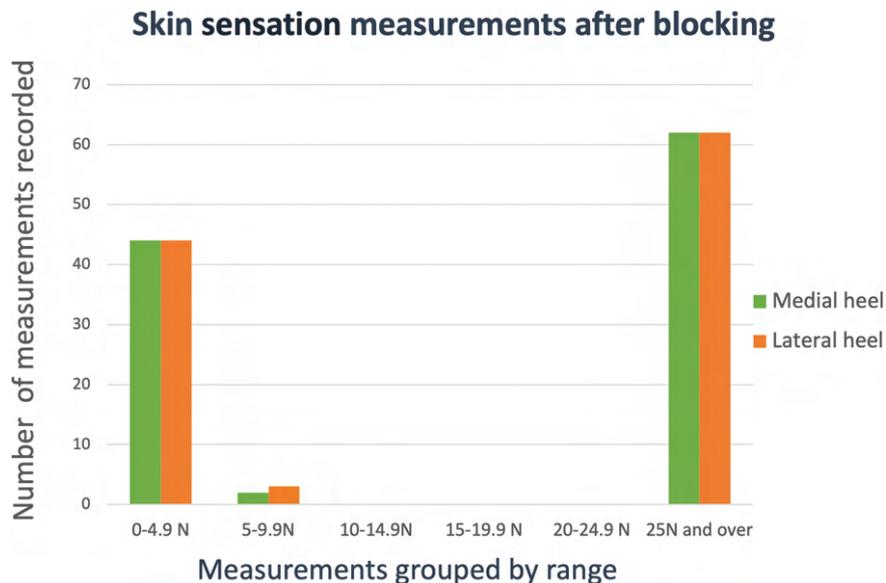


FIGURE 5 Histogram shows mechanical nociceptive threshold (MNT) measurements in Newton (N) for the medial and lateral heel bulb recorded after performing tibial perineural analgesia grouped in ranges.

commonly in research to verify the onset and duration of perineural analgesia (McCracken et al., 2020; Schambourg & Taylor, 2020) and to investigate the diffusion of local anaesthetic agents to nerves in the proximity of injection sites (Hinnigan et al., 2014; Jordana et al., 2014; Miagkoff & Bonilla, 2021). The lateral and medial heel bulbs are autonomous zones (i.e. where testing of the skin sensation provides information on the function of a specific nerve) of the tibial nerve as they are innervated exclusively by the lateral and medial

plantar digital nerves respectively, which are ramifications of the tibial nerve (Labens et al., 2012; Moyer et al., 2011; Prange, 2019; Singh, 2018). Therefore, testing of skin sensation at the heel bulbs was an appropriate assessment method for the tibial perineural injection techniques investigated in this study.

All skin sensation testing was performed by the same operator using a hand-held digital algometer, allowing objective quantification of the effect of tibial perineural analgesia on skin sensation. The

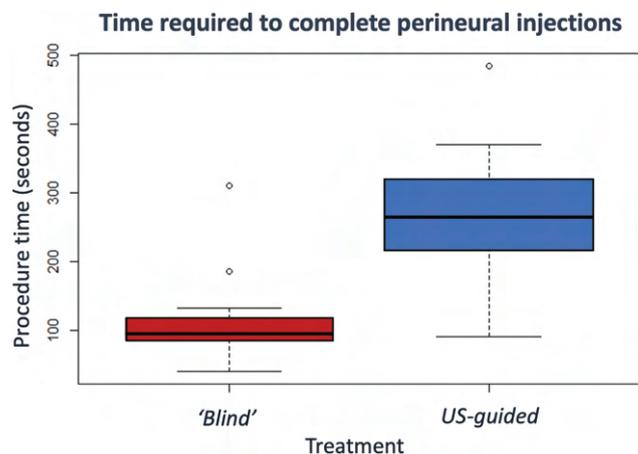


FIGURE 6 Box plot shows procedure (injection) times (s) between the 'Blind' (red) and US-guided (blue) techniques. Lower and upper box lines = 25th and 75th percentiles, respectively; middle box line in bold = median; lower and upper whiskers = lower and upper adjacent values, respectively; open circles = outliers.

operator testing skin sensation was not blinded to the injection techniques being performed.

Algometers are instruments that provide reliable, objective, controlled and safe measurements of mechanical nociception (Gozalo-Marcilla et al., 2020; Luna et al., 2015; Schambourg & Taylor, 2020). The pressures applied using hand-held algometers are manually generated by the operator and are comparable to the pressures that are applied by clinicians when testing skin sensation using a blunt object (e.g. ballpoint pen) in clinical practice.

Previous studies have reported good intraobserver repeatability, interobserver reproducibility and reliability of measurements from algometers, indicating that the use of a single and non-blinded operator would have had minimal effect on the validity of results (Luna et al., 2015).

A binary outcome was observed following tibial perineural analgesia with skin sensation being either present or lost (Figure 5). This pattern of outcome for diagnostic analgesia has been reported by others (Hoerdemann et al., 2017; Schambourg & Taylor, 2020) but partial loss of skin sensation has also been described (Jordana et al., 2014; Miagkoff & Bonilla, 2021). It is possible that the nature of the probe tip used (size and shape) may have played a role in determining the binary outcome observed in this study (Taylor et al., 2016), or that the timing of sensation testing missed cases that had partial loss of sensation.

In 11 out of 27 cases in this study, superficial and deep peroneal perineural analgesia was performed at the same time of tibial perineural analgesia. This is not considered a limitation of this study as the heel bulbs are an autonomous zone of the tibial nerve and therefore skin sensation at this site is unaffected by perineural analgesia of the peroneal nerves.

This study used a US-guided injection technique that differed in a number of respects from the descriptions in the literature (Denoix et al., 2020; van der Laan et al., 2021), although the location of the injection sites was similar. Denoix et al. (2020) described an US-guided

technique using a 25 gauge, 5/8-inch needle and a 6–10MHz microconvex transducer, rather than the linear transducer used in this study. Use of a shorter needle necessitated perineural injection to be performed from two sites (one slightly cranial and the other caudal to the nerve, rather than one). Additionally, 4–10mL less local anaesthetic agent were infiltrated around the nerve. Van der Laan et al. (2021) compared the accuracy of a conventional 'blind' technique and an US-guided technique for perineural injection of the tibial nerve, using cadaveric limbs and a low volume of dye (1mL methylene blue) in the place of local anaesthetic agent. Similarly, to this study, the US-guided technique was performed using a single injection site, a 21 gauge needle inserted cranially to the nerve and a linear transducer (7.5MHz). Ultrasonography, however, was only used to assist in tibial nerve localisation prior to needle insertion and not to guide the insertion of the needle in real time.

The blind injection technique for tibial perineural analgesia selected for this study is one of a number described in the literature. For the majority, the horse is weightbearing on the limb and needle insertion is from the medial aspect. Potentially significant variations include performing the injection with the limb in a flexed position and a lateral approach with the injection being performed from the lateral aspect of the crus (Bassage & Ross, 2010; Carpenter & Byron, 2017).

In their study, Van der Laan et al. (2021) found that perineural injection of methylene blue resulted in successful tibial nerve staining in 85.7% of limbs with the US-guided technique and 47.6% with the 'blind' technique, while 100% of US-guided injections and only 8% of 'blind' injections resulted in successful perineural analgesia in our study. The difference in results suggests that the greater precision and accuracy of needle placement achieved through US guidance is an important factor in successful tibial perineural analgesia, potentially because perineural fat is a barrier to the diffusion of local anaesthetic agent deposited external to this layer (Denoix et al., 2020; van der Laan et al., 2021). The results presented here indicate that the use of 20mL local anaesthetic agent and allowing up to 45min for effect are not sufficient by themselves (blind technique) for adequate diffusion. It seems possible, however, that US guidance might permit the use of a lower volume without impact on the success rate. The use of a lower volume has been described but there are no objective supporting data in relation to success (Denoix et al., 2020).

A longer needle (1.5 inches) was selected for the US-guided injection compared to the needle used for the blind injection (1 inch) and to the needles used by Denoix et al. (2020) and van der Laan et al. (2021). The length facilitated repositioning of the needle for injection of local anaesthetic agent around the nerve without second skin penetration, as well as the shallow angle of tissue penetration helpful to maintaining separation of transducer and needle and to needle visualisation.

An 8–12MHz linear transducer was used to perform US-guided injection in our study, while Denoix et al. (2020) used a 6–10MHz microconvex transducer for the technique. The linear transducer was easy to handle and provided good visualisation of the tibial nerve and needle insertion in all cases, including those horses with

thick skin (cob-type breeds). An advantage of the linear transducer is that it may be more readily available in equine practice.

Performing US-guided tibial perineural analgesia safely in the live horse has been regarded as particularly challenging because of the number of personnel required (van der Laan et al., 2021). Denoix et al. (2020) recommended that two operators restrain the horse, with additional operators responsible for ultrasonographic imaging and for injection of local anaesthetic agent. Despite only one person restraining horses in this study, however, no safety concerns were reported. Nevertheless, the technique requires additional operators (in common with those described in the literature) compared to the blind technique, and the availability of assistance may therefore be a limiting factor for equine practitioners wishing to perform the US-guided technique in the field.

The operators participating in this study, who were experienced in the use of both tibial perineural analgesic techniques, took significantly longer to perform the US-guided technique than the 'blind' technique (275.7 ± 20.6 s vs 115.7 ± 24.9 s; $p < 0.001$). The US-guided injection however was completed in less than 5 min in the majority of cases. Any disadvantage of increased time required to complete the US-guided technique is arguably outweighed by the 100% success rate compared to the 8% success rate for the blind technique given that the need for the injection to be repeated when part of a lameness investigation would be rare, in contrast to the blind technique.

In this study, there were no differences in patient tolerance and operator safety between the two injection techniques, contrary to the expectation that the US-guided technique would be superior in these regards. The good tolerance observed in our study for both techniques may be explained by subcutaneous infiltration with local anaesthetic agent prior to performing tibial perineural injection in all cases. Although not reflected in these results, US guidance reduces the risk of needle puncture of the nerve and the horse suddenly kicking out (Denoix et al., 2020; Rubio-Martinez & Hendrickson, 2021). Whether this reduced risk, together with the decreased requirement for injections to be repeated, outweighs the greater duration of exposure to risk because the US-guided technique takes longer to perform, is not possible with the information available currently. Conclusions about the relative safety of the techniques therefore await further studies.

The study's main limitations are that four different operators performed the perineural injection techniques, that cases were not equally distributed between the two techniques and that the operator testing skin sensation was not blinded. Although no difference in results between operators for the two injection techniques was apparent, case numbers were insufficient and their distribution between techniques was inappropriate to explore intra-operator variability further. A study design with the four operators assigned an equal number of cases for each injection technique may have been preferable.

The absence of pre-existing data meant that sample size calculations were not performed as part of the study design and 20 cases were arbitrarily set as the target. Although additional cases were

recruited, the total number remained relatively low (16 US-guided, 11 blind injection cases). The use of different horse breeds did not seem to influence the results between the two injection techniques; however, no statistical analysis was performed to test the effect of breed, or other independent variables such as, age and sex, due to the small sample size.

In conclusion, the US-guided perineural injection technique for the tibial nerve described in this study was straightforward to perform, well tolerated and resulted in complete tibial nerve analgesia within 30–35 min in all patients.

These results suggest that US-guided tibial perineural analgesia should be used during lameness investigation in preference to blind tibial perineural analgesia when possible. The considerable and significant difference in results observed between the two injection techniques is unlikely to have been greatly impacted by the limitations of the study.

AUTHOR CONTRIBUTIONS

N. Bellitto is the primary author with substantial contribution to study design, data collection, interpretation and analysis of the data and manuscript preparation. L. Voute contributed to study design, interpretation of the data and critical revision of the manuscript. R. Reardon contributed to the interpretation of the data, data analysis and critical revision of the manuscript. J. Withers contributed to study design, data collection and critical revision of the manuscript. All authors approved the final version of the manuscript.

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CONFLICT OF INTEREST STATEMENT

No conflicts of interest have been declared.

FUNDING INFORMATION

None.

ETHICS STATEMENT

The study was approved by the School of Veterinary Medicine Research Ethics Committee, University of Glasgow (Ref EA28/20).

INFORMED CONSENT

Owners gave consent for the horses to participate in this study.

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Static, dynamic and non-weightbearing ultrasound evaluation of the digital flexor tendon sheath improves sensitivity and specificity of manica flexoria tears diagnosis in cobs and ponies

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Summary

Background: Ultrasonography (US) is commonly used as a first-line imaging modality in horses with lameness localised to the digital flexor tendon sheath (DFTS). The reported sensitivity of US for the detection of manica flexoria (MF) tears is low.

Objectives: To report sensitivity (Se), specificity (Sp), positive predictive value (PPV) and negative predictive value (NPV) of US for diagnosing MF tears.

Study design: Prospective observational study.

Methods: Sixty-seven horses (70 limbs) with lameness localised to the DFTS were enrolled. All the horses underwent a standardised US examination of the DFTS including weightbearing (WB), nonweightbearing (NWB) and dynamic NWB US examination. The presence or absence of a MF tear was recorded. All the horses underwent tenoscopic examination as part of the treatment plan. The US diagnosis was recorded and compared with tenoscopic findings. Sensitivity, Sp, PPV and NPV were calculated.

Results: Cobs and ponies were over-represented (46/67 horses). Ultrasonographic examination correctly predicted the presence of a MF tear in 34/37 (92%) limbs (true positive). In 31/33 (94%) limbs, the MF was considered normal during US examination and this was confirmed during surgery (true negative). In two of 33 (6%) cases, US led to a false-positive diagnosis. In three of 37 (8%) cases, US failed to identify the presence of a MF tear (false negative (FN)). In all three cases, the MF was only partially torn. The calculated sensitivity of ultrasonography for the detection of MF tears was 92%, specificity 94%, PPV 94% and NPV 91%. There was no significant difference in the ability of operators to identify MF tears.

Main limitations: Two different operators (both DECVS) performed the ultrasonographic examination. The operator performing the US examination was not blinded to the results of the lameness examination. The tenoscopic examination was performed by the same surgeon who had performed the lameness and ultrasonographic examination.

Conclusions: Ultrasonographic examination of the DFTS is an accurate diagnostic modality to rule in or out the presence of a MF tear. Partial MF tears that do not affect its distal border and/or its attachment onto the SDFT can be challenging to diagnose during US examination.

KEYWORDS

digital flexor tendon sheath, horse, imaging, ultrasound

INTRODUCTION

Digital flexor tendon sheath (DFTS) pathology is a common cause of lameness in horses. The most commonly diagnosed lesions within the DFTS are tears of the deep digital flexor tendon (DDFT) affecting its lateral borders (38%–58% of horses undergoing tenoscopy), superficial digital flexor tendon (SDFT) fibrillation/hyperaemia (14%–38% of horses undergoing tenoscopy) and tears of the manica flexoria (MF) (30% of horses undergoing tenoscopy) (Arensburg et al., 2011; Cender et al., 2023; Findley et al., 2012; McIlwraith et al., 2015; Smith & Wright, 2006; Wright & McMahon, 1999).

The MF is a tendinous collar originating from the medial and lateral borders of the superficial digital flexor tendon (SDFT) and surrounding the DDFT at the level of the distal metacarpal/metatarsal region. The distal free end of the MF is located just proximal to the metacarpo/metatarsophalangeal joint (MCPJ/MCTPJ) (Findley et al., 2012; ICVGAN, 2003). The proposed biomechanical function of the MF is to maintain alignment of the SDFT and DDFT as they run over the palmar/plantar aspect of the MCPJ/MTPJ (Denoi 2003).

Tears of the MF were first described more than two decades ago (Wright & McMahon, 1999). Such injuries are more prevalent in the hindlimbs of ponies and cobs (Findley et al., 2012; Smith & Wright, 2006). Although MF tears are more frequent at the level of the medial attachment to the SDFT, tears of the lateral attachment are not uncommon (Findley et al., 2012; Garcia da Fonseca et al., 2018; Smith & Wright, 2006). Fibrillation and focal tears of the MF can be treated by local trimming and debridement of the torn fibres. Total resection of the MF under tenoscopic guidance is recommended if one margin of the MF is completely ruptured from its attachment to the SDFT (Cender et al., 2023; Smith & Wright, 2006). The prognosis for return to previous level of activity after such procedure is good with 79% of horses returning to pre-injury use (Findley et al., 2012).

Ultrasonography (US) is the first-line imaging modality in horses presented with a DFTS effusion (Schramme & Smith, 2011). The use of US to diagnose MF tears has been reported to have a low sensitivity (38%–64%) (Cender et al., 2023; Smith & Wright, 2006). In recent years, the advantages of nonweightbearing (NWB) and dynamic flexion/extension ultrasonographic examination have been reported (Garcia da Fonseca et al., 2018; Seignour et al., 2012). Namely the reduced tension during NWB ultrasound makes the margins of the MF more easily outlined and increases the diagnostic certainty for MF tears. Furthermore, when the limb is held in NWB position application of digital pressure to the proximal outpouches of the DFTS facilitates identification of the US features of MF tears (Hibner-Szaltys et al., 2022). It is the authors' impression that the sensitivity of US to detect MF tears has significantly improved since last reported.

The aim of this study was therefore to calculate the sensitivity (Se), specificity (Sp), positive (PPV) and negative predictive values (NPV) of US compared with tenoscopy for diagnosing MF tears using the most recently described ultrasonographic techniques. These include NWB ultrasonographic examination, dynamic NWB flexion/extension of the MCPJ/MCTPJ and NWB with digital pressure applied to the proximal DFTS outpouchings.

MATERIALS AND METHODS

Case selection

A prospective observational study was undertaken on 67 client-owned horses referred to Pool House Equine Clinic between January 2017 and July 2022. Twenty-two horses (23 limbs) were previously included in the report by Hibner-Szaltys et al. (2022). Inclusion criteria required the source of lameness to be localised to the DFTS using diagnostic analgesia techniques (perineural and/or intrathecal analgesia). This was performed either by the referring veterinary surgeon or by one of two authors (MM or JW). The degree of lameness at presentation was graded using the AAEP scale. The degree of effusion of the DFTS was graded subjectively on a scale from 0 to 3 (0: no effusion, 1: mild, 2: moderate, 3: severe). In all cases, the ultrasonographic examination was performed prior to tenoscopic evaluation of the DFTS. The tenoscopic examination was performed by the same clinician (Dipl. ECVS) who had performed the ultrasonographic examination; thus, no operator blindness was maintained throughout this study. The US and surgical findings were recorded and compared. Horses presented for investigation of synovial sepsis that underwent US and tenoscopic examination in the same period were excluded from the study. Owners informed consent was obtained for inclusion in the study.

Ultrasonographic examination

All horses that met the inclusion criteria underwent a standardised, US examination of the DFTS. In all cases, the hair was clipped using a No. 40–50 blade and the skin was rinsed using warm water and coupling gel was applied. In cases where a diagnostic image could not be readily obtained the legs were wrapped with soaked bandaging material (Gamgee®, Robinson Healthcare Limited) for at least 1 h before the US examination was performed. The pre-operative US examination was performed following diagnostic analgesia on the same day in all cases in which the lameness was investigated at the Pool House Equine Clinic and prior to surgery for cases where the lameness investigation was carried out by the referring veterinary surgeon. The ultrasonographic examination was performed by the same boarded surgeon (DECVS) performing the lameness investigation and subsequent tenoscopic examination. For US examination performed before January 2019, a GE Logic e R6 machine was used and thereafter a GE Logic e R7 was used instead. The frequency used varied between 8 and 13 MHz depending on the horses' breed and skin thickness.

The standard US examination started with a static examination of the DFTS in a proximodistal direction with the limb in weightbearing (WB) position. This was followed by a NWB examination with the MCPJ/MTPJ resting on the operator's knee or with the dorsal aspect of the hoof wall resting on the floor. In all cases, a dynamic NWB examination was performed as previously described by Garcia da Fonseca et al. (2018). Furthermore, during NWB examination, digital

pressure was applied to the proximal DFTS outpouchings as described by Hibner-Szaltys et al. (2022). Longitudinal and transverse images were acquired during both standing, NWB and dynamic NWB examination (Figure 1). The use of a stand-off pad (HFL38 Tendon Standoff, BCF Ultrasound®) was necessary in some cases to improve probe-skin coupling.

During the US examination, the MF, SDFT, DDFT, synovial membrane, palmar/plantar annular ligament (PAL) straight sesamoidean ligament, oblique distal sesamoidean ligaments and degree of DFTS effusion were assessed. The degree of effusion was subjectively graded on a scale from 0 to 3 (0: no effusion, 1: mild, 2: moderate, 3: severe).

During WB, US examination an asymmetrical appearance of the MF outline on transverse images and the lack of visualisation/shortening of MF outline on longitudinal images were considered suggestive of a MF tear (Garcia da Fonseca et al., 2018; Smith & Wright, 2006). The MF was considered to be asymmetrical on transverse ultrasound images when a difference in thickness, echogenicity or shape was identified between the medial and lateral aspect.

A diagnosis of PAL desmitis was made if the measured thickness of the PAL was greater than 2 mm (Dik et al., 1991). A diagnosis of nonseptic tenosynovitis was made when one or more of the following signs were present: synovial thickening, thickening of the proximal plicae of the DDFT, intrasynovial echogenic debris/adhesions (Smith & Wright, 2006).

During NWB static ultrasonographic examination, the following features were considered to be suggestive of a MF tear (Garcia da Fonseca et al., 2018):

- asymmetrical and/or heterogenous appearance of the MF;
- unattached MF margin; and
- thick irregular edges of the MF.

During NWB dynamic US examination with pressure applied to the proximal DFTS outpouchings, the following features were considered suggestive of a MF tear (Hibner-Szaltys et al., 2022) (Figure 2):

- floating MF fibres floating in the synovial fluid;
- displacement of SDFT in lateral or medial direction relative to DDFT;
- presence of fluid (gap) between SDFT and DDFT at the level of the MF on transverse images; and
- recoiling of the MF.

Tenoscopy

All horses underwent tenoscopic examination of the DFTS under general anaesthesia. The procedure was performed by the same boarded surgeon that had performed the lameness and US examination (MM or JW). In 60 horses, the surgery was performed in lateral recumbency, and in seven horses, the surgery was performed in dorsal recumbency. When the surgery was performed in lateral recumbency, the horses were positioned with the affected limb up-permost. For those cases undergoing surgery in dorsal recumbency,

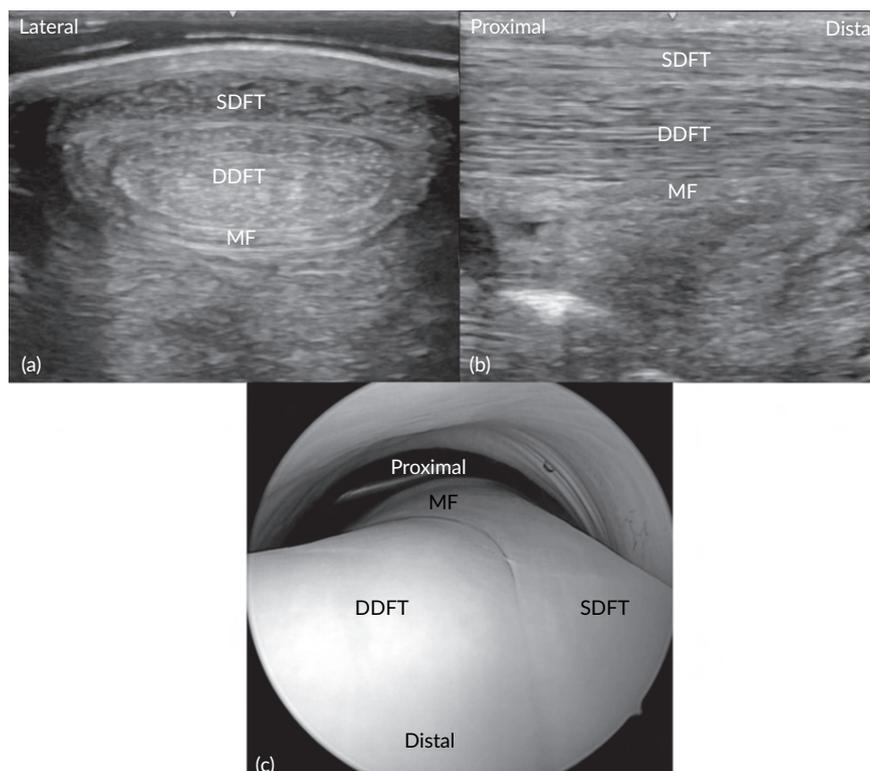


FIGURE 1 Ultrasonographic (a,b) and tenoscopic (c) images showing the normal MF. Transverse (a) and longitudinal (b) ultrasound images of the plantar metatarsal region obtained proximal to the proximal sesamoid bones in a nonweightbearing position.

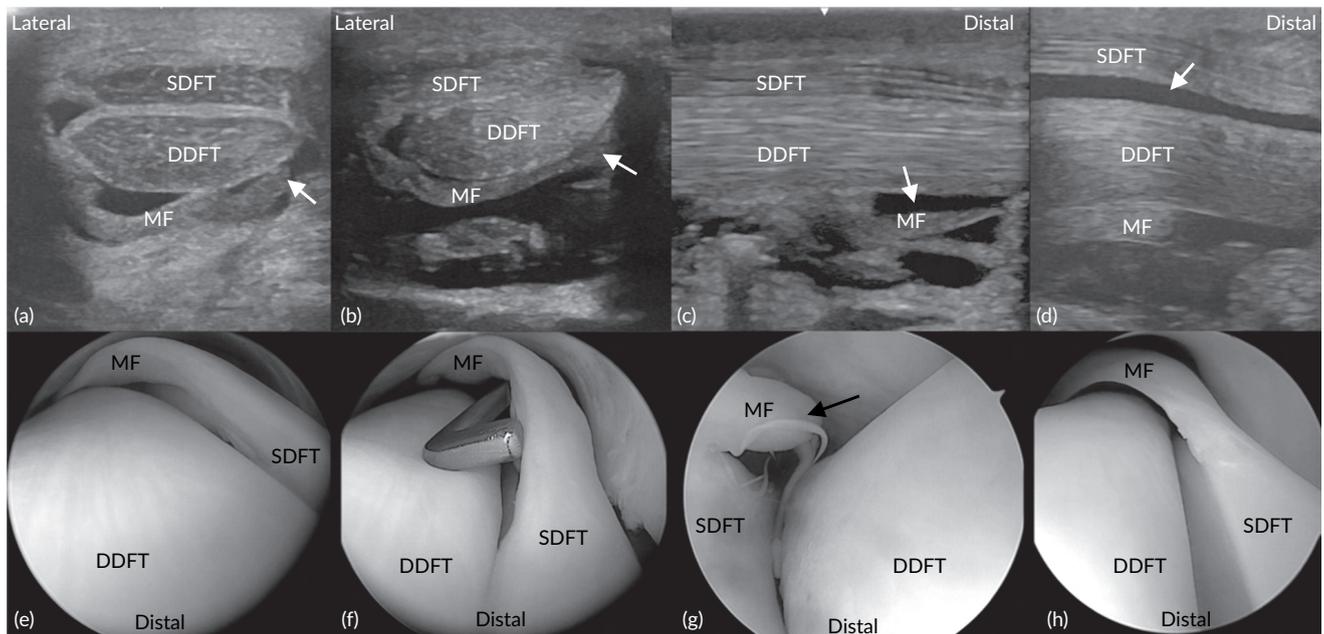


FIGURE 2 Ultrasonographic images showing appearance of abnormal MF (a–d). Transverse ultrasound US images (a and b) of the plantar metatarsal region obtained proximal to the proximal sesamoid bones in a nonweightbearing position (lateral is to the left). Note the recoiling of the MF on the lateral side and the torn MF edge on the medial side (arrow). Longitudinal US images (c and d) of the plantar metatarsal region, obtained proximal to the proximal sesamoid bones in a nonweightbearing position (proximal is to the left). Note the gap present between the MF and DDFT (arrow). In image (d) it is possible to identify a gap between the SDFT and DDFT at the level of MF (arrow). Both these US features on longitudinal images are consistent with MF tear. Tenoscopic images (e–h) obtained with the arthroscope inserted at the level of the base of the lateral proximal sesamoid bone, directed proximally, note a gap between SDFT and DDFT, recoiling of the MF (e, f and h). Image (g) it is possible to see a complete tear of the lateral attachment of the MF to the SDFT is completely torn, note torn fibres at the level of the MF attachment (arrow).

the affected limb/s was hoisted at 120° and positioned with the MCPJ/MTPJ slightly flexed in neutral position. After aseptic preparation of the surgical site, the DFTS was distended with sterile saline. The arthroscope was inserted using the conventional basisesamoid lateral portal (Findley et al., 2012). In one horse with a bilateral MF tear, the medial approach was used in the contralateral limb. The PAL was transected in those cases where the arthroscope could not be easily advanced through the fetlock canal. The tenoscopic examination of the DFTS started from the proximal dorsal recess; the arthroscopic lens was then rotated to assess the dorsal aspect of the MF. The arthroscope was then redirected in order to examine the proximomedial and proximolateral outpouchings. Then, the arthroscope was introduced between the MF and the DDFT to be directed in the proximal cul-de-sac and DDFT mesotendons. The distal part of the DFTS was examined by redirecting the arthroscope distally. In cases where a MF tear was diagnosed, total resection of the MF was performed as previously described (Findley et al., 2012).

Data analysis

Data were analysed using descriptive statistics. Sensitivity, specificity, negative predictive value (NPV) and positive predictive value (PPV) were calculated.

RESULTS

Clinical findings and diagnostic analgesia

Sixty-seven horses (70 limbs) met the inclusion criteria (46 geldings, 20 mares and one stallion). Their age ranged from 5 to 25 years (mean 13.25). Breeds included 46 cobs and ponies, 10 Irish Sport Horses, three Warmbloods, three Thoroughbreds, two Spanish horses, two Friesian and one Irish Draught horse. Ultrasonographic examination was performed on 15 forelimbs (six left forelimbs (LF) and nine right forelimbs (RF)) and 55 hindlimbs (30 left hindlimbs (LH) and 25 right hindlimbs (RH)).

The degree of effusion of the DFTS varied from mild to severe (grade 1–3/3). The degree of lameness observed at presentation varied from a grade 2 to a grade 4/5 on AAEP scale (mean grade 3/5 AAEP for the forelimbs and the hindlimbs).

Diagnostic analgesia of the medial and lateral palmar/plantar nerves at the level of the base of the sesamoid bones (abaxial sesamoid nerve block) did not result in a significant improvement of the lameness in any horse. Intrathecal anaesthesia of the DFTS improved the lameness by 50% or more in 28 limbs (40%). Diagnostic analgesia of the medial and lateral metacarpal/metatarsal nerves, medial and lateral palmar/plantar metacarpal/metatarsal nerves (low 4/6-point nerve block) improved the lameness by 80% or more in 46

limbs (66%). In four cases both intrathecal and low 4/6 point nerve block were performed. Diagnostic analgesia was performed by the same operator that performed the US examination and subsequent tenoscopy when the lameness investigation was performed at the clinic (59/67). Diagnostic analgesia was performed by the referring veterinary surgeon for the cases referred for ultrasonographic examination and subsequent tenoscopy (8/67).

Ultrasonographic findings

The US examination using the above-mentioned US protocol (i.e. WB, WNB and dynamic NWB) was performed following diagnostic analgesia, on the same day, by the same veterinary surgeon. During the US examination a MF tear was diagnosed in 36 limbs (51%) in 35 horses. Of these, 35 were hindlimbs (97%) (20 LH and 16 RH) and one forelimb (3%) (LF). Palmar/plantar annular ligament desmitis was diagnosed in 16 limbs. A SDFT tear was diagnosed in four limbs. A concurrent tear of the SDFT and DDFT was diagnosed in two limbs. Nonseptic tenosynovitis without any tendon pathologic abnormalities (i.e. SDFT, DDFT lesions or MF tears) was diagnosed in two limbs. Desmitis of the proximal digital annular ligament was diagnosed in one case. A synoviocoele of the DFTS was diagnosed in two limbs. An avulsion fracture of the plantarolateral border of the lateral sesamoid bone communicating with the DFTS was diagnosed in one case (1.4%). A longitudinal DDFT lesion alone was diagnosed in five limbs. Nonseptic tenosynovitis secondary to another lesion was present in 61 limbs.

During US examination with the limb in WB position, an asymmetrical appearance of the MF on transverse ultrasonographic images was diagnosed in 18 limbs. The MF outline on longitudinal images was not visible/shorter in 17 limbs. At least one US feature consistent with MF tear could be identified in 25 limbs during US examination with the limb WB. During static NWB US examination, 27 limbs presented asymmetric thickening of the MF, and in six limbs, the MF presented an unattached margin. In 13 limbs, one of the MF edges was irregular. At least one US feature consistent with MF tear could be identified in 27 limbs during US examination with the limb in NWB. During dynamic NWB US examination, floating MF fibres were identified in 16 limbs. The presence of a gap between the SDFT and DDFT was present in 22 limbs. Medial or lateral displacement of the SDFT was present in 13 cases. At least one US feature consistent with MF tear could be identified in 36 limbs during dynamic NWB US examination (Table 1).

Tenoscopic findings

During tenoscopic examination, a diagnosis of MF tear was made in 37/70 limbs. In 11 out of 37 limbs (29.7%), the MF tear affected the lateral attachment of the MF onto the SDFT. Of these 11 limbs, nine (82%) were diagnosed with a partial tear and two (18%) were diagnosed with a complete tear. In 23 out of 37 limbs (70.3%), the MF

TABLE 1 Summary of the ultrasonographic findings consistent with MF tears identified using WB, NWB and dynamic NWB ultrasonographic examination.

	US findings – WB examination		US findings – static NWB examination			US findings – dynamic NWB examination			
	Asymmetrical appearance MF outline	Lack of visualisation/Shortening MF outline on longitudinal images	Asymmetrical/heterogeneous appearance MF	Unattached MF margin	Thick irregular MF edges	SDFT displacement in relation to DDFT	Floating MF fibres	Increased gap between SDFT and DDFT on transverse images	Recoiling of MF
n	18	17	27	6	13	13	16	28	20
%	48.65%	45.95%	72.97%	16.22%	35.14%	35.14%	43.24%	75.67%	50.05%

tear affected the medial attachment of the MF onto the SDFT. Of these 23 limbs, 12 (52%) were diagnosed with a complete tear and 11 limbs (48%) were diagnosed with a partial tear.

In one limb (2.7%), the MF tear was located axially (i.e. dorsal sagittal position). In two limbs (5.4%), the MF tear started proximal to the distal lateral attachment of the MF onto the SDFT. In one of these limbs, the tear started proximal to the distal lateral attachment of the MF and it was longitudinally orientated. In the other limb, the MF tear originated laterally proximal to the distal border of the MF and was transversally orientated leaving the distal border of the MF intact.

Additional lesions were identified in five horses. In two cases, a DDFT tear was diagnosed; in two, a SDFT tear was diagnosed, and in one case, both tendons (SDFT and DDFT) were diagnosed with a longitudinal tear.

SENSITIVITY AND SPECIFICITY CALCULATIONS

Ultrasonography accurately predicted the presence of a MF tear in 34/37 limbs (true positive (TP)). In 31/33 cases, the absence of a MF tear was correctly diagnosed during US examination (true negative (TN)). In two cases, US led to a false-positive (FP) diagnosis. In both cases, a hindlimb was affected. In three cases, US failed to identify the presence of a MF tear (false negative (FN)). Two of these were hindlimbs and one forelimb. The calculated sensitivity for the detection of MF tears was 92%, specificity 94%, PPV 94% and NPV 91% (Table 2).

DISCUSSION

The results of this study confirm that a recently described modified US technique improves sensitivity, specificity and overall detection of MF tears in a predominantly cob/pony population compared with the values reported in previous studies (Cender et al., 2023; Smith & Wright, 2006). The calculated sensitivity, specificity, PPV and NPV in this study are 92%, 94%, 94% and 91%, respectively. In contrast to previous investigations reporting a poor sensitivity of US for the detection of MF tears (38%–64%) (Cender et al., 2023; Smith & Wright, 2006) in the current study, the sensitivity of US for the detection of MF tears was significantly improved (92%).

Although US examination with the horse in WB position remains an important part of a complete US protocol for examination of the

DFTS, a feature consistent with MF tears could only be identified in 25 out of 37 limbs during WB examination. During static NWB examination, a feature consistent with a MF tear could be identified in 27 out of 37 limbs. However, when dynamic flexion/extension and digital pressure were added a feature consistent with MF tears was identified in 34 out of 37 limbs. The advantages of a NWB and dynamic flexion/extension ultrasonographic technique have previously been reported (Garcia da Fonseca et al., 2018; Seignour et al., 2012). A wider contact surface (probe/skin) allows for better visualisation of the tendons and ligaments borders as well as MF contours (Seignour et al., 2012). Furthermore, during flexion of the MCPJ/MTPJ, the tension on the flexor tendons (SDFT and DDFT) is reduced making the margins of the MF more readily identifiable (Garcia da Fonseca et al., 2018). An added advantage of performing NWB ultrasonographic examination in cob breeds is the ability to use a more proximal ultrasonographic window where the skin is usually thinner, and a better image quality can be obtained. Application of digital pressure to the proximal DFTS outpouchings during NWB examination further facilitates the identification of US features consistent with MF tears. The increased turbulence of the synovial fluid created within the DFTS facilitates identification of floating torn MF fibres (Hibner-Szaltys et al., 2022). The results of this study confirm that inclusion of NWB US examination, including dynamic flexion/extension and digital pressure applied to the proximal DFTS significantly improve sensitivity, specificity, positive and negative predictive value of US for diagnosing MF tears compared with previously reported (Cender et al., 2023; Smith & Wright, 2006).

In two limbs (both hindlimbs), the presence of a MF tear was diagnosed during ultrasonographic examination; however, during tenoscopic examination, the MF was found to be intact (FP). In both cases, a lesion of the SDFT at the level of its plantarolateral border was identified during US examination (Figure 1). The presence of such lesion could have misled the operator due to the proximity of the lesion to the lateral attachment of the MF onto the SDFT. Furthermore, in one case, adhesions originating from the medial DFTS wall extending in a dorsolateral direction were identified during tenoscopy (Figure 2). In this case, the presence of floating adhesions replicating the direction of the MF in a medio-dorso-lateral direction could have contributed to a FP diagnosis.

In three limbs (two hindlimbs and one forelimb), the MF was considered normal during US examination; however, during tenoscopic examination, the MF was found to be torn. In all cases, the MF was only partially torn. In one case, the tear was proximal to the distal attachment of the MF onto the lateral border of the SDFT and was longitudinally orientated. Therefore, the appearance of the distal profile of the MF was not significantly altered. In the second case, the MF was torn at the level of its most sagittal portion and extended 5mm proximally. In the third case, the MF was transversally torn at the level proximal to its lateral insertion onto the SDFT. The distal border of the MF was only minimally affected by the tear. The incomplete nature of these lesions combined with the unusual location of the MF tear could be considered the main factors leading to a false-negative US diagnosis.

TABLE 2 Summary of the results of the ultrasonographic examination and tenoscopic examination.

	MF torn	MF intact
US findings confirm presence MF tear	34	2
US findings rule out MF tear	3	31

The sensitivity and specificity of ultrasonography for diagnosing MF tears calculated in this study compares favourably to those reported for contrast tenography (92% vs. 85% and 94% vs. 72% respectively) (Kent et al., 2019). Although the use of US and contrast tenography are not mutually exclusive, the results of this study support the use of US to rule in or out the presence of MF tears when this lesion is suspected, and contrast tenography is not readily available.

We believe that several factors contributed to the improved sensitivity of ultrasonography to diagnose MF tears compared to previously reported:

- Technological improvements in the last decade have provided US equipment able to deliver images of improved definition and quality.
- Introduction of NWB, dynamic NWB and application of digital pressure to the proximal DFTS outpouchings as part of routine ultrasonographic examination of the DFTS (Garcia da Fonseca et al., 2018; Hibner-Szaltys et al., 2022; Seignour et al., 2012).
- Description of ultrasonographic features of MF tears (Garcia da Fonseca et al., 2018).
- Increased veterinary surgeons awareness of the condition (MF tears).

There are several limitations the authors would like to acknowledge, the main one is related to the fact that the lameness examination, the US examination and the subsequent tenoscopic examination, in each case, were performed by the same clinician. As a consequence, the operator performing the US examination was not blinded to the results of the lameness investigation, which likely resulted in some level of interpretation bias. Additionally, a sample size estimation based on the most recently reported sensitivity and specificity of US for the detection of MF tears (Cender et al., 2023) suggest that 602 limbs would be needed (Hajian-Tilaki, 2014) to achieve statistical significance. Based on such sample size, the calculated statistical power of the current study is 21%. Therefore, in order to meet the sample size required, undertaking a multicentre study should be considered in future.

In conclusion, this prospective study has demonstrated that US evaluation of the DFTS using the above mentioned protocol (WB, NWB and Dynamic NWB), is an accurate diagnostic tool to rule in or out the presence of a MF tear in cases with lameness localised to the DFTS. Partial MF tears that do not significantly affect the integrity of the distal border of the MF and its attachment onto the SDFT remain challenging to diagnose during ultrasonographic examination.

AUTHOR CONTRIBUTIONS

M. Marcatili, J. Withers, F. Cantatore and M. Hibner-Szaltys contributed to study design, study execution, data analysis and interpretation. All authors contributed to the preparation of the manuscript and gave their final approval of the manuscript.

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CONFLICT OF INTEREST STATEMENT

No conflicts of interest have been declared.

ETHICS STATEMENT

All aspects of case examination, diagnostic investigation and case management were within the best standards of medical care. No institutional animal care protocol was required.

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SO EFFECTIVE... IT'S LIKE MAGIC

Clinical perspective: The perils, pitfalls and pride of developing an equine in vitro fertilisation (IVF) laboratory in private practice

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Summary

The use of intracytoplasmic spermatozoa injection (ICSI) to produce equine embryos has significantly increased over the past decade, leading to the creation of laboratories specific for ICSI and related in vitro fertilisation (IVF) techniques. However, the commitment, resources and skills necessary for developing a viable IVF laboratory can be underestimated or underappreciated. The purpose of this article is to share the difficulties our clinic encountered when developing an IVF laboratory.

KEYWORDS

horse, embryo transfer, ICSI, in vitro fertilisation

INTRODUCTION

The use of in vitro fertilisation (IVF)—especially intracytoplasmic spermatozoa injection (ICSI)—in horses has significantly gained in popularity over the past decade (Squires, 2019). The ICSI method involves the insemination of a mature (MII) oocyte with a single spermatozoa (Figure 1), followed by in vitro culture to generate a viable blastocyst (Figure 2; Choi et al., 2002, 2016; Galli et al., 2013). These in vitro produced (IVP) embryos can either be transferred fresh or cryopreserved for transfer at a later time (Claes & Stout, 2022; Galli et al., 2007, 2014; Gelo et al., 2019). Current estimates suggest at least 5000 IVP equine embryos were produced worldwide in 2022, and the reality exists that more IVP embryos will be produced in the forthcoming years compared to those derived in vivo.

Presently, ICSI is the most common method of in vitro fertilisation (IVF) in equids but not the only form of IVF. Conventional or traditional IVF is another method and involves the co-incubation of spermatozoa with oocytes, and thus differs from ICSI by instead relying on the spermatozoa's inherent ability to fertilise an oocyte. In humans and cattle, traditional IVF is commonly used with good results (Buratini et al., 2021; Ferré et al., 2020). In horses, consistent results using IVF have historically been elusive, but a recent article described a repeatable method that led to the birth of at least five live foals (Felix et al., 2022). Other IVF techniques of commercial use in horses include preimplantation genetic diagnoses (PGD) for

sex determination or monogenetic disease testing and blastocoele collapse of large (>300 µm) embryos (Choi et al., 2009).

The future appears both bright and burgeoning for IVF techniques in horses. Recognising this trend, our clinic undertook the task of developing an IVF laboratory in 2020. We are now a few years in, and, while we are far from experts in the field of equine IVF, the project has granted enough perspective to offer counsel on what *not to do* when undertaking such a venture. The purpose of this article is to share the perils and pitfalls we encountered when starting an IVF laboratory, hoping the information-sharing lightens the load and lessens the anxiety for future equine embryologists and IVF laboratories. With respect to this last point, we did not want to hinge our laboratory's future outlook solely on the ICSI technique. Thus, while ICSI will be referred to throughout this article, we deemed it an appropriate starting point to explore and develop other IVF techniques, thereby designating us as a true in vitro fertilisation laboratory (Table 1).

THE DO-NOTS

Do not skimp on the budget

Whether it be time, money or space, starting an IVF laboratory 'from scratch' requires significant investments of all three of these things. With respect to time, the commitment is sizeable and—

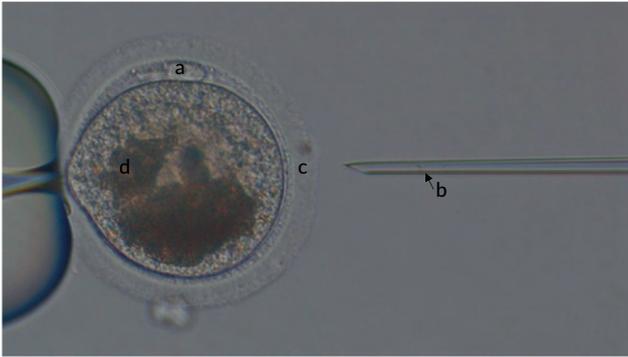


FIGURE 1 Intracytoplasmic spermatozoa injection of an equine oocyte. Note the presence of the polar body (a) and an immobilised spermatozoa (b) within the tip of the injection pipette. This tip is used to breach the zona pellucida (c) to allow for deposition of the spermatozoa into the oolemma (d).

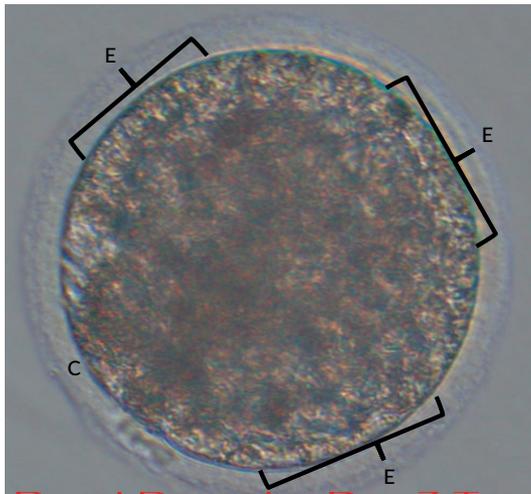


FIGURE 2 An equine blastocyst 7 days after Intracytoplasmic spermatozoa injection. Distinguishing characteristics include circumferential thinning of the zona pellucida (C) and presumptive trophoblast cells (E).

times—ceaseless. In relating this experience to a road race, we knew we were involved in a marathon but the route to the finish line was littered with roadblocks. Delays were encountered with sourcing a suitable laboratory space, equipment delivery, product backorders, installation and quality control. Laboratory design, construction, organisation and maintenance required a 7-day-a-week schedule, which proved particularly demanding during the breeding season. The duties of private practice also had to be prioritised, pushing the time needed to work in the laboratory into the later hours of the day.

In our case, approximately 2–8 h per day were initially devoted to the start-up. Now that we have transitioned to offering clinical IVF, veterinarians spend 20–40 h per week performing ICSI, cleavage and blastocysts checks, preparing embryos for transfer or vitrification, and conducting experiments. We also have a technician working 40 h per week in the laboratory, as well as other part-time assistants

working ~10–20 h per week. These technicians are well trained and motivated and have been instrumental in our success, especially with respect to laboratory maintenance and quality control (QC). In addition to assisting the veterinarians, technicians have the added duties of media prep, cleaning, performing daily QCs, ordering, billing, laboratory organisation and, to some extent, client communication. When taken as a whole, significant people hours were and still are required to organise and maintain an IVF laboratory.

The finances associated with an IVF laboratory are also substantial. Design, construction, equipment, consumables and salaries run into the hundreds of thousands of dollars. Several pro forma drafts (Figure 3) were submitted to project the costs involved, as well as future expenses and profits. Mistakes—which invariably occur—were also costly, whether they were due to time delays or replacing damaged parts. Intellectual value and skills of a proficient equine embryologist also represents a significant financial investment yet has the upside of accelerating the speed with which a laboratory can provide commercial quality results. We thus explored means to offset costs and gather technical expertise by seeking collaboration with outside private organisations, public institutions and qualified embryologists. The majority of embryologists we encountered had backgrounds with either cattle and/or human IVF. While their knowledge and intellectual property can be significant. Equine gametes behave differently *in vitro* than those of other species, thereby necessitating further time, training, commitment and resources regardless of one's background in IVF. We ultimately forged ahead by investing in continuing education of current veterinarians and employees, but, concurrently, developed relationships with clinical and research embryologists, as well as distributors and suppliers of IVF materials, which we continue to utilise to this day. Also worthy of mention were two textbooks we found highly useful for the inception, development, maintenance and improvement of our IVF laboratory. *In Vitro Fertilisation: A textbook of Current and Emerging Methods and Devices* (Nagy et al., 2019) and *Clean Room Technology in ART Clinics* (Esteves et al., 2017).

Identifying a space and constructing a laboratory within it was another significant issue. The current specs of our IVF laboratory are 24' × 19' with 8' high ceilings. It was a space previously housing medical records and required extensive renovation and remodelling. We designed it to contain ample counter space, as well as below-counter space, which can be used to keep the incubators, warming trays, refrigerator, freezer, etc. It also contains two islands that provide additional countertop and cabinet spaces. A notable design error we made was, despite installing numerous electrical outlets throughout the laboratory, all were placed above the countertops, and the islands were not electrified. Additionally, this space has been adequate for starting the laboratory but has also quickly filled up and could have benefited from more forethought. In retrospect, we would have preferred a larger space and designed it with two separate laboratory spaces connected by a hallway. The first room would be the 'embryo room' where the majority of IVF procedures would be performed, while the second room would be for processing of semen and storage of both frozen semen and embryos.

TABLE 1 Basic items needed to start equine ICSI.

Resources	Examples	Comments
Equipment	Stereo-microscope Inverted microscope CO ₂ incubators Multi-gas incubators Micromanipulators Microinjectors Biosafety cabinet Centrifuge Computers and monitors Pipettors Viasensor gas analyser VOC meter pH meter Warming trays or incubators Antivibration platforms Chemistry scale/balance	Should have heating elements Will require CO ₂ and N ₂ gas tanks; recommend installing a manifold Will need two each (left and right) Can also use a laminar flow hood or IVF station Recommend one used for small samples Should be equipped with image-acquisition software Handheld and electric Used for quality control measures
Consumables	Laboratory cleaner Pipette tips Serological pipettes Conical tubes Petri dishes Cryotubes Filters Flourinert Microholding pipettes Microinjection pipettes	Ensure these items are suitable for use in cell culture and or an IVF laboratory; mouse embryo assay (MEA) or sperm survival tests are recommended; these can be performed by the distributor or in-house
Media	Fetal bovine serum M199 Earls M199 Hanks GMOPS Oil overlay Global media DMEM/F-12 media Hyaluronidase Polyvinylpyrrolidone Sterile water for tissue culture	By no means an exhaustive list but contains most items necessary to initiate basic ICSI

Finally—and as will be discussed later—the location and dimensions (especially the height) of our laboratory created challenges in maintaining proper air quality, which can adversely affect embryonic development rates in IVF laboratories (Agarwal et al., 2017; Heitmann et al., 2015; Urman & Yakin, 2012).

Do not purchase cheap equipment

While obviously related to the budget, this consideration deserves added emphasis. The motto 'you get what you pay for' has rung true in our experience. We explored equipment options with different vendors, as well as used equipment. Quality was a big factor and so too were customer service and support. Forming relationships with our installation technicians and company representatives facilitated information exchange and troubleshooting, especially during the nascent stages of the laboratory.

Specific equipment acquired included a stereoscope, inverted microscope, micromanipulators and -injectors, piezo drill, heated

stages, warming trays, balance/scale and single channel pipettes. A commercial and combination refrigerator and freezer were also purchased. However, smaller, individual units would likely have been a better use of space. Antivibration platforms (AVP) were also necessary due to the fine movements required during micromanipulation. Whether it be bumping the table or a horse stomping its foot upstairs, vibrations can be transmitted from various sources—and from various distances—which can obscure the field of vision and bungle an injection. Solid granite, pneumatic or a combination of both are all available as AVPs. We currently have both a solid granite table and a combination unit for our inverted microscopes. A major advantage of the combination units is their portability, which is useful given their overall weight.

Both CO₂ and tri-gas box incubators were initially purchased for oocyte maturation and culture respectively. Benchtop incubators have since been obtained for culturing embryos; these incubators have grown in ubiquity and appear to provide superior performance relative to traditional box incubators (Gelo et al., 2019; Martino et al., 2019; Meyers et al., 2019). Also purchased was a biosafety

	Year 1	Year 2	Year 3	Year 4	Year 5
Revenue					
Embryos Produced	-	?12???	?245???	567???	??&*??
Vitrification Fee		&*???	#VALUE!	78?90?	90???
ICSI Session	-	76?76?	85??5??	90?87?	34???
Total Revenue	-		#VALUE!		
Cost of Goods Sold					
Drugs & Medical Supplies	??&?7	#VALUE!	#VALUE!	#VALUE!	#VALUE!
Total Cost of Goods Sold	-	#VALUE!	#VALUE!	#VALUE!	#VALUE!
Profit Margin	-	#VALUE!	#VALUE!	#VALUE!	#VALUE!
Salary Expense					
Compensation - Director	-	-	-	-	-
Compensation - Vets	-	-	-	-	-
Total Salary Expense	-	-	-	-	-
Other Employee Expense					
Professional Liability	78?92?	76?9??	90???	23??56	??90??
Continuing Education	87?90?	??9*??	90?87?	67?5??	??????
Total Other Employee Expense	-	-	-	-	-
Facility Expense					
	34,000	34,000	34,000	34,000	34,000

FIGURE 3 Example of a pro forma used to evaluate the economic impact of starting and maintaining an equine in vitro fertilisation laboratory.

cabinet (BSC) for media and plate preparation. Either an IVF workstation (similar footprint as a BSC) or a regular laminar flow hood (smaller footprint than a BSC) would likely have sufficed, but—with its built-in UV light and visor window—allows for sterilisation of plastic-ware and an added barrier of protection.

Consumables are another important consideration and dependent on several factors, including size of maturation and culture drops/system, incubator type and technician preference. Plastic-ware labelled 'mouse embryo assay (MEA)' tested are preferred, media and supplements should be 'suitable for cell culture' and we order the majority of consumables from IVF-specific distributors. Alternatively or in addition, in-house bioassays can be performed using sperm survival tests with each new lot of plasticware (De Jonge et al., 2003).

Given the many moving parts involved in starting the laboratory, media preparation proved to be a time-consuming and mistake-laden task, especially when a designated technician was not available. We thus purchased the majority of the media from a commercial source (IVF Limited T/A IVF Bioscience) that came pre-made and with quality assurance. This 'ready-made' media significantly reduced prep time and proved highly convenient. Now that we have a full-time technician and more experience, these are no longer as significant concerns as they once were, relying more and more on our own media prep. We recommend checking pH on all new lots of media and for troubleshooting purposes (Pool, 2004). We use an iStat machine (Abbot Labs), which is a point of care blood-gas analyser often used for critical care cases.

Do not underestimate the importance of air quality

Air particulates, micro-organisms and volatile organic compounds (VOCs) are all substances that can hamper embryo development rates within an IVF laboratory. Several studies from human laboratories report a direct correlation between improved air quality and fertility, cleavage, blastocyst and pregnancy rates (Agarwal et al., 2017; Heitmann et al., 2015; Morbeck, 2019; Mortimer et al., 2018; Urman & Yakin, 2012). Evidence also exists regarding the significance of good air quality in equine ICSI laboratories, particularly as it relates to blastocyst rates (Herrera et al., 2023). High efficiency particular (HEPA) filters combined with solid phase filtration through carbon filters are commonly used within IVF laboratories to improve and maintain good air quality. Positive pressure systems can also be installed and likely represent the best (but most expensive) means of maintaining good air quality (LifeAire Systems LLC).

Unfortunately, our laboratory has none of these. Instead, it has a 'split' unit that only conditions the air already present within the room. There are carbon filters through which this air runs, but the initial air supply is unfiltered and instead trickles in from either the ceiling (above which is an office and living quarters) or the outside basement area. This situation is not desirable and is still a struggle. We initially used air purifying systems sold at local home improvement stores. These were effective at removing a lot of air-borne particulates, but they were loud and generated uneven air currents.

We have moved to a single air unit (Zandair) within the laboratory, which is somewhat effective but unable to scrub completely the entire air supply. They are not able to trap VOCs and/or create positive pressure within the laboratory. Consequently, the laboratory is vulnerable to changes in and contaminants from the outside environment. As for trying to retrofit our current laboratory, our structural engineers found the laboratory was 'imprisoned' by steel beams and concrete supports, requiring further remodelling to re-configure the ventilation system. Also, purchasing and installing a commercial VOC-trap (LifeAire Systems) are expensive, and total costs have been estimated to be in the hundreds of thousands of dollars. It will also lead to significant down-time in the laboratory.

We obviously regret not factoring in air quality during the initial development of the laboratory. Workarounds we currently employ include: placing activated-carbon traps inconspicuously inside the laboratory; as well as running air purifiers throughout the building in which the laboratory is housed. We measure $[VOC]_{\text{laboratory}}$ one to two times a day with a commercial VOC monitor (WW Grainger Inc) and maintain a level ≤ 0.5 ppm. Deviations above this limit are taken seriously and the source(s) identified and eliminated.

Do not rely on the equipment companies to troubleshoot

Stated another way: Become very familiar with your equipment. From studying the product manuals to watching videos on YouTube™ to cracking open a college physics book: knowing how and why the equipment works provides several advantages. First, it allows you to troubleshoot quickly, saving time and money without waiting on a service call. Troubleshooting is a key component of maintaining an IVF laboratory; yet, its importance is seldom disclosed. Whether it be addressing temperature fluctuations on heated surfaces, contamination of culture dishes or elevated $[VOC]_{\text{laboratory}}$ —issues are commonly encountered but seldom discussed in the literature. In addition, some issues can be subtle or a function of the individual laboratory, requiring individual sleuthing and retracing of steps to identify the problem(s).

Familiarity with the equipment also allows for an educated dialogue with colleagues, company representatives and service technicians, which can expedite information exchange and fixing equipment issues. The pioneers of these techniques are also educators, so they seem immune to trivial questions and thus willing to share their insights. Sales reps and techs are equally helpful, but most of their experience is limited with respect to handling and processing *equine* gametes and embryos. They will rely on you for knowing proper incubator settings, surface temperatures, sizes of pipettes, type of maturation/culture dishes, etc. They will also rely on you for placement of the equipment, which can affect workflow and functionality of the laboratory.

Lastly, a working knowledge of the equipment inherently increases your skill and dexterity at both micromanipulation and the various nuances of cell culture. As a consequence, workflow and

efficiency are improved, which translates into improved embryo development rates. Obtaining this knowledge requires further reading and, occasionally going back to basic sciences. For example, we initially experienced issues in using our piezo drill (Hiraoka & Kitamura, 2015). It was not until we got a better understanding of angles of alignment and the physics behind cellular piercing devices did we start to see significant improvements in IVF efficiency and embryo development rates (Ru et al., 2016; Sadak et al., 2019).

Do not rely on your memory

It is likely that >99% of humans do not have a photographic memory. As such, activities within a laboratory—from monitoring surface temperatures to checking media pH—should be manually recorded and logged for future reference. There are many factors to account for, and some have a relatively narrow margin of error. Keeping accurate records allows for review of past performances and facilitates troubleshooting. It is also crucial when multiple individuals are working within the laboratory to avoid overlap and mistakes. Examples of systems routinely monitored in our laboratory include:

- Temperatures (indoor, outside, surfaces, incubators, warm trays/heating stages, refrigerator and freezer)
- Humidity (indoor and outside)
- Gas concentrations within the incubators
- $[VOC]_{\text{laboratory}}$

The above are all components of our quality control (QC) programme, which is an essential part of our daily operations and allows for reliable maintenance and reduction of error. When someone quipped, 'Variability is the nemesis of consistency', they were likely referring to an IVF laboratory. We thus take numerous measures to reduce variability, and we are always searching for more.

To this end, we keep individual laboratory notebooks for experimental methods and materials, brainstorming, diagram sketching and general laboratory notes. Also, our laboratory utilises whiteboards and clipboards to keep track of daily duties including: injection schedules, cleavage/blast checks, media prep, embryo transfers and vitrifications (Figure 4). These are helpful for not only keeping us on task, but also to forecast what may be coming to the laboratory in the near future. Moreover, they provide an added layer of communication among members of the laboratory, improve efficiency and reduce human error.

Along these same lines, it is important to have some quantitative measures of laboratory performance. Common metrics we use are:

- Oocyte recovery rate = No. oocytes recovered / No. follicles punctured
 - Goal > 50%
- in vitro maturation (IVM) rate = No. oocytes matured (MII) / No. oocytes placed in maturation media
 - Goal > 55%

Last Updated: 5/29/23								
Daily Duties List - Week of _____								
Duty	MON	TUE	WED	THU	FRI	SAT	SUN	Notes
Turn Up								
Turn Down								
QCs								
Move Oocytes								
pH Testing								
Cleavage/Blast Checks								
Plate Prep								
Label Prep								
Vitrification								
Order Semen								
Semen Prep								
Denudation								
Aliquot								
Update Boards								
ICSI Sessions								
Wipe Down All Surface								
Client Updates								
Clean BSC								
Empty Trash								
Swiffer								
Sweep and Mop								
Clean Inside Air Filters								
Dust								
Change Footpads								
Fill Cleaning Bottles								

FIGURE 4 Template of daily duties commonly performed in an in vitro fertilisation laboratory.

- $\text{Cleavage rate} = \frac{\text{No. oocytes cleaved}}{\text{No. oocytes inseminated}}$
 - Goal $\geq 70\%$
- $\text{Blastocyst rate} = \frac{\text{No. blastocysts produced}}{\text{No. oocytes inseminated}}$
 - Goal $\geq 20\%$
- $\text{Blastocyst per OPU} = \frac{\text{No. blastocysts produced}}{\text{No. OPUs performed}}$
 - Goal > 1.0

We analyse this data on a monthly basis, evaluating it on a ‘rolling’ basis (e.g. ‘Rolling 120Days’, ‘Rolling 90Days’, etc). Though not fool-proof, this method of evaluation allows for surveillance of recent and past trends, especially for tracking laboratory improvement.

Do not be afraid of change

On the surface, this pitfall may seem contradictory to the previous concept of consistency and the importance thereof. However, the difference lies in the execution and the evolution of proficiency within the laboratory. Commonly referred to as workflow, this concept embodies the daily sequence of events used for procedures within the laboratory, including ICSI, cleavage/embryo checks, vitrification and media prep. Our equipment did not arrive all at once, and, with time and the addition of new items, we had to adjust our workflow. For example, we realised the importance of setting up the laboratory, so the stereoscope used for moving

gametes and embryos between plates were in close proximity to the incubators, minimising the amount of time they were exposed to room air. The micromanipulator was placed on an AVP in a low draught area with ample space to prevent interference from personnel or other equipment. Our refrigerator was within an arm's length of the BSC, allowing for quick exchanges of media between these two.

As alluded to earlier, another evolution that occurred was learning how to use the piezo drill for ICSI (pdICSI) in addition to 'conventional' ICSI (cICSI). In our experience, cICSI had a shorter learning curve and did not seem to take as much prep time as pdICSI (Yoshida & Perry, 2007). However, the literature suggested pdICSI afforded several advantages, including: improved blastocyst rates, ability to collapse the blastocoele cavity and biopsy of the trophectoderm (Costa-Borges et al., 2020; Salgado et al., 2018). As a result, painstaking work was put into developing and optimising the piezo drill settings for these various techniques.

With time and practice, we developed preferences for certain brands or manufacturers as they relate to pipettors, plasticware and media. Sometimes, backorders or shortages forced our hand, requiring us to look for alternatives and/or replacements for items we had previously developed preferences. In these instances, a major or temporary change in instrumentation or materials may be necessary. As mentioned previously, we prefer disposable products that are MEA-tested, but can run in-house bioassays if necessary. The same holds for pipettes and tips. We also realised that what worked best for another laboratory does not necessarily work best for you, but usually requires trial-and-error. To account for changes, standard operating protocols (SOPs) are prepared for all procedures and accessible in both hard and digital copies. They are periodically reviewed and updated. Notable changes are placed on a clipboard and discussed at monthly meetings.

Do not try to set up the laboratory without a consistent supply of oocytes and semen

Our recipient mares are the unsung heroes of our IVF laboratory, generously donating their time and oocytes to practice ICSI, develop culture systems and test embryo viability. Their contributions should not be downplayed, and we continue to use them, whether it be for QC purposes, evaluating new media or developing new techniques (Foss et al., 2013; Lewis et al., 2016). Throughout the development of our laboratory, we have made every effort possible to aspirate at least one mare a day during weekdays, so we could perform ICSI for five consecutive days. Doing so provided consistent motivation and practice to hone our skills.

Our recipient herd was also important for embryo viability testing. In our opinion, the best test of embryo viability was transfer of 'homemade' in vitro produced (IVP) embryos. Well over a dozen transfers were made before successfully establishing a pregnancy, and further transfers were necessary as we sharpened our skills and became more efficient at embryo production.

Transfers have also been necessary for testing new freezing and thawing protocols, as well as evaluating the viability of biopsied embryos.

There is the potential to source oocytes from other places. These would include an abattoir, teaching herd or diagnostic laboratory. Depending on the region or country, some of these sources do not exist, provide only limited access due to welfare concerns or cannot release specimens due to biosecurity. Oocytes of bovine or murine origin can also be used. These are useful for practicing the specific ICSI procedure but have the drawback of not being able to perform viability testing due to their xenographic nature.

With respect to semen, there are several ways to process it for IVF procedures, so plenty is needed to fine-tune protocols (Morris & Maclellan, 2019). Fresh semen was commonly obtained from collections performed earlier in the day, while frozen semen was used from semen destined for disposal. Owner consent was obtained with the understanding the semen was going to be used only for experimental purposes and no live offspring would be produced. Finally, we noticed trends in development rates when certain donors and/or sperm was used. These tendencies had the unexpected benefit of helping train newer members of the laboratory by instilling confidence. We also continue to keep these combinations available to help troubleshoot unexpected drops in rates.

CONCLUSION

The purpose of this discussion was to highlight some of the major pitfalls we experienced when starting up an IVF laboratory. While some of these have been big problems, nothing has been insurmountable. Therefore, we are hopeful that sharing our experience will not only provide confidence and wherewithal for future laboratories, but also promote further information-sharing among those with an active interest in equine IVF techniques.

AUTHOR CONTRIBUTIONS

C. Scoggin was responsible for compiling the information, reviewing references and preparing the bulk of the article. E. Bradecamp, E. Lohbeck, P. Sheerin and M. Schnobrich were responsible for providing data for analysis, reviewing the article and making edits. A. Barhorst and C. Howard assisted with data analysis and reviewing the article. All authors have approved this article.

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CONFLICT OF INTEREST STATEMENT

No conflicts of interest have been declared.

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ETHICS STATEMENT

Animals used during the developmental stages of our laboratory were owned by our company and treated humanely, receiving peri-procedure pain management and monitoring. Pregnancies were terminated between 28 and 60 days of gestation.

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CASE IMAGE

Multimodal imaging and surgical repair of a dorsal-oblique plane, proximal third metacarpal fracture and the diagnostic challenge of radiographic visualisation

Owen Fletcher  | Craig Osborne | Jonathon Dixon Rainbow Equine Hospital, Old Malton,
North Yorkshire, UK**Correspondence:** Jonathon Dixon
Email: imaging@rainbowequinehospital.co.uk**Summary**

A 10-year-old Connemara mare presented with persistent 3/5 (AAEP) right forelimb lameness following a traumatic incident whilst hunting, which was localised to the proximal metacarpal and distal carpal regions with diagnostic anaesthesia. Radiography revealed non-specific moderately increased mineral opacity at the proximomedial margin of the third metacarpus, dorsal to the entheses of the proximal suspensory ligament. However, subsequent computed tomography (CT) performed under general anaesthesia, identified an incomplete, dorsal - obliquely orientated third metacarpal fracture arising from the interosseous space between the third and second metacarpals, also involving the carpometacarpal joint. Using CT guidance, internal surgical fixation was performed under the same anaesthetic episode and resulted in a shortened post-operative recovery period with a successful return to previous athletic function. Surgical fixation can be performed using cross-sectional imaging guidance to optimise recovery and facilitate a positive outcome.

KEY WORDS

horse, computed tomography, incomplete fracture, third metacarpal, traumatic

SIGNALMENT, HISTORY AND CLINICAL FINDINGS

A 10-year-old Connemara mare presented to Rainbow Equine Hospital for evaluation of right forelimb lameness, of 8 weeks' duration. The lameness was acute in onset following a fall while hunting, whereby the limb became fixed in a caudal position with residual forward momentum leading to perceived supraphysiological extension of the carpus. Lameness assessment identified a consistent grade 3/5 right forelimb lameness (AAEP), with the lameness most marked on the lunge on a soft surface on the right rein. No response was elicited with hoof testers, however, carpal flexion exacerbated the lameness. Limb palpation was otherwise unremarkable. A 50% improvement was observed to anaesthesia of the lateral palmar nerve, and on a separate occasion, the lameness was abolished 5 min post instillation of 10 mL of mepivacaine (Intra-epicaine, Dechra Veterinary Products) into the middle carpal joint.

Right carpal radiographs (Canon Medical Systems Ltd) were obtained (70kVp, 0.15 s), revealing increased bone opacity, with loss of

trabecular detail of the proximomedial third metacarpus (Figure 1). An osteochondral fragment was suspected adjacent to the radial carpal bone, though inconsistently appreciated. Ultrasonography showed non-specific heterogeneity of the proximal suspensory ligament, with an altered fibre pattern, though without overt enlargement adjacent to the origin at the palmaroproximal aspect of the third metacarpus. The owner elected conservative management and therefore the middle carpal joint was aseptically medicated with 10 mg of triamcinolone acetonide (Adcortyl, 10 mg I.A., Bristol Myers Squibb), with 1 week of box-rest prescribed, followed by in-hand walking and small paddock turnout.

IMAGING, DIAGNOSIS AND OUTCOME

The horse re-presented 3 weeks later, due to a perceived lack of response to intra-articular medication. On examination, the lameness remained grade 3/5 (AAEP) on the right forelimb and was now most notable on the right rein while lunging on a hard surface.



FIGURE 1 Lateromedial and dorsopalmar radiographs of the right carpus acquired 8 weeks post-trauma, upon initial presentation. Note the increased mineral opacity within the proximomedial aspect of the third metacarpus.

Ultrasonography and radiography of the carpal region were repeated. A subtle hyperechoic osseous protrusion was identified on the palmar margin of the radial carpal bone ultrasonographically and the palmar recess of the middle carpal joint was moderately effused with synovial proliferation. Given the lack of response to intra-articular medication and lack of definitive aetiology, computed tomographic (CT) imaging was recommended for further assessment.

The horse was premedicated with acepromazine (AceSedate, 0.02 mg/kg bwt I.V., Jurox) and romifidine (Sedivet, 0.06 mg/kg bwt I.V., Boehringer Ingelheim Animal Health UK Ltd.). Anaesthesia was induced using ketamine (Ketamidol, 2.5 mg/kg bwt I.V., Chanelle Pharma) and diazepam (Ziopam, 0.05 mg/kg bwt I.V., TVM UK Animal Health Ltd.), and maintained using a continuous rate infusion at 1–1.2 mL/kg/hr of guaifenesin (Myorelax 100 mg/mL, Dechra Veterinary Products UK), ketamine (14 mL–Ketamidol, 2.7 mg/kg bwt I.V., Chanelle Pharma) and romifidine (3 mL – Sedivet, 0.06 mg/mL I.V., Boehringer Ingelheim Animal Health UK Ltd.). Non-contrast computed tomography was performed in right lateral recumbency using a 16-slice multidetector CT scanner (GE RT 16 [GE Healthcare]). Images were acquired from the level of mid radius to the middle phalanx using the following technical variables; 120 kVp, 200 mAs, slice thickness 0.625 mm, field of view 25 cm, matrix of 512 × 512. Images were reconstructed using bone and soft tissue algorithms.

On CT, an incomplete, hypoattenuating plane (non-displaced, monoarticular fracture) extended 15 mm in a palmaromedial to dorsal-axial orientation from the proximal-medial articular surface of the third metacarpus (at the carpometacarpal joint), tracking distally and abaxially within the medial epiphysis and the medial

cortex of the proximal diaphysis (Figure 2). The lesion was present within the medial cortex opposing the second metacarpus, communicating with the interosseous space and was orientated in a dorsal-oblique plane. Moderate regional increased bone attenuation (densification/endosteal sclerosis) surrounded the lesion within the third metacarpus, and abaxially, roughened periosteal bone was evident. The lesion's proximo-distal length extended 22 mm from the carpometacarpal joint. Surrounding the hypodense plane at the proximal margin there was focal osseous resorption, whereas distally there was more consistent hyperattenuation and loss of trabecular architecture. Abaxial to the medial cortical fracture was a millimetric mineral attenuating fragment within the interosseous space. Mild middle carpal joint osteoarthritis and synovial effusion were present. The proximal suspensory ligament was not enlarged, and the central hypodense fat-muscle-connective tissue bundles were well-defined. However, there was mild focal roughening of the proximomedial cortices of the third metacarpus.

Following fracture identification, surgical repair was elected under the same anaesthetic and therefore, to avoid prolonging anaesthesia duration, no contrast media was used to complement the non-contrast CT examination. Due to the inability to radiographically visualise the lesion, and to aid surgical management, metal skin staples were placed using CT guidance to identify the optimum instrument placement for internal fixation (Figure 3).

Procaine benzylpenicillin (Depocillin, 22,000 IU/kg bwt I.M., Intervet UK Ltd.), gentamicin (Genta Equine, 8.8 mg/kg bwt I.V., Dechra Veterinary Products UK), flunixin (Finadyne, 1.1 mg/kg bwt I.V., MSD Animal Health) and morphine sulphate (0.1 mg/kg bwt

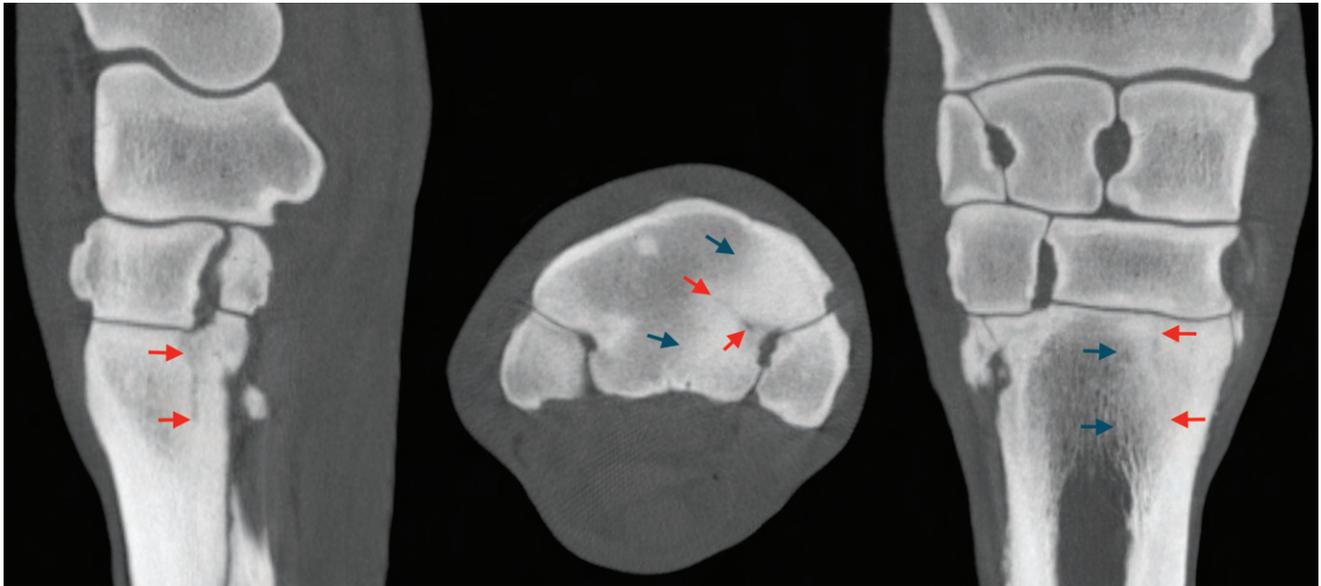


FIGURE 2 Sagittal, transverse and frontal computed tomographic reconstructions of the right proximal third metacarpus. Red arrows denote the fracture plane located medially. Dark blue arrows outline surrounding increased mineral attenuation.

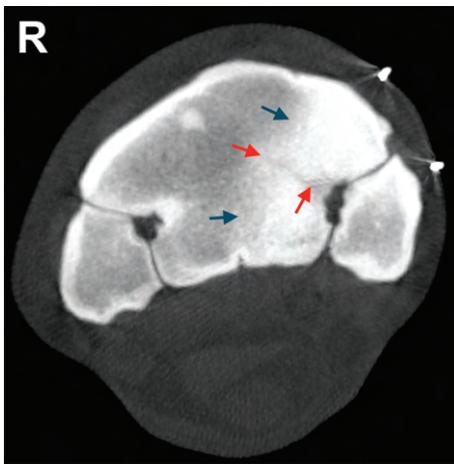


FIGURE 3 A transverse computed tomographic reconstruction with skin surface staples denoting the dorsal margin of the second metacarpus and a second staple indicating the desired entry point for subsequent screw placement. Red arrows denote the fracture plane. Dark blue arrows outline the surrounding increased mineral attenuation.

I.V., Martindale Pharma) were administered. The mare was moved into theatre and positioned in dorsal recumbency. An endotracheal tube was placed and general anaesthesia was maintained using isoflurane in 100% oxygen, delivered by a large animal breathing system. Routine aseptic skin preparation was performed with a 4% chlorhexidine gluconate solution. An 8mm skin incision was made immediately dorsal to the CT-positioned staple. A 2.5mm pilot hole was drilled followed by a 3.5mm glide hole to the depth of the fracture line, measured on CT examination. A 2.5mm insert sleeve was placed into the glide hole and the 2.5mm thread hole drilled across the remainder of the third metacarpus. The thread hole was tapped

by hand using a 3.5mm tap with tap handles, and a 3.5mm 36mm cortical screw placed in lag fashion. Radiographs obtained prior to final tightening showed appropriate screw positioning. The incision was flushed and closed with 3 metric poliglecaprone (Monocryl Ethicon). The leg was flexed to approximately 70 degrees, and the middle carpal joint arthroscopically approached with a standard dorsolateral approach via a stab incision mid-way between the extensor carpi radialis tendon and common digital extensor tendon. This revealed small score lines on the intermediate and third carpal bones with no further abnormalities identified. The middle carpal joint was injected with 30mg of morphine at the completion of surgery and a two-layer bandage applied prior to a rope-assisted recovery. Perioperative antibiotics and flunixin (Finadyne, 1.1mg/kg bwt I.V., MSD Animal Health) were discontinued after 72h. The horse was discharged with 6 days of phenylbutazone (Equipalazone, PO, Dechra Veterinary Products UK), and 14 days of box rest, followed by hand walking for 5 weeks, small paddock turnout for 5 weeks and reassessment after 3 months.

Twelve weeks following hospital discharge, repeat radiography confirmed satisfactory implant position (Figure 4). There was no evidence of right forelimb lameness at the walk or trot. The horse was unshod, and a very mild left forelimb lameness was noted, both in a straight line and when lunged on the left rein on a hard surface. The lameness dramatically improved on a soft surface and was attributed primarily to the absence of shoes. For confirmation, a palmar digital nerve block was placed in the left forelimb, which resolved the lameness, rendering the horse completely sound.

The horse was subsequently shod in the forelimbs, and a gradual introduction to ridden work was undertaken, starting with ridden walking exercise for 10min per day, increasing incrementally to 25min of ridden walking exercise per day over 4 weeks. Trotting exercise was subsequently introduced starting with 5min per day



FIGURE 4 Right carpal radiographs obtained 12 weeks post-surgery, with a single lag screw in situ.

increasing by 5 min per week for a further 4 weeks. These were undertaken without complication and the owners' perception was that the horse remained sound, returning to full exercise at week 20.

Final reassessment 25 weeks following surgery revealed no lameness under any circumstances, including under saddle at walk, trot and canter. The horse had been shod for this examination. Full work had been resumed and the horse was performing successfully in hunting exercise.

DISCUSSION

Fractures of the proximal third metacarpus in a sagittal plane (Beccati et al., 2019; Lloyd et al., 1988; Morgan & Dyson, 2011) and fractures of the dorsal cortex (Jalim et al., 2010) have been described. To the authors' knowledge, there is only one prior report of an incomplete, frontal/dorsal plane fracture of the proximal third metacarpus (Paschke & Walliser, 2016). The distinguishing features in the case presented here include an acute lameness following known trauma, computed tomography and CT-guided surgical repair description and the subsequently shortened rehabilitation period resulting in a successful return to full exercise.

Similarly, to the report by Paschke and Walliser (2016), the horse in this case was an older, non-Thoroughbred. However, rather than an accomplished dressage mare with an increasing workload, this case was a leisure horse used for hunting with a known history of trauma. The limb had been caught in a hole in the ground leading to caudal and extended limb positioning with perceived carpal hyperextension. The oblique orientation of the fracture plane is suspected to have arisen as a consequence of supraphysiological compression and/or torsion. In previous reports, including that of Paschke and Walliser (2016), a stress-fatigue injury was hypothesised (Beccati et al., 2019), extrapolated from stress-related fractures identified at other sites, for example, those seen in the young Thoroughbred

racehorse. Therefore, irrespective of partial similarity of location, the presenting features, and thus likely management and outcome factors are likely to be different in a known traumatic event.

A moderate grade of lameness was observed in both this horse and the case by Paschke and Walliser (2016), and in both cases, radiographic evaluation was of limited benefit. In this instance, complete superimposition of the fracture plane with the head of the second metacarpus prevented radiographic diagnosis, further supporting the use of cross-sectional imaging modalities such as MRI or CT. Despite limited documentation in the literature, it is considered that similar fractures have likely previously occurred but remained undetected or a presumptive diagnosis (Lloyd et al., 1988). The medial origin of this fracture resulted in regional densification, which had also extended to surround the entheses of the proximal suspensory ligament by the time of presentation. It is presumed that given the traumatic history, this sclerosis/densification was not pre-existing and the horse had no history of forelimb lameness to suggest otherwise. These findings are interesting however, especially considering the partial response to anaesthesia of the lateral palmar nerve and subsequent resolution of the lameness with intra-articular anaesthesia of the middle carpal joint. On the basis of a non-contrast CT examination, the proximal suspensory soft tissues were normal. Computed tomography was performed to investigate possible middle carpal articular fragmentation and for further assessment of potential proximal suspensory ligament enthesiopathy, but instead proved vital both to rule these out and for the subsequent diagnosis and to guide surgical treatment.

Radiographic examination did identify osseous abnormalities within the region affected, though not the specific injury this horse had sustained. Increased mineral opacity within the palmaroproximal aspect of the third metacarpus is otherwise associated with enthesiopathy of the proximal suspensory ligament. Similarly, arthroscopic evaluation alone in this case would have found evidence of osteoarthritis (cartilage score lines apparent on examination),

however, would have failed to identify the fracture leading to an incorrect diagnosis and potentially inappropriate management. As with previous radiographically discrete fractures at this level (Beccati et al., 2019), CT facilitated a thorough assessment of osseous anatomy while extending the anaesthesia length by only 10 min; with a total general anaesthetic episode of 150 min. Consequently, the fracture plane location was identified, and the extent was accurately determined. Skin staples were placed under CT guidance due to the lack of radiographic visibility facilitating timely surgical intervention. Surgical repair was performed with a singular 3.5 mm cortical screw placed in a lag fashion across the fracture. This was performed in part due to the success of management of dorsal cortical fractures in third metacarpal/metatarsal bones with cortical screw fixation (Jalim et al., 2010), and for the risk of propagation of the fracture with the forces experienced during the recovery from general anaesthesia; although this was at the time deemed to be low risk. To the authors' knowledge, no previous literature documents the use of a cortical screw in a lag fashion for a fracture of this configuration. We suggest that as is seen with dorsal cortical fractures, surgical management may result in a more rapid return to function than conservative management. In addition, it was hypothesised that internal fixation would reduce the extent of callus formation, which may have occurred with conservative treatment (Paschke & Walliser, 2016) and could have impacted on the other local structures such as the carpometacarpal joint.

In the report by Paschke and Walliser (2016), their metacarpal fracture required coaptation of 4 weeks duration and a total of 8 weeks strict box rest prior to discharge, with ridden exercise not being reinstated until 22 weeks post admission. With our fracture, stabilised by surgical repair, the horse was discharged 4 days post-surgery, began walking exercise 2 weeks later following suture and bandage removal and remained sound at walk with no lameness seen. A controlled exercise programme was reinstated with increasing walking exercise and small paddock turnout over a period of 12 weeks to allow adequate time for bone healing. The mare was reassessed 12 weeks following discharge, was sound under all conditions on the affected limb and had returned to ridden exercise, increasing to full exercise by week 20. At the owners' request, further assessment at week 25 confirmed the horse remained sound, and she has continued to perform at the desired level since then, without further interventions.

The 10-min CT examination proved invaluable for determining the unidentified pathology and enabling screw placement accurately, under the same general anaesthetic. The ability to obtain a definitive diagnosis and perform internal fixation resulted in a shorter time to discharge and a more rapid return to walking exercise than previously reported. This also facilitated shortened hospitalisation and reduced the overall recovery period; with a return to full exercise taking only 20 weeks. Hypothetically, without CT, the failure of combined radiographic and arthroscopic examinations to identify the fracture may have led to a seemingly persistent lameness following surgery. This reinforces the benefit of early cross-sectional (CT or magnetic resonance imaging) studies in atypical lameness cases

(Beccati et al., 2019), for both diagnostic and prognostic purposes. It is the authors' consideration that such merit justifies the additional time and financial expense. Additionally, CT clearly identified an osteophyte rather than the osteochondral fragment within the middle carpal joint that was suspected based on radiographs. In the context of the pre-existing middle carpal joint osteoarthritis, persistent lameness would have encouraged a more guarded outlook for future soundness. Meanwhile, limited evidence would suggest that athletic prognosis in these fractures could be considered good (Paschke & Walliser, 2016).

A limitation of this study is that, whilst the fracture is the most pronounced finding on CT imaging, it remains impossible to definitively differentiate respective contributions towards lameness from the other minor findings. In the authors' opinion, the intra-articular anaesthesia of the middle carpal joint could desensitise the fracture given the articular component, due to communication between the middle carpal and carpometacarpal joints. High palmar perineural anaesthesia may have provided additional information prior to surgery in this case, as it could be expected from the subsequent findings that this would have significantly improved or abolished the lameness in this case.

In conclusion, this report supports the use of cross-sectional imaging in cases where there are no definitive clinical or conventional imaging findings or where there is a lack of correlation with the presenting complaint. Additionally, internal fixation of incomplete dorsal plane third metacarpal fractures may facilitate a more rapid return to exercise than conservative management alone.

AUTHOR CONTRIBUTIONS

All authors contributed to the study design, study execution and preparation of the manuscript. All authors gave their final approval of the manuscript.

CONFLICT OF INTEREST STATEMENT

The authors have no conflicts of interest to declare.

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Not required for a retrospective case report.

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Continued from page 80

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Zycosan has been shown to prolong coagulation parameters up to 24 hours after injection, therefore caution should be used when administering this drug before or after strenuous activities (see Target Animal Safety). Due to the anticoagulant effects, this drug may exacerbate Exercise Induced Pulmonary Hemorrhage (EIPH). The concurrent use of NSAIDs with Zycosan has not been evaluated.

Due to the anticoagulant effects of Zycosan and known anticoagulant effects of some NSAIDs, caution should be used if NSAIDs are concurrently administered. Horses concurrently treated with Zycosan and NSAIDs should be monitored for hemorrhage or other clinical signs of abnormal bleeding (e.g., petechiae, ecchymosis, or epistaxis). The safety of long-term repeat use of Zycosan has not been evaluated. Pigmentary changes in the retina (pigmentary maculopathy) have been reported in human patients following long-term oral use of pentosan polysulfate sodium. It is not known if a similar finding occurs in horses. The safe use of Zycosan has not been evaluated in breeding, pregnant, or lactating horses.

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ADVERSE REACTIONS:

Injection site reactions were the most frequently reported adverse reactions in the field study. Injection site reactions were associated with clinicopathology changes in some cases. Other adverse reactions reported in more than one horse were prolongation of coagulation parameters (activated partial thromboplastin time (aPTT) and prothrombin time (PT)), lethargy, behavior changes, and colic.

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REVIEW ARTICLE

A review of prevention and management of castration complications

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Summary

Despite castration being one of the most frequently performed elective equine surgical procedures, complication rates are high and range from severe life-threatening conditions to mild complications that are of no consequence to the horse. This article will briefly review strategies to prevent complications and surgical castration techniques before reviewing, in-depth, the following complications: excessive scrotal swelling and seroma formation, scrotal infection, septic funiculitis, septic peritonitis, haemorrhage, evisceration, omental prolapse, pyrexia, tetanus, penile trauma, hydrocele, signs of colic and continued stallion-like behaviour. Whilst this list of complications can be daunting, an understanding of male anatomy, castration techniques and an awareness of possible complications can reduce both the incidence and mortality of complications, should they occur. This article will consider castrations performed in both a hospital and field setting and will review complication prevention, surgical techniques of castration and the management of intra-operative and postoperative complications.

KEYWORDS

horse, castration, complication, evisceration, haemorrhage, infection

INTRODUCTION

Bilateral orchiectomy (castration) is one of the most frequently performed elective equine surgical procedures (Kilcoyne et al., 2013; Schumacher, 2019). Despite the relatively straightforward nature of the procedure and the frequency at which it is performed, reported intraoperative and postoperative complication rates range from 10%–60% (Hodgson & Pinchbeck, 2019; Kilcoyne et al., 2013; Mason et al., 2005; Rosanowski et al., 2018) with 14.5% of castrations having one or more intraoperative complications (Hodgson & Pinchbeck, 2019). Complications can occur within minutes, hours, days, months or even years after castration. Complication severity ranges from those that cause no distress to the horse but are a concern for the owner (hydrocele or continued undesirable behaviour) to mild complications that resolve with minimal intervention (oedema, scrotal infection), to severe and life-threatening complications (haemorrhage, evisceration, septic peritonitis) (Rosanowski et al., 2018).

Although castration is considered a routine surgical procedure, mortality rates are reported between 0.3% and 1% (Kilcoyne et al., 2013; Mason et al., 2005).

PREVENTION

A thorough understanding of anatomy and the various techniques for castration will help prevent complications. Furthermore, the ability to quickly recognise and manage complications may reduce mortality and morbidity. Clinical examination of the colt prior to castration should focus on answering the following questions.

- Are there any systemic conditions that may increase the risk of complications (pyrexia indicating systemic infection, infected wounds, harsh lung sounds, heart murmur, poor body condition, suspicion of a high parasitic burden etc...)?

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- Are there two testis present?
- Is there evidence of a scrotal or inguinal hernia?
- Are the facilities and assistants available during the surgery sufficient for a safe castration?
- Which castration technique is most suitable? Is the horse's conformation and temperament suitable for a standing castration? Is a field anaesthetic safe and feasible?

Horses that react violently to scrotal or inguinal palpation, small equids or equids with poorly developed testes may be best castrated under general anaesthesia (GA).

As an elective surgical procedure, the use of perioperative antimicrobials must be considered, studies have shown the benefits of peri- and postoperative antimicrobials. Horses that received procaine penicillin and nonsteroidal anti-inflammatory drug (NSAID) had significantly lower serum amyloid A (SAA) and significantly fewer infections postoperatively compared to horses that only received NSAID therapy (Busk et al., 2010). The duration of antimicrobial therapy should also be considered as SAA and fibrinogen concentrations were significantly higher and Beta-haemolytic *Streptococcus* spp. more frequently cultured when horses were only administered a single dose of intravenous sodium penicillin G compared to a 3-day course of daily intramuscular procaine penicillin (Hauke et al., 2017). Depending on the castration technique employed, the author prescribes either procaine penicillin (22 mg/kg bwt IM BID) or oxytetracycline (7.5 mg/kg bwt IV BID) preoperatively and continues these for 24 h postoperatively.

The British Equine Veterinary Association (BEVA) clinical guidelines recommend perioperative, intraoperative and postoperative analgesia, with NSAIDs recommended for 3 days postoperatively (Bowen et al., 2020). As SAA remains elevated for up to 8 days postcastration (Jacobsen et al., 2005), it could be considered reasonable to prescribe NSAIDs for at least one-week postcastration. The author administers phenylbutazone (4.4 mg/kg bwt IV) preoperatively and continues oral phenylbutazone at 2.2 mg/kg bwt BID for 5 days postoperatively.

SURGICAL TECHNIQUE

Broadly speaking there are three castration techniques, open, closed or half-closed, and each procedure has advantages and disadvantages. The open castration technique involves incising the parietal tunic and leaving the parietal tunic open following castration. Incising the parietal tunic exposes the caudal ligament of the epididymis, transecting this ligament frees the testis and epididymis from the parietal tunic. The testis and epididymis are then removed using an emasculator. With this technique, the parietal tunic remains open, creating a potential passage into the abdominal cavity; however, this technique requires the least dissection and can be performed in the standing sedated or recumbent horse.

With the closed castration technique, the parietal tunic is dissected free from the scrotal fascia. The parietal tunic is not incised and is removed along with the testis and epididymis. A closed

castration procedure seals the parietal tunic by either the action of the emasculating device or the use of a proximal ligature. The closed castration technique eliminates any potential communication between the abdominal cavity and the scrotal incision. Closed castration with a proximal transfixing ligature is advisable in cases that are at higher risk of evisceration (Schumacher, 2019), the author uses size 1 or 0 polyglactin 910 for ligation of the spermatic cord and parietal tunic depending on the size of the horse.

The half-closed castration technique involves isolating the parietal tunica from the scrotal fascia (as for a closed castration) and then making an incision through the parietal tunic at the distal end of the spermatic cord. The testis, epididymis and a portion of the spermatic vasculature and cord are prolapsed through the incision where the spermatic vasculature and cord are emasculated or ligated and transected. The parietal tunic is then removed along with the testis and epididymis. The parietal tunic can be crushed and severed separately from the testicular vessels and the ductus deferens or sutured closed. This technique has the highest incidence of complication (Kilcoyne et al., 2013; Moll et al., 1995) likely due to increased tissue handling, contamination and surgical time. The closed and half-closed castration techniques can be performed standing but are far more frequently performed with the horse in lateral or dorsal recumbency under anaesthesia.

The surgical approach to the testis is most commonly performed via a scrotal incision, which is left open to heal by second intention. Primary closure of the scrotal incision, when performed under aseptic conditions, reduces infection, oedema and signs of pain (Barber, 1985; Palmer & Passmore, 1989) and is also particularly useful for horses that cannot be exercised after castration, such as when there is concurrent lameness or when castration is performed at the same time as another procedure that requires box rest.

Castrations can be performed on the standing or recumbent horse. For horses castrated standing the author sedates the horse with detomidine (0.01 mg/kg bwt IV) and butorphanol (0.01 mg/kg bwt IV) as an initial bolus and assesses the degree of sedation during aseptic scrotal preparation and local anaesthetic injection of the testis. Often additional sedation is given before the start of the surgical procedure and this dose is judged based on the initial response to the sedation already administered. A twitch may also be applied to facilitate restraint. For horses castrated recumbent with total intravenous anaesthesia (TIVA), the author sedates the horse with romifidine (0.04 mg/kg bwt IV) and butorphanol (0.01 mg/kg bwt IV) to facilitate placement of an intravenous jugular catheter. The horse is then premedicated with an additional 0.04–0.08 mg/kg bwt romifidine and TIVA induced with ketamine (2.5 mg/kg bwt IV) and diazepam (0.05 mg/kg bwt IV). A 1 mg/kg bwt dose of ketamine and a 0.04 mg/kg bwt dose of romifidine is prepared ready if the plane of anaesthesia is insufficient for the duration of the surgery. Regardless of a standing or recumbent castration the author always injects local anaesthetic, such as lidocaine or mepivacaine along the scrotal incision site and into the testicle or spermatic cord. The author uses 20–30 mL of local anaesthetic per testicle and 5–10 mLs for the scrotal incisions. The advantages of standing castration include the eliminated risks associated with recovery following anaesthesia and

standing castrations are less expensive (Mason et al., 2005). However, Hodgson and Pinchbeck (2019) found standing castrations were associated with an increased risk of discharge and/or infection and Mason et al. (2005) reported a 22% complication rate for standing castrations compared to 2.1% complication rates for recumbent castration with primary closure.

COMPLICATIONS

Scrotal swelling and seroma

Preputial and scrotal oedema (Figure 1) is the most common complication following castration (Mason et al., 2005; Moll et al., 1995; Rosanowski et al., 2018) and has been reported to occur in up to 70% of castrations (Rosanowski et al., 2018). The incidence of postoperative swelling is multifactorial. Inadequate drainage, inadequate postoperative exercise, excessive tissue trauma, poor surgical technique, postoperative infection, excessive movement during surgery and bleeding in the first 24h are all risk factors for postoperative swelling (Hodgson & Pinchbeck, 2019; Mason et al., 2005). Oedema is usually greatest on the fourth day postcastration (Cox, 1987) and normally resolves within 14 days (Mueller, 2015).

Prevention centres around an aseptic and atraumatic surgical technique with adequate postoperative drainage and exercise. Owners should be advised that turning a horse out into a large field does not guarantee the horse will undertake adequate postoperative exercise. Perioperative antimicrobials have been shown to reduce postoperative swelling (Mason et al., 2005). Sufficient analgesia is also important as pain from postoperative swelling results in the gelding becoming unwilling to move, creating a negative cycle of further swelling, more pain, less movement and premature closure of the surgical site. Postoperative swelling can result in the

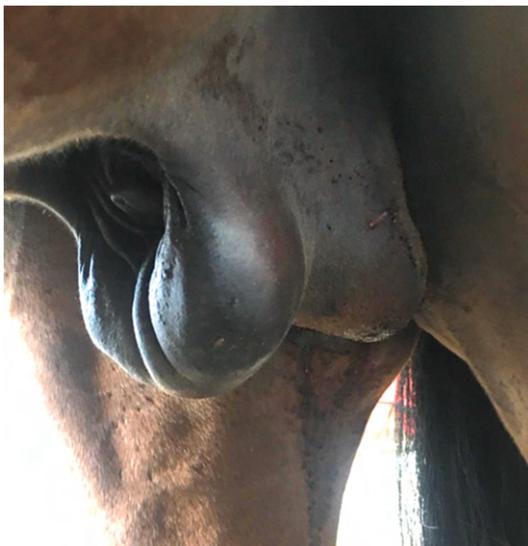


FIGURE 1 This horse has moderate scrotal and penile sheath oedema following castration. *Image Courtesy of Helen Braid.*

development of other complications such as infection, phimosis and paraphimosis, highlighting the importance of adequate postoperative management.

Treatment centres around re-establishing drainage, enforcing suitable exercise and providing nonsteroidal anti-inflammatory drugs (NSAIDs). To re-establish drainage the horse should be adequately restrained, sedated and aseptically prepared. A sterile gloved finger can be introduced into the sealed incision to release the accumulated fluid. The scrotal incision can then be stretched in both directions to widen the scrotal incision, preventing repeat premature incisional closure. Incisions that are not made at the most ventral aspect of the scrotum but are made laterally or caudally are at increased risk of excessive fluid accumulation as the drainage point is not at the most dependant part of the scrotum (Figure 2). The administration of NSAIDs helps to resolve excessive oedema both directly, by suppression of the inflammatory pathways and indirectly as an analgesic, increasing the horse's comfort so the gelding is more willing to move and exercise. Antimicrobials can be administered prophylactically in cases of seroma formation to prevent infection and if the horse displays clinical signs of infection (pyrexia, dullness, inappetence or discharge) then antimicrobial therapy is indicated. Prior to re-establishing drainage, one should take the opportunity to sterilely introduce a bacteriology swab into the scrotum and sample the discharge for bacteriological culture and sensitivity, so appropriate antimicrobials can be prescribed.

Lavage and cold hosing of the scrotal wound remains controversial. It may assist in cleaning, decreasing inflammation and keeping the scrotal wounds open but high-pressure lavage should be avoided as this can force bacteria deeper into the surgical site, which may lead to infection of the spermatic cord (septic funiculitis) or peritoneal cavity (septic peritonitis).

Scrotal/local infection

Scrotal swelling and scrotal infection are often incorrectly considered a single problem as a gelding can have a swollen, but not clinically infected scrotum. Horses typically present 2–6 days postoperatively and can present with just local signs infection, including a warm painful scrotal swelling with discharge, or horses can present with local and systemic signs of illness and infection including dullness, inappetence, pyrexia, cellulitis, lameness and discharge from the scrotal incision. Kummer et al. (2009) reported 20.2% of horses were pyrexia for 1–2 days postcastration. No treatment was required, and the pyrexia resolved spontaneously, but five horses with pyrexia for >2 days were diagnosed with a respiratory infection. So, whilst transient pyrexia might be considered a normal response to soft tissue surgery, clinicians should rule out other possible pathologies including infection.

Performing castrations standing has been associated with an increased risk of infection in multiple studies (Hodgson & Pinchbeck, 2019; Kilcoyne et al., 2013; Mason et al., 2005; Rosanowski et al., 2018). Treatment is similar to management of scrotal



FIGURE 2 A castration site with scrotal swelling, exudative discharge and a very lateral incision in the scrotum. The placement of a scrotal incision (arrowed) this far lateral hinders ventral drainage (arrowhead) and may have increased the risk of scrotal infection in this case. *Image Courtesy of Leahurst Equine Hospital.*

swelling; cold hosing, NSAIDs and analgesia, aseptic re-opening of the scrotal incisions and forced exercise to promote drainage. Broad spectrum antimicrobials should be administered and adjusted according to bacteriology culture and sensitivity results. If the infection does not resolve in a timely manner, early referral for surgical resection of the infected spermatic cord is indicated (Claffey et al., 2018).

Septic funiculitis

Infection of the spermatic cord is termed septic funiculitis and may be a sequela to scrotal infection. A 'scirrhous cord' is a chronically infected spermatic cord with pyogenic bacteria (Schumacher, 2019). Bacteria seed either from a contaminated surgical instrument or ligature or ascends from a scrotal infection. Bacteria isolates include *Staphylococcus*, *Streptococcus equi zooepidemicus*, Beta-haemolytic *Streptococcus* spp., *Staph. aureus*, *Streptococcus parauberis*, *Proteus* spp., *E.coli*, *Acinetobacter* spp., *Enterococcus* spp., *Pasteurella* spp., *Achromobacter* spp., *Actinomyces* spp., *Bacteroides* spp. and gram-negative coccus (Claffey et al., 2018; Duggan et al., 2021). Fungal hyphae (suspected to be *Aspergillus*) have also been isolated from infected tissue (Claffey et al., 2018).

Risk factors include older horses (Duggan et al., 2021), intra- or postoperative bleeding, an open castration technique (Schumacher, 2019) and a scrotal incisional infection (Claffey et al., 2018). The typical presentation for septic funiculitis are recurrent scrotal oedema, scrotal incision(s) that fail to heal, chronic discharging sinus tract(s), painful scrotal or inguinal regions, pyrexia and occasionally ipsilateral hindlimb (Schumacher, 2019). Most geldings present within 4 months of castration, but horses can present with septic funiculitis up to 10 years after castration (Duggan et al., 2021).

Examination of the scrotal and inguinal area can be diagnostic if abnormal tissue is palpable. Thickening of the spermatic cord

palpable in the inguinal region may progress proximally through the inguinal ring where enlargement may be detected on rectal examination (Claffey et al., 2018). Both transcutaneous and transrectal ultrasonography are extremely useful to evaluate for inguinal or peritoneal abscessation and to determine the extent of spermatic cord that may require resection (Kilcoyne & Spier, 2021). In a case series of 12 horses with chronic septic funiculitis, abdominal adhesions were identified in 50% of horses, including adhesions to the ascending and descending colon (Comino et al., 2022).

Treatment is surgical excision of the affected cord either under GA, laparoscopically, or as a two-stage procedure (Comino et al., 2022). If this surgery is performed in the acute stages the fibrous attachments can be readily broken down; if surgery is delayed the fibrous adhesions develop a significant vascular supply, making dissection of the affected tissues more challenging. Additionally, if the initial castration was performed very proximal then the infected portion of the cord is likely to be deep in the inguinal region, making access challenging. Surgical treatment includes resection of the infected spermatic cord segment by emasculatation with or without ligation and the wound is left open to heal by second intention (Claffey et al., 2018; Duggan et al., 2021). If the infected portion of the spermatic cord has been successfully excised, then healing of the scrotal incision should be quick and without complication. Prognosis is good with complete resolution of clinical signs and return to previous exercise in 14/16 horses using a traditional approach (Claffey et al., 2018) and 12/12 with the combined two-stage approach (Comino et al., 2022).

Clostridial infection

Tetanus and botulism can occur in unvaccinated horses and infections with clostridial species can result in necrotising cellulitis, endotoxaemia, paralysis of the voluntary muscles, sepsis and death. Anaerobic peritonitis caused by *Clostridium septicum* has been reported as a complication of a closed castration performed in the field under intravenous general anaesthesia in a 2-year-old Warmblood horse (Shearer et al., 2017) and *Clostridium botulinum* and type-B C botulinum toxin were isolated from a necrotic wound that developed subsequent to castration in a 2-year-old Thoroughbred gelding (Bernard et al., 1987). It is vital that appropriate vaccination or administration of tetanus antitoxin and tetanus toxoid is established in all horses prior to castration. Horses infected with *C. tetani* postcastration should be treated with radical necrotic tissue debridement, high doses of penicillin, tetanus antitoxin, NSAIDs, analgesia and intravenous fluid therapy (Bernard et al., 1987; Muylle et al., 1975). Despite intensive care the mortality rate remains over 70% (Ribeiro et al., 2018).

Haemorrhage

The incidence of excessive postcastration haemorrhage is 1.8%–2.4% (Carmalt et al., 2008; Kilcoyne et al., 2013; Moll et al., 1995).

The most frequent source of postcastration haemorrhage is the testicular artery (Cox, 1987), but the testicular vein and large dermal vessels can also result in substantial haemorrhage.

Preoperative risk factors include older horses and donkeys, as these animals have larger spermatic vessels and a higher incidence of excessive haemorrhage following castration (Hodgson & Pinchbeck, 2019; Sprayson & Thielmann, 2007). Intraoperative risk factors include breaks in sterility, difficult surgical access, intrascrotal or tunic haematomas, poor surgical technique, poorly functioning emasculators or inappropriate emasculator use (Hodgson & Pinchbeck, 2019; Mason et al., 2005; Schumacher, 2019). Inappropriate emasculator use includes inverted emasculator application, excessive tissue placed within the emasculator jaws (including scrotal skin), oblique application of the emasculators and insufficient application time. Incorrectly constructed or serviced emasculators, such as those that are too sharp, may result in the spermatic cord being severed before it can be adequately crushed.

Different types of emasculators have different jaw profiles and different mechanisms of haemostasis and resection (Comino et al., 2018). A recent study by Comino et al. (2018) found that in contrast to previous studies (Kilcoyne et al., 2013), the Reimer castrator had a higher-pressure resistance than the Serra castrator in open castrations. The Henderson Equine Castrating Instrument is a pliers-like instrument attached to a power drill, that twists the spermatic cord, rather than cutting, resulting in sealing the severed vessels. In one report using the Henderson emasculators, haemorrhage was not reported as a complication (Hinton et al., 2019) and in another only 1% (3/300) cases had postoperative haemorrhage (Racine et al., 2019) and 2 of these cases were in donkeys.

Logically one might presume that ligation of the spermatic cord would reduce the incidence of haemorrhage, but Carmalt et al. (2008) found the placement of ligatures did not significantly reduce the incidence of postoperative haemorrhage. Despite this, the author would recommend placing absorbable ligatures in all donkeys and mules undergoing castration and in any horse with a perceived increased risk of haemorrhage. Practitioners perceive an increased risk of infection following ligation of the spermatic cord (Moll et al., 1995) but two studies have found no association between the application of absorbable ligatures and the development of infection (Carmalt et al., 2008; Kilcoyne et al., 2013).

Dripping of blood from the scrotal wound for several minutes after castration is expected but treatment should be implemented if bleeding is a drip rate faster than one can count (>1 drop per second), a drip rate that is increasing or a stream of blood for >15 min after castration. Direct treatment is aimed at identifying the spermatic cord stump and clamping, re-emasculating or ligating the cord and testicular artery. If clamping the spermatic cord is to be attempted in the standing horse further application of local anaesthetic is recommended due to the potential for violent reactions from the horse during clamping as the spermatic cord may not be anaesthetised that far proximal and other tissues that are not anaesthetised may be inadvertently grasped. The author will topically



FIGURE 3 A 3-year-old Thoroughbred gelding with haemorrhage following castration. The scrotum has been packed with two sterile Knit-firm (Millpledge Veterinary) dressings tied together and the scrotum has been closed with the application of towel clamps. This image is taken immediately prior to packing removal.

spray local anaesthetic into the scrotal incision if clamps are to be applied. Long, right-angled forceps, are easier to apply than straight forceps and forceps should be left in place for 12h. The severed end of the spermatic cord may be difficult to locate, especially if the cord was transected very proximally. Occasionally the cord cannot be found; if this is the case then the scrotum can be packed (Figure 3), or the stump must be located with the horse anaesthetised in dorsal recumbency. Alternatively, the mesorchium can be ligated laparoscopically with the horse standing or anaesthetised (Trumble et al., 2000; Waguespack et al., 2001).

If the source of the bleeding cannot be identified, then the scrotal incision can be packed with large sterile swabs (laparotomy swabs) or sterile Knit-firm (Millpledge Veterinary) bandage(s) for 24–48h and the scrotum tacked closed with two or three large sutures or multiple towel clamps (Figure 3). If more than one swab or Knit-firm is placed within the scrotum the packing should be tied together to facilitate removal. The use of multiple small swabs should be avoided, however, if used they should first be sutured together in a large bundle to eliminate the risk of a swab(s) being left behind. Cotton wool or gamgee should never be used to pack a scrotum as these materials shed within the scrotum and can result in a granulomatous foreign body reaction. If the scrotum is packed the gelding should be prescribed broad spectrum antimicrobials as a precautionary measure. Geldings should be maintained on box rest for 3–5 days to allow the clot to stabilise.

Topical agents that facilitate haemostasis include chitosan (Tucker et al., 2020) and absorbable haemostatic gauze (Ramey et al., 2021). Systemic coagulation agents have been used with variable success and include; epsilon-aminocaproic acid (30–40 mg/kg bwt IV, q6–8h), tranexamic acid (5–25 mg/kg bwt slowly IV, q8–12h), conjugated oestrogens (0.05–0.1 mg/kg bwt IV, q12–24h) and 10% formalin (10–50 mL of 10% formalin in 1 L isotonic fluids q12–24h) (Kilcoyne & Spier, 2021; Moreno et al., 2021).

Horses should be referred to a hospital if blood loss is excessive and the horse shows signs of cardiovascular compromise and hypovolaemic shock (tachycardia, tachypnoea, pale mucous membranes, cold extremities, a weak pulse). It is important to remember that packed cell volume (PCV) and total protein (TP) concentration will not change significantly in the first 12–24h following an acute haemorrhage due to the horse's capacity for compensation by splenic contraction and fluid redistribution (Getman, 2009). Serial clinical examinations and monitoring of PCV, TP and lactate gives an indication of the extent of blood loss and the necessity for a blood transfusion.

One should also be aware that some horses may haemorrhage into the abdomen, making excessive bleeding difficult to identify (Waguespack et al., 2001). Haemorrhage into the abdomen can be diagnosed and monitored ultrasonographically. Active bleeding results in swirling, hyperechoic abdominal fluid, previous bleeding will appear as a superficial layer of hyperechogenic material on the abdominal floor (large blood clot) and an increased peritoneal effusion.

Eventration

Eventration is the most serious of all complications of castration with an incidence rate of 0.1%–2.96% (Hinton et al., 2019; Hodgson & Pinchbeck, 2019; Hutchins & Rawlinson, 1972; Kilcoyne et al., 2013; Moll et al., 1995; Owens et al., 2018). There are only two options for a horse that has eviscerated: euthanasia or anaesthesia and surgical reduction of the small intestine. Breed is a known risk factor, with Standardbred and draught horses reported to have a higher incidence of inguinal herniation (Moll et al., 1995; Shoemaker et al., 2004). Shoemaker et al. (2004) reported 7.6% of draught colts to prolapse omentum or intestine within 7 days of castration. Other risk factors include pre-existing inguinal hernias (Taylor et al., 2000), large inguinal rings and colts less than 6 months old. The latter is because most congenital inguinal hernias resolve by the time a colt is older than 6 months of age (Marien et al., 2001).

Prevention is a combination of a thorough clinical history and eliminating the above-mentioned risks factors and castrating any high-risk horses under GA with a closed technique and proximal spermatic cord ligation with a ligature. The closed castration technique without the application of a ligature and the Henderson emasculator instrument technique are ineffective in reducing the risk of evisceration (Hinton et al., 2019; Shoemaker et al., 2004; van der Velden & Rutgers, 1990).

Evisceration usually occurs immediately or within 4h of castration (Hunt & Boles, 1989; Hutchins & Rawlinson, 1972) but has been

reported to occur 7–12 days (Boussauw & Wilderjans, 1996; Thomas et al., 1998) after castration. If the owners wish to pursue treatment, then the treating veterinary surgeon needs to administer broad spectrum antimicrobials, NSAIDs and sedation for transportation. The eviscerated intestine should be cleaned with sterile saline and protected from further contamination and trauma. This can be achieved by replacing the intestine back into the scrotum and closing the scrotal incision with sutures or towel clamps (van der Velden & Rutgers, 1990) taking care not to inadvertently traumatise the eviscerated bowel. Alternatively, the eviscerated intestine can be placed into a moist sterile truss support (Figure 4a) to protect the intestine from further damage and to minimise further evisceration. In the author's experience replacement of the intestine back into the scrotum is almost impossible in the standing case and a saline-soaked towel or drape should be made into a sling and used to support the intestine during transportation (Figure 4a). Prompt referral is essential for a successful outcome (Getman, 2013).

At the referral facility, GA is induced to allow the intestine to be cleaned and returned to the abdomen (Figure 4b). A midline laparotomy is almost always required to retract the small intestine back into the abdominal cavity. Although it is sometimes possible to push small intestine back through the inguinal canal, a midline laparotomy approach allows for easier small intestine assessment and resection, if required (Figure 4c). Survival is reported between 44% and 72.2% (Shoemaker et al., 2004; Thomas et al., 1998). Reduced survival rates are associated with an inguinal-only surgical approach, increased length of prolapsed intestine, the condition of the small intestine at the time of surgery and if a resection and anastomosis is required (Shoemaker et al., 2004; Thomas et al., 1998).

Omental prolapse

This is not the dire emergency that intestinal evisceration is and most cases of omental prolapse can be managed by emasculating the prolapsed omental tissue. The horse's vaginal rings should be examined per rectum to ensure that only omentum, and not intestine, has traversed the vaginal ring. Broad spectrum systemic antimicrobials, NSAIDs and sedation are administered prior to aseptic preparation of the site. Gentle traction is applied to the omental tissue until fresh tissue is seen and emasculators are applied to the fresh tissue, resecting the exposed contaminated tissue. The horse should be box rested and closely monitored for 2–3 days to prevent further prolapse of the omentum. If omentum continues to exit the scrotal incision or there is a large amount of omentum prolapsed (Figure 5), the gelding should be referred for omental resection and closure of the superficial inguinal ring under GA. The prognosis for survival is good and complications are rare.

Septic peritonitis

Postcastration infection has the potential to result in an ascending infection leading to peritonitis. Inguinal abscesses that extend into the abdominal cavity, are reported in geldings castrated within

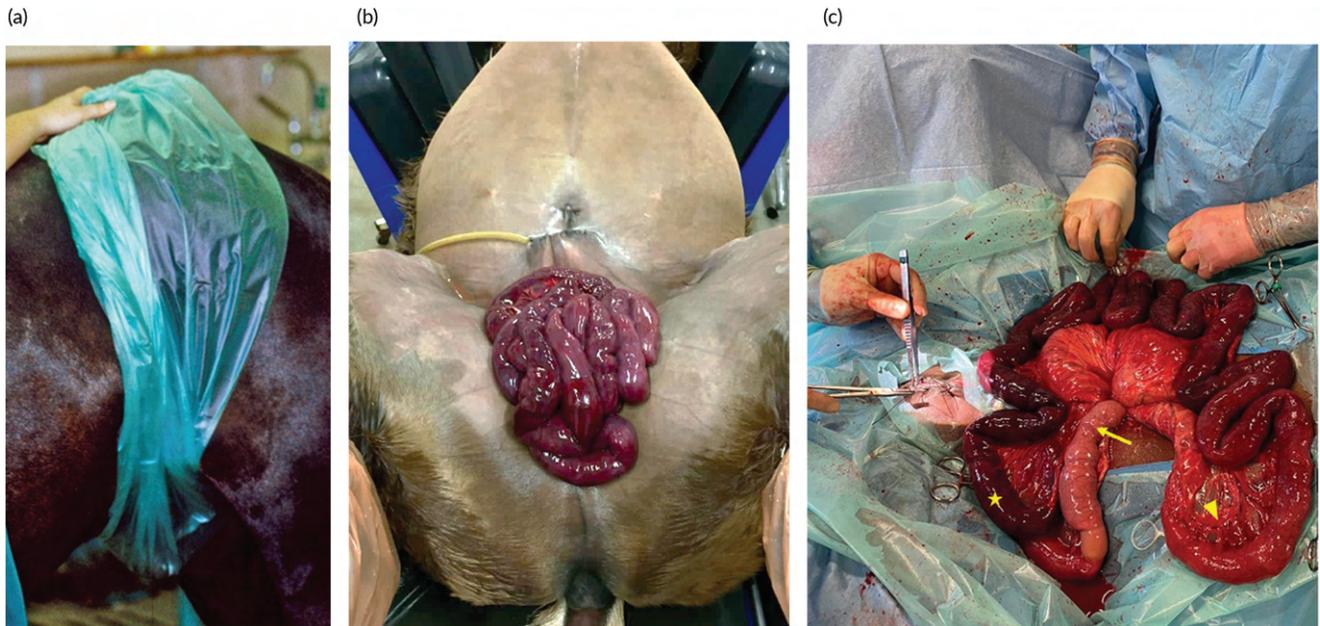


FIGURE 4 (a) A sterile drape applied as a truss support bandage applied to a horse that had small intestinal eversion following castration. *Image Courtesy of Leahurst Equine Hospital.* (b) Small intestinal eversion following castration of a 4-month-old foal. The foal has been anaesthetised at a referral hospital and the small intestine cleaned with sterile saline. The penile sheath has been sutured closed and urinary catheter placed, the abdomen is being aseptically prepared for a ventral midline laparotomy. Note the volume of everted small intestine prohibited any attempts of reduction via the scrotal incisions. Also note the small intestine serosa is a red-purple colour as a result of blood supply strangulation at the vaginal ring and external trauma. (c) The small intestine has been reduced back through the inguinal ring via a combination of intra-abdominal traction through a ventral midline laparotomy and reduction of the small intestine scrotally. The scrotal skin incision is being closed to the left of the image and the small intestine has been exteriorised via a ventral midline laparotomy and arranged to assess intestinal viability. Note the discolouration of the serosal surface (starred) compared to the more normal appearing small intestine (arrowed). Also note the haemorrhagic appearance to the mesentery and mesenteric vessels (arrowhead).

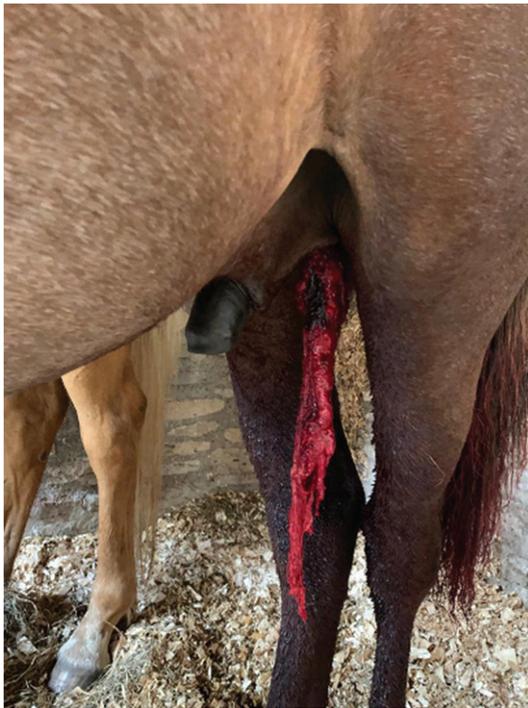


FIGURE 5 This horse has a severe omental prolapse. This degree of omental prolapse may be best managed at a referral centre. *Image Courtesy of Hattie Barnes.*

21 days–2 years (Arnold & Chaffin, 2012; Comino et al., 2022) although these abscesses may remain as a septic funiculitis and not cause a clinical peritonitis.

Horses often develop a subclinical, nonseptic peritonitis after castration, because the vaginal and peritoneal cavities communicate but bacterial septic peritonitis is rare (Schumacher et al., 1988). Clinical signs include, pyrexia, depression, inappetence, diarrhoea and colic. Diagnosis is suspected on clinical presentation and trans-abdominal ultrasonography and is confirmed by abdominocentesis. Peritoneal fluid analysis is complicated as many horses show an increased peritoneal total nucleated cell counts (TNCC) $>10.0 \times 10^9/L$ for up to 5 days following castration (Schumacher et al., 1988) and so peritoneal TNCC alone should not be used as an indicator for septic peritonitis, following castration. Cytological assessment of peritoneal fluid is useful as identification of 'left-shift' neutrophils or intracellular bacteria indicate septic peritonitis (Adams et al., 1980; Dyson, 1983). Treatment entails broad spectrum antimicrobials, NSAIDs, analgesia and supportive therapy. Peritoneal lavage can also be performed.

Penile damage

Penile damage can result from direct surgical trauma (Beavers & Mitchell, 2018) or indirectly, as a result of scrotal oedema or drug

administration leading to paraphimosis, which if prolonged can result in permanent penile paralysis. In cases of paraphimosis the penis should be supported in a sling to prevent damage to the penis and to decrease preputial oedema.

Hydrocele

A hydrocele is a rare, idiopathic accumulation of peritoneal fluid within the parietal tunic, or vaginal sac, that may appear months or years after castration (Schumacher, 2019). Hydroceles only form after open castration techniques due to retention of the parietal tunic (Cox, 1987). The accumulation of fluid within the scrotum can be mistaken for a testis (Figure 6) or inguinal hernia but palpation and ultrasound examination indicate fluid accumulation within the vaginal cavity. If fine needle aspiration is performed a clear yellow fluid is retrieved. This condition does not affect the horse and is only treated if the owner considers the condition unaesthetic. Treatment is surgical resection under GA.

Signs of colic

Remembering the term 'colic' only mean abdominal discomfort or pain, there are a number of reasons why a horse might display signs of colic following castration. Sedative and anaesthetic protocols may result in pelvic flexure or caecal impaction and complications such as haemoabdomen or peritonitis can result in signs of colic. A horse displaying signs of colic after castration should be examined closely to determine if the signs of colic are caused by pain associated with the



FIGURE 6 Hydrocele of a gelding that presented for examination 18 months after castration.

castration procedure or if there is a primary gastrointestinal lesion. A horse that continues to show signs of colic following administration of suitable analgesia should be thoroughly investigated to ensure the signs of colic are not associated with a gastrointestinal pathology that requires prompt surgical intervention.

Continued stallion-like behaviour

Libido slowly declines over 56 days following castration (Thompson et al., 1980), but castration does not guarantee elimination of masculine behaviour, and geldings may display stallion-like behaviour, including mounting and erection after castration (Cox, 1986). This is a common undesirable outcome with 20%–30% of geldings castrated before or after puberty still displaying stallion-like behaviour (Line et al., 1985).

Historically continued stallion-like behaviour was attributed to incomplete resection of the epididymis; however, the epididymis neither produces nor releases androgens and so this is not the source of hormone driven behaviours (Cox, 1986; Line et al., 1985). However, continued stallion-like behaviour can occur if the surgeon mistakenly removes the epididymis instead of the testis (Trotter & Aanes, 1981). When investigating a gelding displaying stallion-like behaviour, one must determine if the behaviour is innate or if an incomplete castration has been performed. This can be done by locating a testis per rectum, ultrasonographically, or by completing a hormonal assay. Antimüllerian hormone (AMH) is a sensitive indicator of unilateral cryptorchid horses (Claes et al., 2013). Treatment of continued stallion-like behaviour when confident of complete castration may be limited to restricting social interaction with other horses and stricter handling (Cox, 1987). The use of progestogen has been reported to ameliorate sexual and aggressive behaviour in geldings (McDonnell, 2007; Roberts & Beaver, 1987).

CONCLUSION

Castration complications are varied in frequency and severity and can be challenging to manage. A good understanding of anatomy, surgical options and complication management will promote better outcomes for our now gelding cases.

AUTHOR CONTRIBUTION

All authors contributed to the preparation of the manuscript and all authors gave final approval of the manuscript.

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Pelvic flexure enterotomy in the horse: A review of current literature

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Summary

Enterotomy of the pelvic flexure is commonly performed during colic surgery in the horse to empty the large colon of its contents. A two-layer closure with absorbable suture material is recommended, but a consensus has not been established. Many different techniques have been described and tested to evaluate surgical time, luminal diameter reduction, bursting pressure and post-operative complications including adhesion formation. The results of these studies vary within and between enterotomy techniques. The objective of this narrative review was to evaluate and summarise relevant published literature on pelvic flexure enterotomy techniques in the horse.

KEYWORDS

horse, colic, enterotomy, pelvic flexure, surgery

INTRODUCTION

The goal of pelvic flexure enterotomy is to allow for evacuation of the large colon of its contents. This procedure is performed most often to remove impacted ingesta in the large colon that may be too large or firm to pass with medical management. While impactions typically are formed by dry or poorly masticated ingesta, they can also be composed of non-digestible substances such as sand and foreign bodies (Foerner, 1982; Markel et al., 1988; Southwood, 2019). An enterotomy is also commonly performed in cases of impactions or foreign body obstructions in the small colon, to reduce bulk of ingesta moving from the large colon through the inflamed or injured bowel aboral and avoid recurrence of such lesions. In cases of caecal impactions, a pelvic flexure enterotomy may facilitate outflow of ingesta from the intestinal tract oral to it and help minimise the chances of recurrence. In cases of enterolithiasis or fecaliths, if the enterolith(s) or fecalith(s) can be moved to the pelvic flexure, they are removed through a pelvic flexure enterotomy as well (Foerner, 1982; Rakestraw & Hardy, 2012; Southwood, 2019).

Another indication for performing a pelvic flexure enterotomy is to facilitate manipulation of the large colon for correcting a displacement or a volvulus. Additionally, pelvic flexure enterotomy has been linked to a decreased incidence of post-operative ileus in some studies (Mair & Smith, 2005; Roussel Jr et al., 2001).

Although generally considered benign, the procedure is not without risk. Enterotomy increases surgical time as well as manipulation

of the bowel. It implies opening the bowel thus changing a clean procedure into a clean-contaminated procedure (Provost & Bailey, 2019). It can also leave exposed suture material on the serosal surface of the bowel, which can cause local inflammation and adhesions (Aldrich et al., 2017; Alonso et al., 2014; Freeman, 1997; Mueller, 2002).

TECHNIQUE

For the procedure, an impervious drape, and a water hose with Y-shaped extensions or alternatively two water hoses, are needed. A tray to lay the drape and exteriorised colon on facilitates the procedure, especially if the colon is very full and, therefore, heavy.

For the procedure, the colon is exteriorised on the colon tray to one side of the horse, generally lateral to the celiotomy incision to the side opposite from where the surgeon is positioned, or between the horse's hind legs. If a table is not available, the colon is best positioned between the horse's hind limbs using the abdomen and pelvis to support the exteriorised organ (Marien et al., 2000; Markel et al., 1988; Rakestraw & Hardy, 2012; Southwood, 2019).

Care is taken to ensure that all surfaces are draped aseptically. A second drape is generally placed over the exteriorised colon to act as a splash barrier between the enterotomy and the peritoneal cavity. The colon can be coated with sodium carboxymethylcellulose (Marien et al., 2000; Southwood, 2019) or with diluted heparin (Ellis

et al., 2007) to help reduce the adherence of faecal material to the serosa.

The enterotomy is performed with a scalpel blade on the anti-mesenteric border of the pelvic flexure. The full thickness incision is made as long as necessary to easily remove the colon contents. If it is only feed material, generally a 6–7 cm incision is sufficient (Markel et al., 1988; Rakestraw & Hardy, 2012; Southwood, 2019). In the case of foreign bodies, enteroliths or faecaloliths, depending on their size, the incision may have to be longer to allow for the object to be exteriorised without trauma to the intestine.

Evacuation of the colon content is then performed by irrigating its lumen with tap water at body temperature (Southwood, 2019). Since contamination of the pelvic flexure adjacent to the enterotomy site is unavoidable, copious concurrent lavage of its surface is also necessary.

The evacuated contents are collected in a container to reduce contamination of the room and sterile field. The ingesta is typically strained to allow drainage of the liquid part but retention of solid particles, to avoid obstruction of the tubes and drains (Markel et al., 1988). Such systems can be custom made to accommodate surgeons' preferences and the available facilities.

Once evacuation is completed, the enterotomy incision is closed according to the surgeon's preferences. Stay sutures at the ends of the enterotomy incision may facilitate suturing while decreasing contamination. Regardless of the preferred suture pattern, irrigating the sutured enterotomy with sterile isotonic solution after each suture layer, changing gloves, employing a separate suturing kit for the pelvic flexure enterotomy and cleaning instruments with a wet gauze between layers are strategies that have proven useful to minimise contamination in an *ex vivo* model, and are, therefore, recommended (Giusto et al., 2017).

The sutured enterotomy is then checked for leakage and contamination, the pelvic flexure is copiously lavaged and returned to the abdomen.

One of the reasons why the pelvic flexure is the preferred location to access the large colon lumen is the fact that the majority of the large colon lacks mesenteric attachments to other organs or to the body wall. This allows the surgeon to readily exteriorise the organ and perform the enterotomy at a location remote from the horse's abdominal cavity and incision. Therefore, the chances of substantial peritoneal contamination are minimised with proper technique. Furthermore, access to both ventral and dorsal colon is possible from a pelvic flexure enterotomy.

The procedure is ideally performed by three people. The surgeon is responsible for manipulating the colon within the abdomen, guiding the enterotomy hose inside the lumen from outside the intestine, and milking the contents and ingesta towards the enterotomy site. A sterile assistant keeps one hand sterile to support the colon and prevent retraction into the abdominal incision. The other, unsterile hand is used to manipulate the enterotomy hose within the lumen as directed by the surgeon (Rakestraw & Hardy, 2012; Southwood, 2019). An unsterile assistant holds the other arm of the Y hose and lavages the ingesta off the serosal surface and colon tray.

Placement of the colon to the side of the horse or between the hindlimbs depends on surgeon's preference, availability of a tray and how much of the colon can be exteriorised prior to the enterotomy.

CLOSURE TECHNIQUES

Most commonly, a two-layer hand-sewn closure with size USP 2-0 absorbable monofilament material is recommended. The first layer should be a simple continuous, penetrating full thickness and oversewn by an inverting continuous Cushing pattern (Markel et al., 1988; Southwood, 2019).

Other described closure techniques include a one-layer Utrecht (Young et al., 1991), one-layer Cushing (Aldrich et al., 2017; Gandini et al., 2013), two-layer with suture reversal (Aldrich et al., 2017), double inverting closure (Doyle et al., 2003), three-layer closure (Marien et al., 2000; Young et al., 1991), thoracoabdominal stapling device (Ellis et al., 2007; Gandini et al., 2013; Rosser, Brounts, Livesey, & Wiedmeyer, 2012; Rosser, Brounts, Slone, et al., 2012), skin staples (Gandini et al., 2013) and one-layer seromuscular Cushing with barbed sutures (Sinovich et al., 2020).

While several authors have compared different techniques *ex vivo* (Aldrich et al., 2017; Gandini et al., 2013; Sinovich et al., 2020), *in vivo* (Young et al., 1991) and clinically (Ellis et al., 2007; Rosser, Brounts, Livesey, & Wiedmeyer, 2012), an evidence-based consensus on the best technique to be applied clinically is lacking.

Hand-sewn, two-layer closures

Reported advantages of hand-sewn, two-layer closure consisting of a full-thickness simple continuous followed by an inverting suture are a high bursting strength, resistance to leakage, effective haemorrhage prevention and cost-effectiveness. Its main disadvantage is that it is time consuming (Aldrich et al., 2017; Gandini et al., 2013; Rosser, Brounts, Slone, et al., 2012; Sinovich et al., 2020).

It is difficult to fully compare results of *ex vivo* studies because of discrepancies in their methods; however, in all studies, bursting pressure recorded for two-layer closures was higher than intraluminal colonic pressure recorded *in vivo* (up to 58 mmHg, Mathis et al., 2006; Moore et al., 1996). When tested for leakage, none of the closures in any of the studies leaked. Luminal percentage reduction after closure ranged widely, between 2.07% and 14.18%, depending on the location and technique of measurement (Aldrich et al., 2017; Gandini et al., 2013; Rosser, Brounts, Slone, et al., 2012; Sinovich et al., 2020). A reduction in lumen diameter at the pelvic flexure may cause complications in the immediate post-operative period, when motility may be impaired due to inflammation (Aldrich et al., 2017; Koenig & Cote, 2006). The reduced intraluminal diameter could hamper the passage of ingesta after normal feeding has been resumed (Lopes & Pfeiffer, 2000).

In vivo, the two-layer closure resulted in good apposition of all layers of the colonic wall with mucosal healing, and it was deemed

superior to the other two techniques tested, namely a one-layer Utrecht and three-layer (simple continuous mucosal layer, simple continuous seromuscular layer and continuous Cushing; Young et al., 1991).

Two-layer closure with suture reversal, consisting of a full-thickness simple continuous pattern oversewn with a Cushing pattern beginning by reversing at the end knot of the first layer, without cutting the needle end of the suture, is also described. Although this technique results in an equivalent resistance to bursting pressure to the two-layer closure, it does not appear to be any less time consuming (Aldrich et al., 2017).

Double inverting closures are used by a surprisingly high percentage of surgeons, according to a recent survey (Comino et al., 2015), although they have never been tested, they have been reported only once, and they have been associated with post-operative haemorrhage in a clinical study (Doyle et al., 2003). Based on available evidence, these patterns may not effectively occlude submucosal vessels in horses. In dogs, gastrotomies are recommended to be closed in a full-thickness simple continuous pattern, oversewn with a Cushing pattern, to prevent intraluminal bleeding from mucosal and submucosal vessels (Rasmussen, 2003). In horses, hysterotomy closure for caesarean section also necessitates attention to a secure mucosal and submucosal closure to prevent post-operative haemorrhage and death (Vandeplassche, 1980). This may explain the increased risk of haemorrhage in enterotomies using a two-layer inverting technique.

Hand-sewn, one-layer closures

One-layer inverting Cushing constructs, investigated *ex vivo*, had bursting pressures higher than intraluminal colonic pressures recorded *in vivo* (up to 58 mmHg; Aldrich et al., 2017; Gandini et al., 2013; Sinovich et al., 2020). In two studies, bursting pressures were the same or lower than those of two-layer closures (Aldrich et al., 2017; Gandini et al., 2013; Sinovich et al., 2020). As expected, one-layer closures were faster to perform than two layers in all studies, but luminal diameter reduction was not statistically different between constructs.

A one-layer Utrecht, consisting in a serosubmucosal inverting suture with bites placed at 45° to the incision, was associated with post-operative colic *in vivo*. Upon necropsy and histological examination, evidence of leakage and adhesions formation was found. Histologically, this single-layer technique resulted in serosa-to-serosa contact that extended almost to the luminal surface in most enterotomies. The muscularis and submucosal layers were not apposed at the enterotomy site, and a large gap was present at the mucosal surface, which may have prevented mucosa and submucosa to heal properly (Young et al., 1991).

Furthermore, it can be speculated that one-layer closures also be susceptible to haemorrhage due to the lack of effective closure of mucosal and submucosal vessels. However, this aspect has not been directly investigated in this literature.

Hand-sewn, three-layer closures

Although three-layer closures are associated with submucosal haematomas and ingesta trapped within the submucosal space in an experimental *in vivo* study from Young et al. (1991), the successful use of this technique has been reported in a clinical study on 30 horses (Marien et al., 2000). However, three-layer closures may be more time consuming, and were not used by any of the respondents to Comino's 2015 survey of large animal veterinary surgeons.

TA-90 stapled closures

The use of a thoracoabdominal stapling device (TA-90) in over 200 clinical cases was reported for the first time by Ellis et al. in 2007. No complications were recorded and the closure was much faster than the two layer (3 min vs. 15 min), likely due to the fact that the stapled closure was not oversewn. Rosser, Brounts, Livesey, and Wiedmeyer (2012) also reported the safe use of the same stapled technique in 70 clinical cases, with complications and outcomes similar to published literature on general colic surgery and large colon enterotomy.

Rosser, Brounts, Slone, et al. (2012) also compared *ex vivo* constructs of hand-sewn two-layer closures to TA-90-stapled closures. They concluded that both constructs were safe, as they did not result in leakage, and measured bursting pressures were equivalent. Luminal diameter was reduced significantly more by the two-layer technique than by the stapled technique, and the latter was significantly faster to perform.

Gandini et al. (2013) had somehow different findings compared to the Rosser, Brounts, Slone, et al. (2012). They also found stapled closures to be significantly faster than hand-sewn one- and two-layer closures, but only if the enterotomy was small and could be closed with just one cartridge. If two cartridges were to be used, as it was the case for longer incisions, the time required to achieve full closure was equivalent for stapled and hand-sewn techniques. Furthermore, bursting pressures appeared to be higher for hand-sewn two-layer closures than for stapled constructs in this study. Luminal diameter reduction, on the other hand, did not appear to differ between techniques.

Skin staples closures

The use of skin staples to close pelvic flexure enterotomies has been reported anecdotally in clinical cases. Gandini et al. (2013) found the technique to be easy, fast and economic to perform *ex vivo*. Bursting pressure was lower than for hand-sewn two-layer closure, but still higher than intraluminal colonic pressures recorded *in vivo* (up to 58 mmHg). Luminal diameter reduction was equivalent to the other techniques tested.

It can be speculated that skin-stapled closures may also be susceptible to haemorrhage, since mucosal and submucosal vessels may not be effectively obliterated. However, no in vivo or clinical studies have tested this technique to date.

Complications and outcome

Complications following pelvic flexure enterotomy are rare and likely associated with the primary disease than with the procedure itself (Southwood, 2019). Cohen and Honnas (1996) reported that horses undergoing pelvic flexure enterotomy were 1.5 times more likely to develop diarrhoea than horses with similar lesions, but that did not have an enterotomy performed. The same group reported a significant association between enterotomy and post-operative incisional infection (Honnas & Cohen, 1997), whereas other studies (Anderson et al., 2015; Colbath et al., 2014; Coomer et al., 2007; Galuppo et al., 1999; Ingle-Fehr et al., 1997; Isgren et al., 2017; Kobluk et al., 1989; Phillips & Walmsley, 1993; Torfs et al., 2010) found no association.

While undergoing a standard pelvic flexure enterotomy is not consistently a risk factor for developing incisional infections, cases with higher contamination, such as enterotomy not in the pelvic flexure, multiple enterotomies, large colon resection and anastomoses, larger enteroliths removal, do appear to have a higher risk (Crosa et al., 2020; Darnaud et al., 2016).

Interestingly, geriatric horses were less likely to undergo a pelvic flexure enterotomy than mature horses in a study (Gazzerro et al., 2015), but complications rate and prognosis were equivalent for both groups. Performing an enterotomy does not directly impact return into sporting activity (Christophersen et al., 2011).

CONCLUSIONS

Many techniques are described to close pelvic flexure enterotomies in horses. Of them, the most commonly used clinically is a hand-sewn two-layer closure consisting of a simple continuous followed by a continuous Cushing pattern. This technique has tested optimally ex vivo and is associated with the best healing scores in vivo. TA-90-stapled closures are a safe and fast, although costly, alternative.

Ex vivo studies test constructs technical features, such as speed and cost, and biomechanical characteristics such as leakage, bursting pressure and reduction in luminal diameter. Although important, care must be taken to extrapolate clinical significance from these parameters.

In vivo experimental studies, such as the one from Young et al. (1991), offer the opportunity of examining such aspects, albeit in healthy horses. Such studies are, however, very expensive, and increasingly difficult due to ethical concerns.

Clinical studies such as those from Marien et al. (2000), Ellis et al. (2007) and Rosser, Brounts, Slone, et al. (2012) currently give the most valuable insights on the benefits and pitfalls of enterotomy techniques, including survival and complication rates associated with their use both long and short term. They are, however, plagued by design variability and confounding factors such as the horse's primary disease process and co-morbidities. It is therefore difficult to compare both within and between studies.

The gold-standard technique for performing a pelvic flexure enterotomies could be established ex vivo through a systematic review of the available evidence. Using this data, a prospective longitudinal clinical study should be planned to follow clinical cases subjected to this procedure both short and long term. Using best practices for clinical trials, such as the PetSORT or CONSORT models, will ensure the technique is consistent and reduce interference from confounding variables such as underlying disease process and co-morbidities (Moher, 2005; Ruple et al., 2023; Sargeant et al., 2023; Turner et al., 2012).

AUTHOR CONTRIBUTIONS

V. Albanese designed the study and prepared the article. P. Straticò and A. Munsterman valuably edited the submission and approved its final version.

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No conflicts of interest have been declared.

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*Overall, horses in the treatment group had a statistically significant decrease in pain score (2.87 decrease compared to a 0.72 decrease in the control group) from the beginning to the end of the trial (44 days total).

Thermal imaging also demonstrated a significant impact on temperature readings both at the time of the trial as well as after exercise and application of Performance Ultra. Near the end of 30 days, horses in the treatment group had a lower average back temperature.

**Reference:
Walter, K.W., Altman, J. & Haussler, K. (2023) Reducing chronic back pain and inflammation in horses using a commercial herbal liniment. Equine Veterinary Education, 35, e499-e506.