EQUINE VETERINARY EDUCATION/AMERICAN EDITION

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AAEP®

EQUINE VETERINARY EDUCATION

American Edition | May 2022

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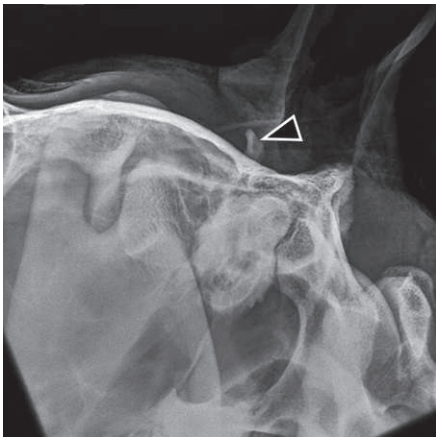

AAEP NEWS In this issue

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

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Success Story: Ambulatory practice spins off emergency service into standalone practice

"We burned out a veterinarian, and we didn't see it coming," lamented Dr. James Beckman when reflecting on the abrupt resignation of a cherished associate several years ago.

Dr. Beckman and his wife, Dr. Kerry Beckman, own Gas Light Equine Veterinary Practice, a three-doctor ambulatory practice that services the areas in and around Louisville, Ky. Since its establishment in 2006, practice doctors rotated on-call responsibilities on weeknights and weekends. An informal arrangement with several other practitioners in the area enabled spot emergency coverage on weekends if a doctor needed a couple of hours off for personal reasons such as to attend a child's sporting event.

"She was phenomenal," Dr. Beckman said of his former associate. "We had her for three years and she was doing great. Then she came to us one day and said she was done, she was burned out, the on call was killing her and she was going to corporate medicine. At that point, we vowed to come up with a different plan."

That plan culminated in the Beckman's establishment of a separate, after-hours emergency practice in January 2021 following several years of planning, preparation and COVID-related delay. Bluegrass Equine Emergency Service is an "independent," two-doctor ambulatory practice that services only after-hours emergencies for Gas Light Equine and other equine veterinarians in the Louisville area who have chosen, or would like, to reduce or relinquish their on-call coverage.

The two employed veterinarians are not affiliated with Gas Light Equine, and each works two nights per week from 5:00 p.m. to 7:00 a.m. and every other weekend. They do not see patients during the day, nor provide routine services during emergency visits. Compensation is straight salary along with profit sharing at the end of the year if deemed available.

The Beckmans are diligent to distance themselves and their Gas Light Equine practice from the client-facing aspect of Bluegrass Equine Emergency Service. Their hands-on involvement is concentrated on the back-end bookwork, including inventory. On rare occasions when the emergency veterinarian is slammed, however, Dr. Beckman serves as the backup and responds as a representative of Bluegrass Equine Emergency Service.

Emergencies are assessed a basic call charge and set fee structure. Payment by cash, check or credit card is due at the time of service. At the end of their shift, the emergency veterinarian returns each patient to its regular veterinarian, along with an email of medical records and a full report of everything they did, saw and recommend. A phone call is made for cases deemed as needing prompt attention.

The Beckmans' initial concerns over client reaction to new veterinarians seeing their emergencies proved unfounded. Quite the contrary. "They felt bad about calling us out in the middle of the night because we had been there all day," he said. "Now, they don't think twice about calling with an emergency after hours because they know that is the practice's job."

Bluegrass Equine Emergency Service has seen patients for seven or eight different practices in the Louisville area, several of which are subscribers. Clients of subscriber practices receive preferred scheduling and a discount on the service call. Additionally, subscribing practices can promote no on-call responsibilities when advertising for new associates, providing a leg up in the highly competitive veterinary labor market.

"Any subscriber can turn off their phone at 5:00 p.m. and rest assured that their patients are covered until 7:00 a.m., at which time they'll have an email detailing everything that was done," said Dr. Beckman. "With this practice up and running, there's no reason for any veterinarian in the Louisville market to have to see emergencies if they don't want to."

For veterinary subscribers, improved daytime revenue is offsetting lost emergency revenue. "We're able to be much more efficient and do more daytime work because we don't have to deal with being out all night," he said. "That's something that is being found in the practices utilizing the service—yes, they're losing the emergency funds, but the daytime work is more lucrative because of the nighttime coverage."

Spinning off Gas Light Equine's after-hours emergency service into a separate independent practice is a calculated business strategy by the Beckmans and one more example of how practices might tackle the all-too-common pain point of emergency coverage to help make equine practice more sustainable. Resulting improvements in work-life balance have enabled the Beckmans to work normal hours, enjoy more family time and increase their joy of equine practice.

"There are many options out there on how to cover emergencies without causing problems to your daytime practice—co-ops, partnerships, independent practices like this—but we need to work together so we can all have normal lives," he said. "This has been a great experience for us, and I want others to love their jobs as much as we love ours."

5 things to know about AAEP this month

1. Recognize a colleague for their volunteer service by nominating them for the AAEP's Good Works for Horses campaign beginning June 1 at aaep.org/good-works-horses.
2. Renew your AAEP membership by May 31 and be eligible to win a free annual convention registration. Renew at aaep.org/membership-dues-renewal.
3. U.S. members: Administer compassionate service to horses without incurring financial stress through Vet Direct Safety Net. Learn more at aaep.org/vet-direct-safety-net.
4. Graduate students, fellows and residents: Apply for up to $20,000 in research funding from The Foundation by June 8 at foundationforthehorse.org/graduate-student-fellow-resident-research-grants.
5. Second- and third-year students: Apply for Merck Animal Health, Oakwood Foundation and Zoetis Foundation scholarships using a single application by June 1 at aaep.org/scholarships.

Nominate an altruistic colleague during summer 2022 'Good Works' campaign

We want to know about members performing selfless acts of giving back to the horse, for the sake of the horse and people they serve. The AAEP's Good Works for Horses campaign, sponsored by Educational Partner Zoetis, will recognize AAEP-member veterinarians whose volunteer efforts are having a positive effect on the equine community.

Practitioners, horse owners and others are invited to nominate AAEP-member veterinarians who have contributed veterinary services or resources to benefit horses and the local equine community. Nominations can be made online beginning June 1 at aaep.org/good-works-horses. The last day to submit an entry is Aug. 31.

Three veterinarians will be selected for recognition each month, June through August, in a Win-Place-Show order. Monthly honorees and their nominators will be acknowledged across AAEP's media platforms, and each month's winner will receive a prize package from AAEP and Zoetis. A grand prize winner will be selected from among the three monthly "Win" honorees and honored Nov. 19 during the AAEP's 68th Annual Convention in San Antonio, Texas.

For more information about the Good Works for Horses campaign, contact Sally Baker, AAEP director of marketing and public relations, at sbaker@aaep.org.

Honor an exemplary colleague with an AAEP award nomination

Nominations due by June 1

Reward an outstanding colleague, industry professional or organization for exemplary service to the horse or betterment of the profession by nominating them for a 2022 AAEP award. The nomination deadline is June 1, and winners will be announced and recognized during the President's Luncheon at the AAEP's 68th Annual Convention in San Antonio, Texas, Nov. 18–22.

Nominations are being accepted in the following categories:

AAEP Research Award
Distinguished Educator – Academic Award
Distinguished Educator – Mentor Award
Distinguished Life Member Award
Distinguished Service Award
George Stubbs Award
Sage Kester Beyond the Call Award
The Lavin Cup (The Equine Welfare Award)

Visit aaep.org/about-aaep/annual-awards for nomination forms and additional information about the awards and selection process. You may also request a nomination form from Sue Stivers at sstivers@aaep.org or (859) 233-0147.

Dr. Joy Tomlinson receives the AAEP Research Award from 2021 AAEP President Dr. Scott Hay during the 67th Annul Convention in Nashville, Tenn.

Transforming Equine Practice: Tips for minimizing aged accounts receivable

Aged accounts receivable (AR) are a scourge on small businesses such as equine practices. These outstanding debts not only cut into cash flow and profits, but they create uncertainty and divert staff resources away from other more productive activities.

So what's an equine practice to do? We posed the question in the March 15 edition of the Spur e-newsletter and received responses from changing payment policy to working empathically with struggling clients to prioritizing AR monitoring.

A number of practices have transitioned from invoicing to payment at time of service. This includes Steele Equine Veterinary Services & Performance Horse Center in Zolfo Springs, Fla., which until two years ago offered clients the option to pay at time of service or be invoiced. Practice growth and launch of a facility expansion prompted practice co-founder Dr. Liz Steele to take a more active interest in business management, and she was startled by what she discovered.

"I almost fainted when I realized our AR over 30 days past due was $65,000, and this was the average normal the deeper I dug," she said. "$65,000 of work I sweated out that I had not been paid for was enough for me to get a little mad at myself and say, 'If you can walk in the feed store and know you must pay before you walk out, then there is no reason you should expect to come to the vet and pay them later when you feel like it.'"

Dr. Steele implemented a policy requiring every client to keep a credit card on file with payment due at time of service. She permits two large active clients to accumulate 30 days' worth of charges but runs their card at each 30-day mark. The policy shift has reduced accounts receivable to zero with minimal impact on the client roster.

"Clients expect to pay at the time of service," said Dr. Steele. "On the rare occasion that someone refuses to put a card on file, we kindly ask them to go elsewhere for services. It has cut out all of our headaches and saved a lot of time. I would say we lost less than five clients, but these are the type of clients that drain your energy anyways."

Meanwhile, Dr. Eleanor Green, a former practice owner and hospital director/chief of staff at two different university teaching hospitals, worked individually with clients with unpaid bills by showing sincere interest in them and their limiting circumstances. It's a philosophy that worked for her just as it did her father, an MD surgeon whose payment collection rate was well above the industry norm.

"I remember some of my clients who were strapped, but when I showed empathy for them and their challenges,

they paid me what they were able to every month," she said. "For one client, it was $2 per month until he was back on his feet. He paid in full and became a long-term, loyal client. This was a typical response. Of course, there are bad actors who intend to cheat, but I found them in the minority and dealt with them as was necessary."

For larger, multi-doctor practices like McKee-Pownall Equine Services in Ontario, Canada, it can be beneficial to regularly monitor AR from both overall practice and individual practitioner standpoints.

Excluding its race and show horse business, where the standard industry practice of billing the trainer who then bills the owner still applies, McKee-Pownall advises new clients of payment at time of service with a credit card on file. Before switching to payment at time of service, legacy clients who consistently paid their bills on time were given the option to continue being invoiced so long as they maintained timely payments.

According to practice co-founder Dr. Mike Pownall, the practice examines its average days accounts receivable each month to stay on track toward meeting its established goal for the year which, for 2022, is to be below 20 days. "We'll sit down and go through our aged AR and put a plan in place," he said. "When we do that, our average days AR drops significantly because we're paying attention to it. When we neglect it because we think we're doing well or we're too busy, things start creeping up again."

In addition, the practice looks at each veterinarian's AR on a quarterly basis, not as a potentially punitive measure but instead to help any veterinarian with a higher-than-average AR become better at managing it.

"I look at AR as a preventable disease," said Dr. Pownall. "If you do it right, you really don't have a balance. If you measure it, you can monitor it. And if you monitor it, you need to act upon it."

Explore rehabilitation, emergency coverage approaches during June Round Tables

Optimize recovery of injured equine athletes and discover different approaches to emergency coverage—a pain point for many practitioners—by tuning in to the AAEP's Virtual Wednesday Round Tables in June.

The Round Tables are offered on the second and fourth Wednesday of every month through October. The sessions are free for members, who simply need to register in advance through AAEP Anywhere at aaepanywhere.org.

Following is the upcoming schedule of tentative session topics:

May 25: Three Laws of Financial Planning
June 8: Rehab: At a Facility vs. at Home
June 22: Innovative Models for Emergency Coverage

As a reminder, sessions are recorded and made available for viewing on-demand through AAEP Anywhere, the association's free-to-members online learning platform.

On-demand sessions are available 48 hours following the live session and include mentioned resources such as PowerPoint slides, images and more. CE credit is not offered for the Round Tables.

The Virtual Wednesday Round Tables are sponsored by:

FDA issues final guidance on animal drug compounding from bulk drug substances

The U.S. Food and Drug Administration (FDA) on April 13 published final guidance clarifying when the agency does not intend to take enforcement action against those who compound drugs for veterinary patients from bulk drug substances.

Final "Guidance for Industry #256 (GFI #256), Compounding Animal Drugs from Bulk Drug Substances," accessible at fda.gov/media/132567/download, establishes the conditions under which veterinarians may use compounded drugs that are prepared from bulk drug substances.

The AVMA has summarized what the guidance means for veterinarians, including when compounding for nonfood-producing species and compounding for food-producing animals and free-ranging wildlife species. The summary is available at https://tinyurl.com/4d8s78bw.

The final guidance includes many changes made in response to concerns expressed by the AVMA and provides veterinarians considerable latitude when compounding in a practice setting, as well as when writing patient-specific prescriptions.

However, for a pharmacy to provide compounds prepared from bulk drug substances for office stock or dispensing, the compound must be on one of three lists to be maintained by the FDA:

- List of Bulk Drug Substances for Compounding Office Stock Drugs for Use in Nonfood-Producing Animals (https://tinyurl.com/2f3w8hd6)
- List of Bulk Drug Substances for Compounding Drugs for Use in Food-Producing Animals or Free-Ranging Wildlife Species (https://tinyurl.com/ycydezcn)
- List of Bulk Drug Substances Currently Under Review (https://tinyurl.com/2p8hfex3)

An AAEP task force is currently reviewing the guidance document and will be seeking input soon for the addition of other equine products to be included in the list of bulk drug substances for compounding office stock.

Members in the News

Dr. Amy Johnson

Dr. Amy Johnson appointed to endowed professorship at Penn Vet

Dr. Amy Johnson, associate professor of large animal medicine and neurology at the University of Pennsylvania School of Veterinary Medicine's New Bolton Center, has been appointed the Marilyn M. Simpson Associate Professor of Equine Medicine effective July 1.

Dr. Johnson received her veterinary degree from Cornel University and was the first American veterinarian granted dual certification in neurology and large animal internal medicine through the American College of Veterinary Internal Medicine. She currently serves on the AAEP's Infectious Disease and Scientific Review & Editorial committees, and she has co-anchored the Kester News Hour at the annual convention since 2019.

Purdue's equine sports medicine center named for Honor Roll member

Purdue University's board of trustees on April 8 approved the naming of the Donald J. McCrosky Equine Sports Medicine Center in honor of the AAEP Honor Roll member. Dr. McCrosky's $3 million gift will provide current and future research funds to address issues of equine health and performance.

Dr. McCrosky received his veterinary degree from Purdue in 1968. He went on to open his own practice treating both small and large animals. In 2004, he sold his small-animal practice to focus solely on equine medicine, with an emphasis on reproduction.

Purdue University

Dr. Donald and Lois McCrosky

Don't let your AAEP membership expire

Recruitment and retention of equine practitioners is the highest priority for AAEP and a challenge that affects the entire AAEP family: veterinary students seeking a viable and rewarding career path; young practitioners buckling under student debt, emergency coverage demands and feelings of burnout; and veteran practitioners struggling to find a new associate, partner or buyer for their practice.

After extensive research and listening throughout 2021, the AAEP is helping lead the necessary transformation of equine practice by strategizing solutions to bolster the sustainability and vibrancy of a career as an equine practitioner.

We invite you to help us accomplish this mission by renewing your membership by June 30. DVM dues will increase from $320 to $345 this year. The $25 increase is the first in seven years and will help offset investments in the future of equine practice while continuing to provide you with knowledge- and network-boosting resources for your practice. These include free on-demand CE when you need or want it through AAEP Anywhere; clinical and non-clinical insights by way of the twice-monthly Virtual Wednesday Round Tables; case advice and support through AAEP Member Vet Talk on Facebook; and adoption of more hands-on CE opportunities.

Flexible payment options are available if you are unable to renew in full by June 30. You may use a quarterly option

Early-career practitioners advance their ultrasound skills at the inaugural New Practitioners Symposium hands-on CE event in February.

that spreads dues over four installments—$86.25 now and on Sept. 30, Dec. 30 and March 30. A monthly option is also available. If you renew in full by May 31, you will be entered into a drawing to receive a voucher for a free registration for a future AAEP Annual Convention of your choice.

Membership renewal can be completed at aaep.org/membership-dues-renewal. We appreciate your commitment to the AAEP and look forward to serving you in the year ahead.

AAEP mourns the loss of two Honor Roll members


Dr. Fred Lewis

Dr. Fred Lewis

Longtime member Dr. Fred Lewis, who in 1957 opened the first veterinary hospital in Howard County, Md., died March 13 at the age of 95.

A World War II veteran, Dr. Lewis received his veterinary degree from Cornell University in 1953. Although he sold his Lewis Veterinary Hospital in 1995, Dr. Lewis continued to practice medicine from the family farm until several months prior to his passing. He served on the AAEP's Membership Committee in the late 1990s. With his late wife, Mary Agnes, Dr. Lewis also bred and raced Thoroughbreds under the Lewis Family Racing Stable monikor.


Dr. Roman Garza

Dr. Roman Garza

Longtime member Dr. Roman Garza, who spent a combined 35 years of service as the show veterinarian and a member of the board for the Rio Grande Valley Livestock Auction in Mercedes, Texas, died Feb. 25 at age 86.

Dr. Garza received his veterinary degree from Texas A&M University in 1964. With a passion for racehorses and rodeo, he opened his first veterinary practice the following year at Del Camino Downs in Mission, Texas, while concurrently serving in the U.S. Army Reserve Medical Corps. In 1970, Dr. Garza opened McAllen Animal Hospital, where he practiced until his retirement in 2016.

Welcome to new AAEP members!

We are delighted to welcome the following practitioners who joined the AAEP between Jan. 1–March 31, 2022:

Kate Allen, DVM, Axbridge, Somerset, United Kingdom
Amy Alston, DVM, Dade City, FL
Cristian Amavizca, DVM, MVZ, Douglas, AZ
C. Collins Anderson, DVM, San Antonio, TX
Claudine Anen, DVM, Krefeld, NRW, Germany
Camille Arcand, DVM, Saint-Lazare, QC, Canada
Randi Armand, DVM, Trujillo Alto, Puerto Rico
Mohamed Atef, DVM, Dubai, United Arab Emirates
Anje Bauck, DVM, Gainesville, FL
Chandler Bradford, DVM, Midland, TX
Stacy Caffey, DVM, Decatur, TX
Ross Chewning, DVM, Willow Creek, MT
Crystal Christman, BSc, DVM, Okotoks, AB, Canada
Elizabeth Clementson, DVM, Bastrop, TX
Germaine Daye, DVM, Gallup, NM
Jacqueline DeDeo, DVM, Auburn, NH
Kira Epstein, DVM, Athens, GA
Sarah Escaro, DVM, Lexington, KY
Charlie Fahy, DVM, Poulsbo, WA
Rebecca Fitzgerald, DVM, Cypress, CA
Benjamin Fox, DVM, Dripping Springs, TX
Emma Fox, DVM, Staunton, VA
Raemi Gipson, DVM, College Station, TX
Lutz Goehring, DVM, PhD, DACVIM, Lexington, KY
Olivia Hardebeck, DVM, Kentland, IN
Heath Heyden, DVM, Inwood, IA
Elizabeth Holmes, DVM, Geneva, FL
Olivia Jones, DVM, Sha Tin, New Territories, Hong Kong
Earnest Kearns, DVM, Kenton, OH
Leticia Kelly, BVSc, Hendra, QLD, Australia
Jamie Kopper, DVM, Ames, IA
Kayla Le, DVM, DACVS-LA, Madison, WI
Christopher Lesbines, VMD, Gambrills, MD
Madison Levitsky, DVM, Greeley, CO
Ido Lipnick, DVM, Magal, Israel
Samantha Loeber, DVM, Evansville, WI
Kimberly MacKinnon, DVM, Brewster, NY
Meleah McMillen, DVM, Brooksville, FL
Mannes Meijer, DVM, Gaanderen, Gelderland, Netherlands
Christopher Merren, DVM, Seffner, FL
Patrick Meyers, DVM, BS, MS, DACT, Rockwood, ON, Canada
Kaylee Montone, DVM, Palm Harbor, FL
Joshua Morris, DVM, Hugo, OK
Tate Morris, DVM, Elmont, NY
Keith Murch, DVM, Stittsville, ON, Canada
Jess Murray, DVM, Melville, SK, Canada
Melvin Newell DVM, Albany, GA
Katelynn Pierce, DVM, Beaufort, SC
Tyler Powers, DVM, Live Oak, FL
Eric Robinson, DVM, Gilmanton, NH
Ryan Ruegsegger, DVM, Hazel Green, WI
Juan Sanchez, DVM, Seffner, FL
Lee Schaufler, DVM, Millstadt, IL
Steven Scott, DVM, Linden, TN
Renee Seaton, DVM, Terrace, BC, Canada
Glenn Smith, DVM, Winchester, ON, Canada
John Snyder, DVM, Santa Fe, NM
Katlyn Tomschin, DVM, Wilsall, MT
Rebecca Turney, DVM, Tennessee Ridge, TN
Munehiro Umemoto, DVM, Ushiku-shi, Ibaraki-Ken, Japan
Susan Vanderjagt, DVM, Holland, MI
Stijn Vinckier, DVM, Leuven, Vlaams Brabant, Belgium
Jasmyn Virgo, DVM, Kennesaw, GA
Corinne Waddell, DVM, Blaine, WA
Annina Widmer, DVM, St. Moritz, Switzerland
Ingrid Wolff, DVM, Santa Ynez, CA

Member benefit: Fill the information gaps with document retrieval services

Dr. Lydia Gray

Without institutional support for accessing veterinary literature, the road to discovery can be expensive and often paved with detours and dead ends. Whether you're seeking a specific article to assist with a case or simply following a trail of medical curiosity, enlist the help of the AAEP's document retrieval service.

Over 300 members have received more than 600 articles through the service during the past year, including Dr. Lydia Gray of Elburn, Ill.

"Not being at a university, it can be expensive to gain access to full scientific journal articles, not just abstracts on PubMed," she said. "I'm thrilled that AAEP is now making it quick, easy and free for its members to request research papers."

Request a mediated database search on a particular veterinary topic by contacting Megan Gray, member concierge, at membership@aaep.org.

For copies of articles, book chapters and conference papers, email membership@aaep.org and include the following required information:

- Journal/Proceedings/Anthology Title *(Do not abbreviate unless your citation is abbreviated)*
- Article Title
- Volume
- Year
- Pages

If available, please also include:

- Article Author
- Issue Number or Designation
- ISSN/ISBN
- Call Number/DOI
- OCLC Number

If possible, please copy and paste the requested information or embed a screenshot of the article citation into the body of the email. The requested item(s), if available, will be delivered to your inbox as a PDF file in 5 to 7 business days. There is no cost for the first 25 articles each year; beyond 25 articles, a fee applies. For additional information on this valuable benefit of your membership, email Megan Gray at mgray@aaep.org.

The Foundation, BEVA partner on Ukraine equine relief

Initial $50,000 donation kicks off joint effort

The Foundation for the Horse in mid-April awarded $50,000 to help horses in Ukraine and neighboring countries. The aid comes from individual donors who responded generously to The Foundation's appeal for assistance in March as well as a matching donation by The Foundation.

The funds were distributed through The Foundation's partnership with the British Equine Veterinary Association and its charitable arm, BEVA Trust. The groups are collaborating on equine relief efforts in and around Ukraine with two additional organizations: British Vet Professionals for Ukraine and British Equestrians for Ukraine.

The goal of this partnership and initial $50,000 gift is to support the veterinary and equestrian communities that have "boots on the ground" and are making an immediate impact for all horses and working equids in Ukraine and in neighboring countries to which animals have been evacuated, including Poland, Slovakia, Hungary, Romania and Moldova.

To make a disaster gift in support of this combined effort to help horses in Ukraine, visit foundationforthehorse.org/give-now or justgiving.com/bevatrust.

Courtesy of BEVA

A pony in poor condition is reunited with its owner in Poland after five weeks apart and 23 hours in transit from Dnipro, Ukraine.

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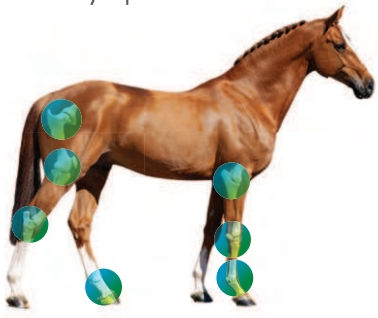
Spryng™ also provides a natural scaffold, potentially protecting the joint from further injury, unlike other lameness management options that may only mask symptoms.



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Highlights of recent clinically relevant papers

Small intestinal strangulation survival

This retrospective clinical study by Meredith Rudnick and co-workers at the University of Florida, USA, examined the effects of age, disease and type of surgery on long-term survival in horses after surgical treatment of small intestinal strangulating diseases over periods relevant to the expected lifespan of a horse.

Post-operative data were gathered from medical records and owner contact for 89 horses with small intestinal strangulation. Survival times from surgery to date of death or date of last follow-up were analysed by Kaplan–Meier statistics. Variables of interest were age, type of strangulating disease and surgical correction. Cox proportional hazards regression was used to evaluate these variables.

Short-term survival was not affected by any of the variables measured. For long-term survival with Kaplan–Meier statistics, horses ≥16 years old had significantly shorter median survival times (72 months; 95% CI 32.0–96.0) than younger horses (121.7 months; 95% CI 90.0–162), horses without resection had significantly longer survival times (120 months; 95% CI 86–212) than horses that had jejunocaecostomy (76.8 months; 95% CI 24–125) and horses with miscellaneous diseases had significantly longer median survival times (161.9 months; 95% CI 72.0–M; note: the upper limit could not be computed by the software package so -M is displayed) than horses with strangulating lipoma (79.8 months; 95% CI 32.0–120.0). In the multivariable Cox Proportional Hazard model, age (HR = 2.67; 1.49–4.75) and anastomosis (HR = 0.65; 0.46–0.92) had the most significant effect on median survival time.

The remaining lifespan of older horses at the time of surgery had the greatest effect on survival. Horses that did not require resection and anastomosis had favourable outcomes, underscoring the potential importance of early intervention to reduce the need for resection.

Pain severity scores

This cross-sectional study by Debra Sellon and co-workers in the USA and Qatar compared estimates of pain experienced by horses as provided by veterinarians and horse-owners. Factors associated with individuals who perceive horses to be experiencing extreme pain or minimal pain were also determined.

Internet-based questionnaires which included items related to recognition of pain in horses, estimated degree of pain experienced by horses and demographic information were completed by horse-owners and veterinarians. Variables associated with perception of a high or low degree of pain were investigated using logistic regression analyses.

Final data sets included responses from 553 horse-owners and 263 veterinarians. Pain scores varied widely and differences in median scores from horse-owners and veterinarians were small. Horse-owners providing high pain ratings were more likely to have <10 horses (OR 2.0; 95% CI 1.1–3.5) and to not have a college degree (OR 1.5; 95% CI 1.0–2.2). Those providing low pain ratings were less likely to own <10 horses (OR 0.6; 95% CI 0.4–0.9). Veterinarians providing high pain ratings were more likely to be employed in a mixed animal practice (OR 2.8; 95% CI 1.3–5.9) and to lack board-certification in a veterinary specialty (OR 2.1; 95% CI 1.1–4.2). Veterinarians providing low pain ratings were more likely to be male (OR 2.4; 95% CI 1.3–4.2).

These results indicate that assessments of the degree of pain that horses are experiencing vary widely among horse-owners and equine veterinarians.

CT of the elbow

In this retrospective study, Marieke Zimmerman and co-workers in France and Belgium evaluated the feasibility of computed tomography (CT) as an imaging technique for detecting lesions in horses with elbow lameness.

Medical records from one centre were reviewed to identify horses that had undergone elbow CT. Subchondral bone sclerosis; resorption of the radius, ulna and humerus; osteophyte; and enthesophyte lesions were graded. A total of 139 elbows of 99 horses (16 with elbow pain and 123 control elbows) were included (median age, 9 years). Osseous cyst-like lesions (n = 13), only seen in the proximomedial radius and medial humerus, were the most common cause of lameness in horses with elbow pain (n = 16), with significantly higher grades of bone resorption (including osseous cyst-like lesions) in this group. One elbow had an avulsion fracture of the lateral epicondyle, two others showed signs of osteoarthritis. Significantly higher grades of sclerosis in the proximomedial radius were seen in horses with elbow pain; however, mild-to-moderate subchondral bone sclerosis was seen in all horses at the medial aspect of the joint. Osteochondral fragmentation lesions of the weightbearing surface of the medial radius (2/16 vs. 1/123) and intra-articular gas (4/16 vs. 2/123) were significantly more common in horses with elbow pain compared with control horses. Mild linear resorptive subchondral bone lesions were often not clinically relevant (32/123 vs. 5/16 in medial humerus; 19/123 vs. 2/16 in medial radius).

These results indicate that elbow CT is a feasible method for detecting clinically relevant lesions in adult Warmblood horses with elbow pain.

Acute synovial structure involvement

This study by Anke-Charlotte Müller and co-workers in Germany investigated the clinical usefulness of serum amyloid A (SAA) in the initial diagnosis of synovial structure involvement caused by acute (<24 h) penetrating limb injuries in horses and aimed to correlate SAA with standard diagnostic parameters.

Fifty-five horses with acute limb injuries were divided into two groups: Group 1 (n = 26) with a diagnosis of penetrating synovial trauma and Group 2 (n = 29) without synovial structure penetration. On admission SAA, white blood cell (WBC) count and fibrinogen as well as clinical criteria and synovial fluid parameters were assessed. The two groups were compared using a two-sample t-test (metric parameters) or a

Wilcoxon–Mann–Whitney test (ordinal parameters). Correlation was determined between SAA and the following parameters: WBC count, fibrinogen, synovial total nucleated cell count and percentage of neutrophils (%N), body temperature and the degree of lameness. Concentrations of SAA were not different between groups; however, there were statistically significant differences in general health, the degree of lameness and synovial fluid parameters. In Group 1, serum SAA concentrations positively correlated with fibrinogen concentrations and synovial fluid %N.

The authors concluded that SAA cannot be used as a sole tool to diagnose synovial structure involvement caused by limb injuries. Synovial fluid parameters remain the most important tool in the diagnosis of synovial penetration. In cases where synoviocentesis fails or is not possible, SAA might support diagnosis.

Equine ophthalmic pain scale

This study by Flaminia Ortolani and co-workers in Italy describes the development and preliminary validation of a composite pain scale, the Equine Ophthalmic Pain Scale (EOPS), to assess ocular pain in horses.

Indicators associated with ocular pain were selected and classified as behavioural, physiological or ocular expressions. Eight horses diagnosed with ocular or adnexa diseases that required medical or surgical treatment were enrolled in the study (Group P). The developed EOPS was applied at baseline (T0) and 1 week later (T7). The EOPS was also applied twice, 1 week apart, to 15 healthy control horses (Group C). Videos of all assessments were retrospectively analysed by seven masked observers, who scored items included in the behavioural and ocular expression categories of the EOPS. The inter- and intra-observer reliability was excellent (intraclass correlation coefficients ≥0.75) for most of the scored items. Cronbach's alpha (0.76) indicated that the EOPS had good internal consistency. The total score (TS), calculated as the sum of all scores, differed between groups at T0 and reduced after medical/surgical treatment in Group P, indicating the responsiveness of the EOPS. The area under the curve (AUC 0.918, 95% CI 0.815–1.000) indicated that the EOPS was very accurate for distinguishing healthy from pathological animals. Sensitivity and specificity of EOPS to identify horses with ocular pathology (at the optimal cut-off, i.e. TS ≥7) were 81.3% and 100.0% respectively. However, 'overall behaviour', 'position inside the box', 'ear movements' and 'head position' items as well as physiological parameters, showed suboptimal reliability, consistency and/or item-total correlation, suggesting further improvement could be made to this composite scale.

Tissue predictability of elastography

Elastography is an emerging imaging modality for characterising tendon injury in horses. In this study, Sherry Johnson and co-workers in the USA (a) investigated differences in glycosaminoglycan, DNA and soluble collagen levels in mesenchymal stem cell (MSC) treated limbs compared to untreated control limbs using a collagenase model of tendinopathy; (b) compared elastographic features between treatment groups; and (c) determined tissue-level predictive capabilities of elastography in relation to biochemical outcomes.

Bone marrow was collected for MSC culture and expansion. Tendinopathy of both forelimb deep digital flexor tendons (DDFTs) was induced with collagenase under ultrasonographic guidance. One randomly assigned limb was treated with intralesional MSC injection with the opposite limb serving as an untreated control. Horses were placed into a controlled exercise programme with elastographic evaluations performed at baseline (0) and 14, 60, 90 and 214-day post-treatment. Post-mortem biochemical analysis was performed. MSC-treated limbs demonstrated significantly less (42%) glycosaminoglycan. Significant differences in elastographic region of interest (ROI) percent hardness, ROI colour histogram and subjective lesion stiffness were appreciated between treatment groups at various study time points.

Elastographic outcome parameters were weak predictors of biochemical tissue analysis, with all R^2 values ≤0.50. Within this range of differences in glycosaminoglycan content between treatment groups, elastography outcomes did not predict biochemical differences. Tissue-specific differences between DDFTs treated with MSCs compared with controls were apparent biochemically, but not predicted by elastography.

S. WRIGHT
EVE Editorial Office

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Case Report

Use of computed tomography angiography for the evaluation of a cutaneous haemangioma in a Standardbred horse

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Keywords: horse; cross-sectional; fetlock; vascular; benign neoplasia

Summary

Cutaneous haemangiomas are benign vascular neoplasms that arise from endothelial cells of blood vessels. Haemangiomas account for 0.6–4% of all equine cutaneous neoplasms, and the fetlock is the most commonly affected site. We describe the use of computed tomography angiography (CTA) for the evaluation of a cutaneous haemangioma located on the plantarolateral aspect of the left hindlimb fetlock of a 9-month-old Standardbred colt. Computed tomography angiography of the affected fetlock was performed under general anaesthesia. The medial plantar artery was catheterised, and a total volume of 50 ml of iodinated non-ionic contrast medium (Iopamidol, 300 mg I/ml, Bracco Imaging Canada) was injected at a rate of 2 ml/s. Following contrast medium administration, the dorsal metatarsal artery and branches including the lateral and medial digital arteries were well demarcated. Two smaller lateral and medial arteries were also identified, forming the vascular network of the metatarsophalangeal joint. At the level of the haemangioma, two tortuous arteries arising from the lateral digital artery were identified, in addition to multiple small branches from nearby cutaneous arteries. These vessels supplied the homogeneously strongly contrast-enhancing cutaneous mass. The initial goal of the CTA study was to map the vascular anatomy for arterial embolisation in conjunction with pharmacological therapy. Considering the involvement of multiple small arterial branches, complete surgical excision along with ligation of the two main supplying arteries was alternatively elected, resulting in a successful long-term outcome (**Figs 1** and **2**).

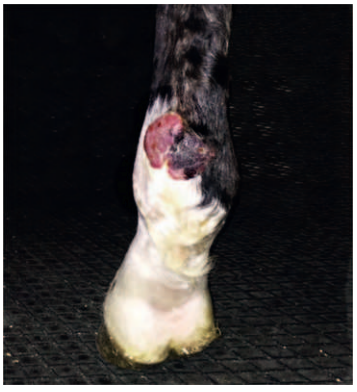

Fig 1: Photograph of the left hindlimb showing a 5.7 cm dorsoplantar × 3.4 cm proximodistal × 1.1 cm lateromedial mass on the plantarolateral aspect of the metatarsophalangeal joint. The lesion is dark purple, well-demarcated and exhibits a combination of verrucous and smooth surfaces.

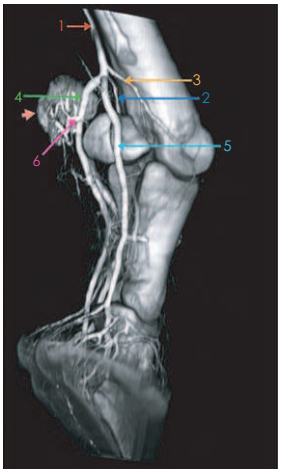

Fig 2: Computed tomography angiography image of the left hindlimb following catheterisation of the medial plantar artery. Lateral is to the left. Three-dimensional reconstruction (plantaromedial view) of the left hindlimb depicting the dorsal metatarsal artery (1) dividing into the lateral (2) and medial (3) arteries which form the vascular network of the metatarsophalangeal joint, the lateral (4) and medial (5) digital arteries, and one of the main arteries (6) providing blood supply to the cutaneous mass (short arrow).

Key points

- Cutaneous hemangiomas are benign vascular neoplasms that arise from endothelial cells of blood vessels and account for 0.6–4% of all equine cutaneous neoplasms. The fetlock is the most affected site.
- Histopathology and immunohistochemistry are required to obtain a definitive diagnosis.
- The use of cross-sectional imaging to assist with treatment planning is currently poorly documented in the literature. Computed tomography angiography can be used to map the vascular anatomy of cutaneous haemangiomas for accurate surgical planning.

Abstract submitted to the Large Animal Resident Forum, 2020 ACVS Surgery Summit Scientific Abstracts, poster presentation.

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Case Report

Dentigerous cysts with exostosis of the temporal bone in horses – A new variant diagnosed by computed tomography

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Keywords: horse; temporal teratoma; dermoid cyst; temporal odontoma; heterotopic polyodontia

Summary

Two mares were presented with mucoid discharge from a draining tract at the margin of the right pinna. Ultrasonographic examination revealed a draining tract originating from a cavity filled with a small amount of hypoechoic fluid. A smoothly contoured, bar-shaped formation of bone opacity was visible on oblique radiographs, originating from the temporal bone of the affected side in both mares (**Fig 1**). There was no evidence of an ectopic tooth on the radiographs. Computed tomography (CT) combined with positive contrast sinography of the draining tract revealed bone formation arising from the supramastoid crest of the right temporal bone extending towards a cyst-like structure but without direct connection in both cases (**Fig 2**). This bone formation was located at a site on the supramastoid crest, close to the external acoustic meatus, where ectopic teeth may also occur. The surgical excision of the cyst and the draining tract was performed immediately after the CT examination under general anaesthesia in both mares. No signs of recurrence were observed for up to 6 months after surgery. Histopathology in one horse revealed a sinus-like mass extending from the skin surface via a narrow canal to a cystically enlarged cavity. The dentigerous cyst or temporal teratoma in horses is a well-known congenital malformation that occurs in the temporal region and usually contains dental tissue. In the two cases presented here, neither classical temporal dentigerous cysts nor dentigerous cysts, dermoid type were detected. The Hounsfield Units (900–1300 HU) of the exostosis were more indicative of bone formation than for dental structures. Following the currently used terminology, we suggest a new classification of 'dentigerous cysts' in horses with the following subgroups: the first type is the '(temporal) dentigerous cyst' (including dental tissue); the second one is the '(temporal) dentigerous cyst, dermoid type (no dental tissue detected)', and the last, one which is described here for the first time, is the (temporal) dentigerous cyst associated with an exostosis in the temporal region (no dental tissue detected). For the latter, removal of the cyst and draining tract resulted in good outcome.

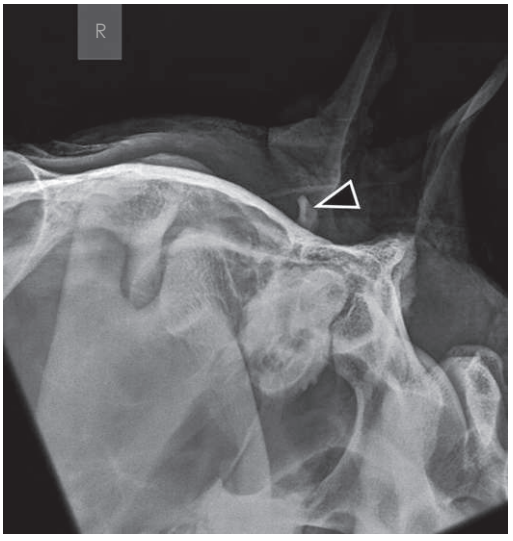

Fig 1: Radiograph of the head: Oblique projection, left dorsolateral 60° right ventrolateral. Rostral is to the left. A structure of bone opacity (arrowhead) in the right temporal region with smooth contour and slight heterogeneous structure extends towards the auricle.

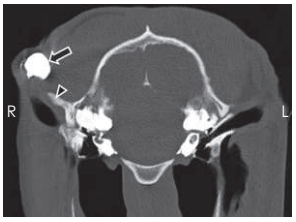

Fig 2: Transverse CT image after injecting contrast medium into the sinus canal. Right is to the left. The exostosis (arrowhead) derives from the supramastoid crest of the right temporal bone and extends in the direction of the cyst (arrow), but without connection. The structure of the exostosis is homogeneous, and the contour is smooth.

Key points

- There are three types of the (temporal) dentigerous cyst: It can be associated with dental tissue, without dental tissue or with an exostosis in the temporal region.
- Oblique radiographs may be indicative of the type of cyst, but a CT and sinography are more reliable.
- Differentiation of the types is crucial for the surgical procedure: Surgical removal of the cyst, without removal of the exostosis, resulted in good long-term outcome.

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Case Report

Paratracheal air cyst in a foal

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Keywords: horse; cervical cyst; congenital anomaly

Summary

A 1-day-old American Paint Horse was presented for a large 19 cm × 10.5 cm, cranio-ventral cervical mass extending from Viborg's triangle to the mid-cervical region (**Fig 1**). The foal was observed to be normal after parturition, able to stand and nurse well. Shortly after birth, the owners observed swelling along the ventral neck. The swelling enlarged gradually over the first 12 h of life according to owner observation. After this initial rapid growth, the swelling remained static in size until hospital presentation. The foal was systemically healthy, and the mass was fluctuant, nonpainful or warm upon digital palpation. Ultrasonographic examination of the mass revealed an encapsulated cyst-like structure filled mostly with gas and a small amount of hyperechoic fluid in the most dependent portion of the cyst. The foals' breathing was normal, and no nasal discharge or coughing during or after nursing was observed. Bronchoscopy and oesophagoscopy revealed no abnormalities in the pharynx, larynx, trachea or oesophagus. Radiographs and a computed tomography (CT) scan of the neck identified a communicating tract between the lumen of the cystic mass and mid-trachea (**Fig 2**). The foal developed temporary blindness and difficulties nursing following anaesthetic recovery from CT, and delayed removal of the cyst was recommended to allow further maturation of the foal prior to undergoing additional general anaesthesia. Upon discharge, the cyst continued to grow in size and became more fluid than air-filled requiring repeat centesis and draining. The foal was then re-presented at 3 weeks of age for surgical removal. In surgery, direct communication with the trachea was identified and ligated. Histopathology demonstrated that the cyst lining was composed of squamous epithelium with goblet cells and occasional ciliated cells. The location, morphological features and congenital presentation of the mass were consistent with a paratracheal air cyst (PAC). Surgical resection resulted in excellent functional and cosmetic outcome. Although not previously reported in horses, PAC should be included in the differential diagnosis of an air-filled ventral neck mass in equine neonates. Complete surgical excision may result in a successful outcome.

Fig 1: Preoperative image of the head and neck of a 3-week-old foal. A large fluctuant, nonpainful mass is evident at the cranial, ventral neck.

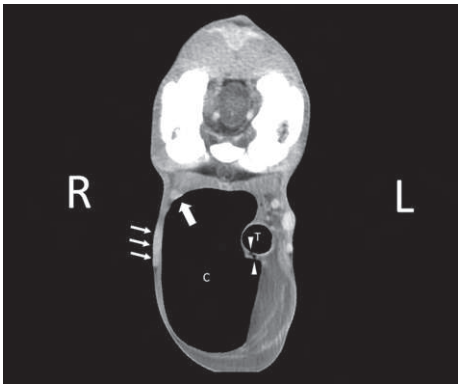

Fig 2: Transverse CT image of the foal's neck at the level of the atlantoaxial articulation. The communication between the right ventral cervical cyst (C) and trachea (T) was located at the ventral luminal margin between tracheal rings 3 and 4 (arrowheads). The right common carotid artery (wide arrow) and external jugular vein (thin arrows) were displaced dorsolaterally and laterally, respectively. Dorsal (top) and ventral (bottom).

Key points

- Although not previously reported, paratracheal air cyst should be considered in the diagnosis of a cystic mass of the ventral cranial neck in equine neonates.
- CT examination can provide valuable information on the origin of a cystic mass of the ventral cranial neck and assist in subsequent surgical planning, but histopathology is necessary to confirm the diagnosis of a paratracheal air cyst.
- Complete removal of a paratracheal air cyst can result in excellent patient outcome.

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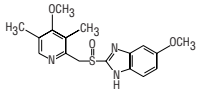
- Chemical name: 5-Methoxy-2-[[(4-methoxy-3,5-dimethyl-2-pyridinyl) methyl]sulfinyl]-1H-benzimidazole. Empirical formula: C17H19N3O3S. Molecular weight: 345.42. Structural formula:



How Supplied

- GastroGard (omeprazole) Paste for horses contains 37% w/w omeprazole and is available in an adjustable-dose syringe. Each syringe contains 2.28 g of omeprazole. Syringes are calibrated according to body weight and are available in boxes of 7 units or 72 units.

Storage Conditions

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Indications

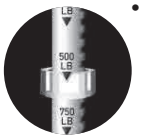
- For treatment and prevention of recurrence of gastric ulcers in horses and foals 4 weeks of age and older.

Dosage Regimen

- For treatment of gastric ulcers, GastroGard Paste should be administered orally once-aday for 4 weeks at the recommended dosage of 1.8 mg omeprazole/lb body weight (4 mg/kg). For the prevention of recurrence of gastric ulcers, continue treatment for at least an additional 4 weeks by administering GastroGard Paste at the recommended daily maintenance dose of 0.9 mg/lb (2 mg/kg).

Directions For Use

- GastroGard Paste for horses is recommended for use in horses and foals 4 weeks of age and older. The contents of one syringe will dose a 1250 lb (568 kg) horse at the rate of 1.8 mg omeprazole/lb body weight (4 mg/kg). For treatment of gastric ulcers, each weightmarking on the syringe plunger will deliver sufficient omeprazole to treat 250 lb (114 kg)body weight. For prevention of recurrence of gastric ulcers, each weight marking will deliver sufficient omeprazole to dose 500 lb (227 kg) body weight.
- To deliver GastroGard Paste at the treatment dose rate of 1.8 mg omeprazole/lb body weight (4 mg/kg), set the syringe plunger to the appropriate weight marking according to the horse's weight in pounds.
- To deliver GastroGard Paste at the dose rate of 0.9 mg/lb (2 mg/kg) to prevent recurrence of ulcers, set the syringe plunger to the weight marking corresponding to half of the horse's weight in pounds.



- **To set the syringe plunger:**
  1) While holding plunger, turn the knurled ring on the plunger ¼ turn to the left and slide the knurled ring along the plunger shaft so that the side nearest the barrel is at the appropriate weight marking, aligning the arrows on the ring and plunger as shown in the pictogram.
  2) Lock the ring in place by making ¼ turn to the right. Ensure it is locked.
- Make sure the horse's mouth contains no feed. Remove the cover from the tip of the syringe, and insert the syringe into the horse's mouth at the interdental space. Depress the plunger until stopped by the knurled ring. The dose should be deposited on the back of the tongue or deep into the cheek pouch. Care should be taken to ensure that the horse consumes the complete dose. Treated animals should be observed briefly after administration to ensure that part of the dose is not lost or rejected. If any of the dose is lost, redosing is recommended.
- If, after dosing, the syringe is not completely empty, it may be reused on following days until emptied. Replace the cap after each use.

Warning

- Do not use in horses intended for human consumption. Keep this and all drugs out of the reach of children. In case of ingestion, contact a physician. Physicians may contact a poison control center for advice concerning accidental ingestion.

Adverse Reactions

- In efficacy trials, when the drug was administered at 1.8 mg omeprazole/lb (4 mg/kg) body weight daily for 28 days and 0.9 mg omeprazole/lb (2 mg/kg) body weight daily for 30 additional days, no adverse reactions were observed.
- To report suspected adverse drug events, for technical assistance, or to obtain a copy of the Safety Data Sheet (SDS), contact Boehringer Ingelheim Animal Health USA Inc. at 1-888-637-4251. For additional information about adverse drug experience reporting for animal drugs, contact FDA at 1-888-FDA-VETS, or online at www.fda.gov/reportanimalae.

Precautions

- The safety of GastroGard Paste has not been determined in pregnant or lactating mares

Clinical Pharmacology

- Mechanism of Action: Omeprazole is a gastric acid pump inhibitor that regulates the final step in hydrogen ion production and blocks gastric acid secretion regardless of the stimulus. Omeprazole irreversibly binds to the gastric parietal cell's H+, K+ ATPase enzyme which pumps hydrogen ions into the lumen of the stomach in exchange for potassium ions. Since omeprazole accumulates in the cell cannaliculi and is irreversibly bound to the effect site, the plasma concentration at steady state is not directly related to the amount that is bound to the enzyme. The relationship between omeprazole action and plasma concentration is a function of the rate-limiting process of H+, K+ ATPase activity/turnover. Once all of the enzyme becomes bound, acid secretion resumes only after new H+, K+ ATPase is synthesized in the parietal cell (i.e., the rate of new enzyme synthesis exceeds the rate of inhibition).
- **Pharmacodynamics:** In a study of pharmacodynamic effects using horses with gastric cannulae, secretion of gastric acid was inhibited in horses given 4 mg omeprazole/kg/day. After the expected maximum suppression of gastric acid secretion was reached (5 days), the actual secretion of gastric acid was reduced by 99%, 95% and 90% at 8, 16, and 24 hours, respectively.
- **Pharmacokinetics:** In a pharmacokinetic study involving thirteen healthy, mixed breed horses (8 female, 5 male) receiving multiple doses of omeprazole paste (1.8 mg/lb once daily for fifteen days) in either a fed or fasted state, there was no evidence of drug accumulation in the plasma when comparing the extent of systemic exposure (AUC0-∞). When comparing the individual bioavailability data (AUC0-∞ , Cmax, and Tmax measurements) across the study days, there was great inter- and intrasubject variability in the rate and extent of product absorption. Also, the extent of omeprazole absorption in horses was reduced by approximately 67% in the presence of food. This is evidenced by the observation that the mean AUC0-∞ values measured during the fifth day of omeprazole therapy when the animals were fasted for 24 hours was approximately three times greater than the AUC estimated after the first and fifteenth doses when the horses were fed hay ad libitum and sweet feed (grain) twice daily. Prandial status did not affect the rate of drug elimination. The terminal half-life estimates (N=38) ranged from approximately one-half to eight hours.

Efficacy

- **Dose Confirmation:** GastroGard (omeprazole) Paste, administered to provide omeprazole at 1.8 mg/lb (4 mg/kg) daily for 28 days, effectively healed or reduced the severity of gastric ulcers in 92% of omeprazole-treated horses. In comparison, 32% of controls exhibited healed or less severe ulcers. Horses enrolled in this study were healthy animals confirmed to have gastric ulcers by gastroscopy. Subsequent daily administration of GastroGard Paste to provide omeprazole at 0.9 mg/lb (2 mg/kg) for 30 days prevented recurrence of gastric ulcers in 84% of treated horses, whereas ulcers recurred or became more severe in horses removed from omeprazole treatment.
- **Clinical Field Trials:** GastroGard Paste administered at 1.8 mg/lb (4 mg/kg) daily for 28 days healed or reduced the severity of gastric ulcers in 99% of omeprazole-treated horses. In comparison, 32.4% of control horses had healed ulcers or ulcers which were reduced in severity. These trials included horses of various breeds and under different management conditions, and included horses in race or show training, pleasure horses, and foals as young as one month. Horses enrolled in the efficacy trials were healthy animals confirmed to have gastric ulcers by gastroscopy. In these field trials, horses readily accepted GastroGard Paste. There were no drug related adverse reactions. In the clinical trials, GastroGard Paste was used concomitantly with other therapies, which included: anthelmintics, antibiotics, non-steroidal and steroidal anti-inflammatory agents, diuretics, tranquilizers and vaccines.
- **Diagnostic and Management Considerations:** The following clinical signs may be associated with gastric ulceration in adult horses: inappetance or decreased appetite, recurrent colic, intermittent loose stools or chronic diarrhea, poor hair coat, poor body condition, or poor performance. Clinical signs in foals may include: bruxism (grinding of teeth), excessive salivation, colic, cranial abdominal tenderness, anorexia, diarrhea, sternal recumbency or weakness. A more accurate diagnosis of gastric ulceration in horses and foals may be made if ulcers are visualized directly by endoscopic examination of the gastric mucosa. Gastric ulcers may recur in horses if therapy to prevent recurrence is not administered after the initial treatment is completed. Use GastroGard Paste at 0.9 mg omeprazole/lb body weight (2 mg/kg) for control of gastric ulcers following treatment. The safety of administration of GastroGard Paste for longer than 91 days has not been determined. Maximal acid suppression occurs after three to five days of treatment with omeprazole.

Safety

- GastroGard Paste was well tolerated in the following controlled efficacy and safety studies.
- In field trials involving 139 horses, including foals as young as one month of age, no adverse reactions attributable to omeprazole treatment were noted.
- In a placebo controlled adult horse safety study, horses received 20 mg/kg/day omeprazole (5x the recommended dose) for 90 days. No treatment related adverse effects were observed.
- In a placebo controlled tolerance study, adult horses were treated with GastroGard Paste at a dosage of 40 mg/kg/day (10x the recommended dose) for 21 days. No treatment related adverse effects were observed.
- A placebo controlled foal safety study evaluated the safety of omeprazole at doses of 4, 12 or 20 mg/kg (1, 3 or 5x) once daily for 91 days. Foals ranged in age from 66 to 110 days at study initiation. Gamma glutamyltransferase (GGT) levels were significantly elevated in horses treated at exaggerated doses of 20 mg/kg (5x the recommended dose). Mean stomach to body weight ratio was higher for foals in the 3x and 5x groups than for controls; however, no abnormalities of the stomach were evident on histological examination.

Reproductive Safety

- In a male reproductive safety study, 10 stallions received GastroGard Paste at 12 mg/kg/day (3x the recommended dose) for 70 days. No treatment related adverse effects on semen quality or breeding behavior were observed. A safety study in breeding mares has not been conducted.

For More Information

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Clinical Commentary

A diagnostic approach to congenital neck masses in foals

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Keywords: horse; juvenile; neonatal; cyst

Congenital abnormalities of foals create a particular concern to veterinarians and owners and it is not always intuitive to know how to proceed when these uncommon lesions are encountered. In particular, masses of the neck region can all look strikingly similar yet have a wide array of aetiologies, involved structures and clinical ramifications. Masses of the neck region of foals may be associated with the guttural pouches, larynx, trachea, branchial arches, oesophagus and thyroid and yet all appear as a similarly sized lump of the ventral neck. However, it is important to obtain a definitive diagnosis, as conditions that involve the vital structures of the neck can be life threatening if not appropriately and promptly addressed. The veterinarian should have a sense for the differential diagnosis associated with these masses to guide diagnostic evaluation, treatment and owner expectations. Here, we will discuss congenital conditions of foals in approximate order of frequency of occurrence, all of which present as clinically similar masses of the cervical region and suggest a diagnostic strategy to discriminate between these lesions.

Guttural pouch tympany

Guttural pouch tympany presents clinically as an air-filled mass of the throatlatch, which may be unilateral or bilateral (**Fig 1**). The cause of the condition is undetermined, but it has been suggested that it may be the result of an abnormally large or dysfunctional mucosal fold covering the pharyngeal ostium. This creates a one-way valve into the guttural pouch and subsequent inflation. The masses usually appear within the first few weeks of life. In particular, German Warmbloods, Paint horses and Arabian horses are over-represented (Blazyczek *et al*. 2004). Concurrent clinical signs often include dyspnoea, dysphagia and stridor occurring as a result of structural displacement of the laryngeal structures. Aspiration pneumonia may occur secondary to the dysphagia. Diagnosis of guttural pouch tympany is accomplished via endoscopic evaluation of the guttural pouch or via radiography. Though breed-specific major gene loci have been identified in both German Warmbloods and Arabians, a genetic test is not yet available (Metzger *et al*. 2012). Surgical resection of the pharyngeal ostium of one or both pouches is often curative with a reported recurrence rate of 30% (McCue *et al*. 1989; Blazyczek *et al*. 2004). Alternatively, placement of a Foley catheter through the pouch opening for a period of 3 weeks appears to be curative in the reported cases without recurrence (Caston *et al*. 2015) and may represent a cost-effective alternative.

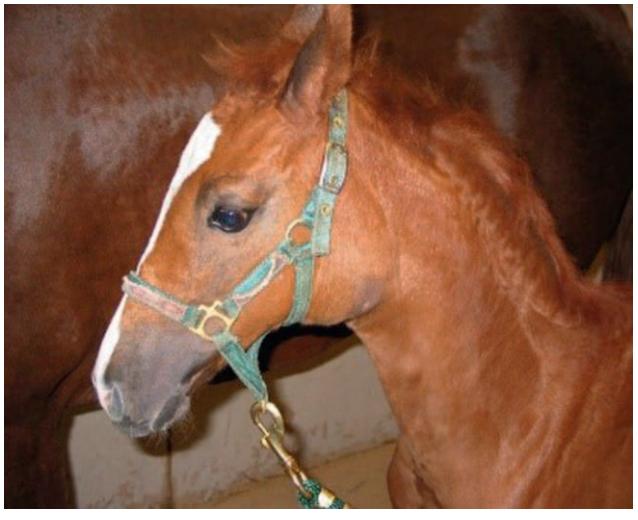

Fig 1: A foal with retropharyngeal swelling as a result of guttural pouch tympany. Image courtesy of Eric Parente, DVM, DACVS.

Branchial arch cyst

Branchial arch cysts are the result of congenital errors of development during differentiation of the branchial arches, the embryologic neural crest cell structures from which develop into portions of the head and neck during development. These generally present clinically as retropharyngeal, nonpainful, fluid-filled swellings (**Fig 2**). The masses may grow slowly or rapidly over a period of several months and can present from birth or shortly thereafter, but also in old age. Definitive diagnosis can be reached based upon histopathologic confirmation after surgical removal, however, presence of stratified squamous epithelial cells in the cystic fluid should increase suspicion of this aetiology. In humans, the absence of malignant transformation or degenerative change in cytologic analysis of the aspirated fluid aids in the diagnosis of branchial arch cysts (Agaton-Bonilla and Gay-Escoda 1996). Magnetic resonance imaging, or radiographic or sonographic assessment of the throatlatch may also be useful in characterising the nature of the mass. On occasion, secondary changes such as laryngeal or arytenoid dysfunction are identified on endoscopy. Surgical resection of the cysts is reported, however, post-operative complications including laryngeal hemiplegia are common (Nolen-Walston *et al*. 2009). Sclerotherapy and marsupialisation have also been reported as possible treatment techniques with mixed success (Estrada and Schumacher 2013; Rinnovati *et al*. 2018).

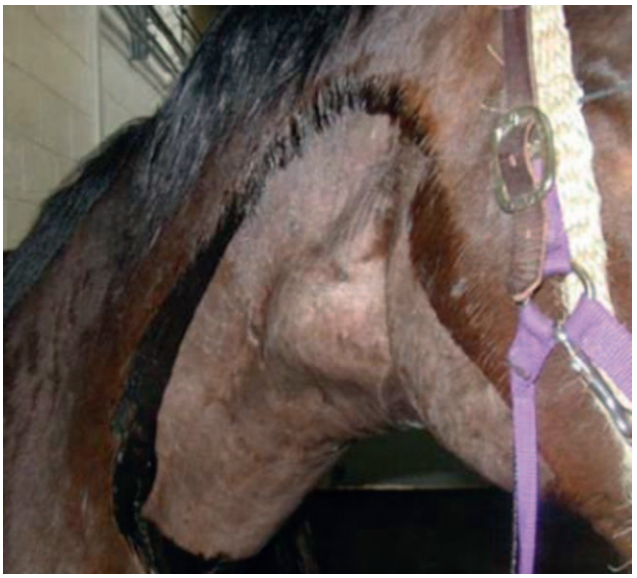

Fig 2: A horse with a throatlatch mass the result of a branchial arch cyst. Image courtesy of Rose Nolen-Walston DVM, DACVIM.

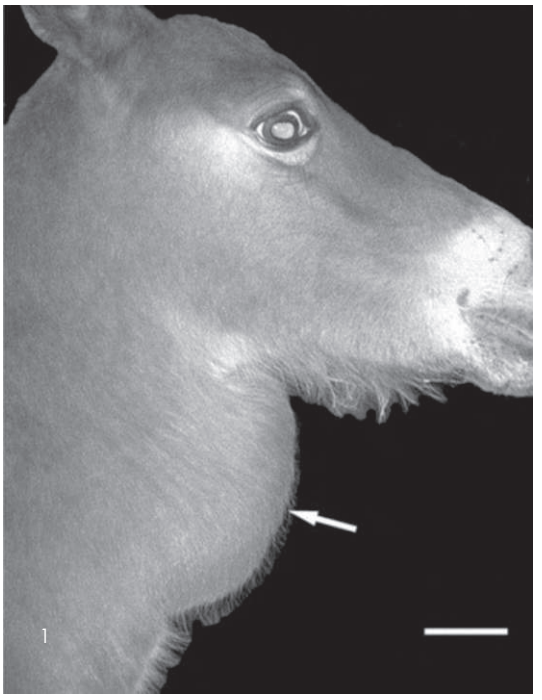

Fig 3: A foal with a ventral cervical mass (arrow) as a result of rhabdomyoma. Image courtesy of Joseph Haynes, DVM, PhD, ACVP.

Congenital goitre

While uncommon, congenital goitre (noninflammatory, non-neoplastic enlargement of the thyroid gland) is a well-reported condition of newborn foals (Allen *et al*. 1994; Nieth *et al*. 2017). Hyperplastic goitre occurs in fetal life when there is either an excess or lack of iodine in the maternal diet, when thyroxine synthesis is lacking, or ingestion of goitrogens such as certain plants of the family *Brassicaceae* (Drew *et al*. 1975; Doige and McLaughlin 1981; Breuhaus 2011). In particular, neonatal goitre is associated with Congenital Hypothyroidism and Dysmaturity Syndrome (CHD) which has been identified in regions of Western Canada and, more recently, in Europe (Breuhaus 2011; Koikkalainen *et al*. 2014). Foals with this syndrome are usually dysmature, with concurrent musculoskeletal abnormalities including mandibular prognathism. In most reported cases, foals either died or were euthanised by one week of life. Mares do not have to show signs of clinical thyroid enlargement or hypothyroidism. Alternatively, dietary iodine in excess of 48 mg/day in pregnant mares has also been associated with congenital goitre in foals (Baker and Lindsey 1968). Thyroidal hyperplasia may, but is not always, associated with an externally visible or palpable mass-like enlargement of the thyroid located in the ventral cranial 1/3 of the neck in the neonate. In humans, the current gold standard for diagnosis of thyroidal hyperplasia is via ultrasound, combined with confirmatory cytologic evaluation of fine-needle aspirates (Tamhane and Gharib 2016). There is one report of a foal with thyroid hyperplasia and respiratory disease that was treated symptomatically with eventual regression of the goitre (Neith *et al*. 2017).

Oesophageal cysts

Both oesophageal duplication cysts and intramural inclusion cysts are uncommonly described horses; however, both are generally palpable as masses of the ventral cervical region.

Intramural inclusion cysts are those located within the wall of the oesophagus and are lined by squamous epithelium (Bezdekova 2012). These cysts are not surrounded by the oesophageal musculature layer, which characteristically distinguishes them from oesophageal duplication cysts. The diagnosis is generally made based on identification of a fluid-filled defect on barium contrast radiography of the oesophagus (Bezdekova 2012). Both surgical removal and marsupialisation of the mass followed by ablation have been described with varied success (Sams *et al*. 1993). Both intramural inclusion cysts as well as oesophageal duplication cysts may present with similar clinical signs including oesophageal obstruction; however, duplication cysts are histologically dissimilar from intramural inclusion cysts. Successful surgical excision of a tubular duplication cyst in a foal has been described (Gaughan *et al*. 1992).

Accessory lung

Accessory lung development is suggested to be the result of embryological development of an extra lung bud at an aberrant site, either intrathoracic or extrathoracic. In calves, intrathoracic accessory lung is commonly reported. The condition is less common in horses, however. Four case reports exist in total; two were identified in live foals and two were identified on necropsy. Of these, three were intrathoracic lesions and one could be externally visualised in the caudal cervical region (Smith and McEntree 1974; Crowe and Swerczek 1985; Davis *et al*. 1991). This mass was eventually found to be associated with an accessory cervical tracheal bronchus and air-filled diverticulum. Closure of the tracheal defect and diverticulum was performed, though the foal was later euthanised for unrelated reasons (Davis *et al*. 1991).

TABLE 1: Differential diagnosis for masses of the neck in foals

	Typical location	Diagnostic modalities	Histopathologic characterisation
Guttural pouch tympany	Pharyngeal region: unilateral or bilateral	Guttural pouch endoscopy, radiography	No diagnostic utility.
Branchial arch cyst	Retropharyngeal: cranial half of the neck	Cytologic presence of squamous epithelium in aspirated contents. Radiography, sonography, computed tomography. Sonographically, dependent hyperechoic structures consistent with blood clots may be identified.	Thick fibrous capsule, the majority with a discrete lining of simple columnar to stratified squamous epithelium.
Congenital goitre	Ventral cranial third of the neck	Sonography, radiography, computed tomography (though usually not required for diagnosis). Cytologic analysis of fine-needle aspirate for confirmation.	Thyroidal hyperplasia. Follicles are small with a minimal amount of colloid.
Oesophageal cyst	Anywhere along the ventral cervical region	Contrast radiography, sonography.	Duplication cyst: stratified squamous epithelium lining cyst-like cavity. Wall contained smooth muscle with few submucosal glands. Intramural cyst: stratified squamous keratinised epithelium with a loose fibrovascular stroma.
Paralaryngeal bronchial cyst	Laryngeal region	MRI, computed tomography, radiography. Confirmation based on histopathology after removal.	Cyst lining is pseudostratified ciliated columnar epithelium underlaid with submucosal glands and a layer of smooth muscle consistent with the respiratory system.
Accessory lung	Reported extrathoracic case: ventral to the trachea. Most are intrathoracic.	Cervical tracheal bronchus diagnosed histopathologically after removal. Likely to be visualised on endoscopy or by computed tomography.	Cervical tracheal bronchus consistent with tracheal origin, diverticular lining consistent with respiratory epithelium.
Rhabdomyoma	May arise anywhere where there is striated muscle.	Diagnosed histopathologically after removal. Suspicion may be guided by radiography, sonography and computed tomography.	Intersecting bundles of spindle and strap cells in a myxomatous stroma. Nuclei were small with a low mitotic rate. Mass margins were well defined and bordered normal skeletal muscle and collagen. Stained strongly positive on IHC for actin, myoglobin and desmin.
Paratracheal air cyst	Reported cranial ventral neck.	Computed tomography, radiography.	Tracheal origin – squamous epithelium, goblet cells, occasional ciliated cells.

Paratracheal air cyst

The cervical tracheal bronchus and air-filled diverticula described by Davis *et al*. (1991) bear much in similarity to the paratracheal air cyst (PAC) in the accompanying case report (Zetterström *et al*. 2022). Like the cervical tracheal bronchus, it is postulated to be the result of a malformed supernumerary lung or early lung bud division. In this case, there was no accessory bronchus described alternatively suggesting that this lesion could be the result of failed closure of the membranous portion of the trachea or tracheal rings during development. In both cases, diagnosis was made initially via recognition of the communication between trachea and air-filled region using radiography. Bronchoscopy was not successful in identifying the origin of the PAC, although in the case of the cervical tracheal bronchus, a 2 mm defect in the trachea was identified on tracheoscopy. The PAC contained purulent material culturing *Streptoccucus zooepidemicus*. While no infection was identified in the case of the cervical tracheal bronchus and air-filled diverticulum, they are frequently septic in reports of the condition in humans (Doolittle and Mair 2002). In both cases, surgical closure of the communication was achieved and curative in the short term. In humans, cervical tracheal bronchi and vestigial tracheal diverticula and subsequent air cysts are often grouped as variations of congenital tracheal bronchi, however, full embryologic characterisation of these clinical entities has not yet been completed

Paralaryngeal accessory bronchial cyst

One report exists of a paralarygneal accessory bronchial cyst in the horse (Baxter *et al*. 1992). The anomaly was recognised during surgical correction of left laryngeal hemiplegia in a 3-year-old Thoroughbred. The cyst was presumed to be the cause of the laryngeal dysfunction in this case rather than recurrent laryngeal nerve axonopathy. Due to the size and location of the cyst, the authors suspect that retrospectively, identification of the lesion should have been possible based on careful palpation of a mass near the larynx. In man, radiographic evaluation along with magnetic resonance imaging or computed tomography is useful when clinical suspicion for these lesions arises. Histopathologic evaluation is necessary for confirmatory diagnosis (Mehta *et al*. 2004).

Rhabdomyoma

Rhabdomyomas are benign tumours of the striated muscle which are rare in both humans and domestic animals. Middle-to-older age animals are more commonly affected, although there is one clinical case report of a congenital rhabdomyoma diagnosed in an 11-day-old Appaloosa filly (Meyerholz *et al*. 2004). These tumours are generally well-delineated soft tissue masses that are typically 1–2 cm in size. However, in this case, the mass was 8 × 15 cm and located in the ventral cervical region, arising from the sternothyroideus and sternohyoideus muscles overlying the trachea (**Fig 3**). Interestingly, while most reported cases of rhabdomyoma in domestic species are similar to the human 'adult-type' tumours, this lesion was histologically most similar to the human 'fetal type' rhabdomyoma and may represent a separate clinical entity from previous reports of rhabdomyoma in domestic animals. Diagnosis of the mass was made histopathologically after surgical excision. No concurrent clinical signs were reported.

The table provided (**Table 1**) can be used for quick reference for generation of a differential diagnosis and provide guidance for diagnostic planning. Though many masses identified in the neonatal period are often assumed to be congenital, trauma may cause mass lesions such as haematomas and abscesses even at a very early age and should be included in potential diagnoses. However, careful imaging and cytologic evaluation where appropriate will yield a diagnosis for the majority of aetiologies described.

Authors' declarations of interest

No conflicts of interest have been declared.

Ethical animal research

Not applicable to this clinical commentary.

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Authorship

This commentary was written by A. Oliver and edited and amended by R. Nolen-Walston.

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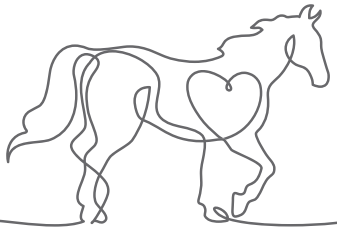

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Case Report

Co-occurrence of papillomas related to *Equus caballus* papillomavirus type 2 and cutaneous habronemiasis

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Keywords: horse; habronemiasis; immunohistochemistry; papilloma; skin

Summary

A 2-year-old female Pura Raza Española horse born and raised in Sicily (Italy), living both outside and inside, was referred in June 2017 for a dermatological evaluation. The mare had cutaneous multifocal, nodular lesions around the left lower eyelid and the left labial commissure. Macroscopically, the lesions were characterised by a warty and partially ulcerated overlying epidermis. These lesions had been noted since February and developed over approximately 3 months but had worsened rapidly in the last days before the consultation by increasing in thickness and causing ulceration of periocular skin. The cutaneous lesions were treated by excisional surgery. Only minimal restraint was required, performed with halter and lead rope. Lidocaine was injected into the peri-lesional area. The lesions were surgically removed (the excised tissue was submitted for histology). After removing the lesions, the wounds were clamped for 3 min with Klemmer forceps previously immersed in liquid nitrogen for about 5 min. The wounds were closed with simple interrupted sutures. Ivermectin was administered systemically as a larvicide (200 μg/kg orally, two doses were given 3 weeks apart) and topically applied for 10 days. The surgery was curative, and no local recurrence of lesions during a 3-year follow-up period was observed. The excised lesions were submitted for histology, and cutaneous viral papillomas with a concomitant severe focal eosinophilic dermatitis with intralesional Habronematidae nematode larvae were diagnosed. The morphology of these nematodes was consistent with L3 larvae of Habronematidae nematodes. It was impossible to assess whether the larvae were of *Habronema muscae*, *Habronema microstoma* or *Draschia megastoma*. Immunohistochemistry revealed papillomaviral capsid protein L1 in the epidermal proliferative lesion, confirming the presence of a productive infection. Rt-qPCR confirmed the presence of EcPV type 2. This study shows that EcPV-2-related papillomas and cutaneous habronemiasis can coexist (this coexistence has not been previously reported). This study's clinical relevance lies in underlining that EcPV-2-related cutaneous or mucocutaneous lesions can undergo worsening with co-occurrence of habronemiasis. We suggest reporting other cases in which these two conditions occur concomitantly, in order to better understand the connection between the two pathological entities (**Figs 1** and **2**).

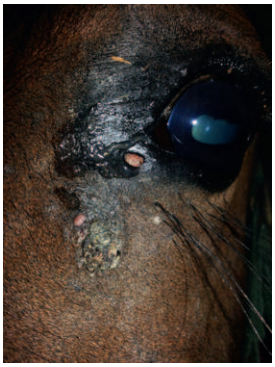

Fig 1: Horse, haired skin. Co-occurrent EcPV2-related papillomas and habronemiasis. Nodular lesions with a warty and partially ulcerated overlying epidermis around the left lower eyelid.

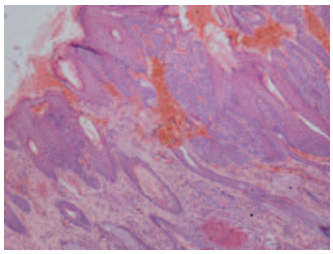

Fig 2: Horse, haired skin. EcPV2-related lesion characterised by severe epidermal thickening, with co-occurrent abundant dermal predominantly eosinophilic infiltrate due to cutaneous habronemiasis and flame figure formation (asterisk). HE, 10× magnification.

Key points

- EcPV-2-related papillomas and cutaneous habronemiasis can coexist.
- EcPV-2-related cutaneous or mucocutaneous lesions can undergo worsening with co-occurrence of habronemiasis.
- Further studies and reports are needed to better understand the connection between EcPV-2-related lesions and habronemiasis and the flies' role in transmitting EcPVs and their associated lesions.

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Clinical Commentary

Papillomaviruses in equids: A decade of discovery and more to come?

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Keywords: horse; papillomavirus

Introduction

The case report that this clinical commentary accompanies (Vichi *et al*. 2022) describes the diagnosis and treatment of a 2-year-old horse with cutaneous papillomatosis lesions, attributed to *Equus caballus* papillomavirus 2 (EcPV2) infection, and concurrent cutaneous habronemiasis. The additional pathology caused by the presence of Habronematidae larvae necessitated surgical removal of the lesions. Without this coinfection, the papillomavirus infection and lesions could have progressed in several ways.

The number of known papillomaviruses is increasing for many species, including equids, as is the range of pathologies in which they are implicated. This article summarises known presentations of papillomavirus-associated infections in equids and outlines the underlying host-viral biology.

Progress in papillomavirus discovery

Papillomavirus (PV) infections may be latent, subclinical or result in lesion development. Active infections may cause self-resolving benign proliferative lesions, more severe or persistent benign disease, or malignant cancers (**Fig 1**). To date, nine *Equus caballus* papillomaviruses (EcPV1 to 9) and two asinine papillomaviruses (EaPV1 and 2) have been identified (Papillomavirus Episteme). Additionally, bovine papillomavirus (BPV) types 1, 2 (Chambers *et al*. 2003) and 13 (Lunardi *et al*. 2013) infect horses and donkeys and cause equine sarcoids, in an unusual example of a cross-species infection by this host-specific family of viruses.

In humans, 225 papillomaviruses (HPVs) have been identified (Papillomavirus Episteme), of which at least 14 can cause cancer with metastatic potential (high-risk HPVs (hrHPVs)) (World Health Organization 2020). An infectious cause of human cervical cancer was first suspected from the 1950s onwards, and hrHPVs 16 and 18 were characterised as causes of cervical cancer in the 1980s (Dürst *et al*. 1983; Boshart *et al*. 1984), a discovery for which Harald zur Hausen was awarded a Nobel Prize (Nobel Prize 2008). It is now known that hrHPVs are the cause of almost all cases of cervical cancer, as well as a significant proportion of other anogenital cancers and approximately 25% of head and neck cancers (de Martel *et al*. 2017). Moreover, hrHPVs are implicated in over 5% of human cancers worldwide (Berman and Schiller 2017). Given the much lower numbers of PVs so far discovered in veterinary species, for example, 23 in dogs and 27 in cattle (Papillomavirus Episteme), it seems there are many more veterinary PVs to discover, both of low and high clinical significance. Additionally, already known veterinary PVs could become newly implicated in pathological conditions.

There has been a considerable increase in the number of known equine-infecting PVs in the last decade, prior to which only EcPV1 (Ghim *et al*. 2004) and BPV1 and BPV2 (Chambers *et al*. 2003) were fully characterised. PVs were previously suspected to be involved in genital papillomas and carcinomas and aural plaques, but EcPV2 and EcPV3, respectively, were not fully characterised from samples of these lesions until 2011 (Lange *et al*. 2011). EcPVs 4 to 7 were first reported in 2013 (Lange *et al*. 2013), EcPV8 in 2018 (Linder *et al*. 2018) and EcPV9 in 2019 (Li *et al*. 2019). In 2013, BPV13 was linked with sarcoids after being detected in samples in Brazil (Lunardi *et al*. 2013). *Equus asinus* papillomavirus 1 (EaPV1) was discovered in 2014 in Asinara white donkeys with no proliferative lesions and appears to have low pathogenicity (Lecis *et al*. 2014), whereas EcPV8 was discovered in horses with severe, generalised papillomatosis (Linder *et al*. 2018). Furthermore, EcPV2 has recently been associated with an increasing range of squamous cell carcinomas (SCCs), its DNA having been detected in a subset of SCCs of the stomach (Alloway *et al*. 2020) and head (Sykora *et al*. 2017) as well as of the genitalia.

Papillomaviruses in benign proliferative disease

Most PVs are strictly epitheliotrophic, infecting dermal keratinocytes or squamous epithelium. A subset of PVs also infects mesenchymal cells; for example, BPV1, BPV2 and BPV13 infect and transform dermal fibroblasts as well as keratinocytes (Bocaneti *et al*. 2016). The life cycle of PVs is linked to epithelial differentiation, a strategy that enables virions to be shed during desquamation and helps the virus escape the immune response (**Fig 2**). PVs have a double stranded circular DNA genome, encoding early (E) genes involved in viral genome replication and oncogenesis, and late (L) genes which encode the capsid proteins (**Fig 3**). The early genes are expressed in deeper tissue layers and the late genes only more superficially (Nasir and Campo 2008).

Papillomavirus infections are productive, with virions shed by infected hosts, in classic papillomatosis. An example is EcPV1-associated "warts" which mostly occur on the face and distal limbs of young horses. These typically spontaneously regress within a few weeks, when the host clears the infection and becomes immune to reinfection (Dong *et al*. 2017). Benign dermal fibropapillomas in cattle induced by BPV1 and BPV2 follow the same course, but the growths include proliferating fibroblasts as well as keratinocytes. In a minority of cases, for example if the host is

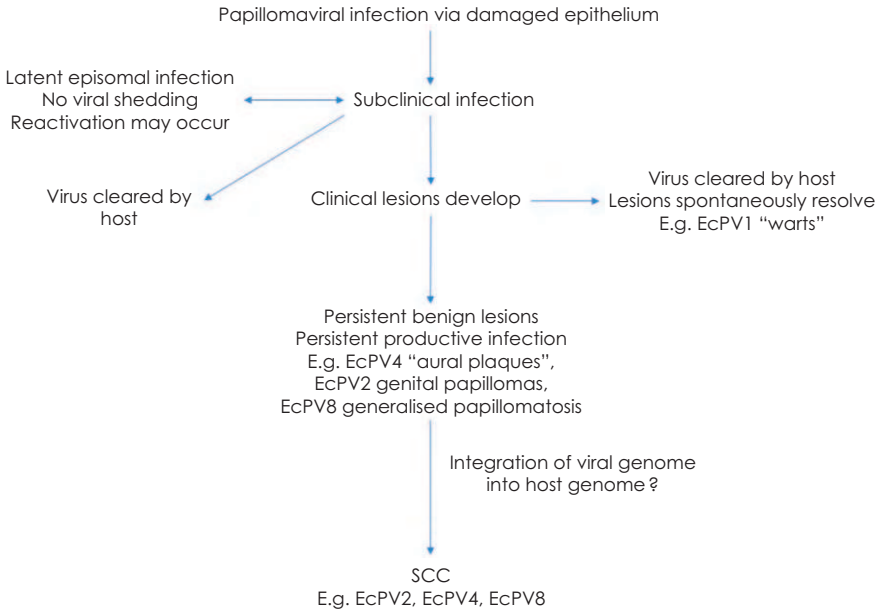

Fig 1: Possible outcomes for* Equus caballus *papillomaviral (EcPV) infections. NB more than one state may exist within a host, for example, there may be simultaneous productive and nonproductive infection.

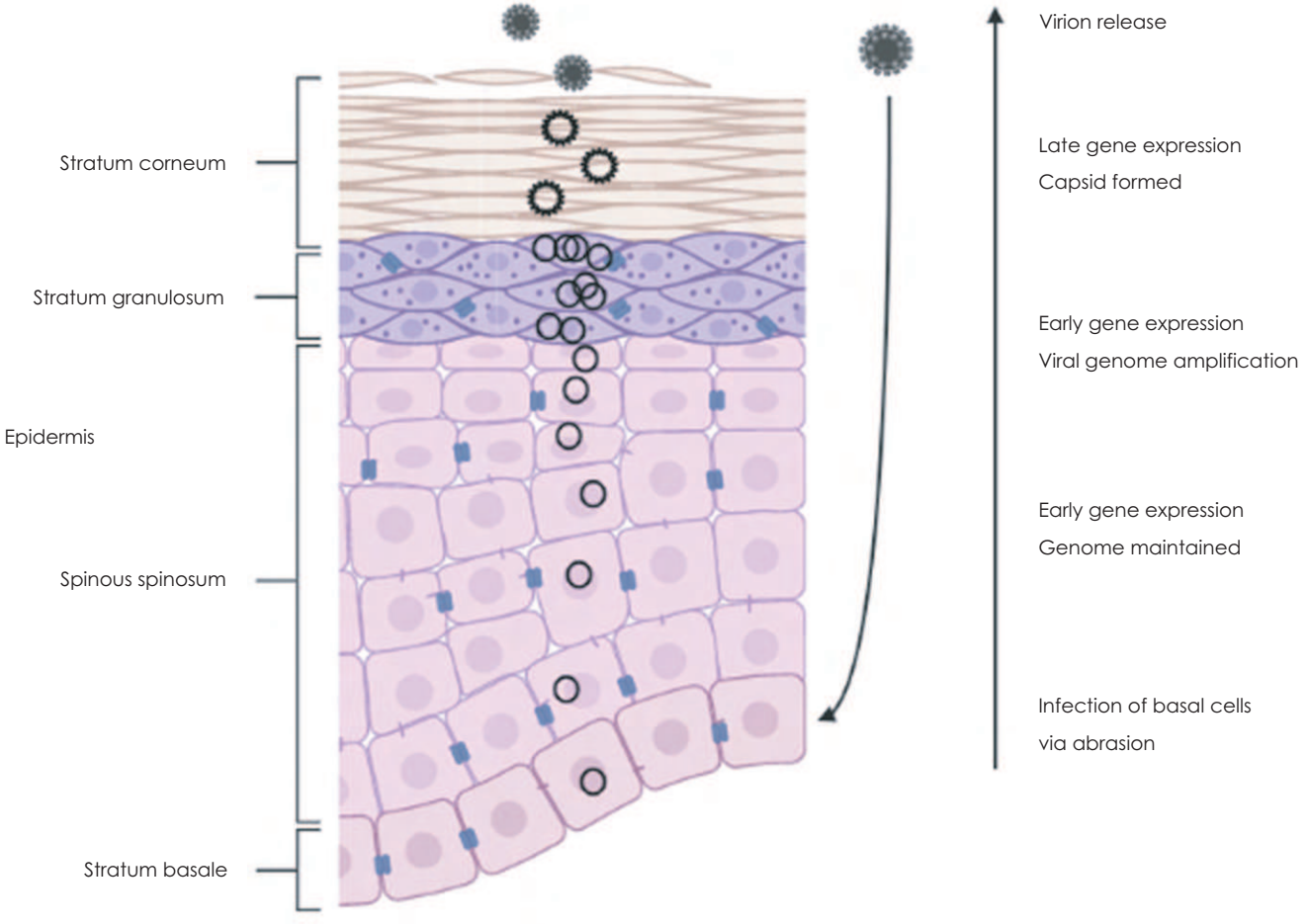

Fig 2: Schematic diagram of the productive life cycle of papillomaviruses (Created with BioRender.com).

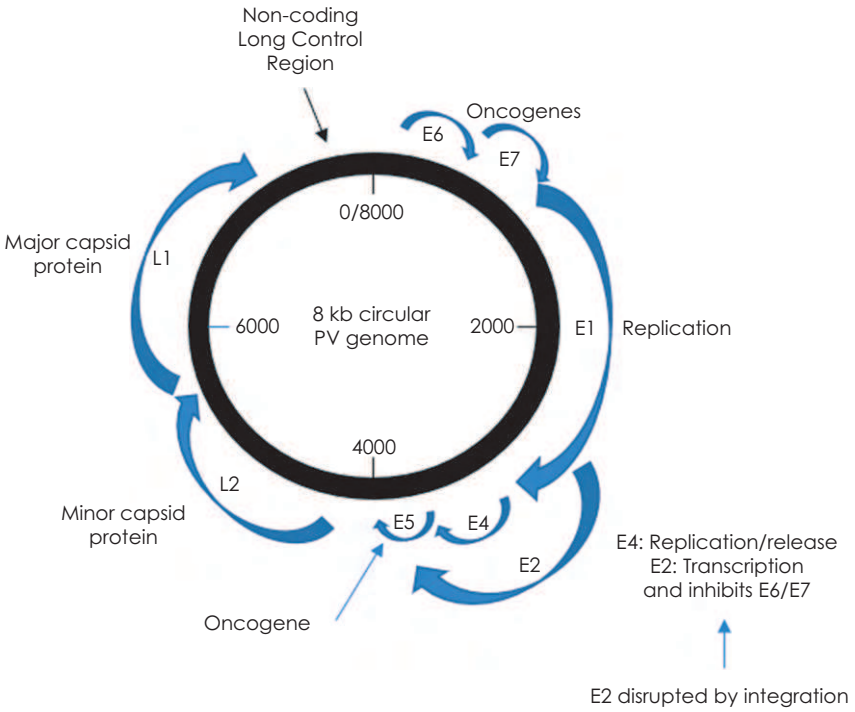

Fig 3: Schematic diagram of a papillomaviral genome, showing the open reading frame and function of each gene and the area disrupted if the viral genome integrates into the host genome.

immunocompromised, these infections are not cleared and the lesions become more severe and widespread (Turk *et al*. 2005). As discussed in the case report, co-infection with other pathogens may influence viral survival and pathogenesis.

Some equine PV infections involve persistent productive infections and lesions, for example, chronic genital papillomas associated with EcPV2 infection (Knight *et al*. 2011). Equine aural plaques are thought to be caused by EcPV3, EcPV4 and EcPV6, and co-infections with these viral types and with EcPV1 and EcPV5 appear common (Mira *et al*. 2018). As mentioned in the case report, the presence of one skin pathogen may aid invasion by another. Although not usually clinically significant, aural plaques typically do not regress and the finding of PV-like structures in the lesions by electron microscopy (Fairley and Haines 1992) suggests virion production. EcPV8 was reported to cause thousands of widespread coalescing hyperkeratotic papules and plaques, particularly on the trunk, in three horses. The lesions persisted for over a year in all three, after which one was euthanased and the others were lost to follow-up. Viral capsid protein was detected in the lesions, suggesting a productive infection in these cases too (Linder *et al*. 2018).

Papillomaviruses in invasive/metastatic disease

EcPV2 is being increasingly associated with some SCCs of the horse. Additionally, an aural SCC containing EcPV4 DNA was reported in an aged horse with multiple aural plaques (Peters-Kennedy *et al*. 2020) and a horse with EcPV8-containing lesions was reported to have multiple SCCs as well as extensive papillomas in the inguinal region (Peters-Kennedy *et al*. 2019).

Integration of the PV genome into the host genome is a dead end for the virus because the infection becomes nonproductive. However, it is an important step in hrHPV-induced oncogenesis and is seen in most, although not all, human cervical SCCs (Groves and Coleman 2015). It is also possible to have nonproductive infections in which the viral genome is maintained in its circular episomal form, as occurs in latency (De Leo *et al*. 2020). In equine sarcoids, the BPV genome appears to remain episomal and not to integrate, meaning expression of the viral genome alone is able to transform cells (Amtmann *et al*. 1980). Equids have traditionally been considered dead end hosts of BPV because infective virions have not been isolated from equine sarcoids as they can be from bovine papillomas (Hainisch *et al*. 2017). However, there are reports and evidence of apparent equid-equid transmission and sarcoid outbreaks (Ragland *et al*. 1966; Reid *et al*. 1994; Nasir and Campo 2008) and of equid-adapted BPV1 variants (Nasir *et al*. 2007). It is unclear how virions could be produced from fibroblasts, given virion production is linked to epithelial differentiation, and sarcoids are primarily tumours of fibroblasts with variable epidermal involvement (Martens *et al*. 2000). However, BPV1 infection has been found in the epidermis as well as the dermis of sarcoids, viral L1 capsid protein has been detected in sarcoid epidermis (Brandt *et al*. 2011), and approximately half of tested sarcoids are positive on an assay which detects BPV DNA complexed to L1 capsid protein (Brandt *et al*. 2008), suggesting at least some equids may be able to shed BPV1 and BPV2.

The case report unusually identified EcPV2 in cutaneous lesions on the face (Vichi *et al*. 2022). EcPV2 is usually associated with lesions in the genital area and more recently of the nasal and oral cavities (Sykora *et al*. 2017). However, it is possible that some cases of facial warts presumed to be caused by EcPV1 could contain EcPV2. EcPV2 DNA has been detected in normal samples and papillomas as well as in

precancerous lesions and SCCs (Bogaert *et al*. 2012). However, it is found in a much higher proportion of samples from SCCs than from healthy animals, suggesting it is an "oncogenic rather than a commensal virus" (summarised in Sykora and Brandt 2017). Additionally, an in situ hybridisation study using probes for EcPV2 detected viral material in 80–100% of neoplastic cells in a subset of genital SCCs, and in vessels of regional lymph nodes and metastases, providing stronger evidence that this virus is causative for SCCs than PCR positivity alone (Zhu *et al*. 2015). There is also some evidence that EcPV2 may integrate into the host genome, as hrHPVs do. In a qPCR (quantitative PCR) study, a subset of EcPV2-positive equine genital SCCs contained more copies of the viral E6 oncogene than the regulatory E2 gene, which is disrupted by integration (**Fig 3**), per host cell (Sykora *et al*. 2017).

Papillomavirus transmission

The anatomical distribution of many PV-induced diseases is suggestive of direct/fomite transmission. For example, EcPV1 lesions mostly occur on the face, and cattle (fibro)papillomas are often on the head and udder. PVs are nonenveloped viruses, resistant to freezing and desiccation (Roden *et al*. 1997), and may therefore persist in the environment for some time. Virions infect new hosts via abrasions as they cannot invade intact epithelium.

It is thought, although not yet proven, that EcPVs implicated in aural plaques are transmitted by flying insects, such as black flies (*Simulium* spp.), which are often seen in horses' ears (Fairley *et al*. 2014). The principle of flying insects acting as mechanical vectors for PVs has been experimentally proven in rabbits (Dalmat 1958). The anatomical distribution of sarcoids is also suggestive of an insect vector, and BPV1 and BPV2 DNA has been found on UK biting and nonbiting flies (Finlay *et al*. 2009). However, infective BPV virions have not been detected on flies to date.

Transmission mechanisms of other EcPVs to other anatomical sites are unclear. HPVs causing tumours of the head, neck and genitalia in humans are considered to be primarily sexually transmitted, but numerous other routes, both horizontal and vertical, have been proposed (Tumban 2019; Mchome *et al*. 2021). BPV1 and BPV2 DNA has been detected in the blood and semen of healthy equines (Silva *et al*. 2014), EcPV2 in smegma (Sykora *et al*. 2017) and EcPV9 in semen (Li *et al*. 2019). Additionally, given EcPV2 has now been found in gastric SCCs (Alloway *et al*. 2020) and SCCs of the equine head including of the larynx and guttural pouch (Hibi *et al*. 2019), there are multiple potential transmission mechanisms.

Vaccination

Effective prophylactic PV vaccinations have been developed for human patients, but post-exposure vaccination does not appear effective. HPV vaccines contain virus-like particles (VLP) which are empty viral capsid protein shells. Over 90% efficacy against persistent infections of HPV 16 and 18 has been demonstrated, and the vaccines also appear to offer cross-protection against similar HPV types (Cutts *et al*. 2007). BPV1 VLP vaccines have been shown to be safe and immunogenic in horses (Hainisch *et al*. 2012), and to protect horses from an experimental BPV1 or BPV2 challenge (Hainisch *et al*. 2017). Given that it may be possible to protect horses from PV-associated diseases, further research into the number of PVs affecting the horse, and the range of diseases they cause, is warranted. The potential market for such vaccinations should also be explored.

Author's declarations of interest

No conflicts of interest have been declared.

Ethical animal research

Not applicable to this clinical commentary.

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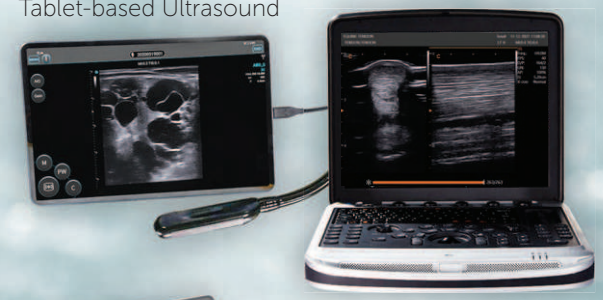

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Original Article

Effects of supplements containing turmeric and devil's claw on equine gastric ulcer scores and gastric juice pH

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Keywords: horse; stomach; equine gastric ulcer syndrome; gastric ulcers; turmeric; devil's claw; nonsteroidal anti-inflammatory drugs

Summary

Supplements containing turmeric (*Curcuma longa*) and devil's claw (*Harpagophytum* species) are commonly fed to horses to decrease inflammation and pain, but because these supplements contain spices and plant irritants, warning labels such as 'these supplements might cause gastric irritation' are required. The purpose of this study was to determine whether supplements containing turmeric and devil's claw cause or worsen gastric ulcers in stall-confined horses, as suggested in required cautions that appear on the labels of these animal products. Twelve clinically healthy Thoroughbred horses with naturally occurring equine gastric ulcer syndrome (EGUS) scores >0 were included in a noncrossover study design. Horses were stratified by EGUS score and assigned to either the treatment (supplements containing turmeric and devil's claw) or control (same supplements without active ingredients) group and fed the supplements for 28 days. Gastroscopy was performed on Days 0, 14 and 28. The EGUS score, nonglandular ulcer number (NGN) and severity (NGS) scores, and glandular number (GN) and severity (GS) scores were recorded during each gastroscopy by a masked investigator (F.M.A.). In addition, bodyweight, gastric juice pH, packed cell volume (PCV), total protein (TP) and blood biochemical results were measured on Day 0 and Day 28. Mean EGUS and NGS scores were significantly lower in both treatment and control groups by Days 14 and 28, when compared to Day 0. NGN score was not different among groups. Bodyweight, gastric juice pH and blood parameters did not change during the study. In conclusion, supplements containing turmeric and devil's claw did not cause or worsen gastric ulcers or alter health parameters after 28 days of feeding.

Abbreviations

ANOVA	analysis of variance
CBC	complete blood count
cHgb	corrected haemoglobin
EGGD	equine glandular gastric disease
EGUS	equine gastric ulcer syndrome
GN	glandular ulcer number
GS	glandular ulcer severity
HCT	haematocrit
NGN	nonglandular ulcer number score
NGS	nonglandular ulcer severity score
NSAID	nonsteroidal anti-inflammatory drugs
OA	osteoarthritis
PCV	packed cell volume
TS	total solids

Introduction

Commercial supplements containing turmeric (*Curcuma longa*) and devil's claw (*Harpagophytum* species) have been used to ameliorate pain and inflammation attributed to osteoarthritis (OA) and other disease processes in horses (Gupta *et al*. 2013; Axmann *et al*. 2018). In practice, these supplements are often used as a substitute for nonsteroidal anti-inflammatory agents (NSAIDs) which may be prescribed for treatment of these conditions. The anti-inflammatory properties of turmeric extract and *Harpagophytum* spp. have been described and include inhibition of both cyclo-oxygenase-2 (COX-2) and prostaglandin-E2 (PGE2) production (Lantz *et al*. 2005; Fiebich *et al*. 2012). Because turmeric is a spice and devil's claw contains plant irritants, there is anecdotal concern that their use might result in adverse effects including gastric ulceration, hypoproteinaemia, azotaemia and loss of body condition as may be seen with prolonged use of NSAIDs, which inhibit both COX-1 and COX-2 enzymes (Khan and Lee 2011; Knych 2017). The National Animal Supplement Council currently requires that supplements containing turmeric include the label cautions 'may be GI irritant' and 'not to be used in patients with stomach ulcers', while supplements with devil's claw include the label caution 'use with caution in animals at risk for GI ulcerations'. However, there is a lack of supporting evidence for the validity of these claims in horses. In contrast, studies in humans and other species provide evidence that turmeric has gastroprotective properties and may be considered a therapeutic modality for treatment of gastric ulcers in people (Yadav *et al*. 2013). In the light of these contradictions, data demonstrating the safety of supplements containing turmeric and devil's claw in horses would be of great clinical value to equine veterinarians who may prescribe them for use in patients with inflammatory conditions.

The purpose of this study was to determine whether feeding a supplement containing turmeric and devil's claw would worsen gastric ulcer scores, change gastric juice pH, decrease body weight or alter blood parameters in horses. We hypothesise that a supplement containing turmeric and devil's claw fed once daily for 28 days will not increase gastric ulcer scores, alter blood parameters or result in weight loss in stall-confined horses.

Materials and methods

Animals

All procedures performed on horses during the study were approved by the Louisiana State University (LSU) Institutional

Animal Care and Use Committee (LSU IACUC Protocol Number 19-073). Horses used for the study were Thoroughbreds of average size, bodyweight (median 483 kg, range 433–525 kg) and competition age (median 8 years, range 4–17 years) randomly selected from the resident herd at the LSU School of Veterinary Medicine, Equine Health Studies Program, which were not enrolled in ongoing studies. A complete physical examination was performed on all horses to exclude the presence of clinical disease. To determine the effect of the supplements on blood parameters, a CBC (Siemens Advia 120 Hematology System)[1] and plasma blood gas and biochemical panel (epoc® Blood Analysis System)[1] were performed on Days 0 and 28. Packed cell volume (PCV) and total protein (TS) were measured on Days 0, 14 and 28 prior to gastroscopy examinations. In addition, bodyweight was measured weekly using a digital livestock scale.

Gastroscopy examinations were performed on nineteen horses to arrive at the final twelve horses with EGUS scores (Andrews *et al*. 1999; Sykes *et al*. 2015b) (**Table 1**) of >0 that met the inclusion criteria (nine geldings, three mares). Horses were stratified by EGUS score and randomly assigned to one of two groups: treatment or control.

Experimental design

The study was performed as a single-period noncrossover design of 28 days in duration. Horses in both the treatment and control groups were brought in from nearby pastures and placed in box stalls (3 m × 3 m) for a 2-day acclimatisation period prior to the start of the study. Diet consisted of locally derived mixed grass square-bale hay fed at 1.5% bodyweight and a commercial concentrate feed (2.2 kg, daily; Omolene® 200; Purina Mills, LLC)[2]. Horses were fed twice daily on a consistent schedule. Those allocated to the treatment group were fed two separate supplements (Smart & Simple™ Turmeric Pellets[3] [30 g] and SmartComfort™ Pellets[3] [56 g]) containing a total dose of 12,000 mg of turmeric root powder and 2500 mg of devil's claw extract. Horses in the control group were fed the same supplements without the active ingredients. Both the treatment and the control were administered once daily by top dressing on the concentrate feed. The supplements were received in containers labelled A or B so that all personnel at the study site were masked to treatments.

Gastroscopy was performed on all horses on Days 0, 14 and 28 of the study period. To improve visualisation of the stomach, food was withheld beginning 16–18 h prior to examination, and water was not withheld. A muzzle was placed on each horse at the time when food was withheld to prevent ingestion of shavings or other environmental material. Horses were sedated with xylazine (XylaMed™)[4] [0.4 mg/kg bwt, i.v.] prior to the gastroscopy procedures. Gastroscopic examinations were performed using a 3-m video endoscope[5]. The stomach was insufflated with air using an electric air pump (Airhead 120V Hi-Pressure Air Pump)[6] until the rugae of the stomach were no longer visible. Mucosal surfaces of the stomach were cleansed of mucus and debris with tap water flushed through the biopsy channel of the endoscope. Lesions in the nonglandular mucosa were scored by size using an EGUS scoring system (**Table 1**) (Andrews *et al*. 1999; Sykes *et al*. 2015b) and number and severity of the nonglandular (NGN, NGS) and glandular (GN, GS) mucosa using a validated equine scoring system (**Table 2**) (MacAllister *et al*. 1997). Scores were assigned by a single-blinded investigator (F.M.A.). Gastric fluid was aspirated upon entry and prior to flushing fluid into the stomach. The gastric juice pH was measured within 1 h using a bench top pH metre (Thermo Orion pH Meter Model 410A)[7]. Suction was used to remove insufflated air from the stomach at the completion of the examination.

Data analyses

Data analyses were performed using SAS 9.4 software (SAS Institute Inc.)[8]. A mixed analysis of variance (ANOVA) model was used to analyse the variables measured with treatment, day and their interactions as the fixed effects and each animal as the random effect. Assumptions of these models (linearity, normality of residuals and homoscedasticity of residuals) and influential data points were also assessed by examining standardised residual and quantile plots. When a fixed effect was detected, Tukey post hoc comparisons were performed with least square means for the effect. Significance was set at $P < 0.05$.

Results

All 12 horses completed the study period, and horses readily consumed the supplements. Two horses fed the turmeric and devil's claw supplements experienced mild colic signs one time for approximately 1 h and were treated medically with

TABLE 1: Grading system for nonglandular equine gastric ulcer syndrome (EGUS) (Andrews *et al*. 1999)

Grade	Squamous mucosa
0	The epithelium is intact, and there is no appearance of hyperkeratosis
1	The mucosa is intact, but there are areas of hyperkeratosis
2	Small, single or multifocal lesions
3	Large single or extensive superficial lesions
4	Extensive lesions with areas of apparent deep ulceration

TABLE 2: Number and severity grading system for nonglandular and glandular equine gastric ulcer syndrome (EGUS) (MacAllister *et al*. 1997)

	Description
Lesion number	
0	No lesions
1	1–2 localised lesions
2	3–5 localised lesions
3	6–10 lesions
4	>10 lesions
Lesion severity	
0	No lesions
1	Appears superficial, mucosa only missing
2	Deeper structures involved, greater depth than grade 1
3	Multiple lesions and variable severity (1,2 and/or 4)
4	Same as 2 and has active appearance, active = hyperaemic and/or darkened lesion crater
5	Same as 4, plus haemorrhage or adherent blood clot

tap water given via nasogastric tube. Both horses received a single dose of xylazine (200 mg, i.v.) for sedation, and one horse received a single dose of flunixin meglumine (500 mg, i.v.) and N-butylscopolammonium bromide (136 mg, i.v.). One horse sustained a laceration which was sutured and then, after dehiscing, and was managed by second intention. No medications were administered to this horse apart from sedation and local lidocaine analgesia, subcutaneously. All horses remained in the study.

Equine gastric ulcer syndrome scores (EGUS)

On Day 0, mean EGUS score for treatment and control groups was not significantly different (**Fig 1**). However, mean EGUS score significantly decreased in both treatment and control groups by Day 14 ($P = 0.0060$) and Day 28 ($P = 0.0112$) compared to Day 0.

Nonglandular and glandular ulcer severity and number (NGS, NGN, GS, GN)

Mean NGS scores were not significantly different between treatment groups on Day 0 (**Fig 2**). However, by Days 14 ($P = 0.0064$) and 28 ($P = 0.0064$) mean NGS scores significantly decreased in both treatment groups. In addition, mean NGN scores decreased ($P = 0.0689$) by Days 14 and 28, but were not significantly different compared to Day 0 scores (**Fig 3**).

Mean GN and GS showed no treatment, day or day-by-treatment effect during the study. Glandular ulcers were observed in two horses from the treatment group on Day 0. In both of these horses, glandular ulcers healed by Day 14 and remained healed by Day 28. In addition, glandular ulcers were observed in one untreated control horse on Day 14, and these lesions were still present by Day 28.

Gastric pH, bodyweight, blood analyses

Mean gastric juice pH (mean 2.20, range 1.46–5.28) was variable and low throughout the study period in both treatment and control groups, and no treatment, day or treatment-by-day effects were seen throughout the study period ($P > 0.05$; **Fig 4**).

Mean bodyweight increased from 479 kg on Day 0 to 498 kg by Day 28 of the study, but was not significantly different between treatment groups or days ($P > 0.05$). Three horses had mildly decreased haematocrit (HCT) values on Day 0, and lactate was below the detected analyser range in five samples. Potassium showed a day effect, with serum K increasing significantly ($P = 0.006$) in all groups by Day 28, but remained within reference range. A day effect was also observed for lactate (decreased), BUN (decreased) and creatinine (increased) [$P < 0.0001$, $P = 0.0096$, $P = 0.0310$, respectively], but these values were not significantly different between treatment groups and remained within the reference ranges. The haematocrit (Hct) and corrected haemoglobin (cHgb) were significantly lower ($P = 0.0287$, $P = 0.0321$) in the treated group compared to controls by Day 28; however, values remained within the reference range in both groups during the study.

Discussion

The supplements containing turmeric and devil's claw were readily consumed by all horses and appeared safe. In the study reported here, mean EGUS and NGS scores decreased significantly in the both control and treated horses by Days 14 and 28 of the study period. These findings of lower ulcer scores were not due to the turmeric and devil's claw in the supplements, as scores showed no treatment-by-day effect. Haematocrit and corrected haemoglobin decreased over time in the treated group, but remained within reference intervals. Thus, we accept the hypothesis that the supplements containing turmeric and devil's claw fed to horses did not worsen gastric ulcers, lead to bodyweight losses or significantly alter blood parameters.

Turmeric (*C. longa*), a spice belonging to the ginger family containing the polyphenol curcumin (diferuloylmethane), is known to have antioxidative effects

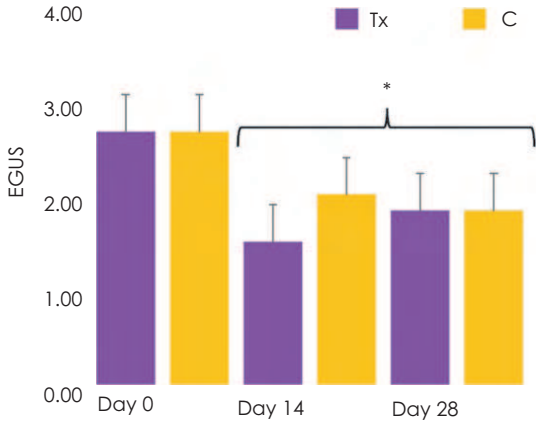

Fig 1: Equine gastric ulcer syndrome (EGUS) scores (Mean ± s.e.m.) (Andrews et al. 1999) in horses (n = 6) fed supplements (Tx) containing turmeric and devil's claw (purple), Day 0, before treatment, and Days 14 and 28 of treatment and horses (n = 6) fed control (C) supplements before treatment (gold) Day 0 and Days 14 and 28. *Significant (P < 0.05) differences compared to Day 0.

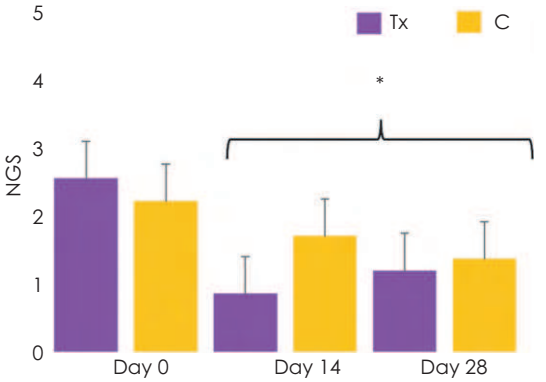

Fig 2: Nonglandular gastric lesion severity (NGS) scores (Mean ± s.e.m.) (MacAllister et al. 1997) in horses (n = 6) fed supplements (Tx) containing turmeric and devil's claw (purple), Day 0, before treatment, and Days 14 and 28 of treatment and horses (n = 6) fed control (C) supplements (gold) before treatment and Days 14 and 28. *Significant (P < 0.05) differences compared to Day 0.

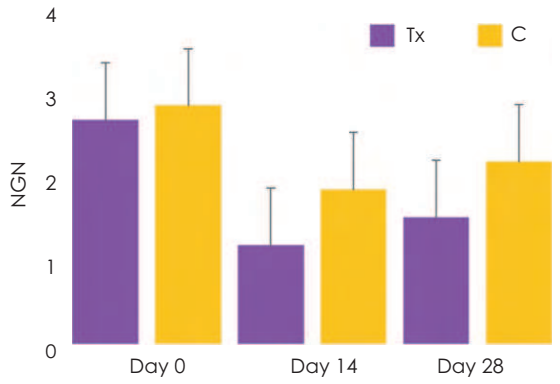

Fig 3: Nonglandular gastric lesion number (NGN) scores (Mean ± s.e.m.) (MacAllister et al. 1997) in horses (n = 6) fed supplements (Tx) containing turmeric and devil's claw (purple), Day 0, before treatment, and Days 14 and 28 of treatment and horses (n = 6) fed control (C) supplements (gold) before treatment and Days 14 and 28. The NGN scores were lower (P = 0.0689) on Days 14 and 28, when compared to Day 0, but this was not significant.

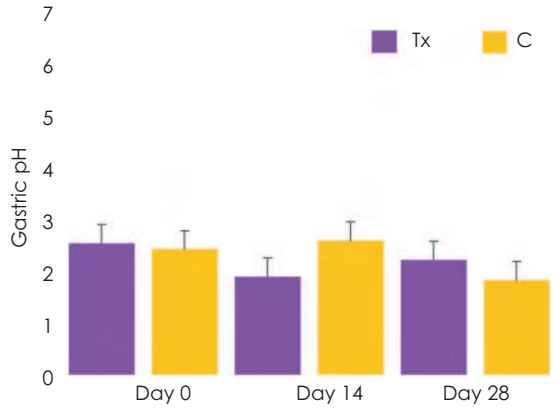

Fig 4: Gastric juice pH (Gastric pH) (Mean ± s.e.m.) in horses (n = 6) fed supplements (Tx) containing turmeric and devil's claw (purple), Day 0, before treatment, and Days 14 and 28 of treatment and horses (n = 6) fed control (C) supplements (gold) before treatment, Day 0 and Days 14 and 28. There were no day or treatment-by-day effects.

via its properties as an oxygen free radical scavenger. Curcumin has also been shown to have potent anti-inflammatory and anticatabolic effects on a number of cell types through its effects on the NF-κB pathway and AP-1 transcription factor activation (Brouet and Ohshima 1995; Singh and Aggarwal 1995; Liacini *et al*. 2002; Clutterbuck *et al*. 2009). Curcuminoids have been shown to reduce the IL-1β-mediated upregulation of NF-κB targets, such as MMP-1, MMP-3, MMP-9 and cyclo-oxygenase-2 (COX-2) (Schulze-Tanzil *et al*. 2004; Jackson *et al*. 2006; Shakibaei *et al*. 2007; Clutterbuck *et al*. 2009). Its inhibition of COX-2 may result in higher total gastric acid output and decreased mucus and blood flow in stomach mucosa due to subsequent inhibition of prostaglandin (PGE) concentrations, while its effect on the downregulation of COX-1 has not been validated (Lantz *et al*. 2005; Rajagopal *et al*. 2018). Lower PGE tissue concentrations could lead to nonglandular and glandular lesions. However, a recent study showed that phenylbutazone, a potent NSAID with nonselective COX inhibitory properties, induced glandular gastric ulcers in horses without inhibition of gastric tissue PGE-2 concentrations (Pedersen *et al*. 2017). In contrast, in humans and other species, curcumin was reported to have gastroprotective properties and has been postulated as an antiulcerogenic therapy in people (Yadav *et al*. 2013). Curcumin also upregulated mucin, PGE 2 and zinc, modulators of gastric health (Rajagopal *et al*. 2018). Since both treatment groups showed decreased ulcer scores, it is unlikely the turmeric had a positive effect on gastric health. However, a larger study population might have elucidated the gastroprotecive effects of these ingredients.

Devil's claw (*Harpagophytum procumbens*) is a South African botanical belonging to the sesame family. Devil's claw has effects on inhibition of inflammatory mediators including TNF-alpha and COX-2, similar to turmeric (Fiebich *et al*. 2012). Recent data have shown evidence that devil's claw extracts have a wider mechanism of action than NSAIDs (Chrubasik and Pollak 2002; Chrubasik *et al*. 2003). *Harpagophytum procumbens* extracts were shown to inhibit COX-2 mediated prostaglandin synthesis (Fiebich *et al*. 2001; Jang *et al*. 2003; Huang *et al*. 2006; Georgiev *et al*. 2011; Mncwangi *et al*. 2012; Fiebich *et al*. 2012) and suppress leukotriene biosynthesis (Loew *et al*. 2001). In addition, *H. procumbens* inhibited COX-1 and COX-2 activity and nitric oxide (NO) production in humans (Anauate *et al*. 2010). As with turmeric, devil's claw's effect on COX enzymes did not seem to have an adverse influence on gastric mucosa in horses in the study reported here. It could be that the antioxidant properties of *H. procumbens* might have a sparing effect on gastric mucosa in the face of COX and PGE inhibition.

The finding of decreased nonglandular ulcer scores in both groups when stall-confined was unexpected, as previous studies have shown that pastured horses typically have less severe nonglandular ulcer scores when compared to stall-confined horses (Feige *et al*. 2002). The exact reason for the decrease in gastric ulcer severity in the study reported here was likely due to the higher plane of nutrition during the stall confinement. Typically, pasture grass in Louisiana has poor nutritional quality during the late fall and winter. Once horses were stall-confined, there was likely a decrease in competition for feed and increased nutritional value of the square bales fed during the period, as reported in a previous study (Woodward *et al*. 2014). Previous feed analyses showed that the round bales and pasture grass had lower nutritional value when compared to the nutritional value of the square bales and grain fed to the horses during stall confinement (F.M. Andrews, unpublished data). It should also be noted that once horses were stall-confined, feed levels were adjusted to maintain bodyweight, which increased slightly over the 28-day study period. It seems likely that the improvement in nonglandular ulcer scores in both groups was related to improved nutrition, rather than stall confinement.

Equine glandular gastric disease (EGGD) is recognised with increased frequency in horses. The prevalence of glandular disease in the study reported here was 21% (4/19), which was similar to the prevalence of glandular ulceration observed in a population of Thoroughbred racehorses examined at a single point in time (Sykes *et al*. 2019).

However, the prevalence of glandular disease has been reported to be higher in a study in racehorses and Warmblood showjumpers (Begg and O'Sullivan 2003; Pederson *et al*. 2018). The pathophysiology remains largely unknown, although a breakdown in mucosal defence mechanisms has been postulated (Banse and Andrews 2019). It is interesting to note that glandular ulcers were only present initially in two horses entered into the study, and these resolved completely by Day 14 and did not reoccur. Conversely, glandular lesions were detected on Days 14 and 28 in one horse in the control group. While the changes in glandular ulcer number and severity scores were not significant in our study, it is interesting that a horse in the control group developed lesions during the stall confinement period, whereas two horses with glandular ulcers in the treated group showed resolution of the glandular lesions. These findings might be attributed to the disease process, certain risk factors, a treatment effect or chance alone. To determine the effect of these supplements on resolving, glandular ulcers would require further investigation in a greater number of horses with glandular lesions.

There were minimal changes in blood parameters observed in the study reported here, and blood parameters, although significantly increased or decreased, were never outside reference ranges. Additionally, there was no treatment-by-time interaction in creatinine or total protein concentration, suggesting the supplements did not cause renal or GI toxicity. In one study, mice administered capsules containing devil's claw at acute daily doses (0.5, 1.0, and 3 g/kg) and chronic daily doses (100 mg/kg bwt) showed increased aminotransferase (AST); however, neither gross nor histopathologic changes were seen at necropsy (Al-Harbi *et al*. 2013). A full toxicological evaluation was not performed in the horses reported here.

Although two horses showed mild signs of colic, daily clinical evaluations did not change over the period of the study. Furthermore, it is unclear how the other active ingredients in the supplements might have interacted or had a synergistic effect on preventing turmeric and devil's claw from causing or worsening gastric ulcer scores. One of the active ingredients (marshmallow root) was found in a supplement that demonstrated some efficacy in maintaining stomach health in horses in a previous work at the authors' institution (Andrews *et al*. 2015). However, as the remainder of the active ingredients differed between these supplements, direct comparison of the effect of marshmallow root is precluded. The use of various extracts of the marshmallow root has been reported in human medicine as an alternative therapy for various ailments including upper respiratory tract irritation; however, to the authors' knowledge, its effect on gastric mucosa has not been reported (Curnow and Owen 2016).

Gastric juice pH remained low throughout the study period, which was similar to other studies where a single sample of gastric juice was taken (Huff *et al*. 2012; Woodward *et al*. 2014). Gastric fluid pH can be affected by stomach contents and the location in which the sample is taken (Murray and Grodinsky 1989). The gastric fluid samples in our study were taken at a single point in time upon entry into the stomach, which may have been more variability than analysis of fluid obtained via continuous or serial collection. However, mean pH values in the study population were similar to those obtained from a continuous pH probe monitoring over a 24-h period (Murray and Schusser 1993; Sykes *et al*. 2015a). A lack of significant change in either group suggests that the supplements had no effect on gastric juice pH; however, serial monitoring of gastric fluid after treatment might have been useful to further investigate its effect on gastric acid production and total acid output, which could be changed without altering pH.

There were several limitations to this study, including the number of horses enrolled and dose and duration of supplement administration. The small number of horses included may have contributed to a type I or type II error. Dose and duration may influence findings of adverse effects. The turmeric mean dose was 24 mg/kg bwt and the devil's claw dose was 5 mg/kg bwt as fed to the horses, which is low compared to doses given to laboratory animals in other studies. Horses in the current study consumed the supplement for a month, while horses being treated for chronic inflammatory disorders may be consuming the supplement for months or years. However, the much higher doses did not lead to adverse effects in other species. Rats, guinea pigs and monkeys showed no adverse effects on health and gross and histologic changes in the liver, kidneys and heart when fed turmeric at a dose of 2.5 g/kg bwt (Shankar *et al*. 1980). Similarly, rats administered *Harpagophytum* extract at 1 g/kg bwt daily for 3 months displayed no clinical abnormalities or significant histologic changes in tissues sampled (Joshi *et al*. 2020). At the doses reported in the study here, no clinical or biochemical adverse effects were noted.

In conclusion, administration of supplements containing turmeric and devil's claw did not cause or worsen gastric ulcers in these stall-confined horses. The supplements were palatable and well tolerated by our study population. The findings from this study suggest that supplements containing these botanicals are safe when administered to horses. As with any supplement or therapeutic agent, caution should be exercised with its use in competition. Consultation with the appropriate regulatory bodies prior to administration is advised.

Authors' declaration of interests

SmartPak Equine supplied the supplements used in this study and have donated to the LSU Foundation in the past.

Ethical animal research

This study was completed in accordance with the Louisiana State University Institutional Animal Care and Use Committee (IACUC), Protocol Number 19-073.

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Authorship

M. St. Blanc, F. Andrews and H. Banse contributed to study design and execution. M. Keowen, F. Garza, M. Retif and N. Arana-Valencia contributed to study execution and data collection. M. St. Blanc and F. Andrews contributed to statistical interpretation and manuscript preparation. M. Keowen, F. Garza, M. Retif, H. Banse and N. Arana-Valencia contributed to the editing and proofreading of the manuscript.

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[6]Kwik Tek, Inc. Denver, Colorado, USA.
[7]Thermo Fisher Scientific, Beverly, Massachusetts, USA.
[8]SAS Inst Inc., Cary, North Carolina, USA.

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Original Article

The effect of porcine hydrolysed collagen on gastric ulcer scores, gastric juice pH, gastrin and amino acid concentrations in horses

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Keywords: horse; gastric ulcers; supplements; porcine hydrolysed collagen; omeprazole

Summary

Gastric ulcers are common in horses. The purpose of this study was to test the effect of porcine hydrolysed collagen (PHC) on gastric ulcer scores and gastric juice pH in horses. We hypothesise that PHC-administration will result in improved gastric lesion scores and act synergistically with omeprazole to improve treatment efficacy. Thoroughbred horses (n = 10) were studied in a 2-period, 2-treatment crossover design, where the PHC (45 g) was administered twice daily. Horses were treated for 56 days. Gastroscopy was performed and gastric juice pH measured on Days 0, 14, 28, 42, 49 and 56. Nonglandular gastric ulcer number (NGN) and severity (NGS) and glandular ulcer number (GN) and glandular severity (GS) scores were assigned by an investigator masked to treatment and serum gastrin and amino acid concentrations. By Day 42, 2 weeks after discontinuing omeprazole treatment, NGN and NGS scores returned to pretreatment values and serum gastrin was higher when compared to values measured on Day 28. By Day 49, after the feed-deprivation period, NGN and NGS were similar to pretreatment values. By Day 56, mean NGN score was significantly lower in PHC-treated horses, compared to controls. Mean gastric juice pH significantly increased in both groups on Day 28 and the pH was significantly (P = 0.0127) higher in the PHC-treated horses. Serum amino acid concentrations were not significantly different 2 h after feeding PHC and hydroxyproline was not detected. Serum gastrin concentration did not increase 2 h after feeding in the PHC-fed horses. The PHC fed to horses enhanced the effects of omeprazole on increased gastric juice pH, inhibited gastrin secretion after feeding and resulted in fewer nonglandular ulcers after long-term feeding (56 days) in stall-confined horses undergoing intermittent feeding.

Introduction

Equine gastric ulcer syndrome (EGUS) describes ulcerations in the nonglandular (equine squamous gastric disease [ESGD]) and glandular regions (equine glandular gastric disease [EGGD]) of the stomach. Ulcers in horses are common and prevalence of both ESGD and EGGD ranges from 45 to 90% (Andrews *et al*. 1999a; Begg and O'Sullivan 2003; Dionne *et al*. 2003; Sykes *et al*. 2015a; Sykes *et al*. 2015b). Risk factors for ESGD include stall confinement, intermittent feeding and intense exercise and the pathogenesis is due to the erosive effects of gastric hydrochloric acid, volatile fatty acids and bile acids (Berschneider *et al*. 1999; Nadeau *et al*. 2003a,b; Sykes *et al*. 2015a; Sykes *et al*. 2015b). In addition, grain feeding has been shown to increase serum gastrin concentration, which leads to increased gastric acid secretion (Smyth *et al*. 1989; Sandin *et al*. 1997; Wickens *et al.* 2013). Horses with this condition perform poorly (Franklin *et al*. 2008; Nieto *et al*. 2009), which has an adverse economic effect. Unfortunately, less is known about risk factors for EGGD. A high prevalence, 47–65%, of EGGD was reported and varied with populations and breeds (Begg and O'Sullivan 2003; Hepburn 2014; Sykes *et al*. 2015a; Sykes *et al*. 2015b; Banse *et al*. 2018; Banse and Andrews 2019).

Omeprazole, a proton pump inhibitor, is effective in treating and preventing ESGD, however long-term treatment is expensive and ulcer recurrence is common when treatment is discontinued (Andrews *et al*. 1999b). In addition, the efficacy of omeprazole in treatment of EGGD is less known and requires longer treatment periods to accomplish healing (Sykes *et al*. 2015a; Sykes *et al*. 2015b; Banse and Andrews 2019). Thus, the development of affordable and natural supplements to improve stomach health or act synergistically with omeprazole during or after appropriate treatment would be desirable.

Historically, biomaterials have played an important role in the treatment of diseases and the improvement of health (Chen and Liu 2015). Natural biomaterials, such as collagen, have been used successfully for the treatment of gastric ulcers (Castro *et al*. 2007; Kumar *et al*. 2013; Bakaeva *et al*. 2016; Sugihara *et al*. 2018). Collagen, a natural biomaterial, is commonly used due to its biocompatibility, biodegradability and weak antigenicity, well-established structure and biologic characteristics (Mano *et al*. 2007). Hydrolysed collagen contains bioactive amino acids (glycine, proline and hydroxproline) and short peptides (e.g. proline-hydroxyproline, glutamine-glycine-alanine-arginine), which are hydrolysed from animal connective tissues. When these amino acids and peptides are administered orally they stimulate local gastric mucus production, coat and form a barrier over ulcerated tissues, provide antioxidant activity and increase the local pH environment within the ulcer bed, improving tissue resistance to acid degradation (Li *et al*. 2007; Ohara *et al*. 2007; Shaw *et al*. 2016; Li and Wu 2018).

Hydrolysed collagen has systemic benefits (Castro *et al*. 2007). One of these benefits includes decreasing plasma

gastrin concentration, which in turn deceases gastric acid secretion and increases gastric pH (Castro *et al*. 2010). In order for these effects to occur, bioactive peptides from collagen hydrolysates must be bioavailable. In other species, bioactive peptides were measured in plasma, confirming absorption through the intestinal barrier (Iwai *et al*. 2005). In people, 95% of amino acids (glycine, proline and hydroxyproline) and dipeptides (proline-hydroxyproline) from collagen hydrolysates were found in blood samples within 12 h of administration (Iwai *et al*. 2005). In another study, hydroxyproline was present in plasma 1–2 h after oral administration of collagen hydrolysates confirming systemic absorption (Zague 2008). There are no reports of the serum amino acid concentration and their effects on serum gastrin in horses fed collagen hydrolysates.

The purpose of this study was to evaluate the effect of PHC added to feed on nonglandular and glandular gastric ulcer scores, gastric juice pH, serum amino acid concentration and serum gastrin in stall-confined horses treated with omeprazole and undergoing intermittent feeding. We hypothesise that PHC enhances the effect of omeprazole on gastric juice pH during treatment and prevents nonglandular and glandular ulcer recurrence after omeprazole treatment and during intermittent feeding in stall-confined horses. The secondary hypothesis is that amino acid concentrations will increase in plasma after PHC feeding and there will be an association with decreased feed-induced gastrin stimulation.

TABLE 1: Validated equine nonglandular and glandular ulcer scoring system used to score gastric ulcers in the study reported here

Lesion Number Score	Descriptions
0	No lesions
1	1–2 localised lesions
2	3–5 localised lesions
3	6–10 localised lesions
4	>10 lesions or diffuse (very large) lesions

Lesion Severity Score	Descriptions
0	No lesions
1	Appears superficial (only mucosa missing)
2	Deeper structures involved (>depth than Number 1)
3	Multiple lesions and variable severity, (1,2 and/or 4)
4	Deeper structures involved (>depth than Number 1) and has active appearance (hyperaemic and/or darkened).
5	Same as number 4 plus haemorrhage or blood clot

Scores reflect the entire area of each region of the stomach and each horse's stomach received 4 scores: nonglandular lesion number (NGN), nonglandular lesion severity (NGS), glandular lesion number (GN) and glandular lesion severity (GS) (MacAllister *et al*. 1997).

Materials and methods

All procedures performed on the horses were approved for ethical care and use of animals by the Louisiana State University Institutional Animal Care and Use Committee (LSU IACUC Protocol #15-001). All horses used in this study were Thoroughbreds (6 geldings and 4 mares, 7–17 years of age, weighing 431–615 kg bodyweight [bwt]) from the resident herd at Louisiana State University School of Veterinary Medicine, Equine Health Studies Program. A physical examination, complete blood count (CBC) and plasma biochemical panel were performed on all horses before the beginning of the study to exclude horses that had clinical and laboratory evidence of disease.

Gastroscopy examination was initially performed on 18 horses. Ten horses with the most severe nonglandular number (NGN) scores, based on a validated gastric ulcer scoring system (**Table 1**) (MacAllister *et al*. 1997) were selected for inclusion into the trial. Horses were then stratified by the NGN score and assigned by a coin-flip to the two groups: treatment (PHC[1]) and untreated control (C). Stratification of the animals by NGN to random allocation allowed selection of more homogeneous groups, therefore reducing group bias.

Experimental design

The experiment was performed as a 2-period, 2-treatment (PHC, 45 g, twice daily mixed in grain feed or C) crossover design, so that all horses received treatment and served as their own controls. The treatment period lasted 56 days (**Fig 1**).

Two days before the beginning of each study period (Day-2) horses were put in covered nonenvironmentally controlled stalls (3 × 3 m) and fed mixed grass hay (1.5% bwt) and grain concentrate (Omelene®100[2], 1.0 kg, twice daily, 08.00 and 16.00 h). Horses had free access to potable city water in buckets at all times. This experimental design of multiple treatments manipulations over the 56 days was used successfully in previous studies (Loftin *et al*. 2012; Andrews *et al*. 2016). In the study presented here, Days 1–13 were used to allow pretreatment of the PHC. From Days 14–28, both groups were treated with omeprazole (Gastrogard® paste, 4.0 mg/kg, orally, once daily)[3] to determine if PHC might enhance healing or improve ulcer scores over administration of omeprazole alone. This period simulated clinical treatment of horses diagnosed with EGUS. From Days 29–41, omeprazole was discontinued in both groups of horses to simulate discontinuation of clinical treatment of EGUS. Days 29–41 were used to determine if the PHC could maintain stomach health after discontinuation of omeprazole treatment. From Days 42–49, a feed-deprivation period (Murray 1994) was instituted, designed to evaluate the effects of bolus feeding and feed stress to determine if PHC might prevent ulcers from worsening. From Days 50–56, horses were allowed to recover and horses were fed their normal diets. All horses remained stall-confined for the study but were able to see other horses in nearby stalls and in adjacent pastures.

The feed-deprivation model was slightly modified from the original report (Murray 1994; Murray and Eichorn 1996). Briefly, on Day 41, horses were muzzled and feed was withheld for 12 h, then on Day 42, horses underwent gastroscopy. Horses were fed their normal ration for 24 h and feed was withheld for 24 h, until a total of 96 h of cumulative feed-deprivation was achieved over the 7-day period. During this period, horses in the treatment group continued to receive the PHC

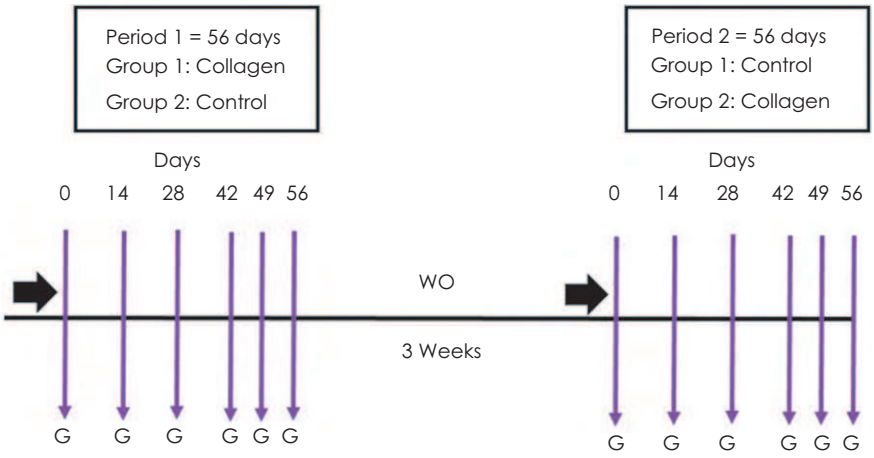

Fig 1: Timeline of experimental events. ➡ ***= 2-day acclimation period, G = gastroscopy; WO = Washout; Collagen = porcine hydrolysed collagen treated group; Control = untreated group; FD = feed-deprivation period, Days 42–49; OME = omeprazole treatment.***

twice daily and the control group received grain only twice daily. Between treatment periods, horses were turned out into a pasture for at least 3 weeks to washout residual PHC treatment before beginning period 2.

Gastroscopic examinations were performed on all horses on Days 0 (before treatment), 14, 28, 42, 49 and 56. Endoscopy of the stomach was performed using a 3-m endoscope (G34-300 Endoscope)[4]. Feed was withheld for 16–18 h and water was not withheld before gastroscopy. Horses were sedated with xylazine (AnaSed® injection; 0.4 mg/kg bwt, i.v.)[5] before gastroscopic examination. To enable observation of the nonglandular squamous mucosa (fundus ventriculi, margo plicatus and glandular mucosa), the stomach was insufflated with air using an air compressor (Airhead 120V Hi-Pressure Air Pump)[6] attached to the endoscope biopsy chamber (until the rugae or stomach folds were absent). Mucosa was rinsed of adherent food material and mucus with tap water flushed through the endoscope biopsy channel using 60 mL syringes. For each horse's stomach, NGN, NGS, GN and GS were assigned for each entire nonglandular and glandular regions, based on a validated equine scoring system (MacAllister *et al*. 1997) (**Table 1**). Scores were assigned in real-time by one of the authors (F.M.A.), masked to the treatment, but not for day of scoping. In addition, hyperkeratosis (yellow colour and thickening), hyperaemia (mucosal reddening) and desquamation (generalised 'peeling' or shedding of the mucosa) were recorded.

Prior to insufflation and addition of water, gastric fluid was aspirated from the stomach during gastroscopy and pH measured within 2 h using a benchtop pH metre (ThermoOrion® pH Meter Model 410A).[7] In a prior study, gastric juice samples measured immediately after aspiration did not differ from samples measured 2 h after aspiration (Huff *et al*. 2012).

Packed cell volume (PCV) and total solids (TS) were measured in whole blood and horses were weighed using a digital scale prior to each gastroscopy examination. In addition, bodyweight was measured weekly before the morning feeding and diet was adjusted for maintenance during the study.

To determine the effect of PHC treatment on post-prandial inhibition of gastrin secretion, gastrin concentrations were measured by chemiluminescent, enzyme-labelled immunometric assay[8] in serum samples obtained on Days 0, 14, 28, 42, 49 and 56. On Days 42 and 49, serum gastrin concentrations were measured before and 2 h after the morning PHF treatment in grain or grain only feeding. Serum samples were stored at −80°C for 30 days after collection.

Amino acid concentrations (including among others, glycine, proline, hydroxyproline, glutamine, alanine and arginine) were measured in serum samples prior to each gastroscopy and on Days 42 and 49, 2 h after PHC treatment to determine if the amino acids in PHC were bioavailable. Amino acids were measured in serum samples that were stored at −80°C for 30 days, using the Dionex ICS-3000 system[9]. Serum samples were thawed at room temperature and an aliquot (100 uL) of serum was mixed with an aliquot of concentrated HCl (0.1N; 100 uL) and added to a hydrolysis tube. Samples were frozen and connected to a vacuum for 1 min and closed to maintain vacuum. Samples were thawed and this procedure was repeated three times. The hydrolysis tube was then placed on a heating block at 110°C for 24 h. After finishing, the hydrolysate was transferred into a 5 mL volumetric flask and washed and made level. The hydrolysate was diluted 100-fold as an injection sample. A standard curve was generated by diluting a standard amino acid stock solution (16.0 nmol/mL) to 8.0, 4.0 and 2.0 nmol/mL. Amino acid analysis was performed with Dionex ICS-3000 system, which included Dionex GS50 Pump, AS50 Autosampler and ED50 Electrochemical detector. Chromeleon 6.8 software was used to control the system and process data. The sample was separated on a Dionex AminoPac PA-10 column (2 × 250 mm) and Dionex AminoPac PA-10 Guard column (2 × 50 mm). Dionex AAA disposable electrode was used to detect signals. The mobile phase consisted of water, 250 mM sodium hydroxide and 1 M sodium acetate. The flow rate was 0.25 mL/min and injection volume was 25 μL.

Statistical analyses

Least square means and standard errors (SEM) were calculated and used to generate the graphs. Data analyses were performed using SAS 9.4.[10] A crossover design with 10 horses over two time periods was used to determine the effect of the PHC treatment on NGN, NGS, GN and GS, bwt, TS, PCV and gastric juice pH. A mixed analysis of variance (ANOVA) model was used to analyse variables measured with treatment, period, day and all their interactions as the fixed effects and each animal as the random effect. The residuals from all ANOVA models were checked and confirmed for normality with the Shapiro–Wilk test. Assumptions of these models (linearity, normality of residuals and homoscedasticity of residuals) and influential data points were also assessed by examining standardised residual and quantile plots. When a fixed effect was detected, Tukey post hoc comparisons were performed with least square means for the effect. Significance was set at $P<0.05$.

Results

The PHC mixed with grain concentrate and fed twice daily was readily consumed by all horses and no adverse responses were observed. As was expected, bwt was significantly lower in both treatment groups on Day 49, after the feed-deprivation period, compared to other days.

Prevalence of EGSD and EGGD

Of the 18 horses scoped, 10/18 (55%) had ESGD, whereas 6/18 (33%) had EGGD. Of the 10 horses with ESGD enrolled in the study, 6/10 (60%) had EGGD as well. There was no correlation between presence of nonglandular ulcers and glandular ulcers in horses in this study.

Gastric ulcer scores

Nonglandular ulcer number (NGN)

On Day 0, prior to treatment, there was no significant difference in mean NGN between treatment groups (**Fig 2)**. By Day 14, mean NGN scores were lower in both treatment groups, but this was not significant (**Fig 2**). However, by Day 28, after omeprazole treatment, mean NGN scores were significantly ($P<0.0001$) decreased in both groups (**Fig 2**). In addition, nonglandular lesions were healed in 60% (6/10) and 70% (7/10) of horses in periods 1 and 2, respectively, after 2 weeks of omeprazole treatment, which was not significantly different. On Day 42, 2 weeks after discontinuation of the omeprazole treatment, mean NGN scores were increased ($P<0.001$) in both groups compared to pretreatment values, indicating recurrence of ulcers. Two weeks after omeprazole treatment was discontinued, 83% (5/6) and 100% (7/7) of horses had recurrence of nonglandular lesion in periods 1 and 2, respectively, which was not significantly different between treatment groups. At the end of the feed-deprivation period (Day 49), mean NGN scores were significantly higher compared to those recorded on Day 42, indicating that during the feed-deprivation period ulceration recurred in these horses. A significant treatment effect was not observed on Days 42 or 49. By the end of the recovery phase, on Day 56, mean NGN scores were significantly lower in the PHC-treated horses, when compared to untreated controls (**Fig 2**).

Nonglandular severity

On Day 0, mean NGS scores in each group were not significantly different (**Fig 3**). Although there was a trend for lower mean NGS scores in the PHC-treated horses, there was not a significant treatment effect on NGS on any day during the study (**Fig 3**). On Day 28, after 14 days of omeprazole treatment, mean NGS scores decreased significantly ($P<0.0001$) in both treatment groups (**Fig 3**). On Day 42, two weeks after discontinuation of omeprazole treatment, mean NGS scores significantly ($P>0.001$) increased to Day 0 values in both treatment groups. On Day 49, at the end of the feed-deprivation period, mean NGS scores remained significantly increased compared to mean NGS scores seen after omeprazole treatment. On Day 56, after the recovery phase, mean NGS scores were decreased in the PHC-treated group, but there was no treatment effect (**Fig 3**).

Glandular number and severity

Glandular ulcers were observed in many of the horses at various times in the study. Mean GN and GS scores were generally low and GS did not show a day or treatment by day effect. However, mean GN was significantly decreased in both groups after 14 days and GN stayed significantly lower for the study period compared to Day 0 (**Fig 4**). This did not appear to be related to treatment.

Hyperkeratosis, hyperaemia and desquamation

Hyperkeratosis of the nonglandular mucosa was observed in 94/120 (78.3%) of gastroscopy examinations, with 50% of them occurring in horses receiving PHC and 50% occurring in controls.

Hyperaemia of the glandular mucosa and desquamation (peeling of mucosa) of the nonglandular mucosa occurred in 27/120 (22.5%) and 24/120 (20%) gastroscopy examinations, respectively. The distribution of hyperaemia was primarily in the pyloric antrum whereas desquamation was observed throughout the nonglandular mucosa. There was not a significant difference regarding hyperaemia and desquamation between treatment groups (44.4% and 41.6%, respectively) and control groups (55.5% and 58.3%, respectively).

Gastric juice pH

Gastric juice pH was variable and low throughout the study in both treatment groups (**Fig 5**). However, on Day 28, gastric juice pH significantly ($P<0.0001$) increased in both treatment groups due to omeprazole treatment, compared to the other days. Furthermore, gastric juice pH was significantly ($P = 0.0452$) higher in the PHC-treated horses compared to controls. The PHC treatment appeared to have a synergistic effect with omeprazole on increasing gastric juice pH. Gastric juice pH was significantly ($P<0.001$) lower 2 weeks after discontinuation of omeprazole (Day 42) and remained low in both treatment groups for the rest of the study period.

Total solids (TS) and packed cell volume (PCV)

Mean TS values remained within normal reference values (55–81 g/L) with a mean value of 68 g/L. There was no significant difference between treatment and control group at any time point during the study. However, on Day 42, TS decreased significantly 2 weeks after discontinuation of omeprazole and remained significantly lower throughout the rest of the study.

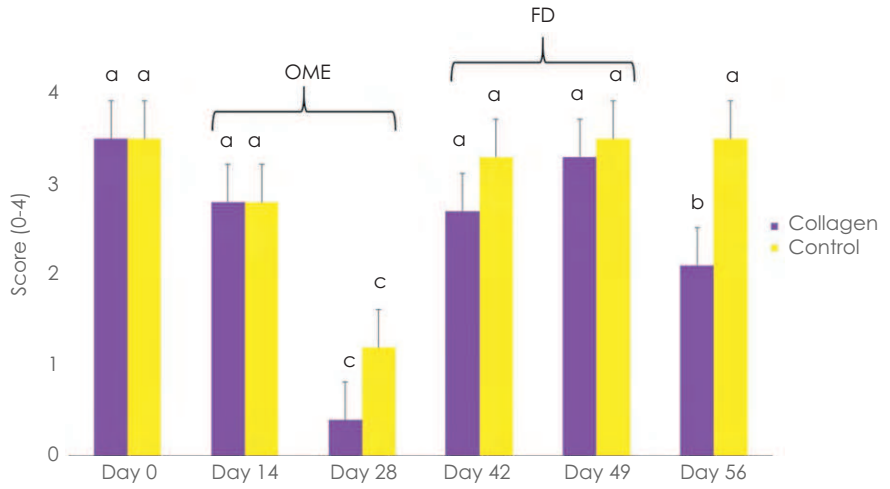

Fig 2: Least square means (SEM) nonglandular number (NGN) scores in PHC-treated and control horses on Days 0 (before treatment), 14, 28, 42, 49 and 56 of treatment. Different lower case letters denote significant (P<0.05) differences when compared to the other days and treatment. Days 14–28: OME = Omeprazole treatment; Days 42–49: FD = Feed-deprivation model.

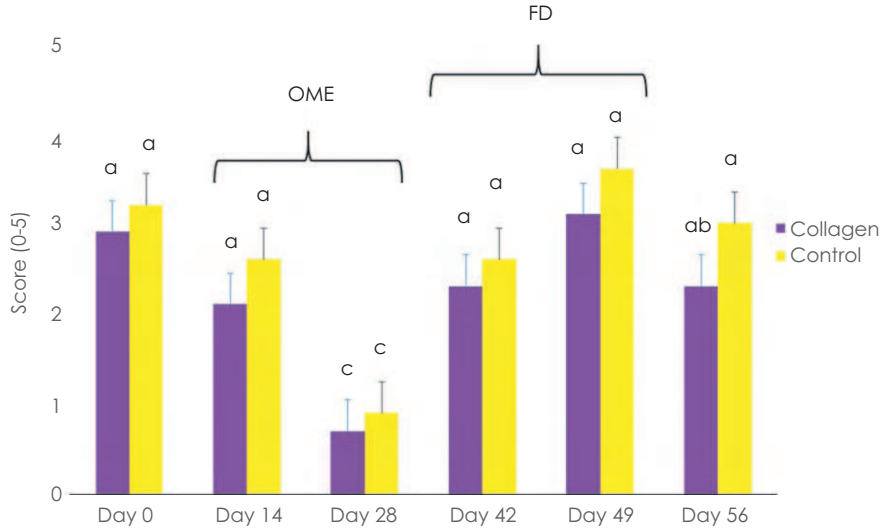

Fig 3: Least square means (SEM) nonglandular ulcer severity in Porcine Hydrolysed Collagen (Collagen) treated and untreated (Control) horses on Days 0 (before treatment), 14, 28, 42, 49 and 56 of treatment. Different lower case letters denote significant (P<0.05) differences when compared to the other days. Days 14–28: OME = Omeprazole treatment; Days 42–49: FD = Feed-deprivation model.

PCV ranged from 30% to 51%, with a mean value of 37.9%. PCV did not differ significantly between PHC-treated and control horses, during this study. However, mean PCV decreased significantly on Day 42, 2 weeks after discontinuation of omeprazole treatment, for both treatment groups and it remained low for the remainder of the study.

Serum gastrin

Mean serum gastrin concentrations were variable throughout the study period and there was a significant (P = 0.0005) day effect in the main model (**Fig 6**). Mean gastrin concentrations were significantly (P = 0.0408) higher in both treatment groups on Day 28, after 2 weeks of omeprazole treatment. On Day 42, mean serum gastrin concentrations significantly (P = 0.0107) increased 119% and 115% in PHC and control groups, respectively, 2 h after grain feeding. However, on Day 49, mean serum gastrin concentration did not change in the PHC-treated horses compared to an increase of 115% in the control, although there was not a significant treatment by day effect.

Serum amino acids

Serum amino acid concentrations (including glycine, glutamine, arginine, alanine and proline) ranged from 38.1 to 168.2 µg/mL. Serum hydroxyproline, a major component of PHC, was not detected in the samples measured. No treatment by day effect was seen in the study. It should be noted that on Day 49, 2 h after feeding, mean serum amino

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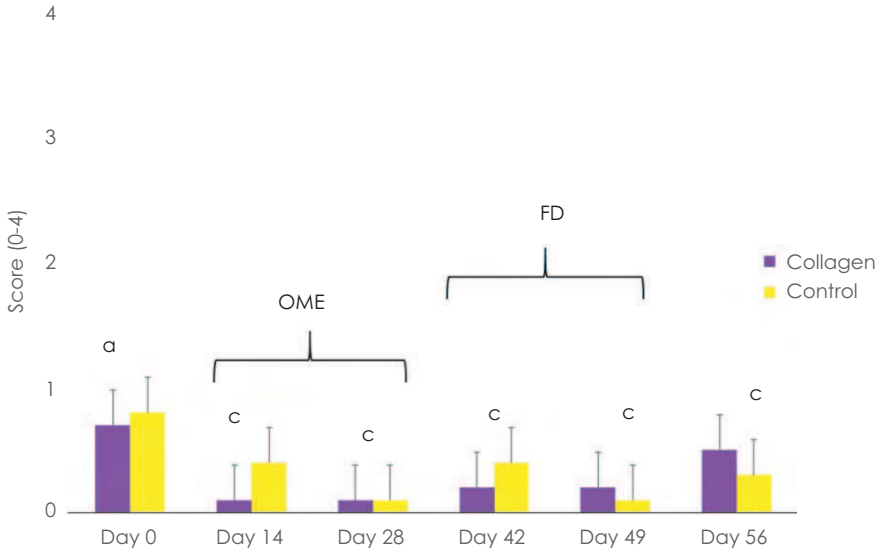

Fig 4: Least square means (SEM) glandular number (GN) Porcine Hydrolysed Collagen (Collagen) treated and untreated (Control) horses on Days 0 (before treatment), and Days 14, 28, 42, 49 and 56 of treatment. Different lower case letters denote significant (P<0.05) differences when compared to the other days. Days 14–28: OME = Omeprazole treatment; Days 42–49: FD = Feed-deprivation model.

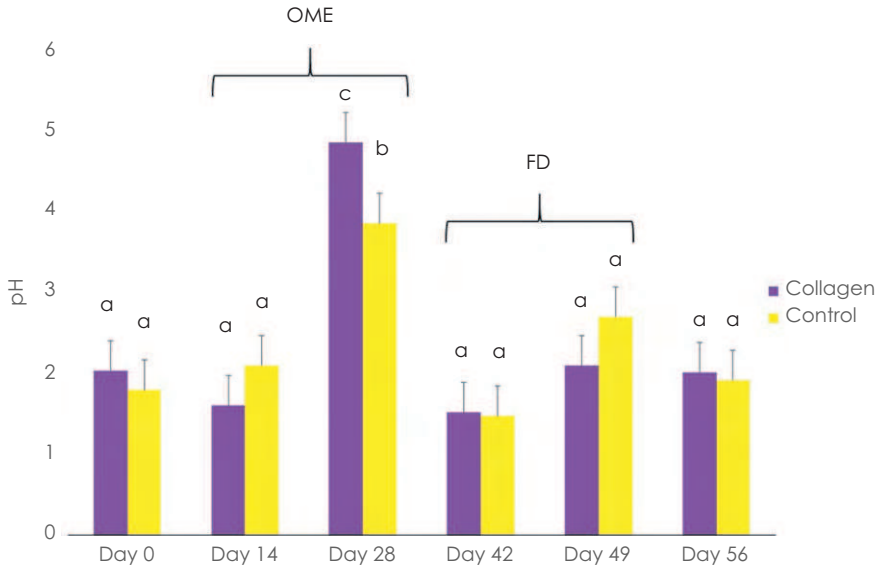

Fig 5: Least square means (SEM) gastric juice pH in Porcine Hydrolysed Collagen (Collagen) treated and untreated (Control) horses on Days 0 (before treatment), and Days 14, 28, 42, 49 and 56 of treatment. Different lower case letters denote significant (P<0.05) differences from each other and treatment effects. Days 14–28: OME = Omeprazole treatment; Days 42–49: FD = Feed-deprivation model.

acid concentrations increased in serum of the both PHC- and control-treated horses and in the PHC-treated horses serum gastrin concentrations did not increase, while gastrin increased by 115%, although this was not significant.

Discussion

The PHC powder[1] fed to horses in this study was associated with fewer nonglandular ulcers (ESGD) in stall-confined horses after 56 days. Fewer ulcers occurred while stomach pH remained low and was not significantly different from untreated controls. Concurrent PHC treatment and omeprazole resulted in significantly higher gastric juice pH when compared to control horses. Nonglandular gastric ulcers were significantly fewer and less severe in both groups associated with omeprazole treatment, but additional effects of PHF treatment on ulcer healing was not observed. Furthermore, grain feeding increased serum gastrin concentrations by 115% in control horses, whereas serum gastrin did not increase in the PHC-treated horses, suggesting that the PHC fed here inhibited serum gastrin secretion.

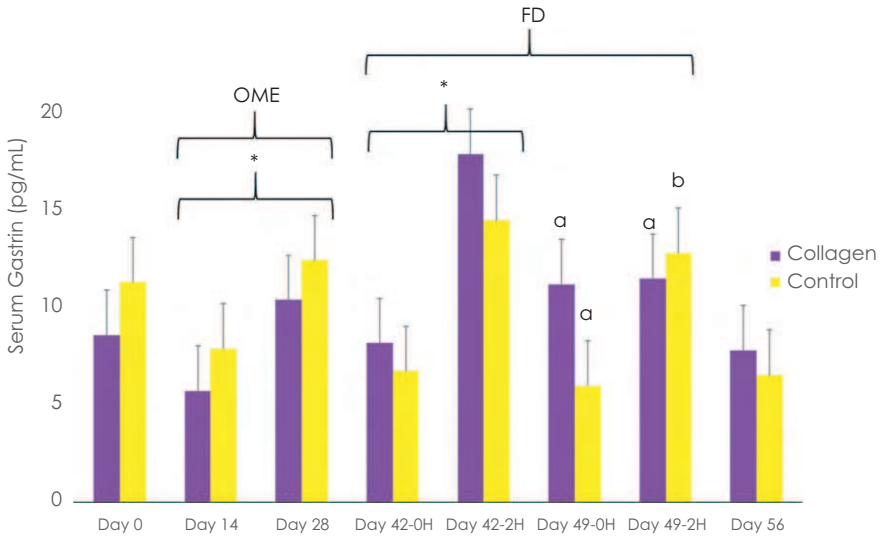

Fig 6: Least square means (SEM) serum gastrin concentrations in horses treated with Porcine Hydrolysed Collagen (Collagen) and untreated (Control) horses at Days 0 (before treatment), and Days 14, 28, 42, 49 and 56 of treatment. In addition, on Day 42 0H and Day 49 0H serum gastrin was measured 2 h after feeding Collagen mixed in grain or Control, 42-2H and 49-2H, respectively. The (*) denotes a significant difference (P<0.05) when compared to the previous day or previous hour. Different lower case letters denote significant (P<0.05) differences from each other and treatment effects.

The PHC used in this study was rich in bioactive peptides, glycine, glutamine, alanine, arginine, hydroxyproline and proline. In the present study, the dose of PHC administered was 45 g twice daily, which corresponded to approximately 100 mg/kg for an average-sized Thoroughbred horse (450 kg). This dose was decided based on the maximum volume that the animals tolerated per feeding and was considered affordable per dose. The dose in rats in one study varied from 100 to 1500 mg/kg. In that study, PHC treatment resulted in a logarithmic dose–response that reduced the ulcer lesion index (ULI) from 12% at 100 mg/kg to 75% at 1500 mg/kg (Castro *et al*. 2007, 2010). From these data in rats, it is possible that a higher dose of PHC might have resulted in fewer and less severe nonglandular ulcers before 56 days of treatment.

The exact mechanism in which PHC resulted in a treatment effect is unknown. However, in other species, the bioactive amino acids and peptides in PHC stimulated gastric mucus production, formed a barrier over ulcerated tissues and increased pH within the glandular ulcer bed, resulting in resistance of the tissue to acid degradation (Ohara *et al*. 2007; Shaw *et al*. 2016). In addition, collagen hydrolysates were shown to have anti-inflammatory and antioxidant activity *in vitro* (Aleman *et al*. 2011; Nakchum and Kim 2016; Song and Li 2017). Antioxidant and immunomodulary properties are important in glandular mucosal healing in other species (Das and Banerjee 1993; Xu *et al*. 2007) and might have contributed to reduction in the number of nonglandular ulcers in the study reported here. Furthermore, glycine and proline both locally and systemically have antioxidant effects by regulating the nuclear factor erythroid 2-related factor 2(Nrf2)-antioxidant response element (ARE) pathway, which might have played a central role in regulating antioxidant enzymes and protected the gastric tissue against oxidative damage triggered by inflammation (Song and Li 2017).

Glycine, on the other hand, a major structural component of collagen, might have the ability to reduce inflammatory responses (Zhang *et al*. 2010; Hartog *et al*. 2013). This effect might be due to inhibition of the production of pro-inflammatory cytokines via glycine-gated chloride channels (GlyR). Inflammation within the ulcer bed might inhibit the healing process and PHC treatment might act locally and decreased inflammation and facilitated ulcer healing by forming a barrier over ulcerated tissues. In addition, glycine might have increased pH within the ulcer bed, making gastric tissue resistant to acid degradation (Ohara *et al*. 2007; Shaw *et al*. 2016). The fact that hydroxyproline was not found in serum samples might support the local effects of PHC in the study reported here.

In horses, the surface of the nonglandular stomach is coated by dense osmophilic surface-active phospholipids (SAPL), similar to the structure to pulmonary surfactant (Ethell *et al*. 2000). Because the nonglandular mucosa has only a very thin to nonexistent mucus layer (Bullimore *et al*. 2001) and no bicarbonate secretion compared to the glandular mucosa, the PHC used in horses in this study might have acted as surface-active peptides and provided a secondary defence mechanism against the erosive effects of hydrochloric and other organic acids. In addition, PHC was shown to increase pH within glandular ulcer beds and might have increased pH in nonglandular ulcers beds and facilitated healing in the study reported here. Healing might be accelerated in the nonglandular and glandular mucosa when omeprazole is administered concurrently with PHC, as gastric juice pH was increased when omeprazole was administered concurrently.

Few studies have been published evaluating hydrolysed collagen in horses with gastric ulcers. In a recent study in horses, a supplement containing hydrolysed collagen among other ingredients, resulted in fewer nonglandular ulcers after 28 days of administration and prevented recurrence of nonglandular ulcers after omeprazole treatment (Andrews *et al*. 2016). In the study described here, the PHC supplement alone was less effective and did not prevent recurrence of nonglandular ulcers 14 days after omeprazole treatment was

discontinued and after feed-deprivation. A treatment effect was not seen until 56 days of treatment. The differences between the study reported here and that previous study was likely due to multiple ingredients found in the supplement used in that study. Ingredients such as sea buckthorn berries, pectin and lecithin, probiotics and antacids might have worked synergistically with hydrolysed collagen to facilitate nonglandular ulcer improvement before Day 56. However, the effect of that supplement on improvement of glandular ulcers was not determined.

Omeprazole is effective in treating ESGD, but ulcers rapidly reoccur after cessation of treatment (Andrews *et al*. 1999b). Serum gastrin concentrations were significantly increased after 14 days of omeprazole treatment, which likely contributed to recurrence of ulcers by Day 42. Hypergastrinemia, after cessation of omeprazole treatment, has been reported in other species (Berlin 1991). It is likely that there was not a synergistic effect of omeprazole and PHC on NG ulcer healing.

In several studies, omeprazole treatment was less effective for treatment of EGGD and might require up to 60 days of treatment to effect improvement and healing of lesions (Sykes *et al*. 2015a; Sykes *et al*. 2015b). In the study reported here, significant differences in glandular ulcer healing were not seen in either treatment group. It should be emphasised that glandular lesions in horses in this study were generally mild and were seen in the horses throughout the study. The prevalence of glandular lesions in Thoroughbred horses is typically low compared to other breeds (Banse and Andrews 2019).

Gastric juice pH was low and variable in the horses during the study. The gastric juice samples in the study reported here were taken at one point-in-time, which, depending on location samples are obtained, the pH can be variable. A more accurate representation of gastric juice pH, stomach pH, could be measured using continuous monitoring with an *in situ* 24 h pH probe (Murray and Schusser 1993; Nadeau *et al*. 2000; Raidal *et al*. 2017; Sykes *et al*. 2017). However, gastric juice pH in the study reported here was similar to measurements in samples collected from the ventral stomach via gastric cannulae and by continuous pH monitoring in unfed and fed horses. The increase in gastric juice pH when PHC was administered concurrently with omeprazole might have contributed to a local effect on lesion healing (Castro *et al*. 2007). In addition, there might be a systemic effect on decreasing serum gastrin activity, although serum gastrin did not significantly decrease in serum during the study period and no significant treatment effect was seen. Thus, it is likely that PHC most effected local mucosal pH within the ulcer bed. Reports in rats showed that PHC increased pH within the ulcer bed in the stomach coating and buffering stomach acids preventing the erosive effects on stomach tissue (Ohara *et al*. 2007; Shaw *et al*. 2016).

Serum gastrin concentrations were variable throughout the study period. On Day 28, after 14 days of omeprazole treatment, serum gastrin was significantly increased. On Day 49, serum gastrin concentration increased 115% in the control horses and was not increased in the PHC-treated horses, 2 h after feeding. Although, there was not a significant treatment effect on ulcer scores, this might indicate that GI absorption of bioactive amino acids in the PHC inhibited feed-induced gastrin secretion leading to less acid secretion by the stomach. Porcine and bovine hydrolysed collagen administered intragastrically to rats, prior to experimental induction of gastric ulcers, reduced the ulcer index scores by 20–55% and 12–75%, respectively, in a dose dependent manner (Castro *et al*. 2007). The mechanism for mucosal protection was the result of decreased plasma gastrin (approximately 40%), and an increased (50–267%) mucus production.

In the study described here, squamous gastric ulcer scores rapidly returned to values observed prior to omeprazole treatment and the intermittent feed-deprivation model (Days 42–49) maintained those ulcer scores. Once omeprazole treatment is discontinued, squamous gastric ulcers quickly recurred in horses, as has been previously reported (Andrews *et al*. 1999b), and this effect was not attenuated by PHC treatment, although a reduced squamous ulcer number score was observed on Day 56. During the feed-deprivation period, mean NGN and NGS did not show a treatment effect in the horses, which is in contrast to the results of a previous study where a supplement containing hydrolysed collagen led to lower NGN ulcer scores in the horses 14 days after omeprazole treatment and during the feed-deprivation period (Andrews *et al*. 2015). The different results between the studies might have been due to the synergistic effects of other components in that supplement.

Both PCV and TS significantly decreased 2 weeks after discontinuation of the omeprazole treatment (Day 42), but there was not a difference between treatment groups. The exact reason for this decrease in PCV and TS was unknown and likely has no clinical importance, since PCV and TS remained within normal reference range during the study period. This is in contrast to a previous study that showed, horses with gastric ulceration had significantly lower RBC counts and haemoglobin concentrations than those without ulceration (McClure *et al*. 1999).

Conclusion

Porcine hydrolysed collagen (45 g, mixed with feed twice daily) was readily eaten by all horses and did not result in adverse reactions in horses in this study. The PHC used in this study was associated with fewer nonglandular ulcers after 56 days of treatment in stall-confined horses undergoing omeprazole treatment and feed-deprivation. In addition, when PHC was concurrently administered with omeprazole, gastric juice pH significantly increased, however there was no added treatment benefit on ulcer healing. The improvement of nonglandular ulcer scores appeared to be a local mucosal effect, since serum bioactive amino acids and peptides from the PHC did not significantly increase in serum during the study. However, there was a mild effect on inhibiting serum gastrin concentrations after feeding, which suggests some systemic effects.

Thus, supplementation with PHC might be a safe supplement to aid in protection of the nonglandular stomach. It should be noted that PHC is not a pharmaceutical agent and is not meant to be substituted for pharmacologic agents in the treatment of EGUS in horses.

Authors' declaration of interests

Brian Lamp and Jos Olijve were employed by Sonac-Darling Ingredients International, which funded the study.

Ethical animal research

All procedures used on the horses were approved by the Louisiana State University Institution Animal Care and Use Committee and all horses were from the Louisiana State University equine herd.

Source of funding

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Authorship

P. Camacho-Luna contributed to study execution, data analysis and interpretation and preparation of the manuscript. F.M. Andrews contributed to study design, study execution, data analysis and interpretation and preparation of the manuscript. F. Garza contributed to study execution, data analysis and interpretation and preparation of the manuscript. M.L. Keowen contributed to study execution and preparation of the manuscript. Chin-Chi Liu contributed to data analysis, interpretation and preparation of the manuscript. Bryan Lamp contributed to study design, study execution, data analysis and interpretation and preparation of the manuscript Jos Olijve contributed to study design and interpretation and preparation of the manuscript. All authors gave their final approval of the manuscript.

Manufacturers' addresses

[1]Sonac, Son, The Netherlands.
[2]Gray Summit, Missouri, USA.
[3]Boehringer Ingelheim Animal Health, Duluth, Georgia, USA.
[4]Karl Storz, Inc., Goleta, California, USA.
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[8]Immulite/Immulite 1000, Seimens, Tarrytown, New York, USA.
[9]Thermo Fisher Scientific, Waltham, Massachusetts, USA.
[10]SAS Institute Inc., Cary, North Carolina, USA.

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Original Article

Angular orientations derived from a portable media device to assess postural stability during quiet standing in the horse

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Keywords: horse; postural sway; inertial sensor; angular orientation; wearable device

Summary

Background
Body-mounted sensors are becoming increasingly popular to measure horse movement with the majority of investigations examining linear movement. Many of these devices can also determine angular orientations, and measuring angular movements may increase the ability to differentiate between conditions.

Objective
To determine whether angular orientations using the sensors within a portable media device (PMD) could be used to differentiate stance conditions in standing horses.

Study design
Cross-over.

Methods
A PMD was mounted over the withers (thoracic vertebra 8–10) using a surcingle. Seven healthy horses were examined during quiet standing, and five trials were collected in a square stance, base-narrow stance and at 5 and 10 min following sedation with xylazine hydrochloride (i.v.). Angular orientations around the mediolateral (ML), craniocaudal (CC) and vertical axes, and range of motion and standard deviations (SD) were extracted. Mixed-model ANOVAs, with stance condition as a fixed effect and horse as a random effect, were performed with significance at $P<0.05$.

Results
There was no significant difference in any angular orientation variable when comparing the base-narrow to square stance condition. At 5 minutes following sedation with xylazine hydrochloride, there were significant differences in the range of motion and SDs for all three angular orientations (pitch, roll and yaw) compared with square stance ($P<0.005$). At 10 minutes following sedation with xylazine hydrochloride, there were significant differences in the range of motion and SDs, respectively, for rotation around the ML (pitch) (0.91°, 0.21°) and CC (roll) (1.07°, 0.25°) axes compared with square stance (pitch: 0.71°, 0.16°; roll: 0.73°, 0.18°; $P<0.036$).

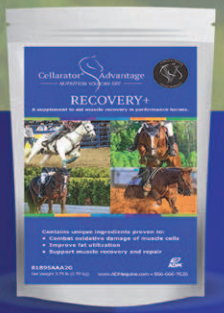
Main limitations
No gold standard was used to determine the accuracy of the angular orientations.

Conclusions
Use of angular orientations derived from sensors within the PMD may be another avenue for evaluating horses' postural sway during stance.

Clinical relevance

- The inertial sensors contained in the portable media device could measure angular orientations while mounted on the horse that were significantly greater compared with those on a flat surface.
- Angular orientations derived from inertial sensors within a body-mounted portable media device could differentiate stance conditions in horses during quiet standing.
- The orientation changes in pitch and roll were more sensitive than yaw in differentiating control versus sedation conditions.

Demands of exercise are a stressor for the performance horse. Reactive oxygen species (ROS) such as free radicals are produced in the muscles during exercise. Heavy work can overwhelm the body's natural ability to deal with ROS resulting in oxidative stress which can damage muscle proteins, lipids, and DNA, release pro-inflammatory cytokines leading to muscle pain, and damage the mitochondrial membrane thus decreasing energy production.



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Review Article

Exertional heat illness in Thoroughbred racehorses – Pathophysiology, case definition and treatment rationale

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Keywords: horse, case definition EHI racehorses; cooling interventions EHI racehorses; exertional heat illness; pathophysiology EHI racehorses; pharmacological interventions rationale; Thoroughbred racehorse

Summary

The combination of extreme heat, humidity and strenuous exercise is potentially challenging to the thermoregulatory system of a Thoroughbred racehorse, and under certain circumstances can cause a condition called exertional heat illness (EHI). It is difficult to predict its occurrence in an individual animal due to the combination on race day of extrinsic and intrinsic risk factors. Education regarding EHI is essential to its prevention, and lack of understanding amongst those responsible for the horse's care may in fact be the most important risk factor. The pathophysiology of EHI is similar across elite athletic species and is directly related to an elevation in core body temperature that exceeds the 'critical thermal maximum', causing widespread destruction of cells. It is not only the high core temperature but also its duration that will dictate outcome. Treatment strategies should decrease the hyperthermia to near normal levels within 30 min of onset, which will minimise adverse consequences. This review article outlines the pathophysiological changes as EHI progresses, and the case definition at each emergent level. Pharmacological treatment strategies and their rationale are presented. Aggressive cooling, however, is the key treatment and requires a technique which is both practical and effective in rapidly reducing core body temperature. The treatment paradigm is early detection, rapid assessment enabling prioritisation of individuals and aggressive cooling. Each stage is critically discussed.

Introduction

Described previously as 'sunstroke', EHI is probably the oldest known medical condition in human subjects (Osler 1892). In recent times, it has been documented worldwide, across species, wherever strenuous exercise is performed in hot or warm and humid environments (Adams and Jardine 2020). EHI has been under-reported and almost neglected in the equine veterinary literature, although an associated illness, 'the exhausted horse syndrome', has been thoroughly described in endurance horses (Nagy *et al*. 2012; Munoz *et al*. 2017). MacDonald and colleagues (2008) described the condition as 'post-race distress syndrome', and this was followed by a review from Brownlow and colleagues (2016), who used the term 'exertional heat illness' after the book of the same name edited by Armstrong (2003), who described the condition in detail in human athletes. Most recently, the incidence of and risk factors for EHI have been reported from Japan (Nomura *et al*. 2019).

There are essential similarities between the human athlete, the racing greyhound and the Thoroughbred racehorse, all of which compete at high levels of physical exertion in hot and humid weather conditions. They tend to exhibit a hyperthermic response to exercise, which in certain circumstances overwhelms the thermoregulatory system, leading to heat-related brain injury and multi-organ dysfunction if not treated appropriately. Some individuals, however, can tolerate substantial hyperthermia without adverse effects. For example, temperatures of more than 42°C have been documented in sled dogs (Phillips *et al*. 1981) and horses (Marlin and Nankervis 2002; McDonough *et al*. 2002), and temperature elevations as high as 41.9°C have been noted in individual soccer players and marathon runners (Maron *et al*. 1977). Nevertheless, a temperature of 40.5°C has been identified as the critical level for cell damage in human subjects (Bynum *et al*. 1978; Armstrong *et al*. 2007), whilst that for horses and dogs has not been definitively established. The clinical manifestations of EHI in all species are those of a neurological disorder and early in the onset of the condition the vague, and sometimes, bizarre manifestations can present a clinical challenge to its diagnosis. The pathological processes, the clinical signs, the treatment regimes and even the prevention strategies are similar across species despite innate differences in their thermoregulatory processes.

This article deals in detail with EHI in the Thoroughbred racehorse, specifically the earliest recognisable signs and the scientific rationale for treatment strategies, with the welfare of both human subjects and horse as absolute priorities. A predisposition to EHI, due to the high levels of metabolic heat produced during racing, makes the Thoroughbred racehorse particularly at risk (Hodgson *et al*. 1993). Lack of education amongst veterinarians, horse trainers and riders is one of the most important determinants of EHI outcomes because misdiagnosis and lack of appropriate treatment can lead to increased levels of morbidity and even mortality.

Pathophysiology of EHI

The three components of the pathophysiology associated with EHI are outlined in **Fig 1**. They tend to act simultaneously and together with the progressive neurological dysfunction, there is also systemic inflammation (SIRS) and disseminated

intravascular coagulation (DIC), the latter being more insidious. Each will be discussed in greater detail.

Direct effect of heat on cells

The direct effects of hyperthermia are concentrated on the denaturation of protein, causing extensive irreversible damage (Lepock 2003). Above the critical thermal maximum, there is generalised cytotoxicity with widespread destruction of membrane components, leading to a decrease in cell viability and ultimately cell death. Injury to the vascular endothelium initiates coagulopathies which readily progress to disseminated intravascular coagulation (DIC). Widespread organ damage follows, with microthrombi deposition in the heart, kidney, lungs and liver. Most importantly, the degree of damage is dependent not only on the absolute temperature peak but also its duration (Goldstein *et al*. 2003).

Relative intestinal ischaemia

In all species, the combination of heat, humidity and exercise causes a substantial reduction in splanchnic blood flow, which can leave the gut in a state of relative ischaemia (Stewart *et al*. 2017). The result has been referred to as 'leaky gut', because the breakdown of tight junctions between cells allows the passage of toxins and bacteria into the bloodstream (Oikawa *et al*. 2007). This can lead to increased concentrations of endotoxin and reactive oxygen species in the circulation, which initiate the production of

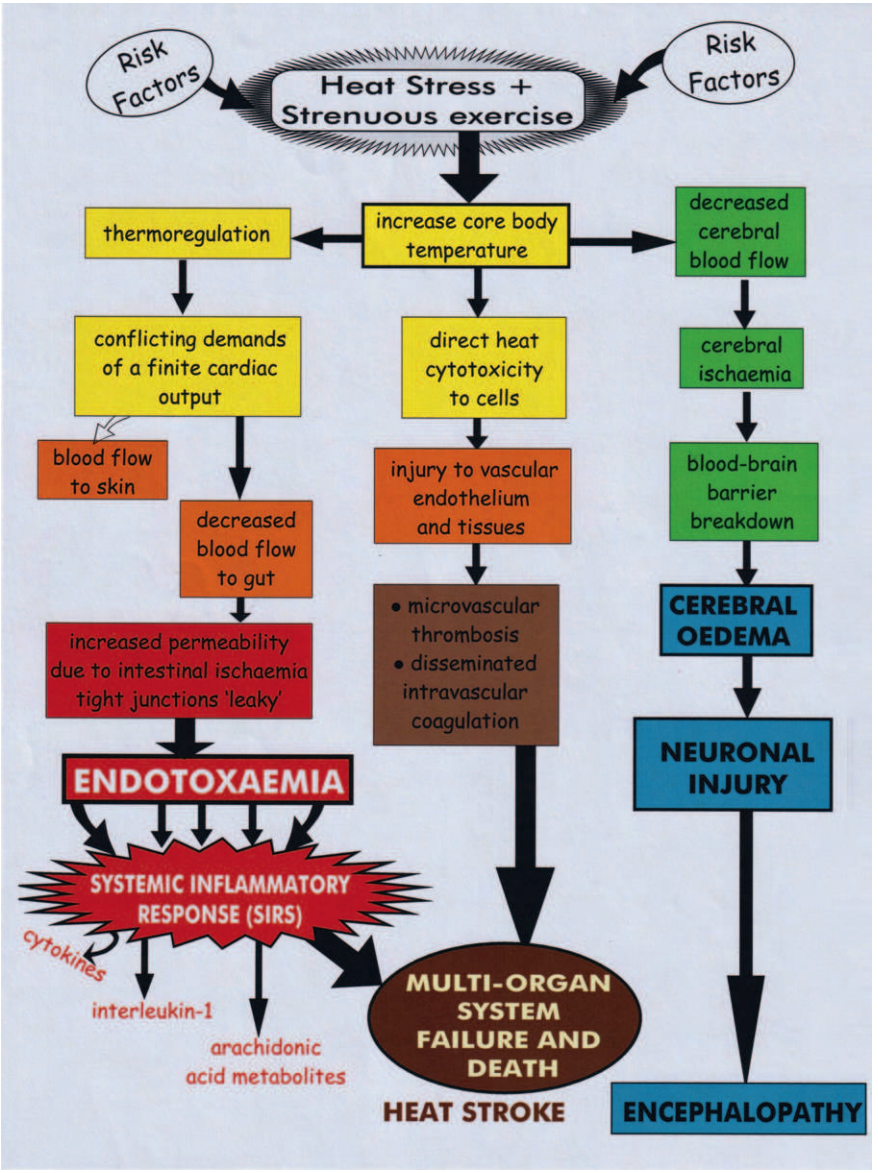

***Fig 1:** Summary of integrated pathway that culminates in multi-organ system failure. Thermoregulation initiates increases in cutaneous blood flow and concomitant decreases to splanchnic perfusion to facilitate heat loss to the environment. Gut ischaemia causes increased permeability and leakage of endotoxin into the systemic circulation and initiates the systemic inflammatory response syndrome of the host. The action of cytokines and other mediators of SIRS are thought to mediate many of the adverse consequences of the heat stroke syndrome that lead to multi-organ system failure and death.*

proinflammatory cytokines and other immune responses, ultimately leading to the systemic inflammatory response syndrome (SIRS). The latter contributes to multiple organ failure, a condition similar to that which occurs in acute sepsis (Lambert *et al*. 2002).

Central nervous system dysfunction

Rodent and dog heat stroke models have clarified the CNS pathophysiology (**Fig 2**) associated with hyperthermia (Sharma and Hoopes 2003). Firstly, there is a reduction in cerebral blood flow, which leads to cerebral ischaemia and in human subjects is probably responsible for the earliest clinical signs: these might include confusion, restlessness, irritability, combativeness and headache. If the hyperthermia continues unabated, there is a serotonin-mediated increase in permeability and breakdown of the blood–brain barrier (BBB), with leakage of plasma proteins from cerebral capillaries, leading to the initiation of cerebral oedema (Sharma 2006a). This causes escalating levels of CNS dysfunction and, if treatment is delayed, cerebral oedema may progress with associated neuronal injury. The cerebellum in human subjects is most susceptible, and the Purkinje cells show a marked and irreversible degeneration. Clinical effects at this stage are leading towards 'heat stroke' and involve stupor and delirium, with the patient unaware of their surroundings. End-stage 'heat stroke' is characterised by a swollen oedematous brain in a closed cranial compartment, causing compression and cellular damage to vital centres with eventual collapse, loss of consciousness, coma and ultimately death (Sharma 2005, 2006b). It has been documented by a number of researchers (Sharma and Dey 1984; Kao and Lin 1996; Sharma and Hoopes 2003) that elevated levels of CNS serotonin are a key driver in the heat stress-associated changes to the permeability of the BBB, leading directly to progressive cerebral oedema and neuronal injury.

The heat illness continuum in Thoroughbred racehorses

In the Thoroughbred racehorse, EHI presents along a severity scale from Level 1 to 4 (**Figs 3–6**), each corresponding to a particular manifestation of CNS dysfunction, as outlined in **Table 1** (adapted from Brownlow *et al*. 2016). Misdiagnosis is common, and the fact that EHI can emerge at any level, perhaps due to differences in the rapidity of escalation or duration of the hyperthermic response, also adds to the confusion in diagnosis. Common to all levels is a 'hot' skin, described as 'burning hot to the touch' (**Fig 7**), with skin surface temperature (SST) >39°C, as measured by infrared thermometer (Digitech Dual-IR)[1] (IRT – **Fig 8**) (Brownlow and Smith 2021). Horses will also have increased levels of distress, manifested by elevated heart and respiratory rates, and some individuals may adopt a 'panting' type of respiration. These signs are collectively referred to as thermal strain. All horses will be sweating profusely, and in some cases, if the weather is very humid, sweat will be dripping off the animal's body onto the ground. Assigning levels to clinical manifestations of EHI allows a more rational approach to veterinary treatment and enhances education and communication.

Level 1

Early manifestations of CNS dysfunction can be described as vague signs of irritability, restlessness and agitation, atypical for that individual animal. There may be head nodding or head shaking, and the horse cannot or will not stand still and is difficult to restrain (**Fig 3**).

Level 2

Neurological dysfunction has progressed, and often, there is a 'kicking-out' pattern of behaviour, which is not in response to any particular stimulus. This is often misdiagnosed by handlers and inexperienced veterinarians as a colic episode or 'fly worry'. The kicking out can be violent and either continuous or spasmodic, with the associated irritability and agitation tending to escalate (**Fig 4**).

Level 3

Horses at this level can display various bizarre neurological behaviours, and misdiagnosis is most common. Horses now have a more depressed demeanour, often described as having a 'spaced out' or 'glassy-eyed', 'vacant' expression, which is characteristic of an altered mentation. They are disoriented, may lean to one side, may have a head tilt and are often described as being 'wobbly' by handlers. There can be varying levels of ataxia, and horses might walk forward then suddenly stop, rear and throw themselves down, run into objects or people, or through fences. At this stage, they represent a substantial risk to themselves and their handlers. Some horses adopt a strange hindlimb gait, hopping on one hind leg whilst carrying the other. This is referred to as the 'broken leg' syndrome, common at this level and a major cause of misdiagnosis (**Fig 5**).

Level 4

Horses at this stage present with substantial levels of neurological dysfunction and represent a medical emergency. They are very ataxic and tend to be disoriented and unaware of their surroundings. They fall down continuously, may collide with objects and then stagger to their feet with plunging behaviours. Such horses are at high risk of injuring themselves and their handlers. Injuries are common, with fractures to the poll, withers and head. In extreme circumstances, collapse may follow with loss of consciousness and death (**Fig 6a,b**).

Rationale for treatment strategies

Detomidine hydrochloride (Dormosedan[2]) – a synthetic alpha-2 adrenoreceptor agonist with dose-related sedative and analgesic effect in nonheat-affected horses

Out of sheer necessity this author has used a variety of medications in an attempt to control horses with EHI when their behaviour has posed an extreme risk. On one such occasion, detomidine hydrochloride (Dormosedan[2] 10 mg/mL - dose rate of 1.0 mL per 550 kg bodyweight intravenously) was administered, but instead of the expected sedation there was a complete reversal of the neurological manifestations within 2–3 min, with the animal exhibiting essentially normal, calm behaviour with no signs of a sedative response (**Table 2**). Since that time, detomidine has been used routinely for EHI cases and has provided consistent and reliable outcomes, becoming the mainstay of treatment at racing jurisdictions in New South Wales (NSW).

In the medical arena, some researchers have considered the cerebrovascular dysfunction itself an attractive target for

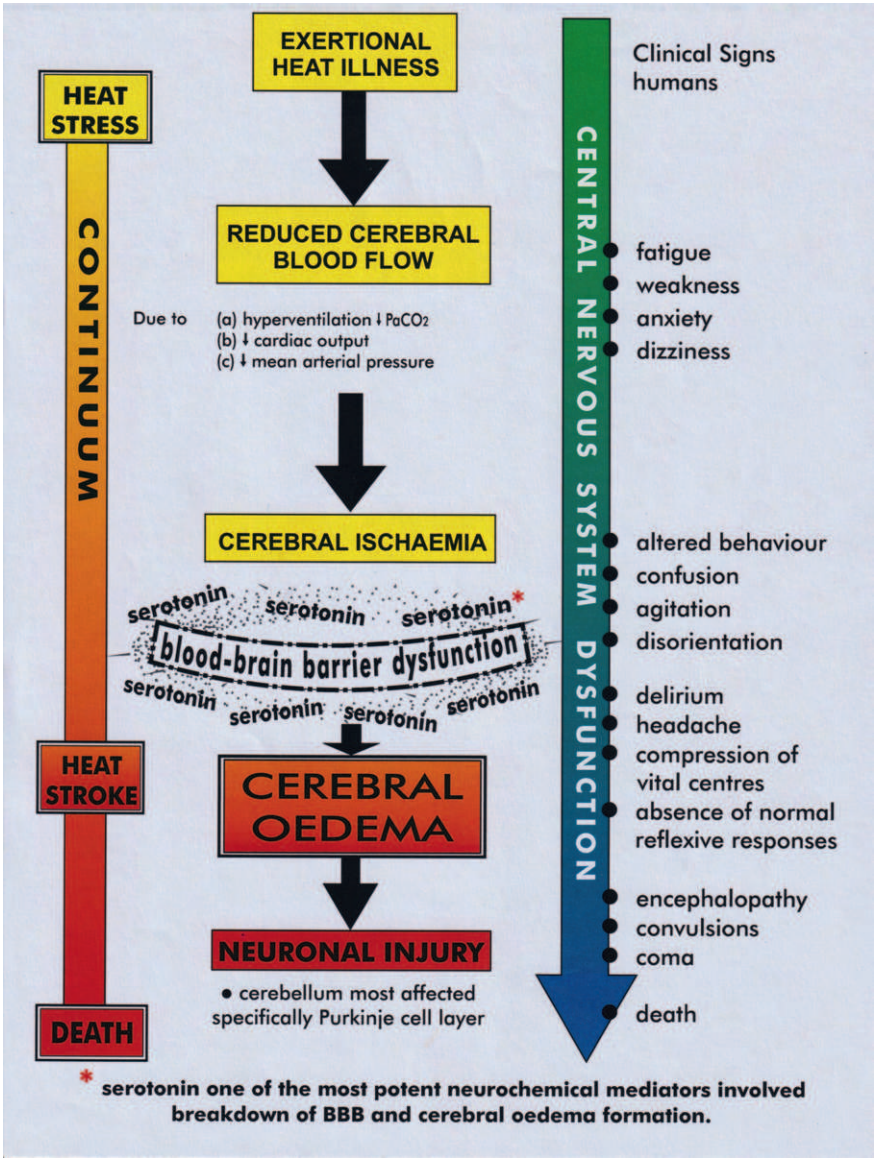

Fig 2: CNS heat stroke pathophysiology from the rat and dog heat stroke models, after Sharma and Hoopes (2003); Sharma and Dey (1984); Sharma (2005); Sharma (2006a); Sharma (2006b).

therapy in 'heat stroke' (Chen *et al*. 2006) and it is argued here that the use of detomidine in heat-affected horses may fulfil that role. In an extensive body of work, Sharma (2005, 2006a, 2006b) has documented that in animal heat stroke models, hyperthermia was instrumental in initiating BBB dysfunction, leading to vasogenic cerebral oedema formation, and that the neurochemical mediators dopamine and serotonin, amongst others, were well known to be involved in its pathogenesis. To support the rationale for using detomidine, an in-depth pharmacological study by MacDonald and colleagues (1988) is cited. In this study, medetomidine (a closely related methylated derivative of detomidine) was found to depress the turnover of biogenic amines in the brain, such as noradrenaline and dopamine, but most importantly, it also significantly decreased serotonin levels. Serotonin is implicated as the key driver in the cerebral pathophysiology associated with EHI (Sharma and Dey 1984; Kao and Lin 1996; Sharma and Hoopes 2003). Chen and colleagues (2006) further reported that drugs which were serotoninergic nerve depletors or receptor antagonists were able to protect against heat stroke reactions. This may explain the excellent results in heat-affected horses observed after using a single dose of detomidine which is used as a 'first-call' medication for Level 1 cases if horses are extremely irritable, to enable cooling without difficulty. From Level 2 up, detomidine is considered almost mandatory to protect the horse, and most importantly, to protect the handlers from harm. It must be emphasised that horses administered detomidine for EHI show no apparent sedation but revert to calm, normal behaviour, and there have been no deleterious

Fig 3: Level 1: EHI – The irritability and restlessness of a horse that is difficult to restrain can scarcely be captured in a single photograph. This horse will not or cannot stand still.

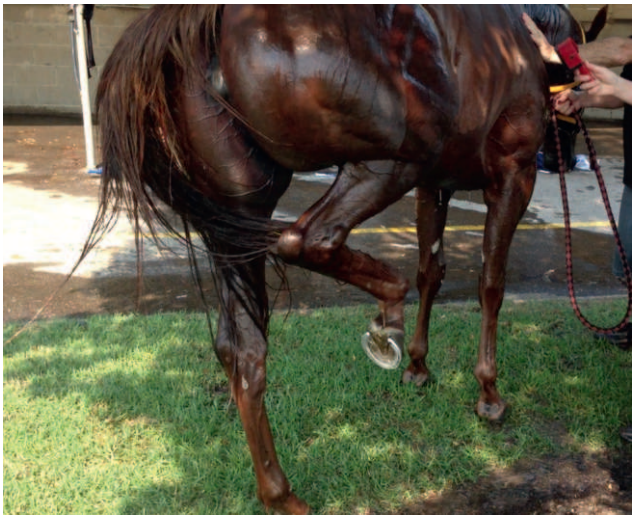

Fig 4: At level 2, horses display 'kicking out' behaviours. These may be violent and continuous or spasmodic. Irritability and restlessness are escalating.

side effects. An empirical study on the efficacy of detomidine and its possible role in inhibition of serotoninergic activity is unlikely because research ethics would preclude the subjection of horses to a hyperthermic environment in which EHI could be reliably induced. Progress therefore depends upon case material such as this, but research in this area needs to be undertaken.

Flunixin meglumine (Flunixin[3]) nonsteroidal anti-inflammatory drug

Part of the pathophysiology of EHI is relative gastrointestinal ischaemia, resulting in leakage of endotoxin into the systemic circulation (Lambert *et al*. 2002). Most researchers agree that the SIRS originates from the gut and that endotoxin is the key driver. This has been reported across species, and circulating endotoxins have been identified in human heat stroke

Fig 5: Horses at Level 3 can display bizarre behaviours which are manifestations of central nervous system dysfunction. Misdiagnosis at this stage is most common.

patients (Bouchama *et al*. 1991), in primate models (Gathiram *et al*. 1988), in greyhound dogs (Bynum *et al*. 1979) and in race and endurance horses (Baker *et al*. 1988; Barton *et al*. 2003; Donovan *et al*. 2007).

Flunixin meglumine is the nonsteroidal anti-inflammatory drug (NSAID) that has most frequently been used for the treatment of endotoxaemia in horses (Shuster *et al*. 1997; Moore 2001). The beneficial effects are most likely due to its ability to reduce eicosanoid production and ameliorate the detrimental effects of endotoxin on cardiopulmonary parameters, oxygenation and plasma volume (Bottoms *et al*. 1981; Semrad *et al*. 1987). It has been documented, however, that flunixin has little effect on the cytokine response to endotoxin and therefore has certain limitations (Cook and Blikslager 2015).

In the case of EHI in racehorses, a single intravenous injection at a dose of 1.1 mg/kg is recommended, based upon the reported beneficial effects of flunixin on the clinical signs of endotoxaemia. An important fact to note is that once SIRS is initiated, cooling will not reverse the cascade of effects, so the argument for using flunixin early and routinely is probably justified.

Particular attention, however, must be paid to the risk of side effects from NSAIDs which potentially include delayed intestinal mucosal repair (Marshall and Bilkslager 2011) and nephrotoxicity (Whelton 1999). Horses most at risk of renal damage are those experiencing volume depletion, and although dehydration is an important risk factor for EHI, it is uncommon in racehorses. Whilst a redistributive volume depletion does occur post-exercise, it normalises very quickly (Lindinger 2014) and the risk entailed in a single dose of flunixin, if weighed against the adverse outcomes of EHI, is considered justifiable.

Glucocorticoids (dexamethasone) dual action – neuroprotection and immune suppression

Glucocorticoids, such as dexamethasone (Dexapent[4] - 5 mg per millilitre; dose rate of 0.1–0.2 mg kg bodyweight as a bolus intravenously), have historically been used to treat cases of EHI (MacDonald *et al*. 2008), and there would

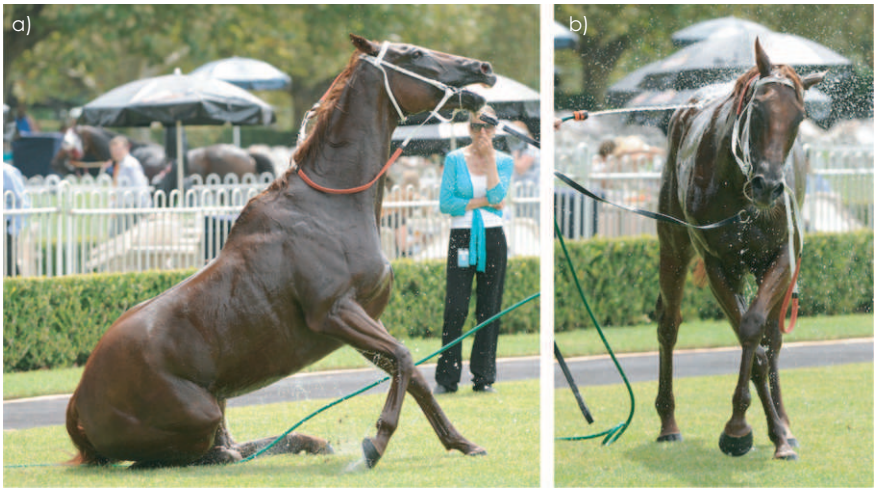

Fig 6: a) Horses at Level 4 have substantial levels of ataxia: they may fall over, stagger to their feet, crash into objects and are a risk to themselves and their handlers. b) Note the 'glazed' expression. This horse is disoriented, has a proprioception deficit and is about to fall over.

appear to be good rationale for their use. Dexamethasone has potent anti-inflammatory and immunosuppressive effects, stabilises cell membranes and decreases vascular permeability (Amsterdam *et al*. 2002). Glucocorticoids have been used for their neuroprotective action in the treatment of brain and spinal cord injuries in human subjects, experimental cerebral ischaemia in animals and cerebral injury and trauma in horses (Hall 1992; Feary *et al*. 2007).

This author has found dexamethasone to be disappointing for the treatment of EHI, especially if used in isolation, because horses do not appear to improve clinically after its administration. This may, however, be a problem with inadequate dose and the time required for corticosteroids to have an effect in modulating inflammation. Recommended dose rates for dexamethasone in horses range from 0.1 to 0.2 mg/kg intravenously, but dose rates recommended for neuroprotection in the dog and rat heatstroke models (Sharma and Hoopes 2003) are in the vicinity of 4.0 mg/kg and best results are achieved if administered prior to the hyperthermic exposure (Liu *et al*. 2000; Whitehouse 2011). This author uses dexamethasone in combination with the other drugs in an emergency situation for Level 3 and Level 4 horses but does not recommend its use as a stand-alone medication in horses which are acting dangerously.

The treatment of EHI – early detection, rapid assessment and aggressive cooling

Early detection

The early detection of the first emergent signs of EHI requires veterinarians and horse handlers to have a knowledge of Level 1 clinical signs, which can be vague, and misdiagnosis at this stage is common (**Table 1**). However, horses at risk of EHI will almost always have skin which is 'burning hot' to the touch, or alternatively, an infrared hand-held thermometer (IRT) can be used to quantify skin surface temperatures. Skin temperatures (Tsk) >39.0°C are indicative of increased risk of EHI and should be treated accordingly (Brownlow and Smith 2021). Belval and colleagues (2018) have shown that in human subjects, aggressive treatment provided at the time of earliest detection, before clinical manifestations even become apparent, can completely abort progression of the condition.

Rapid assessment

It is most important to appreciate that intrinsic risk factors for EHI vary from individual to individual, so that not all horses will display a harmful hyperthermic exercise response, whilst others appear to be able to tolerate high core temperatures without apparent harm (Mitchell *et al*. 2006). Risk assessment of horses is based upon the presence of emergent signs of EHI, and manifestations of a significant level of thermal strain. Horses can then be prioritised for cooling and/or pharmacological interventions. The author considers it best to be pro-active in assessment and interventions, the absolute priority being the welfare of the horse.

Aggressive cooling

When cooling is rapidly initiated and the core temperature returns to a normal range within 30 min, damage to cells is minimal (Casa *et al*. 2012) (**Fig 9**). There is much information concerning cooling methods for horses (Williamson *et al*. 1995; Marlin *et al*. 1996, 1998; Kohn *et al*. 1999; Jeffcott *et al*. 2009; Takahashi *et al*. 2020). **Table 3** summarises the strategies for cooling in the Thoroughbred racehorse, together with the aim and anticipated outcome. Each cooling technique generates a theoretical cooling rate; for instance, the most rapid rates (0.15–0.24°C per minute) are observed with cold water (Proulx *et al*. 2002). As the water becomes warmer, the cooling rate decreases accordingly, but nevertheless can be quite acceptable under certain conditions.

The need for an aggressive approach to cooling will be determined by whether a horse is exhibiting signs of EHI, at which level, and the severity of the weather conditions on the particular race day. The actual technique of cooling can be based on individual preferences. This author prefers cold water (6.0–12.0°C) and a systematic approach to the horse's body, which targets the major vessels and covers as much of the body surface as possible with an adequate volume of

TABLE 1: Case definition: Exertional heat illness in Thoroughbred racehorses

Level	Clinical manifestations	Treatment
1	Head nodding Irritability Restlessness Difficult to restrain Can't or won't stand still Normal mentation	Targeted aggressive cooling intervention with cold water (TACI) Cooling collar *Most horses will respond to cooling but if very irritable use detomidine as 'first-call' drug*
2	Irritability Restlessness 'Kicking-out' behaviours Normal mentation Misdiagnosis common at this stage	Targeted aggressive cooling intervention – Cooling collar *Detomidine* *Flunixin meglumine* *Most horses will require medication*
3	Any bizarre neurological behaviour will fit in this category Altered mentation, towards depression – 'glassy-eyed', 'vacant', 'spaced-out' expression Horses may react to simple stimulus by rearing over backwards or throwing themselves down Varying levels of ataxia – 'wobbly' Broken leg syndrome Misdiagnosis common at this stage	Targeted aggressive cooling Cooling collar *Detomidine* *Flunixin meglumine* *Medication is mandatory for horse and handler safety – welfare must be the absolute priority*
4	Horses have substantial signs of CNS dysfunction Altered mentation – totally disoriented and unaware of surroundings Very ataxic – fall down continuously then stagger to their feet, collide with objects, people. Represent a significant risk of injury to themselves and their handlers.	Targeted aggressive cooling Cooling collar *Detomidine - may have to repeat dose as required* *Flunixin meglumine* *Dexamethasone* *This is a medical emergency –medication is mandatory – welfare must be the absolute priority*

The various levels of EHI that a Thoroughbred racehorse may display after strenuous exercise in hot or warm and humid conditions. Note that the illness may emerge at any level and escalate if intervention strategies are not initiated quickly enough. Dose rates for drugs are provided in the text. This table is the result of 10 years of observations of horses displaying signs of EHI. First published by Brownlow *et al*. (2016) there have been some minor amendments.
All horses are distressed and have elevated heart and respiratory rates. All horses have skins which are described as 'burning hot' to the touch.

water. Monitoring is essential, and the clinician is looking for

Fig 7: Horse handlers need to be educated to monitor their horse's SST by touch. If the skin feels 'burning hot' to the touch, they need to seek veterinary assistance or immediately instigate cooling interventions.

Fig 8: Alternatively, the use of the hand-held infrared thermometer enables a quantification of the SST, which can be used as a risk assessment tool and also as a monitoring tool for the efficiency of cooling interventions.

improvement in the clinical signs of thermal strain and a decrease in skin temperature from >39.0° to around 30.0°C within 15–20 min. The time frame for cooling should not be rigid but based upon the response of the individual animal, and the cutaneous vessels should always remain visible. Once a skin temperature of 30.0°C is reached, the horse can

TABLE 2: Case series of EHI: Use of detomidine hydrochloride * in Thoroughbred racehorses from a weather field study – dose rates and proposed rationale for treatment (Brownlow 2020)

Level of EHI	Number of cases	Dose given	Rationale for treatment
1. Agitation + restlessness	437	0.5 mL	Use as *first line treatment* to decrease agitation and restlessness, enabling cooling to take place without incident.
2. Agitation + restlessness, kicking out	19	1.0 mL	As above. To decrease risk of injury to horse and handler, which appears significant.
3. Increased levels of CNS dysfunction	1	1.0 mL	As above. To decrease and control risk of injury to horse and handler, which is escalating.
4. Substantial levels CNS dysfunction	1	1.0–1.5 mLs to effect	Horse extremely difficult to cool – animal is unaware of surroundings, throwing itself down. Substantial risk to horse and handler. Emergency situation – drug required as soon as possible to control the situation

Detomidine Hydrochloride *(Dormosedan: Zoetis)* (10 mg/mL); recommended dose rates starting at 0.5 mLs (10 ug/kg); 1.0 mLs (20 ug/kg) to effect.

TABLE 3: The choice of cooling strategies will depend on the intensity and duration of the exercise activity, the likelihood of significant hyperthermia (>42.0°C) and signs of EHI, and the presence of adverse weather conditions. The relative priority of each strategy, the overall aim and the desired outcome are presented

Cooling strategy	Relative priority	Aim of strategy	Outcome of strategy when applied
Knowledge (1)	1	To provide education to all stakeholders about EHI and cooling interventions.	Best practice methods used. Welfare of horses ensured.
Early detection of hyperthermia (2)	2	To prioritise horses for cooling because not all horses will need cooling. Assessment by touch or IRT.	Early intervention enabled by early detection; progression of EHI halted.
Cool within the 'golden half hour' (3)	3	To minimise cell damage. *Critical Thermal Maximum* for cell damage depends on both the temperature elevation and its duration.	Core body temperature is reduced below 40.0°C within 30 min. Minimal cell damage.
Water is used as the 'cooling modality' (4)	4	Efficient transfer of body heat. Water has a thermal conductivity 24 times that of air.	Cooling interventions using water deliver fastest cooling rates.
Temperature of the water: tepid water is acceptable but cold water enables faster cooling (5)	5	To transfer heat from skin to water most efficiently. This requires a wide temperature difference: cold water provides the ideal cooling rate.	Time frame of less than 30 min easily achieved with cold water. EHI is halted with minimal cell damage.
Use of dry fans strategically located to increase air movement over horse's entire body (6)	6	To promote heat loss using convective/evaporative heat loss mechanisms. The effects of air flow are related to air speed over the body surface and the temperature of the air flow.	Improved effective cooling with wind speed > 2.5 m/sec. (Hot air fanned over an animal may cause heat gain.)
Use of misting fans (7) + (8)	7	To assist cooling by using a fine mist propelled by air or simple fine water spray	Useful before racing but may be counter-productive after racing.
Use of a cooling collar (9)	8	To assist cooling by reducing temperature in the carotid artery and thence the brain	Not evidence-based as yet but appears to clinically help horses cope with thermal strain.
Scraping (10) and (11)	9	Supposedly removes heated water lying on the skin surface which would otherwise insulate the skin and impede cooling.	Two scientific articles in peer-reviewed journals report that scraping has no effect on cooling.

Major Reference Pertaining to each Priority for **Table 2**:- (1) Pryor et al. (2020); (2) Brownlow and Smith (2021); (3) Casa *et al.* (2012); (4) Proulx *et al*. (2002); (5) McDermott et al. (2009); (6) Casa et al. (2007); (7) Sefton *et al*. (2016) (8) McEntire *et al.* (2013) (9) Brownlow (2018); (10) Marlin et al. (1998) (11) Takahashi *et al*. (2020).

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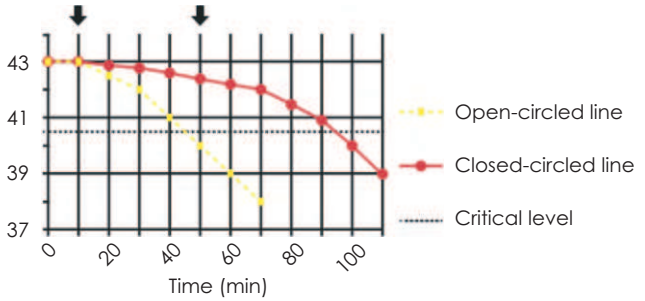

Fig 9: The vertical axis shows the individual's temperature in degrees centigrade, and the horizontal axis represents the elapsed time up to 100 min. The dotted line represents the critical thermal maximum, defined as the temperature above which widespread cell damage occurs. In the human subject, this is close to 40.5°C. The aim of cooling is to decrease the core temperature below that level in the shortest possible time. The yellow line and the attendant arrow at 43.0°C represent an early intervention within 10 min of the appearance of clinical signs. Note that the early intervention reduces the temperature below the critical thermal maximum at approximately 30 min post-intervention. Comparatively, a late intervention (red line and arrow) allows body temperature to remain above the critical thermal maximum for an extended period of time (90 min), in which case there is probably irreversible widespread cell damage (with permission: Armstrong et al.2007).

Fig 10: Horse handlers using mobile iced water spray unit. The unit is located at the tie-up stalls because this is where horses recover after racing and where most cases of EHI actually occur. Horse handlers are encouraged to use these devices on their own volition, but there is always an experienced veterinarian present to supervise the activity and check a horse's status when needed. An educated workforce is probably the most important welfare initiative.

be walked and the addition of a cooling collar seems to provide relief to heat-affected horses. On racetracks, a dedicated cooling station is necessary and best results are achieved when a 'tie-up' stall model is used. Horses are accustomed to being semi-confined, and the cooling units can be separated from the horse by a single rail, so that if the horse becomes ataxic or dangerous, people and horse are easily and safely separated. Fans can be mounted at the back of the stall to provide air flow, increasing evaporative and convective cooling.

The authors have used a purpose-built mobile spray unit (**Fig 10**) based on a 180-L plastic water container with a pump capable of providing 18 L per minute, feeding into a large diameter hose. A 17.5 V battery allows 5–6 h of continuous use. The water is cooled using crushed ice, and the introduction of this technique has been effective in the cooling of horses after racing. Although horses are most likely to experience EHI in the vicinity of the 'tie-up' stalls, the mobility of the unit is essential to cater for the very rare occasion when a horse may experience EHI symptoms on the racetrack or in the winners' enclosure. This device was provided by the major racing body in NSW to the four major metropolitan tracks, 4 provincial tracks and 6 country tracks in that racing jurisdiction.

Problems with the cooling process

A horse may have been cooled effectively, but later when it is allowed to walk or resume its position in the tie-up stalls, a rebound hyperthermia may occur. For this reason, horses must be kept close to veterinary supervision and monitored carefully in case further cooling is necessary. Horses can also be overcooled, usually by over-zealous handlers who have not been taught to monitor the process by touch or IRT readings. Over-cooled horses will cramp up behind and have difficulty walking.

To scrape or not to scrape

Repeatedly scraping in a 'stop-go' fashion after every sequence of hosing heat-affected horses has become accepted tradition, based on the belief that if the remaining water is not removed from the skin surface immediately, it will tend to insulate and inhibit the cooling process; that is, core body temperature will rise. Two equine studies, Marlin *et al*. (1998) and Takahashi *et al*. (2020), have both demonstrated that lack of scraping does not cause core body temperature to rise. Nevertheless, when there are high levels of humidity and the water on the skin surface is not evaporating, scraping at the end of the cooling process may be beneficial. Interrupting the process to scrape may actually be counterproductive with very hot, irritable horses; here, aggressive cooling is an absolute priority and this is best achieved using a continuous water spray. The constant application of water is effectively equivalent to scraping because it disperses the potentially insulating boundary layer of heated water immediately adjacent to the skin surface.

The use of dry fans in the cooling process

The sweating response in horses is the most powerful tool in their thermoregulatory arsenal, and the efficiency of this process depends upon the evaporative capacity of the environment, where air flow is a major determinant. In a study in horses, Takahashi and colleagues (2020) compared five cooling techniques, one of which employed fans. The horse walked on a treadmill at 1.7 m/s, and two fans were placed 4 m ahead of the horse, providing a backward air current of 3.0 m/s, measured at the horse's head. It was reported that fanning as a stand-alone cooling technique achieved a quicker return to baseline temperature than the control, where the animal walked on the treadmill with no additional cooling. It was, however, slower than the other techniques

Fig 11: Sha Tin Racecourse in Hong Kong has an ideal cooling station for horses after racing. Shade cloth protects horses from the effects of radiant heat; long hoses provide copious quantities of water, and strategically positioned fans produce an air flow of 3.5 metres per second on all sides of the animal. Photograph courtesy of Dr Peter Curl, HKJC.

using water. This is an important finding and supports the use of dry fans as an add-on cooling strategy (**Fig 11**). The thermal conductivity of water is approximately 24 times that of air, making water the superior medium for the transfer of heat; nevertheless, air flow over the body surface will have a profound effect on evaporative cooling capacity.

The use of misting fans

Misting fans are of two types. As shown in **Fig 12**, they can provide a fine spray of moisture through an articulated array of fine nozzles, or be combined with air-driven systems at high pressure, which generate smaller droplets and are more effective in wetting surfaces.

Fig 12: This horse has not raced yet and is no doubt enjoying the cool feelings of the misting sprays. However, these sprays will be increasing the humidity in the immediate vicinity by up to 20% and in warm and humid conditions might adversely affect the post-race outcome by reducing the capacity for the evaporation of sweat.

Fig 13: Showing the collar being placed on a horse's neck immediately after racing. The long neck of the horse is well suited to the placement of a cooling collar and the superficial position of the artery and vein in the upper portion of the neck provides an easy target for cooling.

By generating air movement, misting fans assist the evaporation of heated moisture from the skin surface (Farnham *et al*. 2009, 2015) but they also add water to the air, reducing its evaporative capacity. In a study of heat mitigation methods in army trainees, Sefton and colleagues (2016) found that misting fans were ineffective in lowering

Fig 14: The cooling collar is a one-size-fits-all design and is manufactured by Markey Saddlery in Australia. Please note that the author has no pecuniary interest in this product.

body core temperature in hot and humid environments. Core temperature actually continued to rise during 20 min of misting fan cooling, and other researchers (Wong and Chong 2010; McEntire *et al*. 2013) estimated that humidity levels in the vicinity of the fans rose by an additional 10–20%.

Misting fans have been used in horse events, specifically the 2008 Beijing Olympics (Jeffcott *et al.* 2008) based on preliminary observations by Bradbury and Allen (1994). These were high-powered devices, propelling misted water at 8°C in specialised misting tents. It was stated that the combination of fans and chilled water was effective in rapidly reducing horses' rectal temperature, but no data were provided. Marlin (2020) has argued that in dry environments, the efficacy of misting fans is related to the speed of the fans, the amount of mist and the air temperature, and any cooling effect is through evaporation of the mist off the skin surface. This is distinct from hot, humid conditions, where the cooling effect will rely on the temperature of the water supplied to the fans, because evaporation will be low. Marlin makes the point that misting fans are only provided for short-term comfort and are significantly less effective than simple cold water hosing in reducing the body temperature of hot horses.

Use of a cooling collar

Cooling of the brain can only be achieved by conduction through the skull or through the brain's circulation (Chen *et al*. 2006), and animal studies have shown that the temperature of the carotid arterial blood was the major determinant of brain temperature (Baker 1982; Zhu *et al*. 2006).

To exploit this concept, a cooling collar (Chill Time Cooling Collar)[5] was designed for Thoroughbred racehorses (Brownlow 2018). Two pouches filled with crushed ice are positioned, one on either side of the upper part of the neck, over the jugular vein and carotid artery, creating a 'heat sink' effect in the contacting tissues (**Figs 13** and **14**). Although the pouches can accommodate commercially available chemical cold packs, research has shown that crushed ice has a greater cooling capacity and remains colder for longer, maximising the 'heat sink' effect (McMeekan *et al*. 1984; Phan *et al*. 2013).

Cooling collars have been used by human athletes (Tyler and Sutherland 2011) and by the military (O'Hara *et al*. 2008) in the course of sporting competition or battlefield exercises, but in the racehorse the sole purpose is to aid the recovery process. More recently, studies in human subjects have shown that cooling the neck in particular may elicit disproportionately beneficial changes in perceived thermal strain, due to it being an identified site of high alliesthesial thermosensitivity (Cotter and Taylor 2005). There is no doubt that horses appear to gain relief on hot days from the collar, perhaps for that reason, and although the collar's mechanism of action has yet to be determined, it warrants further investigation.

Conclusions

Although this article targets the Thoroughbred racehorse as being particularly at risk, all horses are susceptible to EHI and the greatest risk factor is probably lack of knowledge. It is important, therefore, that everyone who cares for competitive sporting horses should be aware of the condition and be able to recognise the very early emergent signs, because misdiagnosis leading to failure to treat or late commencement of treatment will incur a cost in terms of increased harm.

It must be appreciated, however, that not all horses will require aggressive cooling after strenuous exercise. The majority of racehorses possess a remarkable thermoregulatory capacity that enables them to cope with the levels of thermal strain associated with racing in most weather conditions. For this reason, a blanket policy of cooling all horses is probably counterproductive because of the number of horses competing on a given race day. Therefore, the ability to prioritise some horses over others will provide the best outcomes. Early detection can be achieved by experienced veterinarians; alternatively, an infrared thermometer will enable prioritisation for treatment interventions.

The technique of cooling will depend on individual preference and resources available, but should meet essential criteria: it must be practical, and the cooling modality must be capable of achieving rapid cooling rates. The use of dry fans and perhaps a cooling collar will be additive in their effects.

Finally, it is apparent that the world is experiencing more frequent periods of extreme heat.

Competing in these conditions may become the welfare issue of the future, and racing jurisdictions and event organisers must never assume that there will be zero risk of EHI. Direction for effective prevention and treatment must come from the highest level of the organisations involved in horse sport and should involve the development of awareness training, EHI recognition, education in cooling strategies and risk assessment. EHI must be considered a significant welfare issue and addressed immediately and vigorously, if the public perception of a welfare-oriented industry is to be maintained.

Authors' declarations of interests

No conflicts of interest have been declared.

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M. Brownlow wrote the article and took the photographs. J. Mizzi collaborated in discussion, debate and experience.

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Review Article

The recognition of pain and learned behaviour in horses which buck

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Summary

Bucking behaviour in horses is potentially dangerous to riders. There is limited information about how bucking behaviour should be investigated by veterinarians. The objectives of this article are to define bucking behaviour, to review the literature relating to bucking and allied behaviours in horses and describe personal observations and to describe an approach to clinical investigation and management strategies. A literature review from 2000 to 2020 was performed via search engines and additional free searches. A buck is an upward leap, usually in addition to forward propulsion, when either both hindlimbs or all four limbs are off the ground with the thoracolumbosacral region raised. Bucking often occurs as a series of such leaps and different manifestations include 'pronking', 'bronking' and 'fly bucking'. Causes include excitement, exuberance, defensive behaviour associated with fear, learned behaviour through negative reinforcement or a reaction to musculoskeletal pain. Specific causes of pain include an ill-fitting saddle or girth, thoracolumbar pain, girth region pain, sternal or rib injury, neuropathic pain, sacroiliac joint region pain, referred pain and primary hindlimb lameness. Any of these may be compounded by a rider who is fearful, poorly balanced or crooked. Determination of the underlying cause requires a comprehensive clinical assessment, including assessment of saddle fit for horse and rider and suitability of the horse–rider combination. In some horses, identification of a primary source of pain allows targeted treatment and resolution of pain, but careful retraining is crucial. An understanding of learning behaviour is required for successful rehabilitation. It was concluded that identification of the cause of bucking may enable treatment of primary pain which, when combined with retraining, results in management of bucking behaviour. However, in a minority of horses, dangerous bucking behaviour cannot be reliably resolved, requiring retirement or euthanasia of the horse.

The aims of this commissioned article are firstly to define bucking behaviour and secondly to review the published literature relating to bucking and allied behaviours in horses. There is a scarcity of documented information on the subject, and therefore, personal observations have been included. Finally, an approach to the clinical investigation and the management strategies for causes of bucking behaviour are described.

What is bucking?

Bucking is an instinctive defence mechanism against predators that attack their prey by leaping onto their backs. Bucking behaviour describes quick, sharp movements that can throw a predator off the back; therefore, in order to carry a rider safely, the domesticated horse must be desensitised to the natural instinct to buck. Within the equine industry, different manifestations of bucking behaviour are commonly recognised (**Figs 1**–**5**). A buck may be described as an upward leap, usually in addition to forward propulsion, when either both hindlimbs are off the ground with the thoracolumbosacral region raised (**Figs 1**–**4**), or all four limbs are off the ground. Bucking often occurs as a series of such leaps. The term 'pronking' (also referred to occasionally as 'crow-hopping') may be used to describe a movement in which the horse leaps upwards in the air with little or no forward propulsion, but with flexion of the thoracolumbosacral region (an arched back) and stiff limbs (**Fig 5**). 'Bronking', which is the most dangerous form of bucking for the rider, is a more violent manifestation of 'pronking', whereby the horse propels itself forwards, often at high speed, combined with upward leaps with an arched back and the neck low. Danger to the rider may be increased if the horse stops abruptly or suddenly twists and changes direction. 'Fly bucking' is a forward, upward leap, usually with the head high, the forelimbs on the ground and the thoracolumbar region extended (**Figs 1c**, **2a**, **3b**, **4b**).

Excitement or exuberance may result in bucking, which is unpredictable and inconsistent. Bucking may be a learned defensive behaviour, sometimes instigated by confusing application of cues by the rider provoking bucking, resulting in the rider falling off and the riding session being terminated. Thus, the behaviour is learned by negative reinforcement (removal of an aversive stimulus after a behaviour has occurred). In rodeo horses tightening the bucking cinch around the most caudal aspect of the abdomen provokes bucking behaviour. When the cinch is released, the bucking ceases almost immediately. Some rodeo horses are selected and bred because of their propensity to buck. In other sports and leisure horses bucking is an acquired, undesirable behavioural problem, often as a consequence to pain or of unknown cause, which may result in the horse becoming unsafe to ride. Pain may provoke bucking behaviour and dislodgement of the rider may relieve pressure and pain; thus, bucking may again become a learned behaviour. Such learned behaviour may persist, despite resolution of the underlying pain.

In the development of the Ridden Horse Pain Ethogram (RHpE) (Dyson *et al*. 2018a), the original ethogram comprised 117 behaviours. Bucking was defined as either bucking alone or bucking and kicking out. In addition, bucking was defined with respect to head position (the nose above the withers;

Fig 1: Three bucks from a horse with sacroiliac joint region pain. a) The thoracolumbosacral region is extended, both hindlimbs are extended caudally with the hind feet above the level of the ventral aspect of the thorax. The tail is elevated. The horse's ears are forwards, and there is a relatively normal orientation of the head relative to the neck. b) The thoracolumbosacral region is extended, and both hindlimbs are extended caudally with the hind feet below the level of the ventral aspect of the thorax. The tail is elevated. The horse's ears are back, the lips are separated, the eye has an intense stare, and the head is elevated with extension of the neck. c) The thoracolumbosacral region is extended, both hindlimbs are extended caudally with the hind feet above the level of the ventral aspect of the thorax, and the horse is kicking backwards with the right hindlimb. The tail is elevated. The horse's ears are back, the lips are separated, the eye has an intense stare, the sclera is exposed, and the head is extremely elevated with extension of the neck.

Fig 2: Two bucks from a horse with sacroiliac joint region pain. a) The thoracolumbosacral region is extended, and both hindlimbs are extended caudally with the hind feet above the level of the ventral aspect of the thorax. The tail head is elevated. The horse's ears are back, and the eye has an intense stare. b) The thoracolumbosacral region is extended, the trunk and neck are twisted, and the horse is kicking back and sideways with the right hindlimb. The head is low and turned to the right. The tail is upright.

the nose below the withers but above the ventral aspect of the thorax; the nose below the ventral aspect of the thorax), the position of the hind feet relative to the pelvis (the hind feet below the ventral aspect of the thorax; the hind feet above the ventral aspect of the thorax) and trunk position (straight or twisted ± spinning). These different positions reflect that a horse may buck with either flexion or extension of the thoracolumbosacral region.

Review of the literature

A literature review from 2000 to 2020 was performed, searching via the following keywords: cold-backed behaviour; bucking; girthiness; girth aversion behaviour; sternal pain; rib injury; gastric ulceration; thoracolumbar/back pain; lumbosacral pain; sacroiliac joint region pain, saddle, girth and ridden horse behavioural problems. Google Scholar, PubMed and Scopus search engines were used. Two hundred and forty-six papers were identified, of which 132 had potentially relevant information; only 23 had specific evidence-based observations. The reference lists of key papers were also scrutinised, and a free search performed.

There are many references in the human literature related to injuries sustained by horse riding accidents which are often associated with falls (e.g. Danielsson and Westlin 1973; Bixby-Hammett 1985; Loder 2008; Hawson *et al*. 2010; Hasler *et al*. 2011), some of which specifically cite bucking as a possible causal mechanism for the rider's fall. Rider injuries caused by bucking that do not result in a fall may involve collision with the rigid frame of the saddle, resulting in, for example, separation of the pubic symphysis and male sexual dysfunction (Mulhall *et al*. 2002; Collinge *et al*. 2009; Brouwers

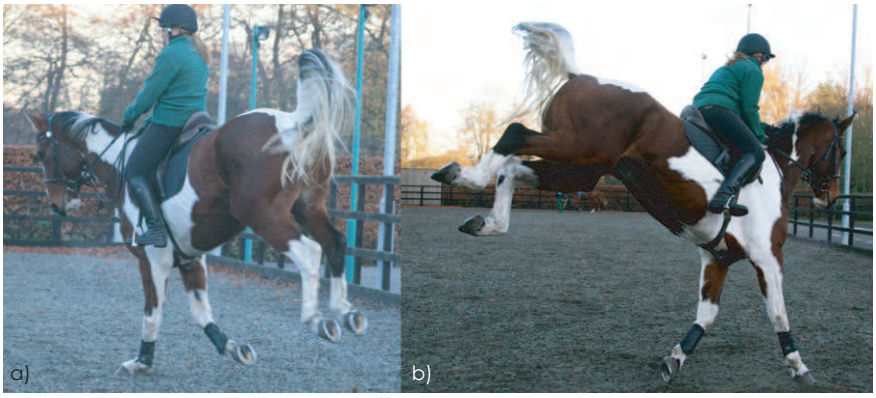

Fig 3: Two bucks from a horse with sacroiliac joint region pain. a) The thoracolumbosacral region is extended, and both hindlimbs are off the ground with the hind feet below the ventral aspect of the thorax. The tail is elevated. The horse's head is in a normal posture relative to the neck. b) The thoracolumbosacral region is extended, both hindlimbs are extended caudally, and the horse is kicking backwards with the right hindlimb, with some rotation of the pelvis. The tail is elevated.

Fig 4: Three horses with sacroiliac joint region pain. a) The horse is bucking with the thoracolumbar region in extension; both hind feet are under the trunk, below the ventral aspect of the thorax. The ears are back, and the horse has an intense stare. b) The thoracolumbar region is extended with both hindlimbs extended caudally with the hind feet above the ventral aspect of the thorax; the tail is upright. The neck is extended, and the head is raised. The ears are back, and the eye has an intense stare. c) The thoracolumbar region is extended with the right hindlimb flexed under the trunk and the left hindlimb kicking back horizontally.

et al. 2012). This emphasises the potential danger to riders associated with horses that buck.

An association between lack of rider experience and falls has been recognised (Hawson *et al*. 2010; Hasler *et al*. 2011). Inexperienced riders often lack core strength and stability (Terada *et al*. 2004; Lovett *et al*. 2005), and if their position becomes compromised, their movements may become progressively out of synchrony with the horse's movement, resulting in uncontrolled bouncing on the horse. This can cause the horse to panic and exhibit fear-induced bucking behaviour, until the rider falls off. The horse may continue to show fear and be extremely apprehensive of being remounted. Fear may rapidly become a persistent feature (LeDoux 1994). If a rider falls off several times, some horses may develop a propensity for recurrent bucking as a learned behaviour and require careful retraining.

In contrast to the human literature, a search of the equine literature revealed a paucity of data directly related to ridden horse bucking behaviour. Several studies have included bucking as an abnormal behaviour which may be used, among others, to monitor a ridden horse's response to an intervention or situation (Rivera *et al*. 2002; Cartier d'Yves and Ödberg 2005; Kaiser *et al*. 2006; von Börstel *et al*. 2009; Quick and Warren-Smith 2009; König von Borstel *et al*. 2011; Kienapfel *et al*. 2014; Waite 2014). It was therefore suggested that in any ridden horse ethogram, bucking should be included (Hall and Heleski 2017). Hyper-reactive escape behaviour to avoid pain may switch to other active coping strategies, such as hyper-reactive predator removal behaviour, including bucking (McLean and McGreevy 2010). Bucking as a manifestation of pain-related poor performance has been described (Dyson 2011, 2016, 2017).

Frequency of occurrence

In a questionnaire-based study of a convenience sample of United Kingdom leisure horses, 17% of 789 horses were reported to have bucked during ridden exercise in the previous week (Hockenhull and Creighton 2013). In a

Fig 5: Several 'pronks' with the thoracolumbar region flexed and the head and neck low. Such bucks are extremely difficult for a rider to sit to, especially if the horse suddenly changes direction. When last ridden the rider had fallen off. The horse had multifocal musculoskeletal pain.

prospective study of 84 Pony Club horses in Australia followed over one year, bucking was described as a potentially dangerous behaviour, but the frequency of occurrence was not recorded (Buckley *et al*. 2013). In a prospective observational study of a convenience sample of 193 sports and leisure horses, in regular work and presumed by their owners to be working comfortably, which were observed during mounting and ridden exercise on a single occasion, two horses pronked repeatedly when moving off after mounting (Dyson *et al*. 2022). In a subset of 148 of these horses with video footage available for retrospective analysis of ridden performance, bucking was observed in one horse (0.7%). In a convenience sample of 40 horses, in normal work and assumed by their owners to be nonlame, which were observed ridden by both the normal rider and a professional rider, transient bucking was observed in three horses (7.5%) when ridden by one of the riders (Dyson *et al*. 2020).

Association with pain

There have been a number of studies highlighting the association between musculoskeletal pain and behaviour (Dyson *et al*. 2018a, 2022; Dyson and Pollard 2020; Dyson and Van Dijk 2020). When horses with and without musculoskeletal pain were compared, bucking was observed significantly more frequently in horses with underlying pain (Dyson *et al*. 2018a). Bucking with the thoracolumbar region in extension ± kicking out is a characteristic feature of horses with sacroiliac joint region pain (Barstow and Dyson 2015). In a retrospective study of horses with sacroiliac joint region pain, eleven of 296 horses (4%) were deemed by their riders to be unsafe to ride because of their behaviour; bucking was observed in 21% of 285 horses assessed when ridden; bucking and kicking out was seen in 17%. Bucking was seen with significantly greater frequency when horses were ridden than when they were evaluated trotting and cantering on the lunge. Bucking occurred more in canter than in trot, both on the lunge and when ridden (Barstow and Dyson 2015). Bucking and kicking out when landing after a show jumping fence was observed in two of a convenience sample of 10 show jumpers in regular competition work, both of which showed other clinical features suggestive of underlying musculoskeletal discomfort (Dyson *et al*. 2018b). In both of the latter studies, the riders did not feel that the horse was bucking to dislodge them from the saddle. In our experience as riders and in our clinical experience of the investigation of bucking horses, some horses bronk vigorously until the rider is dislodged and then stop immediately. Some riders tend to anthropomorphise such behaviour and as a result may not seek veterinary advice in the first instance, but follow alternative management strategies, without trying to determine whether there is an underlying pain-related problem.

Although bucking has occasionally been anecdotally related to primary thoracolumbar region pain (Burns *et al*. 2018), it was not noted as a clinical sign in a recent multicentre study of thoracolumbar region pain (Riccio *et al*. 2018). However, the authors of the current review have shown resolution of bucking behaviour after infiltration of local anaesthetic solution around impinging thoracolumbar spinous processes in some horses. Bucking in these horses was with the thoracolumbar region in flexion and the nose at the level of or ventral to the ventral aspect of the thorax and had historically resulted in multiple rider falls. Bucking has also been observed in association with an acute severe supraspinous ligament tear, and in another horse with spondylosis involving the fifth and sixth thoracic vertebrae (S. Dyson, unpublished data).

There are few other references to the underlying causes of bucking behaviour. Focal intense increased radiopharmaceutical uptake was seen in the mid portion of the left fifth rib, in the girth region of a horse which presented with a history of aversion to girth tightening and bucking behaviour when ridden (Dahlberg *et al*. 2011). The horse returned successfully to full athletic function after rest and local infiltration with isoflupredone acetate and extract of Saracen purpurea. Fragmentation of the caudal aspect of the sternum was associated with bucking behaviour, which resolved following rest and osseous modelling (Butler *et al*. 2017). Chiropractic spinal manipulation resulted in resolution of bucking behaviour in a barrel racing horse (McQueen *et al*. 2017), which the authors related to segmental joint dysfunction, causing increased nociception and autonomic dysregulation, most notably over the sacroiliac joints.

Ill-fitting tack

Bucking behaviour can be considered as an anti-predator response, so it is therefore unsurprising that abnormal pressure

in the withers region, such as that created by excessively tight tree points of a saddle, may cause bucking behaviour with the thoracolumbar region in flexion. Provocation of bucking behaviour has been observed in ridden horses immediately after mounting and moving forwards if a saddle has been incorrectly positioned or if the saddle provokes discomfort (S. Dyson, unpublished data). Horsemen describe a bucking reflex spot; an area on the dorsal aspect of the cranial lumbar region, where manual palpation will provoke some horses to buck. It has been observed that saddles which are too long and exert pressure in this area may provoke bucking (Schleese 2014).

Cold-backed behaviour

'Cold-backed behaviour' is a term used to describe a syndrome comprising one or more of the following behaviours (Dyson 2011; Ahern 2020): girth aversion behaviour while tacking up (Bowen *et al*. 2017); becoming progressively more tense during girth tightening with slight flexion of the thoracolumbar region and then exploding into a series of pronks when moved forwards; extension of the thoracolumbosacral region during mounting and then moving forwards with short steps; normal behaviour during tacking up, but collapsing ('dropping') to the ground when first mounted or vigorous bucking with the thoracolumbar region in flexion and the head down. Often this abnormal behaviour stops within a few minutes and the horse may then work completely normally. However, if the rider falls off, this may frighten the horse and the behaviour may progressively deteriorate, becoming a learned behaviour.

In some horses, no underlying cause can be identified for 'cold-backed behaviour' and the horse can be managed by re-backing if necessary. The behaviour may have been instigated by pain and subsequently become a learned behaviour. If the rider has lost confidence or lacks appropriate skill, then professional help is required. Management changes such as a longer duration of turnout, reduction of carbohydrate intake and increased duration and or intensity of work should be considered. It may be helpful if the horse wears an appropriately fitted roller when not being ridden, which is removed and then replaced several times daily. The horse should be tacked-up slowly, with progressive tightening of the girth to similar holes on the left and right sides, walking the horse forward each time, before tightening another hole. The horse should be lunged with the tack on and made to go forward at the trot and the canter, before being mounted. Some horses appear to behave better if the girth is released by one hole before mounting (D. Marks, Personal communication 2020).

In severely affected horses, their behaviour may be too dangerous to risk a rider, and the use of an Ardall dummy[1], a weighted dummy mimicking a rider, may be helpful (**Fig 6**). This should be done in conjunction with a skilled professional. The trunk of the dummy can be shortened initially, because it appears in some horses that abnormal behaviour is provoked by the sight of the rider's body, rather than the weight of the rider. When re-introducing a rider, they should be 'legged up' onto the horse and should not attempt to mount from the ground; they may need to lie over the horse first before the horse is confident enough to allow the rider to straddle the horse. Some professionals prefer to vault on from the ground while the horse is in a stable, then walk the horse around in the stable. The rider must be able to sit on a bucking horse and is advised to use a neck strap. To halt this bucking behaviour, the rider must have the ability to bring the horse's head up and turn it to one side towards the girth if the horse attempts to buck and to turn the horse in a tight circle. If the horse is able to brace its body without bending the neck and turning the body the bucking may be uncontrollable.

The horse must respect the cues of the handler and rider without fear, be willing to bend the neck towards the girth and to turn in a small circle either to the left or to the right, crossing the hindlimbs. This may require skilled training from the ground initially, without tack or a rider. Conventional trainers may be less suited to this challenge than real 'horsemen', who intuitively can react rapidly to a horse's behaviour and apply and release cues. However, this is a process that usually takes many weeks of careful retraining. Horses with cold-backed behaviour for which no underlying cause can be identified may improve with careful management, but should never be trusted completely, and generally are suitable only for professional riders. Some such horses have been outstanding international athletes.

Some horses which demonstrate 'cold-backed behaviour' show hypersensitivity to light palpation in the girth region (allodynia) or hyper-reactive myofascial trigger points (Bowen *et al*. 2017). Local infiltration with methyl prednisolone acetate (Depo-Medrone™ V[2]) has resulted in resolution of clinical signs (S. Dyson, unpublished data). Paradoxically, horses may behave worse with a girth with elastic insets, rather than a girth constructed of uniform material, so post-treatment management advice includes not using a girth with elastic inserts and not tightening the girth excessively (S. Dyson, unpublished data). A series of 83 horses with 'cold-backed behaviour' and exaggerated responses to digital palpation of latissimus dorsi and caudal deep pectoral muscles were treated by cervical vertebral mobilisation under general anaesthesia, followed by in-hand and ridden exercise to maintain range of

Fig 6: An Ardall dummy, which is weighted, can be used in the retraining of a bucking horse. The torso of the dummy can be made shorter.

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motion of the cervical vertebrae (Ahern 2020). Fifty-one per cent of horses had resolution of clinical signs at 3 and 6 months after treatment. However, there was no control group with which to compare the outcome.

Girth aversion behaviour was associated with gastric ulceration in 32% of 37 horses (Millares-Ramirez and Le Jeune 2019), all of which responded satisfactorily to medication with omeprazole. However, bucking was not reported in any of these cases.

Clinical investigation of bucking behaviour

The principle differential diagnoses for bucking behaviour include thoracolumbar, sternal or rib-related pain, neuropathic pain, lumbosacroiliac joint region pain, referred pain, an ill-fitting saddle or girth, primary hindlimb lameness, fear and behavioural adaptation. Any of these may be compounded by a rider who is fearful, poorly balanced or crooked.

History and clinical assessment

It is important to establish the circumstances under which a horse bucks, for example when first tacked-up and moved forwards, when first mounted, or sporadically during work; only going into canter or in canter; or on landing after a jump. Bucking associated with canter or landing after a jump is most frequently associated with sacroiliac joint region pain. Determine whether the rider feels that the horse is trying to buck them off (highly unusual with sacroiliac joint region pain) or otherwise. Kicking out in association with bucking has only been seen in association with sacroiliac joint region pain, and we speculate that this may be associated with nerve-related pain.

It is also important to establish whether bucking behaviour was sudden in onset or has been part of a slowly progressive deterioration in performance. If sudden in onset, determine whether the onset coincided with any event or change in management. A change in saddle may provoke dangerous bucking behaviour. Alteration in management so that a horse is not being turned out daily may change behaviour. A rider falling off may frighten a horse and provoke subsequent bucking behaviour, which may over time become learned behaviour. Although sacroiliac joint region pain is often insidious in onset and slowly progressive, horses have been seen with no premonitory clinical signs and a sudden onset of bucking $\pm$ kicking out associated with sacroiliac joint region pain. Acute onset sacroiliac joint region pain may occur if the horse has had a fall either in the field or whilst being ridden (Barstow and Dyson 2015). Try to find out whether the rider has recognised any other clinical signs, bearing in mind that some owners are poor at recognising lameness or features of gait or performance that may reflect musculoskeletal pain. Ask whether the horse performs better in canter than in trot or vice versa. Canter being more difficult than trot is a typical feature of sacroiliac joint region pain (Barstow and Dyson 2015). Difficulty in picking up the hindlimbs to be trimmed and shod can also be a feature of sacroiliac joint region pain.

A comprehensive clinical examination is essential, paying particular attention to the development and tone of the thoracolumbosacral epaxial muscles (Girodroux *et al*. 2009; Merrifield-Jones *et al*. 2019). Focal or generalised loss of muscle under the saddle may reflect a chronically ill-fitting saddle. Poor muscle development throughout the thoracolumbar region may reflect primary thoracolumbar region pathology. Muscle atrophy or inadequate muscle development in the lumbar region reflects the way in which the horse has been working and the possible presence of underlying musculoskeletal pain (e.g. hindlimb lameness). The reactions to stimulation to flex and extend the thoracolumbosacral region in the sagittal plane and to flex from side to side should be evaluated, paying attention to range of movement, alteration in muscle tension and changes in the horse's demeanour (e.g. ears back, tail swishing, mouth opening). Assess the alignment of the summits of the thoracolumbosacral spinous processes and the reaction to firm palpation. Application of firm deep pressure using a blunt-ended, rounded metal probe in the cranial saddle region can be helpful to identify deep pain, the result of tight tree points of the saddle. Determine the reaction to light palpation of the girth region (repeated muscle fasciculation is not normal) and the reactivity of myofascial trigger points (Bowen *et al*. 2017), comparing left and right sides. The reaction to palpation of the pectoral muscles, sternum, ribs, thoracolumbosacral and pelvic regions and to pressure applied over the tubera sacrale (Barstow and Dyson 2015) should be assessed.

The horse should be assessed moving in hand, on the lunge and ideally ridden (if safe to do so) with assessment of gait quality in walk, trot and canter, the presence or absence of lameness and the range of movement of the thoracolumbosacral region (Greve and Dyson 2020). A neurological assessment should also be performed. The onset of unpredictable, uncharacteristic behaviour, such as bucking or bolting, has been recognised in adult horses which have developed ataxia associated with equine degenerative myeloencephalopathy, that was confirmed by post-mortem examination (Benedice and Johnson 2018). The alteration in behaviour is thought to reflect functional abnormalities of the brain (Johnson and Reed 2020).

Saddle and girth maintenance and fit (Schleese 2014; Dyson *et al*. 2015; Bondi *et al*. 2020) must be assessed, even if the owner reports recent evaluation by a professional saddle fitter. Note the shape of the girth and the presence of elastic insets at one or both ends of the girth. Observe movement of the saddle during motion. Subtle side to side movement of a saddle is normal, but major left right oscillation of a saddle, the saddle continually slipping to one side or dorsoventral movement (bouncing) of the saddle are abnormal and reflect poor saddle fit (Bondi *et al*. 2020).

If there is a history of cold-backed behaviour when tacked-up or mounted, especially if associated with repeated humping bucks (i.e. the thoracolumbar region in flexion), it is preferable, before tacking up, to assess in an arena the horse's response to application of a roller, leaving it relatively loose initially and then moving the horse forwards and then progressively tightening the roller. The handler should wear an appropriate helmet and gloves, and they must be warned that the horse may 'explode' into a series of bucks and try to escape. If the horse can be controlled and kept on a circle, it should be made to go forwards in trot or canter, and often the bucking will cease. It may not recur that day or thereafter, or it may recur on a subsequent occasion. Then progress to placement of the saddle, repeating the slow tightening of the girth and moving

Fig 7: The use of a specifically designed weighted roller, to mimic the weight of a light rider. It is important that the roller is tight enough; undue movement of the roller can provoke fear.

forwards after each tightening and also observing the horse's behaviour (Dyson *et al*. 2021).

It becomes a judgement call about whether or not a horse is safe to ride, based on the history of the horse, the skill of the usual rider and the availability of a skilled professional rider who is willing and competent to ride a bucking horse. If the horse is deemed unsafe to ride, it can be useful to use commercially available lead weights in a specially designed roller (The Astride[3]) (**Fig 7**) or lead weights in a weight cloth, a weighted-saddle and flasks of lead attached to the front of the saddle in order to mimic the weight of a rider. The use of the RHpE (Dyson *et al*. 2018a) can be useful to determine whether there is an underlying pain-related problem; the display of ≥8/24 behaviours is likely to reflect the presence of pain, although fear could confound the interpretation of some behaviours. Other features which may reflect pain include teeth grinding, tension, sweating disproportionately to the environmental conditions and work intensity, abnormal respiratory noise or elevated respiratory rate and depth of respiration and increased frequency of eyelid movement. If no underlying pain-related cause of bucking can be established, then retraining by a professional, as discussed above, is an option. However, it must be borne in mind that such horses may become safe for a skilled professional, but have the potential to suddenly revert in their behaviour with a less skilled rider, with potentially dangerous consequences.

If an ill-fitting saddle is identified as the likely underlying cause of bucking, then it may be necessary to work with several different professional saddle fitters until a saddle is identified which fits both the horse and the rider optimally and in which the horse is comfortable (Schleese 2014; Dyson *et al*. 2015; Bondi *et al*. 2020). Most professional saddle fitters only have a limited range of stock and may not have a saddle which is ideal for the horse in question. This is a daunting situation for an owner, who can feel pressured into taking the least bad option, and a team approach may provide the best solution, with a veterinarian or physiotherapist providing owner support.

Diagnostic anaesthesia

Assuming that the horse is safe to ride, any lameness should be investigated further using diagnostic anaesthesia, including the sacroiliac joint regions (Barstow and Dyson 2015) where applicable, and the response when ridden should then be assessed. If impinging spinous processes are identified radiographically, then their clinical significance should be assessed by local infiltration of local anaesthetic solution (Denoix and Dyson 2011). If they are the primary cause of bucking behaviour, resolution of this behaviour would be expected. If the horse is unsafe to ride, but clinical signs can be reproduced on the lunge with a weighted roller or saddle, then the horse can be blocked under these circumstances.

Diagnostic imaging

It is suggested that the caudal cervical and entire thoracolumbar region vertebral column should be assessed radiographically (Butler *et al*. 2017), together with the ribs and sternum. Scintigraphy occasionally highlights regions that are difficult to assess using conventional radiographic techniques, but is not reliable for identification of sacroiliac joint region pain (Barstow and Dyson 2015; Quiney *et al*. 2018). Ultrasonographic assessment of the pelvic region per rectum is valuable to assess the caudal lumbar vertebrae, sacrum and sacroiliac joint regions (Nagy *et al*. 2010; Tallaj *et al*. 2017a,b; Boado *et al*. 2020; Vautravers *et al*. 2021).

Management

In some horses, identification of a likely underlying cause can lead to a treatment and management plan, which may involve a team approach with paraprofessionals, such as a physiotherapist or chiropractor. It is beyond the scope of this review to consider the specific treatments for problems identified as primary sources of pain. However, in early post-treatment management, the assistance of a skilled professional horseman, used to working with difficult horses, may be advantageous, as described earlier. There are a number of YouTube video recordings which give a fascinating insight into training, for example Hughes[4]. However, it must be emphasised that retraining needs to be slow and progressive, with an understanding of learning theory (Doherty *et al*. 2017; McLean and Christensen 2017). Rider safety must always be of paramount importance. The importance of matching rider ability with the temperament and movement of the horse (Williams and Tabor 2017) must not be ignored. If an underlying pain-related cause cannot be identified, then working together with a skilled behaviourist (see Society of Equine Behavioural Consultants; www.societyofequinebehaviourconsultants.org.uk) may be of benefit. However, it has to be ultimately recognised that there is a minority of horses in which dangerous bucking behaviour cannot be resolved and for which retirement or euthanasia are the only options.

In conclusion, there is a variety of different sources and causes of pain that may lead to bucking behaviour. In other horses, bucking may have become a learned behaviour, perhaps initiated by previous pain or fear. Identification of the cause of bucking behaviour requires comprehensive clinical assessment. In some horses, targeted treatment and appropriate rehabilitation and retraining may result in a successful outcome.

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Both authors contributed to the manuscript.

Manufacturers' addresses

[1]www.ardall.com
[2]Zoetis UK Ltd., Leatherhead, Surrey, UK.
[3]Eponaire LLC, Loxahatchee, Florida, USA.
[4]YouTube video recording: Solving the root causes of horse bucking. Mike Hughes.

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