2018 Annual Report to IHRC

Equine Sports Medicine Center
COLLEGE OF VETERINARY MEDICINE

PREPARED BY:
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Director, Equine Research Programs
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### Appendix A (Blue)

- **Equine Health Update** - Equine Sports Medicine Center Newsletter  
  Vol. 20, Issue No. 1 – 2018

- **Equine Health Update** - Equine Sports Medicine Center Newsletter  
  Vol. 20, Issue No. 2 – 2018

### Appendix B (Gold) ~ Research Projects In Progress Supported with Pari-Mutuel Funds

- **Dos Santos AP, Taylor SD, Woolcock A, Christian JA, Ruple A. Validation of a Novel Assay to Detect Intraerythrocytic Reactive Oxygen Species (ROS) by Flow Cytometry in Horses.**

- **Figueiredo M, Lescun T, Gimble J. Enhancing the Repair Potential of Equine-derived MSC for Treating Post-traumatic Osteoarthritis.**

- **Hendrix K, Kritchevsky J. Recovery of Salmonella bacterial isolates from pooled equine fecal samples.**

- **Lescun T, Main RP. Validation of an in vivo assessment of fracture risk in equine limb bones.**
• **Lim CK, Pemberton S, Heng HG, Kritchevsky J, Jones-Hall Y.**
Ultrasonographic morphology of the gastrointestinal tract of healthy horses: in vivo, ex vivo and histological comparison.

• **Little D, Lescun T.** Mechanosensitive Channels in Equine Musculoskeletal Soft Tissues.

• **Main RP, Lescun T, Lim CK, Durkes A.** Assessing Fracture Susceptibility in Horse Limb Bones: A Pre-Clinical Study.

• **Taylor S, Grady S, Lescun T, Moore G, Davern A.** Analgesic efficacy and safety of ketorolac, phenylbutazone and flunixin in a model of foot lameness in horses.

**Appendix C (Green) ~ Research Projects Completed Supported with Pari-Mutuel Funds**

• **Lescun T, Breur G, Nauman E, Chandrasekar S, Adams S, Jones Y, Main R.** Finite element modeling and implant nanosurfacing to enhance equine fracture treatment.

• **Taylor S, Cooper B, Grady S, Lescun T, Moore G, Davern A, Brunner T.** Plasma drug concentrations of ketorolac tromethamine, phenylbutazone and flunixin meglumine in horses following single-dose intravenous administration.

**Appendix D (Purple)**

~ Refereed Scientific Articles


~ Abstracts and Proceedings

• **Couëtil LL, Ivester KM, Moore GE.** Relationship between tracheal mucus, exercise-induced pulmonary hemorrhage, and airway cytology in racing thoroughbreds. Proceedings of the Veterinary Comparative Respiratory Society Symposium, Auburn, AL, October 2018.


Appendix F (Gray) ~ Refereed Scientific Publications


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MISSION
To provide first class veterinary diagnostic and investigative support to the horse industry in Indiana and to educate owners, trainers, and veterinarians.

GOALS:
The goals of the ESMC are to pioneer leading-edge research in the area of equine sports medicine, to provide training to future equine veterinarians and veterinary technicians, to offer continuing education to Indiana veterinarians and horsemen, and to diagnose and treat causes of decreased performance in horses.

ACHIEVEMENTS OF EQUINE SPORTS MEDICINE CENTER (ESMC)

Treadmill Evaluations:
Treadmill diagnostic work-ups are an important activity at the ESMC. Twenty one client-owned horses were evaluated on the treadmill in 2018. This brings the total number of horses evaluated since the opening of the ESMC in April 1996 to 503. Treadmill demonstrations at the ESMC continue to be a major attraction for local, national and international visitors to the Purdue campus. In the past year 8 treadmill demonstrations were given to groups or dignitaries who visited Purdue campus including filming sessions that resulted in 2 celebration videos (see links below):
https://takegiantleaps.com/
https://www.youtube.com/watch?v=FuAO_Ks4Vqo&app=desktop

Continuing Education and Extension Service:
• Continuing Education presentations:
  • Buchheit T.
    • Regional and State
      • Updates in equine preventative medicine. Purdue Veterinary Conference, West Lafayette, IN, September, 2018.
  • Couetil L.
    • International
      • Equine Respiratory Days, October 2018, Lusche, Germany
        • Endoscopy of the lower airways
        • Analysis and evaluation of BAL and TW secretions
        • Ancillary respiratory tests
        • Hands-on sessions
      • Calgary International Symposium, Faculty of Veterinary Medicine, Calgary, Canada, September 2018 (Invited lecture):
        • Etiology of equine asthma and effect on racehorses’ performance
    • National
      • The 36th Annual Symposium of the Veterinary Comparative Respiratory Society, October 2018, Auburn University, AL.
- Relationship between tracheal mucus, EIPH, and airway cytology in racing thoroughbreds.
- The American College of Veterinary Internal Medicine, 2nd Annual Large Animal Candidate Boot Camp, October 2018, Texas A&M University, TX.
  - Clinical applications of respiratory function testing
  - Diagnosis and treatment of IAD and RAO
  - Pulmonary function testing laboratory
  - Inhalation therapy laboratory
- Hagyard Bluegrass Symposium, October 2018, Lexington, KY
  - The latest causes and diagnostics for equine asthma
  - How to best manage and treat equine asthma

**Regional and State**

- Farr A.
  **Regional and State**

- Gedehus T.
  **Regional and State**
  - *Centaur Equine Specialty Hospital Continuing Education Meeting*, May 11th, 2018.
    - Stifle pathologies: diagnostic standard in 2018 – MRI/CT, radiography, and ultrasound and the merit of diagnostic arthroscopy.

- Gillespie C.
  **Regional and State**

- Haanen G.
  **Regional and State**

- Hawkins J.
  **Regional and State**

- Koziol J
  **Regional and State**
  - Breeding the Middle-Aged Mare. *Purdue Horseman’s Forum*, West Lafayette, IN, February 10th, 2018.
• Lescun T.

International

- Goulburn Valley Equine Hospital Referring Veterinarians Seminar, Dookie College, University of Melbourne, Victoria, Australia, May 2018.
  - Antimicrobial selection in equine practice
  - Current approaches to synovial sepsis
  - Nerve blocks: Do I trust them?
  - Bone adaptation, fracture and injury
  - Wounds, tumors and other common challenges

Regional and State

- Mississippi Valley Veterinary Medical Association Annual Meeting, Peoria, IL, March 2018.
  - Inertial sensor systems and the lameness exam.
  - What scan is that? Case presentations of advanced lameness imaging.
  - The role of the MRI in the treatment of foot lameness in the horse.
  - Current approaches to synovial sepsis – outcomes matter.
  - Wounds; grafting, dressings and topical stuff.

Local

- Joint disease and other lameness problems in horses. Purdue Rodeo and Western Equestrian teams invited guest speaker. Purdue University, West Lafayette, IN, April 2018.
- Equine joint injection wetlab. Student Chapter of the American Association of Equine Practitioners, Purdue University, West Lafayette, IN, March 2018.
- Tippecanoe County 4-H: Horse and Pony Club/Veterinary Science Club Workshop, Lafayette, IN, March 2018.
  - Discovering lameness in horses
  - Tendons, ligaments and bones – Oh my! Equine lower limb anatomy

• Mielnicki K.

Regional and State


• Mundy L.

Regional and State


• Skelton J.

Regional and State

• Taylor S.

International
  ▪ Equine neonatal foal care (5 lectures)
  ▪ Equine sepsis

National
• Equine neurologic disease and infectious disease (3.5 lecture hours). *Veterinary Meeting and Expo (North American Veterinary Conference)*, Orlando, FL, 2018

Regional and State
• The “Possum Disease”: An Update on EPM. *Purdue Horseman’s Forum*, West Lafayette, IN, February 10th, 2018.

• Tinkler S.

International
• Organizer and lead veterinarian for the Equitarian Initiative in Cusco, Peru, August 2018.
• Equitarian Initiative Workshop, Puerto Jimenez Costa Rica, January 2018.
  ▪ Diverse veterinary models for equid health care delivery in Africa
  ▪ Welfare impacts of the donkey skin trade on donkeys and owners
  ▪ Multiagency cooperative model for improved working equid and community health: Peru model
  ▪ Applied research on working equids
• Rural Working Equid Veterinary Services in a Farming Community Wet Lab 1: Equitarian Initiative Workshop, Puerto Jimenez Costa Rica January 2018.
• International Engagement Methods in Peru Study Abroad (YDAE 43110), March 2018, Cusco, Peru.
  ▪ Organize itinerary with local NGOs and trip details for participants
  ▪ Provide Spanish translation for participants
  ▪ Visit 4 Andean communities and interview community members
  ▪ Help students apply the principles of extension and asset based community development methodology, intercultural communication and community engagement
  ▪ Teach students how to effectively work with local leaders and NGOs (Yanapana Peru, Innovar y Compartir and Alianza Andina)

National
• *AAEP Convention*, San Francisco, December 2018.
  ▪ Improving the Health and Welfare of Working Equids Worldwide: Balancing work in two worlds while striving for success.
  ▪ Equitarian rounds
• Equitarian Initiative Booth Exhibitor
• Mandibular sialadenitis in a donkey gelding. The Donkey Welfare Symposium, UC Davis, October 2018.

Regional and State
• Purdue Youth Development and Agriculture Education, Purdue University and Universidad Nacional de Agricultura (UNALM), Planning for International Engagement Methods, January-March 2018 (YDAE 43100).
  ▪ Challenges of highland agrotourism villages
  ▪ Lifestyles and cultures of highland Andes people
  ▪ Health issues and malnutrition of working equids
  ▪ Interview questions for community-member focus groups
• Donkeys and mules and horses – oh my! A day in the life of a “working equid”. Purdue Horseman’s Forum, West Lafayette, IN, February 10th, 2018.

• Townsend W.
  Regional and State
• Waxman, S
  o Regional and State
    ▪ Purdue Veterinary Conference, West Lafayette, IN, September, 2018.
    • Skin Grafting for the General Equine Practitioner;
    • Immobilization of Equine Limb Wounds;
    • The Effects of Different Needle Type Variables, Hair Preparation, and Insertion Technique on the Contamination of Equine Joints with Tissue and Hair Debris during Arthrocentesis

• Committee service
  International
  - Lim CK:
    Member of the European College of Veterinary Diagnostic Imaging Credentials Committee (2017-2019)
  - Tinkler S:
    Board of Directors, Equitarian Initiative
  - Townsend W:
    Research Committee Member, International Equine Ophthalmology Consortium. 2013-present

  National
  - Couetil L:
    American College of Veterinary Internal Medicine – Case Writing Assessment Committee (2017-2018).
- Kritchevsky J:  
  American Humane Society Scientific Advisory Committee for American Humane Farm Program  
  Equine Endocrinology Working Group

- Lescun T.:  
  American Association of Equine Practitioners, Avenues Task Force, 2012-present  
  American College of Veterinary Surgeons, Examination committee, 2014-present (Chair 2018)  
  American College of Veterinary Surgeons Foundation, Board of Trustees (2018-present)  

- Townsend W.  
  Genetics Committee, American College of Veterinary Ophthalmologists, 2012-present

**State:**  
- Lescun T.:  
  Purdue Liaison to the Board of Directors, Indiana Association of Equine Practitioners 2016-present

**Outreach:**

- Purdue’s Equine Sports Medicine Center Web site dedicated to informing horse owners about equine-related activities at Purdue University has undergone a major update this year. The address of the site is: https://vet.purdue.edu/esmc/index.php

- Outreach activities
  - **Purdue Horseman’s Forum,** Purdue Veterinary Medicine, West Lafayette, IN, February 10\(^{th}\), 2018.  
    - Continuing Education annual meeting for horse owners and veterinarians  
    - Approximately 200 registrations, 13 presentations, treadmill demonstrations and tour of Large Animal Hospital.
  
  - **Centaur Equine Specialty Hospital Continuing Education Meeting,** May 11\(^{th}\), 2018.  
    - "Selected topics in equine orthopedics: Advanced imaging & surgery"

- Lay Publications:
  
  - The Equine Sports Medicine Center continued publication of its newsletter called “Equine Health Update” established as a source of information for Indiana’s horse industry. Dr. Stacy Tinkler is the editor for the newsletter since January 2012. Two issues were released in 2018 and articles are accessible from our Web site. The newsletters are included in Appendix A (Blue).
Adams SB:

Couetil L:

Lescun T:

Stiles J:

Taylor SD:

Tinkler S:
- Edited 2 newsletter articles:

Research:

Research activities from investigators of the Equine Sports Medicine Center are summarized below. The names of members of the ESMC are underlined.

Research projects in progress supported with Pari-Mutual Funds:
Progress reports for the following projects are included in Appendix B (Gold).

Dos Santos AP, Taylor SD, Woolcock A, Christian JA, Ruple A. Validation of a Novel Assay to Detect Intraerythrocytic Reactive Oxygen Species (ROS) by Flow Cytometry in Horses.


Hendrix K, Kritchevsky J. Recovery of Salmonella bacterial isolates from pooled equine fecal samples.

Lescun T, Main RP. Validation of an in vivo assessment of fracture risk in equine limb bones.

Little D, Lescun T. Mechanosensitive Channels in Equine Musculoskeletal Soft Tissues.

Main RP, Lescun T, Lim CK, Durkes A. Assessing Fracture Susceptibility in Horse Limb Bones: A Pre-Clinical Study.


Research projects completed supported with Pari-Mutual Funds:
Complete reports for the following projects are included in Appendix C (Green).


Competitive Equine Research Fellowship supported with Pari-Mutual Funds:
The PVM Equine Research Fellowship is for the recruitment of outstanding M.S. or Ph.D. track students to conduct applied/clinical research in the area of equine medicine at Purdue University to address issues of importance to the health and performance of Indiana racehorses and other equine athletes. The fellowship provides one year (M.S.) or two years (Ph.D.) of stipend support from the PVM Equine Internal Fund and additional years of funding support for degree completion will come from the graduate program of the respective department.

Jesus Hermida, DVM, third-year resident in Large Animal Surgery – PhD student in VCS – Faculty advisors: Drs. Tim Lescun & Russell Main: Application was approved by the ERAB and the Equine Research Fellowship was granted for the 2018-2019 academic year

Externally funded equine research projects conducted in 2018:


Publications supported by the Equine Research Internal Funds:  Appendix D (Purple)
The names of members of the ESMC are underlined.

Refereed Scientific Articles:


Abstracts and Proceedings:


Book Chapters:


Taylor SD. Section editor for the Laboratory Tests section of Blackwell’s 5-Minute Veterinary Consult: Equine. 3rd ed. Authors recruited, sections completed and awaiting my review. Anticipated publication date, June 2018.


Refereed Scientific Publications: [Appendix E (Gray)]


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<th>Year Serving</th>
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APPENDIX A

- **Equine Health Update** - Equine Sports Medicine Center Newsletter
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- **Equine Health Update** - Equine Sports Medicine Center Newsletter
  Vol. 20, Issue No. 2 – 2018
Dismiss the Kiss!
Understanding Kissing Spines and How You Can Help Your Horse

By Jessica Zeiger, DVM (Class of 2018)
Edited by Stephen B. Adams, DVM, MS, Dipl. ACVS

Overriding Dorsal Spinous Processes (ORDSP), or “Kissing Spines” as it is commonly called, is an occasional cause of back pain in horses. Seen predominantly in sport horses, it has become common practice to ask for vertebral radiographs as part of the pre-purchase examination of horses used for practices such as dressage or jumping. Equine practitioners and owners alike need to understand the diagnosis, treatment options, and preventative measures for this condition in order to best help these horses perform comfortably.

Overriding dorsal spinous processes (ORDSP) is the cause of kissing spines. In horses with kissing spines, two DSPs may touch each other in one or more places along the spine. This causes inflammation, back pain, and spasms of the muscles of the back, drawing these spines even closer together. The most common site of this impingement is at the 15th thoracic vertebrae, which is a vertebra where there is an increased rotational motion compared to other vertebrae. The saddle also sits over this vertebra, potentially contributing to the lesion. While we do not know what causes the DSPs to impinge on each other initially, it is known that weakness of the back and core muscles contribute, as do poor saddle fit and poor equitation.

Some horses function well with kissing spines without showing any clinical signs. If it is discovered incidentally or during a pre-purchase examination in a non-painful horse, it should be considered as a predisposition for possible future back pain, but not necessarily an immediate problem. Preventative measures and exercise regimens can be followed preemptively, but horses should only be treated for kissing spines if they become painful.

Horses with back pain may show a variety of signs which include, but are not limited to, lameness, gait abnormalities, decreased flexibility turning to one side, high head carriage, refusal to take correct leads, saddle or rider avoidance, rearing or bucking, and refusal to take jumps. There are many causes of back pain, and your veterinarian should thoroughly evaluate your horse to elucidate all contributing factors to the pain. Kissing spines can be easily diagnosed by taking radiographs of the dorsal spinous processes and looking for areas of contact or impingement between DSPs. Other imaging modalities found to be useful include nuclear scintigraphy and thermography.

(continued on page 2)
Kissing Spines (continued from cover)

Once a diagnosis of kissing spines is made, the treatment options for that horse can be determined. In some horses with only mild pain, saddle fitting can bring significant improvement. However, quite often some form of further treatment will be required. Many horses with kissing spines can be medically managed with injections of corticosteroids into the affected areas of the back. This breaks the cycle of inflammation. This therapy requires a 3 week period following the injection during which time the horse should not be ridden, but should follow a strict exercise regimen to strengthen the muscles of the back and core. Lunge line work with a Pessoa lunging system is one recommendation. Corticosteroid injections in conjunction with physical therapy can show improvement as successfully as the surgical techniques, however, recurrence of clinical signs often occurs, requiring repeated corticosteroid injections.

Another popular non-surgical treatment is SME therapy, which is a multimodal approach combining shockwave therapy, mesotherapy, and exercise. The shockwave decreases the pain associated with the impingement, offering the horse relief, and making them more willing to exercise. The mesotherapy decreases nerve-related pain and also allows for better stretching of the muscles. With the pain decreased and the muscles relaxed, the exercise regimen can work to improve the strength and flexibility of the back muscles, lifting the back and opening the spaces between DSPs. None of these modalities alone will sufficiently treat the signs, but the combination is the key. Like the corticosteroid injections, this technique is often effective, but the clinical signs may recur.

Surgical approaches traditionally have been reserved for those cases which cannot be medically managed. A once popular technique was to use an oscillating saw to remove every other DSP, essentially eliminating the contact points for kissing spines. This procedure is highly invasive, is usually performed under general anesthesia, and requires a 3 month or longer rehabilitation period.

A newer, much less invasive surgery is now available, called the Interspinous Ligament Desmotomy (ISLD), which can be performed in a standing, sedated horse. This approach involves making a small incision and cutting the ligament between two spines, eliminating the pain from the nerve endings where the ligament attaches to the DSP and allowing for a widening of the space between the DSPs. The ISLD is 24 times more likely to result in long term correction than the corticosteroid injections and has a low recurrence rate.

Following ISLD, as with all techniques, a rehabilitation exercise regimen needs to be implemented. Horses who have undergone ISLD can be fully rehabilitated within 6 weeks, compared to 3+ months with spinal processes resection. The faster rehabilitation, lower complications, low cost, non-invasiveness, and high success rates of the ISLD have made this procedure a first-choice treatment for many clients and practitioners. Several recent publications describe another procedure, the subtotal osteotomy of the dorsal spinous process. These surgeries are done standing and may be useful in horses in which the ISLD is not successful, or can be used as a primary treatment for horses not responding to injections.

When faced with a diagnosis of kissing spines, it is important to be informed about the nature of the condition, the treatments available, and what you can do to best help the horse be successful. Remember that not every horse with kissing spines is painful. If the horse requires treatment, there are many options available, but none will be successful without a long-term commitment to physical therapy and rehabilitation. The best way to prevent kissing spines is proper equitation and appropriately building horses’ muscles before pushing them into advanced sporting. By implementing management changes, understanding the importance of proper athletic training, and utilizing innovative treatment options when needed, we can help our horses dismiss this kiss!

References:
5. "Oswaldky Dorsal Spines Prognosis (Kissing Spines)." Equine Neck and Back Pathology: Diagnosis and Treatment, by Frances M. D. Henson, Wiley Blackwood, 2018.

News & Notes

Meet our Staff...

Molly Cripe Birt

I am Molly Cripe Birt, and I have worked as a veterinary technician in the Large Animal Hospital for over ten years. I am a proud graduate of Purdue University’s Veterinary Technology Program in 2007, and earned a Veterinary Technician Specialty in Equine Veterinary Nursing in 2015. My predominant interests lie in equine sports medicine and critical care in post-operative patients. Outside of the hospital, I am an avid endurance cyclist and leisurely hiker with my dog.

Pat Navarre

My name is Shannon Wallace, BS-RVT; I am one of the hospital’s Large Animal Versa Technicians; but mostly stay on the surgery side of the large animal hospital. I graduated from Purdue University with my degree and have been working here for a little over a year. I enjoy spending my free time with my two dogs (Oscar my Newfoundland and Yeti my Golden Retriever), remodeling my house, traveling and working out.

Lynda Lum

Hi! I’m Lynda Lum. I have worked at the Purdue Veterinary Teaching Hospital for 27 years as a Large Animal Medicine Technologist. I enjoy working here because no two days are the same and I learn new things from every patient. I have 6 cats, 3 dogs, 2 children, a bunny and a husband at home. In my spare time I love to hike, crochet, visit wineries and volunteer with a local cat rescue.

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Kissing Spines (continued from cover)

Once a diagnosis of kissing spines is made, the treatment options for that horse can be determined. In some horses with only mild pain, saddle fitting can bring significant improvement. However, quite often some form of further treatment will be required. Many horses with kissing spines can be medically managed with injections of corticosteroids into the affected areas of the back. This breaks the cycle of inflammation. This therapy requires a 3 week period following the injection during which time the horse should not be ridden, but should follow a strict exercise regimen to strengthen the muscles of the back and core. Lunge work with a Pessoa lunging system is one recommendation. Corticosteroid injections in conjunction with physical therapy can show improvement as successfully as the surgical techniques, however, recurrence of clinical signs often occurs, requiring repeated corticosteroid injections.

Another popular non-surgical treatment is SIME therapy, which is a multimodal approach combining shockwave therapy, mesotherapy, and exercise. The shockwave decreases the pain associated with the impingement, offering the horse relief, and making them more willing to exercise. The mesotherapy decreases nerve-related pain and also allows for better stretching of the muscles. With the pain decreased and the muscles relaxed, the exercise regimen can work to improve the strength and flexibility of the back muscles, lifting the back and opening the spaces between DSPs. None of these modalities alone will sufficiently treat the signs, but the combination is the key. Like the corticosteroid injections, this technique is often effective, but the clinical signs may recur.

Surgical approaches traditionally have been reserved for those cases which cannot be medically managed. A once popular technique was to use an oscillating saw to remove every other DSP, essentially eliminating the contact points for kissing spines. This procedure is highly invasive, is usually performed under general anesthesia, and requires a 3 month or longer rehabilitation period.

A newer, much less invasive surgery is now available, called the Interspinous Ligament Desmotomy (ISLD), which can be performed in a standing, sedated horse. This approach involves making a small incision and cutting the ligament between two spines, eliminating the pain from the nerve endings where the ligament attaches to the DSP and allowing for a widening of the space between the DSPs. The ISLD is 24 times more likely to result in long term correction than the corticosteroid injections and has a low recurrence rate.

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Shannon Wallace

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By way of introduction, I am Pat Navarre, the senior technician in the Large Animal Hospital, working primarily in the surgery section. I have had a very enjoyable career here at the College of Veterinary Medicine as I approach the anniversary of my 42nd year of service. There have been some very dramatic changes in veterinary medicine over this time with new modalities, equipment and techniques for treating our patients. Every day is seen as a new day of mentoring, guiding students, both DVM and veterinary technician, in their development as veterinary medical professionals.

On a personal note, I enjoy spending time in the outdoors through conservation efforts and hunting. Through my memberships in conservation organizations, I get enjoyment introducing folks, young and old to the outdoors.

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THO – Oh No! Temporohyoid Osteoarthropathy in the Horse

By Julie Goodman, DVM (Class of 2018) – Edited by Tim Lesun, BVSc, MS, PhD, Dipl. ACVS

Temporohyoid osteoarthropathy (THO) is a disease of the hyoid apparatus in the horse, first reported in 1983. The hyoid apparatus is made up of four paired bones (the stylohyoid, epihyoid, ceratohyoid, and thyrohyoid) and one unpaired bone (the basihyoid); it is located in the throat latch region and helps to stabilize the larynx, pharynx, and tongue. The hyoid apparatus is connected to the skull by the temporohyoid joint, an articulation between the stylohyoid bone and the temporal bone of the skull. THO is caused by ankylosis, or fusion, of the temporohyoid joint, which can then progress to fracturing of the stylohyoid or temporal bone.

It is unknown what exactly precipitates the ankylosis of the joint, but there are many theories. Inflammation caused by otitis media or otitis interna (infection of the middle or inner ear) may cause enough local inflammation to induce osteoarthrits and fusion of the joint. It is also possible that infection from a distant site in the body reaches the temporohyoid joint through the bloodstream. Trauma to events such as passing nasogastric tubes or rough manipulation of the tongue may set the joint up to develop a nonseptic degenerative process. However, owners of horses with THO usually do not report any trauma or infection. THO may be a primary degenerative joint disease, but no other joints are affected.

Symptoms due to osteoarthropathy of the temporohyoid joint include difficulty eating, pain around the ear base, head shaking, and acting out under saddle. If fractures occur, this can result in edema and hemorrhage leading to compression of the nearby nerves or direct tearing or stretching of the nerves. Vestibulocochlear nerve damage manifests clinically as vestibular signs, ranging from a head tilt and mild ataxia (incoordination) to recumbency. Signs of facial nerve damage include drooping of the ear, drooping of the upper eyelid, and muzzle deviation. Because the facial nerve is responsible for blinking of the eye, keratoconjunctivitis sicca, or dry eye, and corneal ulcers can also result.

Horses suspected to have THO should have a complete neurologic exam performed by their veterinarian. The gold standard for diagnosis is endoscopy of the gullet pouch, which will reveal enlargement of the top of the stylohyoid at the temporo-hyoid joint. Radiographs are less sensitive than endoscopy and may not be conclusive as a diagnostic test. A CT scan can also detect fractures or disease of the middle ear. MRI can reveal additional information, such as fluid accumulation or structural changes of the parts of the inner ear, which help determine prognosis. Administering local anesthesia in the external ear canal can help differentiate whether the condition is unilateral or bilateral. Brainstem auditory-evoked response testing (BAER) will reveal partial or complete hearing loss. Tympanocochleitis, or aspirating fluid from the eardrum, can identify potential infections and help determine proper antibiotic usage.

Treatment goals for THO include decreasing inflammation, treating possible otitis or secondary infections, treating secondary eye ulcers and dry eye, performing surgery to remove pressure from the temporohyoid joint, and avoiding further trauma such as ear rubbing, passing a nasogastric tube, and dental floats. Anti-inflammatories (phenylbutazone, flunixin meglate, dimethyl sulfoxide) should be administered for 1-2 weeks. If the horse has severe vestibular signs, the corticosteroid dexamethasone can be given to further assist in decreasing inflammation. There is no consensus on antibiotic treatment, but antibiotics effective against Staphylococcus aureus can be given for 2-4 weeks. Gabapentin can be administered to help with neuropathic pain control. Standard treatment for ulcers is indicated if corneal ulcers are present. In cases of facial nerve dysfunction, temporary tarsorrhaphy (suturing part of the eye lid closed to protect the cornea) may be indicated. Originally, temporohyoid osteoarthropathy was treated surgically with removal of part of the stylohyoid bone, but partial disease recurred was seen frequently with this method due to bony healing along the gap of resected bone. The surgery of choice to treat THO is a ceratohyoidectomy, in which the ceratohyoid bone and its connection between the basihyoid and stylohyoid bone is removed to relieve pressure and tension on the temporal bone. Decreased pain has been noted in horses 24-48 hours after this procedure and the bone does not tend to heal back.

The cornes is the outermost layer of the front of the eye and functions as the first line of defense against any foreign material entering the eye. Since the cornea is exposed to the environment, it is very susceptible to irritation, abrasions and foreign bodies. Horses are notorious for hitting their eye on anything they can find such as a broken water bucket clip, loose piece of fencing, broken boards in the stall or nails sticking out in their environment that can lead to a corneal ulcer.

The cornes itself is about 1 millimeter thick, and composed of several layers. Corneal ulcers occur when the outermost layer of the cornea known as the epithelium is disrupted. You should suspect a corneal ulcer in your horse when you see excessive tear production and runny eye, squinting, or a red or swollen eye. If you see any of these symptoms, you should contact your veterinarian immediately to come evaluate the eye. In order to diagnose a corneal ulcer, your veterinarian will need to apply a stain to the eye. If an ulcer is present, this stain will be taken up by the layer of the corneas below the epithelium called the stroma, and will appear as a bright green spot on the eye.

There are multiple types of corneal ulcers that vary in severity and the way they are treated. One of the most important aspects of corneal ulcer care is to never treat them with steroid medications such as ointment that contains dexamethasone, as these suppress the body’s immune response and can result in infection or worsening of already present infection. Ulcers can vary in their depth into the cornea, and can be non-infected, infected or “melting.” A shallow, non-infected ulcer can usually be treated with topical antibiotic medications, atropine and Banamine at home. Atropine is used in the treatment of corneal ulcers to dilate the eye and prevent painful spasms of the ciliary body within the eye. Banamine is administered for pain relief. Small, non-infected ulcers often heal within 7-10 days when treated and identified quickly.

An ulcer that penetrates deeper into the cornea or is infected is significantly more difficult to treat and requires hospitalization with advanced medical interventions. These horses often require eye medication administered every hour or two hours for multiple days. To facilitate ease of treatment and ensure medications are reaching the cornea, a subpalpebral lavage system can be placed. A “SPL” (subpalpebral lavage) is a small, long catheter that is placed through your horse’s upper or lower eyelid to deliver medication directly onto the eye. The catheter is anchored to your horse’s forehead with a few stitches, and weaved through the mane for further security. Small amounts of liquid antibiotics, antifungals and other medications can then be administered through the small catheter port tied in your horse’s mane, instead of attempting to place ointment in a moving target twelve or more times a day. A melting corneal ulcer occurs when the bacteria in an infected ulcer release enzymes called collagenases that cause the the cornea to break down and appear goozy. This is a severe condition, and requires a SPL in addition to treatment with anti-collagenase containing products on top of antibiotics, atropine and Banamine. Melting ulcers may also require surgical intervention to allow for complete healing. The most severe and urgent form of corneal ulcer is one that is so deep there is only a single layer of cells preventing the eye from rupturing. This type of ulcer is termed a desecemetocle, and even small non-infected corneal ulcers can progress to this severity if not correctly identified and treated.

(continued on page 7)
THO – Oh No!
Temporalhyoid Osteoarthropathy in the Horse
By Julie Goodman, DVM (Class of 2018) – Edited by Tim Lescun, BVSc, MS, PhD, Dipl. ACVS

Temporalhyoid osteoarthropathy (THO) is a disease of the hyoid apparatus in the horse, first reported in 1983. The hyoid apparatus is made up of four paired bones (the stylohyoid, epiphysoid, ceratohyoid, and thyrohyoid) and one unpaired bone (the basihyoid); it is located in the throatlatch region and helps to stabilize the larynx, pharynx, and tongue. The hyoid apparatus is connected to the skull by the temporalhyoid joint, an articulation between the stylohyoid bone and the temporal bone of the skull. THO is caused by ankylosis, or fusion, of the temporalhyoid joint, which can then progress to fracturing of the stylohyoid or temporal bone.

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(continued on page 7)
If your horse has been diagnosed with rain rot, don’t fret! Keeping your horse clean and dry and preventing contact with other horses until the lesions are cleared will be essential to properly managing this disease. Topical products such as lime sulfur and chlorhexidine solution can be used to treat the lesions produced by *D. congolensis*. Limiting moisture and skin damage will be key to preventing your horse from ever obtaining rain rot. Caprylic acid and its derivatives may be useful treatment options for rain rot in the future.

**Take Home Messages**

- If you suspect your horse has rain rot, there are multiple ways a true diagnosis can be obtained. The crusts produced from this infection can be used to perform a cytology or culture. These diagnostic methods can help differentiate from similar diseases such as those caused by ringworm or mange.
- If your horse exhibits any signs associated with temporohyoid osteoarthropathy, contact your veterinarian right away. Many of the earlier signs are often associated with dental issues, but keep in mind this lesser known disease process can be used weekly until the lesions have completely disappeared. Penicillin and trimethoprim sulfonamides are antibiotics that have been successful in treating chronic infections. Research has been conducted revealing different methods of treating *D. congolensis*. In a study by Valipe et al. (2009), it was found that caprylic acid, a fatty acid, is a potential alternative treatment option for rain rot. This could become an important therapeutic option as it eliminates the use of antibiotics and the concern for the development of antimicrobial resistance.

**Corneal Ulcers** (continued from page 5)

Corneal ulcers are one of the most common conditions of the eye in horses. It is important to remember that although some ulcers can be treated at home, all suspected ulcers need to be evaluated by a veterinarian and many require advanced medical treatment and hospitalization. If you have questions about equine corneal ulcers or other medical conditions, please contact your veterinarian today.

**References**

- Cornell-Ulcers

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**Corneal Ulcers** (continued from page 5)

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Rain Rot? Surely Not!
By Audriana Finney, DVM Student (Class of 2019)
Edited by Stacy H. Tinkler DVM, MPH, Dipl. ACVIM

THO (continued from page 4)
Prognosis is good with early detection; if neurologic signs are already present, prognosis is reduced to fair. With certa-
ohyoid Actomy, recurrence of the disease has not been noted, but
fear nerve deficits or vestibular signs may persist. Maximum
improvement can take up to two years, but ataxia usually
resolves with surgery. In a study of 33 cases of temporohyoid
ostearthropathy, 19 of the 20 surviving horses returned to
work after treatment. In another study of 24 cases who were
-treated with ceratohyoidectomy, 89% had improved within
a year; the most significant improvement was made six months
after the procedure, but only 50% returned to work
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Equine Ulcers
(continued from page 5)
Equine ulcers are common, they usually heal quickly if treated
appropriately. Many factors can contribute to the development of equine ulcers, such as
environmental conditions, nutrition, and the horse’s health. Preventative measures can help reduce the risk of equine ulcers, such as
avoiding overworking, providing proper nutrition, and keeping the
environment clean. If an ulcer does occur, prompt treatment
is important to prevent further damage and promote healing.

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If your horse has been diagnosed with rain rot, don’t fret! Keeping your horse clean and dry
and preventing contact with other horses until the lesions are cleared will be essential to properly
managing this disease. Topical products such as lume sulfur and chlorhexidine solution can be used
to treat the lesions produced by D. congolensis. Limiting moisture and skin damage will be
key to preventing your horse from ever obtaining rain rot. Captopril acid and its derivatives may be
useful treatment options for rain rot in the future.

Figure 1. Painbrush lesions seen with Dermatophilosis congolensis.
Photo courtesy of Scott and Miller’s Equine Dermatology

Figure 2. Pus under painbrush lesions seen with Dermatophilosis congolensis.
Photo courtesy of Scott and Miller’s Equine Dermatology

Figure 3. Rain rot can be found in places other than the rump and hair loss can be severe.
Photo courtesy of Scott and Miller’s Equine Dermatology

Rain Rot is caused by the bacteria, Dermatophilus congol-
ensis. This organism requires two factors to be successful and prolif-erate: skin damage and moisture. The skin damage can be
from a small cut obtained while at pasture or from biting flies while the moisture can be from sweat not properly groomed
off after a long ride or chronic rain exposure. Dermatophilosis can be passed on from one horse to the other via the crusts
produced from these lesions. As previously stated, these lesions
look like tufts of hair and are sometimes referred to as looking like paintbrushes (Figure 1). If these clumps of hairs are plucked
off, the underneath skin can have pink areas of irritation, or in more severe cases, a thick creamy pus (Figure 2). When these
lesions heal, it may appear as areas of hair loss or scaling of the skin. Rain rot does not just develop on the rump of horses,
but can be found in the saddle area, nose and legs (Figure 3). Dermatophilosis is rarely transmitted to people and mainly in tropical
areas. If you suspect your horse has rain rot, there are
multiple ways a true diagnosis can be obtained. The crusts pro-
duced from this infection can be used to perform a cytology or
culture. These diagnostic methods can help differentiate from
similar diseases such as those caused by ringworm or mange. The environment your horse is living in will greatly affect
the management of rain rot. For example, horses living in a
more humid climate will be more difficult to manage than a
horse living in a cooler, drier environment. If horses with rain rot are kept in a dry environment for 4 weeks, then most of
these cases will actually regress on their own and no treatment will be needed. More proactive treatment involves removal
of the crusts followed by application of topical treatments.
In more chronic infections, systemic therapy with antibiot-
ics can be used. Common topical products utilized are 2-5%
lime sulfur dip or 4% chlorhexidine solution applied to the
affected areas for 3-5 days. These products can come in either
entire body shampoo or dips to be applied and left on the
skin. Once the lesions are clearing up in a specific area, the
topical products can be used weekly until the lesions have
completely disappeared. Penicillin and trimethoprim sulfon-
amides are antibiotics that have been successful in treating
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References:
The Equine Sports Medicine Center

Purdue’s Equine Sports Medicine Center is dedicated to the education and support of Indiana horsemen and veterinarians through the study of the equine athlete. The Center offers comprehensive evaluations designed to diagnose and treat the causes of poor performance, to provide performance and fitness assessments, and to improve the rehabilitation of athletic horses. Other integral goals of the Center are to pioneer leading-edge research in the area of equine sports medicine, to provide the highest level of training to future equine veterinarians, and to offer quality continuing education to Indiana veterinarians and horsemen. For more information visit our website:

www.vet.purdue.edu/esmc/
A Tale of Foxtail – *Setaria* and its Effect on Equine Husbandry

By Taylor Huffman, DVM Student (Class of 2019)
Edited by Amanda Farr, DVM, Dipl. ABVP – Equine

**Foxtails** are members of the *Setaria* genus in the grass family. The two most common species in Indiana are *Setaria viridis* (green bristle grass) and *Setaria pumila* (pigeon grass). The structure of these plants includes a grassy stem and a seed dispersing aid known as a diaspore, or spikelet, whose appearance resembles a fox’s tail (Figure 1). This diaspore is made up of small barbs that cling to animal fur, skin, or clothing (Figure 2). Once the barb is caught and embedded, it is very difficult to remove.

In the horse world, foxtails are primarily a problem when they are present in hay (Figure 3). Foxtail grows best during times of drought as it is adapted to high temperatures and prolonged sun exposure. Horses can consume the stem of the foxtail without adverse effect, but when the diaspore (seed head) is consumed, the barbs detach and become lodged in the gums, lips, and other oral mucosa. Continued consumption of foxtail-contaminated hay or pasture leads to mucosal irritation with clinical signs including excessive salivation, difficulty in chewing/eating and a foul oral odor. In severe cases it can lead to complete inappetence due to inflammation and ulceration of the gums, tongue and other oral mucosal surfaces (Figure 4).

(continued on page 2)
Foxtail – *Setaria* (continued from cover)

Once foxtail barbs are found in a horse’s mouth, the treatment depends on the severity of the clinical signs and the horse’s level of discomfort. The first step of treatment is the removal of the source, which is primarily hay. If the horse is not displaying signs of discomfort, this may be sufficient to resolve the problem. Once foxtail barbs have become lodged in the gums, lips, and tongue of your horse, they are difficult to remove completely. Studies have shown that following removal of the majority of the foxtail barbs, horses with associated oral ulcers completely resolve within 1-2 weeks. In cases of severe ulcerations, a rinse of the oral cavity using Listerine, combined with the mechanical removal of the barbs with gauze, towels or surgical debridement may be appropriate. While the barbs will eventually work themselves out, a horse with severe mucosal irritation will benefit from a sedated barb removal and flushing of the ulcers, allowing the affected tissue to begin healing more rapidly (Figure 5).

Foxtail consumption is a significant problem that affects the equine community, and it is important to remember that not all horses that consume foxtail-infested hay will be affected in the same way. Just as with bee stings or drug reactions, all horses respond uniquely to the foxtail they ingest, and rarely are all horses in a barn affected. It is important to remember to monitor roughage for the presence of foxtail and be prepared to treat affected animals if foxtail is fed intentionally or accidentally. As hay consumption begins or increases in late fall and winter, this is the most common time to see foxtail barb complications. For this reason, we recommend checking the oral cavity for foxtail ulcerations once hay is fed continuously and contacting your veterinarian if oral pain or ulceration is noted.

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References:

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My name is Ahmed Khairoun. I was a senior clinician at the American Fondouk Working Equid Hospital in Fez, Morocco before I had an offer to start my Large Animal Internship at Purdue University. My ultimate goal is to become a surgeon to bring these skills back to Morocco in order to teach the next generation of Moroccan equine vets. I am happy to know that I can use my knowledge to save equine lives, improve human livelihoods and improve equine surgery capability in my country.

I studied veterinary medicine at the IAV in Rabat, Morocco and after that I completed 3 equine internships. The first was at the American Fondouk in Fez, Morocco, the second was completed at the University of Lyon, France, and my last one was in private equine surgical practice at Milton Equine Hospital in Canada. During my internships on these 3 different continents, I have realized that each presents with its different challenges and I continue to learn a wide array of procedures.

Apart from equine surgery my main passion is playing violin, especially Andalusian music. Performing for people is a truly lovely feeling.

Dr. Fallon Segarra was raised in Clifton, Virginia. She received undergraduate degrees in Biology and Studio Art from Stanford University in 2012, and a Doctorate of Veterinary Medicine from Auburn University College of Veterinary Medicine in 2016. She began her veterinary career with a sports medicine focused internship at Peninsula Equine Medical Center in Menlo Park, California and continued it with a second equine focused internship at the University of Missouri Veterinary Health Center in Columbia, Missouri. This past July marked the start of her Large Animal Surgery Residency at Purdue, for which she is extremely excited. Her professional interests include all aspects of large animal surgery, particularly equine and bovine orthopedics. In her spare time she enjoys hanging out with her fiance and their (four!) dogs, riding her horse, and crocheting.

Jose Ignacio Goñi is a 2015 graduate of Rio Cuarto National University in Argentina. He was born and raised in General Levalle in the center of Argentina. He grew up riding horses on his family’s farm and started working on a Polo horse breeding farm when he was 13 years old. He worked at the Equine Reproduction laboratory at the university, at Kawell, an equine hospital that is located in Buenos Aires, primarily in the Neonatal Intensive Care and Intensive Care Units, and at Park Equine Hospital during 2016. In 2017, he completed a sixth-month fellowship in internal medicine at Hagyard Equine Medical Institute in Kentucky, and then moved to the University of Georgia to complete a one-year rotating internship. In July 2018, he moved to Purdue University to start working as a first-year large animal internal medicine resident. He enjoys riding horses, playing soccer with friends, watching polo and soccer, being outdoors, and spending time with family and friends.

Dr. Kira Tyson is originally from Ridgecrest, California. She grew up with horses, dogs, birds, and reptiles. She has lived in Hawaii, Oklahoma, Washington, and Indiana. She received her undergraduate degree in Animal Science from Oklahoma State University, where she graduated with honors. She received her Doctor of Veterinary Medicine from Purdue University. She remained at Purdue University for a Large Animal Internal Medicine specialty internship following graduation. It was during her internship that she developed a strong interest in large animal internal medicine. She completed her first year of residency at Washington State University. She is happy to be back at Purdue as a second year resident and to complete her training. Her interests include infectious disease, neurology, and neonatology. In her limited spare time, she enjoys hiking, camping, and kayaking.

My name is Ahmed Khairoun. I was a senior clinician at the American Fondouk Working Equid Hospital in Fez, Morocco before I had an offer to start my Large Animal Internship at Purdue University. My ultimate goal is to become a surgeon to bring these skills back to Morocco in order to teach the next generation of Moroccan equine vets. I am happy to know that I can use my knowledge to save equine lives, improve human livelihoods and improve equine surgery capability in my country.

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A Tap at a Time: Recognizing the Risks of Joint Injections

By Cody Schnur, DVM Student (Class of 2019) – Edited by Sarah Waxman, DVM, MS, Dipl. ACVS

Arthrocentesis (the process of injecting or “tapping” a joint) is commonly practiced by veterinarians for two key reasons: (1) to assist in diagnosing lameness by anesthetizing (“blocking”) the joint and (2) to therapeutically medicate an arthritic or painful joint (Figure 1). Joint injections can greatly improve an arthritic horse’s comfort by decreasing active inflammation and pain, improving the overall synovial (joint sac) environment, and protecting existing cartilage. Regardless of the benefits, there are potential complications that accompany arthrocentesis, including joint infection, joint flare, laminitis, and articular cartilage degeneration. It is imperative to understand these risks prior to having your horse’s joint(s) injected and to quickly recognize if these problems become apparent in your horse.

Joint infection (septic arthritis) is a rare complication of arthrocentesis that has been reported to occur at a rate of 2.1 in 10,000 injections (0.021%). Because infection occurs secondary to bacteria entering the joint during the procedure, the risk of infection is kept low when appropriate technique and preparation of the injection site is practiced and the horse is not excessively dirty, sweaty, nor has signs of skin infection (dermatitis). Interestingly, hair removal at the injection site is associated with an increased risk of contaminating the joint with hair fragments. These fragments can increase the chance for an infection so clipping before tapping is not recommended in most situations. Some veterinarians prefer to administer antibiotics into the joint such as amikacin or gentamicin along with the joint medication to help prevent bacterial contamination. Signs of joint infection can include pain, heat, stiffness, and swelling of the joint and surrounding soft tissues (Figure 2) as well as severe lameness most commonly within 2-5 days following an injection, however signs can be delayed up to a week or longer depending on what intra-articular medication was used. It is vital to recognize this risk as it can devastate joint function and potentially end a horse’s career or even life. This condition is treatable with a good chance for successful outcome as long as it is recognized and treated quickly.

Joint flare (reactive synovitis) is an inflammatory reaction that may occur in response to introducing any chemical substance (such as a medication) into a joint. Unlike a joint infection, a joint flare is not caused by bacteria, so it cannot be prevented with antibiotics. Signs are similar to joint infection and include mild to severe lameness, pain, and swelling of the joint typically within 24-48 hours of an injection. Some clinicians suggest administering a concurrent systemic NSAID (non-steroidal anti-inflammatory) at the time of tapping in order to reduce the risk for flare. This condition is much less threatening but does require urgent treatment.

Laminitis (inflammation of the hoof wall) might be linked to injecting high doses and/or frequent doses of corticosteroids (potent anti-inflammatory such as dexamethasone or triamcinolone) or use of corticosteroids in horses with pre-existing metabolic disease (such as Equine Cushing’s (PPID) or EMS).
Human dentistry begins with toddlers and equine dentistry should too! Young horses go through many phases of development which not only includes growing in size and maturity, but also advancing from baby (deciduous) teeth to adult (permanent) teeth. There are four arcades of teeth: two upper (maxillary) and two lower (mandibular) arcades in your horse’s mouth. Between birth and around 2 ½ years of age, 24 baby teeth will erupt. These 24 teeth will be replaced with 36-44 adult teeth between 2 ½ and 5 years of age. With so many teeth erupting, many developmental problems can occur. Your veterinarian should perform an oral exam twice yearly during this developmental phase.

Equine incisors (the front teeth) erupt in the foal as early as prior to birth to several days following birth. The twelve (six upper and six lower) incisors erupt in a fairly specific pattern, with the central incisors erupting first, then the second incisors, and finally the third incisors. Veterinary students are taught the eruption dates of “6 days, 6 weeks, 6 months,” which are roughly accurate in most foals. The adult or permanent incisors erupt in a similar pattern from the central incisors to the outer incisors at 2 ½ years, 3 ½ years, and 4 ½ years. The permanent teeth erupt behind the deciduous (baby) teeth, and should force the deciduous teeth out. When this doesn’t occur, the deciduous tooth is considered “retained”. Retained deciduous incisors can lead to permanent incisor irregularities (Figure 1).

In the interdental space (between the incisors and premolars) canine teeth develop. These erupt primarily in male horses but can also be present in mares. They are sharp, deep-rooted teeth and are frequently present in pairs. They are most common on the lower jaw. Canines that erupt on the upper arcades are often confused with the wolf teeth. The first premolar (behind or caudal to the canine) is called the “wolf tooth,” and due to potential interference with the bit it is commonly removed in young horses prior to training. They are not present in all horses, although most zebras and zebra-crosses develop all four wolf teeth.

Premolars are the first 3 cheek teeth, per side, top and bottom. These also initially erupt as baby or deciduous teeth. They erupt between birth and 2 weeks of age. They are replaced by adult premolars between 2 and 3 ½ years of age. The adult teeth erupt directly above or below the baby teeth. If the baby tooth is not shed, the permanent tooth will push it out further, creating a “tall” tooth. This tall tooth can wear down the opposing (or opposite) tooth, creating a wave or inhibiting normal grinding of feed. Removal of the retained baby tooth, called a “cap,” can reduce this damage and allow normal development of the opposing teeth.

Molars are the back (caudal) three teeth on each side, top and bottom. These teeth only erupt as adult or permanent teeth. They typically appear between 9 months and four years of age. It is not uncommon for young horses around the age of 3 or 4 to develop swelling along their jaw as these teeth erupt, causing minor inflammation in the mandible.

When your veterinarian starts foal vaccinations around six months of age, please make sure they examine your foal’s mouth. Additional abnormalities that can occur include a parrot mouth (over-bite) or a monkey mouth (under-bite) which may result in the incisors becoming overgrown due to abnormal grinding problems (Figures 2a, 2b).

Polyodontia (which means additional teeth) is another abnormality that is most commonly present in the incisors and also behind the last molar of the upper (maxillary) arcade. In most cases, these are not noted until the adult teeth are erupting. Dental dysplasia is another deformity which involves the abnormal development of some aspect of the tooth. It can vary in the degrees of severity and may involve the crown, root, or the entire tooth.

(continued on page 6)
Dental Exams (continued from page 5)

It is clear that with the eruption of 24 baby teeth over the first two years of life, and their replacement with 36-44 adult teeth over the next 2-3 years, young horses have the opportunity for many dental developmental disorders. It is important to involve your veterinarian from 6 months of age onward. Once to twice annual exams can identify and correct many of these abnormalities that can occur during growth, leading to better dentition and fewer problems as they age and become working horses.

References:

Joint Injections (continued from page 4)

Although the exact manner in which corticosteroids induce laminitis has yet to be determined, it is a theoretical risk of which owners should be aware. Signs of laminitis vary but may include an unnatural stance, reluctance to move, excessive weight shifting, a hot hoof wall, anxiety, and sweating. This condition is also considered an emergency and requires urgent treatment.

Articular cartilage degeneration (breakdown of joint cartilage) becomes a concern in horses that receive multiple, high-dose corticosteroid injections (products including Celestone® Soluspan®, Depo-Medrol®, and Vetalog®). Repetitive use of certain steroids and other steroid medications at high concentrations can have a detrimental impact on chondrocytes (cells that make cartilage), causing damage to and loss of the normal cartilage within the joint. Repeated doses of corticosteroids in vigorously exercised horses is particularly unfavorable due to the heightened risk of aggravating performance-related joint wear and tear. To avoid this phenomenon while achieving an optimal result, many veterinarians use lower corticosteroid doses in combination with a few days to weeks of rest following an injection.

Take-home points:
- There are a few risks of having joint injections performed on your horse but fortunately most of them are exceedingly rare.
- Precautions taken by your veterinarian should decrease the odds of your horse getting an infection, flare, laminitis, or articular cartilage degeneration.
- Should your horse develop any of the above mentioned signs following a joint injection, you must contact your veterinarian immediately, as these problems are considered an emergency. For more information about joint injections please get in touch with your veterinarian on an elective basis.

References:
Asthma: A Common Problem in Racehorses and Cause of Poor Performance

By Drs. Laurent Couëtil, DVM, PhD, Dipl. ACVIM and Kathleen Ivester, DVM, PhD, Dipl. ACVS

A study conducted by researchers from the Purdue University College of Veterinary Medicine at the Thoroughbred racetrack in Shelbyville, Indiana (Indiana Grand Racing & Casino) recently shed some light on a frustrating cause of decreased performance: horse asthma. Racehorse owners and trainers have known for some time that when horses have lots of mucus in the airways or cough, performance is typically not optimal. However, a study led by Dr. Laurent Couëtil from PVM Equine Sports Medicine Center has shed new light on this disease that is for the most part “subclinical,” that is to say, difficult to detect because horses look healthy otherwise. They eat well and train fine, but they just can’t win!

The study was funded by the Grayson Jockey-Club Research Foundation and the state of Indiana and PVM research account funded by the total wager tax. The researchers hypothesized that exposure to high levels of dust was responsible for lung inflammation and that inflammation in turn would cause decreased performance. The goal was to enroll 100 Thoroughbred racehorses and to examine them approximately 1 hour after racing. This exam included a “lung wash” in order to collect cells from the deep lung to determine airway health. A few days later each horse was equipped with air sampling devices in order to collect dust around the horse’s nose for about 6 hours while the horse was going about its normal daily routine in the stall. The purpose was to compare dust exposure to lung health and evaluate the impact on race performance.

The researchers were able to examine horses after 98 races thanks to the tremendous support from 8 trainers. The finding showed that approximately 80% of racehorses had evidence of low grade inflammation in their lungs and that the greater the lung inflammation, the poorer horses performed. Importantly, they found that the greater the dust exposure, the worse the lung inflammation. It was not just any type of dust, but what is called “respirable dust” or particles that are less than 4 microns in diameter and are too small to be seen with the naked eye. Also, mold levels in airborne dust were associated with a certain type of airway inflammation. Because respirable dust particles are small, they can penetrate deep into the lung, so it makes sense that exposure to high levels would be associated with lung irritation. The difficulty is that if you can’t see those small particles, it is difficult to know when levels are too high unless sophisticated equipment is placed on horses. Findings from this study were published in the Journal of Veterinary Internal Medicine last September (https://onlinelibrary.wiley.com/doi/full/10.1111/jvim.15226).

Additionally, the study examined the potential role of infectious agents such as viruses and bacteria. Preliminary results showed that common respiratory viruses were not associated with lung inflammation or performance. Work is still ongoing to examine the potential role of bacteria in lung inflammation.

Drs. Couëtil and Ivester have again received funding from the Grayson Jockey-Club Research Foundation to continue this work by examining the effect of low-dust forage on lung health. They started this second study at the Shelbyville racetrack this past summer and recruited 28 horses to be fed either regular hay or low-dust forage such as haylage or steamed hay for 6 weeks and evaluated lung health before and 3 and 6 weeks after being fed the different type of forages while measuring dust exposure of horses. The research team will be back to Shelbyville racetrack next summer 2019 to complete the study and they hope to be able to enroll a total of 60 horses. If you wish to participate in the study, please contact Dr. Couëtil at the Purdue Large Animal Hospital 765-494-8548.

In conclusion, studies conducted by Purdue researchers showed that exposure to small dust particles is responsible for airway irritation in racehorses and in turn, this is likely to impact horse’s performance negatively. Future work is aimed at finding ways to mitigate dust exposure allowing horses to compete at their optimal level. Stay tuned…

References:
The Equine Sports Medicine Center

Purdue’s Equine Sports Medicine Center is dedicated to the education and support of Indiana horsemen and veterinarians through the study of the equine athlete. The Center offers comprehensive evaluations designed to diagnose and treat the causes of poor performance, to provide performance and fitness assessments, and to improve the rehabilitation of athletic horses. Other integral goals of the Center are to pioneer leading-edge research in the area of equine sports medicine, to provide the highest level of training to future equine veterinarians, and to offer quality continuing education to Indiana veterinarians and horsemen. For more information visit our website:

www.vet.purdue.edu/esmc/
Research Projects in Progress Supported with Pari-Mutual Funds:

- **Dos Santos AP, Taylor SD, Woolcock A, Christian JA, Ruple A.** Validation of a Novel Assay to Detect Intraerythrocytic Reactive Oxygen Species (ROS) by Flow Cytometry in Horses.


- **Hendrix K, Kritchevsky J.** Recovery of Salmonella bacterial isolates from pooled equine fecal samples.

- **Lescun T, Main RP.** Validation of an in vivo assessment of fracture risk in equine limb bones.


- **Little D, Lescun T.** Mechanosensitive Channels in Equine Musculoskeletal Soft Tissues.

- **Main RP, Lescun T, Lim CK, Durkes A.** Assessing Fracture Susceptibility in Horse Limb Bones: A Pre-Clinical Study.

**Preliminary report:** Validation of a novel assay to detect intraerythrocytic reactive oxygen species (ROS) by flow cytometry in horses

Dos Santos, Andrea; Taylor, Sandra; Woolcock, Andrew; Christian, John; Ruple, Audrey

1. Development of the proposed methodology

Only healthy horses as determined by physical examination and absence of significant abnormalities from laboratory data (complete blood count and chemistry panel) were included in this study. Whole blood EDTA samples were collected from jugular venipuncture and analyzed within two hours of blood collection. All samples for specificity levels two and three, precision, and stability assays were identically prepared. The samples were divided in:

- Unstimulated vehicle control
- Stimulated vehicle control
- Unstimulated DCFH-DA
- Stimulated DCFH-DA

Stimulation was obtained with 2 mM hydrogen peroxide (H₂O₂, Sigma Aldrich, St. Louis, MO, USA). Unstimulated samples were obtained by the addition of phosphate saline buffer (PBS). DCFH-DA (Sigma Aldrich) was diluted to 50 mM stock solution with dimethyl sulfoxide (DMSO, American Bioanalytical, Natick, MA, USA). Ten microliters of DMSO (vehicle control) or DCFH-DA at 500 µM were added to 5 mL round bottom tubes (brand). The blood samples were centrifuged 3,000 x g for 5 min at room temperature (Sorvall Legend X1R, Thermo Scientific, Whaltam, MA, USA). Plasma anduffy coat were removed with a Pasteur pipette. Ten microliters of red blood cells were diluted in 5 mL of PBS supplemented with 1% bovine albumin (PBSA, Calbiochem, Darmstadt, Germany). One hundred microliters of the red blood cell (RBC) solution were added to the respective tubes. The cells were incubated at 37°C for 20 min.

After incubation, 10 µL PBS were added to the unstimulated cells and 10 µL 20mM hydrogen peroxide solution were added to the stimulated cells. After 20 min incubation at room temperature, the samples were quenched with 300 µL 1% PBSA and immediately analyzed by flow cytometry (BD Accuri™ C6 flow cytometer, Becton, Dickinson and Company, Franklin Lakes, NJ). ROS-dependent fluorescence intensity were detected by green fluorescence with an excitation wavelength of 488 nm (FL1 channel) with gating around RBCs only (gates were determined in specificity level one assay).

1.1 Specificity assays

1.1.1 Specificity level one

Because DCFH-DA is not cell-specific, the fluorescence specificity to mature erythrocytes was determined based on correct gating and appropriate removal of “contaminant” cells, in this case, leukocytes and platelets. For this purpose, three EDTA whole blood samples were collected from three clinically healthy horses. Platelet-rich plasma (PRP) was obtained after the blood was allowed to settle for 20 minutes at room temperature. The plasma was transferred to another tube and centrifuged at 300 x g for 10 minutes at room temperature, and the supernatant corresponded to the PRP. Leukocytes were obtained after the buffy coat was removed from a second EDTA sample and transferred to 10 volumes of a RBC lysis solution (0.15 mM NH₄Cl, 10 mM NaHCO₃, 0.1 mM EDTA, pH 7.4). After incubation on constant shaking for 10 minutes at room temperature, the sample was centrifuged at 3,000 x g for 5 minutes at room temperature and the pellet was resuspended again in 10 volumes of RBC lysis solution. The incubation and centrifugation was repeated and the supernatant was resuspended in 5 mL PBSA 1%. One third tube was used to obtain a RBC solution as described above. Samples from each horse were ran in duplicates and divided as described above (unstimulated vs. stimulated, vehicle vs. DCFH-DA). The samples were used to determine correct gating. The gates were saved as a template and used to analyze all remaining samples in this study.

1.1.2 Specificity level two

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To ensure that the cellular fluorescence reflects ROS generation, a series of triplicate samples from three different horses were incubated with 5 mM of sodium azide (NaN₃, Sigma Aldrich) for 10 minutes after incubation with vehicle or DCFH-DA. Sodium azide inhibits CAT, the enzyme responsible for the transformation of H₂O₂ to H₂O and O₂. After incubation, the erythrocytes were stimulated with H₂O₂ (as described above) or remained unstimulated.

1.1.3 Specificity level three
Level three determined the assay specificity in a dose-dependent manner. Samples from three different horses were incubated with 0 (vehicle only), 1, 5, 10, 50 or 100 µM DCFH-DA. Secondly, samples were incubated with increasing dilutions of H₂O₂ from 0 (PBS) to 6 mM with 2 mM increments.

1.2 Precision assay:
Precision were determined based on the closeness of agreement between independent test results of intra and inter-assay repeatability. Stimulated and unstimulated samples and samples with and without DCFH-DA (as described above) from three different horses were run in triplicate for intra-assay precision, and five different analytical runs were performed for inter-assay precision.

1.3 Stability assay
The capability of the samples to retain the initial measurement over time was determined by measuring fluorescence at 3, 6, 24, 36 and 48 hours post-collection of three different horses. The samples were kept refrigerated (4°C).

1.4 Statistical analysis
Data were compared with two-way ANOVA corrected for multiple comparisons with Tukey's post-hoc. Alpha was set at 0.05. Statistical software used was Prism 7.03 for Windows (GraphPad Software Inc, La Jolla, CA, USA). Nonlinear regression using a robust fit method was used to compare increasing concentration of DCFH-DA. For precision assay, acceptable coefficient of variation (CV) was ≤10% for intra-assay and ≤15% for inter-assay. For stability assay, acceptance criteria were 20% change from baseline.

2. Preliminary results
2.1 Specificity assays
2.1.1 Specificity level one
In the PRP samples (Figure 1), most of the platelets were activated, which can be observed by the narrowed shape of the events in the corresponding logarithmic forward (FSC-A) and side scatter plots (SSC-A), therefore, the gate were determined based in previous experiments (data not shown). To confirm the correct location of the platelet gate, non-activated platelets need to be obtained by a new blood sample, slowly collected into a 3.8% citrate anticoagulant syringe, maintaining a ratio of 9:1 blood:citrate, followed by immediate process without refrigeration to avoid activation.

In the leukocyte samples, in the logarithmic FSC-A vs. SSC-A plots, it is possible to observe an overlap within the RBC cloud and a large amount of either platelets or small fragments generated during lysis procedure. When the events are plotted in a linear FSC-A vs. SSC-A, the characteristic clouds of granulocytes and lymphocytes can be easily identified (Figure 1).

2.1.2 Specificity level two
It was expected that the presence of sodium azide would cause a marked increase in fluorescence. Indeed, the percentage of cells positive for green fluorescence (Table 1) after stimulation with hydrogen peroxide was increased in comparison with cells that was not incubated with azide (p = 0.0027), while the azide exerted no effect on unstimulated cells.
Figure 1: Logarithmic forward (FSC-A) and side scatter plots (SSC-A) with platelet gate (PLT, blue), red blood cells gate (RBC, red), and leukocyte gate (Leukocyte area A, green) in a representative sample of PRP (upper left plot), RBCs (upper middle plot), and leukocytes (upper right plot). Linear forward (FSC-A) and side scatter plot (SSC-A) from the events only within the leukocyte gate from the upper right plot (lower left plot) is represented, where the round cloud represent granulocytes and the oval lower cloud represent lymphocytes. In the RBC samples (lower middle plot), a constant cloud of events were observed in the same FSC vs. SSC location along all horses and repeats. A variable number of smaller events are noted, and they most likely represent remaining platelets in the sample or RBC fragmentation. When hydrogen peroxide is used to stimulate the cells, morphologic changes in the cells can be observed (lower right plot).

Table 1: Percentage of cells (mean ± standard deviation) positive for 2′,7′-dichlorofluorescein (DFC) in a flow cytometric evaluation of erythrocytes incubated with or without 5 mM of sodium azide and stimulated with 2 mM of hydrogen peroxide or unstimulated (PBS).

<table>
<thead>
<tr>
<th></th>
<th>Unstimulated</th>
<th>Stimulated</th>
</tr>
</thead>
<tbody>
<tr>
<td>With sodium azide</td>
<td>0.23 ± 0.09</td>
<td>59.29 ± 6.56</td>
</tr>
<tr>
<td>Without sodium azide</td>
<td>0.21 ± 0.14</td>
<td>74.19 ± 12.61</td>
</tr>
</tbody>
</table>

2.1.3 Specificity level three

The specificity level three assay evaluated the effects of increasing concentrations of DCFH-DA and hydrogen peroxide in the generation of fluorescence. Increasing concentrations of DCFH-DA caused increasing percentages of cells positive to DFC (Table 2). However, the increased fluorescent signal was not linear to the concentration of DCFH (Figure 2). Also, the DCFH-DA concentration effect is statistical significant (p < 0.0001). The effect of increasing concentrations of hydrogen peroxide is not statistical significant (p = 0.3011) in the cells located within the RBC gate.
Table 2: Percentage of cells (mean ± standard deviation) positive for 2′-7′-dichlorofluorescein (DFC) in a flow cytometric evaluation of erythrocytes incubated with increasing concentrations of hydrogen peroxide (H$_2$O$_2$, columns, 0, 2, 4, and 6 mM) and increasing concentrations of 2′-7′-dichlorodihydrofluorescein diacetate (DCFH-DA, rows, 0, 1, 5, 10, 50, and 100 µM). Non applicable (N/A) indicate combinations of peroxide and DCFH-DA concentrations that were not performed.

<table>
<thead>
<tr>
<th>DCFH-DA</th>
<th>H$_2$O$_2$</th>
<th>0 mM</th>
<th>2 mM</th>
<th>4 mM</th>
<th>6 mM</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 µM</td>
<td>0.47 ± 0.68</td>
<td>N/A</td>
<td>N/A</td>
<td>0.52 ± 0.10</td>
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</tr>
<tr>
<td>1 µM</td>
<td>N/A</td>
<td>6.27 ± 0.11</td>
<td>5.25 ± 1.60</td>
<td>3.95 ± 0.37</td>
<td></td>
</tr>
<tr>
<td>5 µM</td>
<td>N/A</td>
<td>19.70 ± 1.51</td>
<td>18.70 ± 1.60</td>
<td>15.66 ± 0.32</td>
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</tr>
<tr>
<td>10 µM</td>
<td>N/A</td>
<td>23.85 ± 1.65</td>
<td>23.68 ± 3.84</td>
<td>23.28 ± 2.43</td>
<td></td>
</tr>
<tr>
<td>50 µM</td>
<td>N/A</td>
<td>46.53 ± 4.08</td>
<td>45.41 ± 3.08</td>
<td>45.43 ± 1.72</td>
<td></td>
</tr>
<tr>
<td>100 µM</td>
<td>0.48 ± 0.35</td>
<td>68.15 ± 5.96</td>
<td>66.37 ± 3.54</td>
<td>71.47 ± 6.71</td>
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</tbody>
</table>

Figure 2: Nonlinear robust fit curve of percentage of cell positive for 2′-7′-dichlorofluorescein (DFC) with incremental concentration of 2′-7′-dichlorodihydrofluorescein diacetate (DCFH-DA; 0, 1, 5, 10, 50, 100 µM) stimulated with 6 mM hydrogen peroxide evaluated by flow cytometry.

2.2 Precision and Stability

Data were collected, but statistical analysis was still not performed.

3. Conclusions

Even though there is overlap of platelets and leukocytes within the RBC gate, the procedure of plasma and buffy coat removal in addition to the high number of events collected (50,000 events within the RBC gate) ensures specific analysis of erythrocytes only with the adopted protocol in this study of ROS detection in equine erythrocytes. Although DCFH-DA is not specific for any cell, it is specific for ROS formation within the cell, as demonstrated by the results of the specificity assays level two and three.

4. Future directions

The 32 horses that belong to the Purdue Teaching Herd are being currently tested in order to evaluate the variability of baseline measurements in a clinically healthy population. The herd is composed of 23 mares and nine geldings, mean age 16±7.5 years old (range 1-26), and from 11 different breeds (9 Quarter Horse, 8 Thoroughbred, 6 Standardbred, 2 Appaloosa and Tennessee Walking Horse, and 1 each American Miniature Horse, Arabian, Paint Horse, Pinto, Saddlebred, and mixed breed). So far, 19 animals were tested and the data was still not analyzed. The last data collection is expected to occur between 12/02/2018 and 12/21/2018, while the data analyses are expected to occur on January 2019. This pilot study will be the base for a next proposal where horses will be evaluated at baseline and after submaximal exercise (treadmill or race) in order to evaluate the production of intraerythrocytic free radical during intense exercise.
Title: Enhancing the repair potential of equine-derived MSC for treating post-traumatic osteoarthritis (PTOA)

Investigators: Marxa Figueiredo (PI, BMS), Tim Lescun (Co-I, VCS), and Jeff Gimble (Co-I, LaCell, Inc.)

Date: 12/14/2018

PROGRESS REPORT

Our hypothesis in this project is that equine mesenchymal stromal/stem cells (eqMSC) can be primed for enhanced chondrogenic and anti-inflammatory activity using novel small molecules for preventing post-traumatic osteoarthritis (PTOA) progression. This hypothesis was proposed to be tested with the following specific aims.

Aim 1. To promote enhanced chondrogenesis, we will examine whether eqMSC can be primed using novel small molecules or chondrocompounds (ChC); and Aim 2. To enhance anti-inflammatory activity, we will examine whether eqMSC can be primed using novel ChC.

Preliminary Results from the Pilot Study

In the past seven months of this Pilot Project in Equine Clinical and Translational Research, we have collaborated with Dr. Lescun to identify horses euthanized for unrelated reasons to the study, and have worked with him to collect tissues from sternum and flank for BM-MSC and ASC isolation, as well as synovium. MSC tissues were sent to LaCell, and ASC and BM-MSC were isolated by Dr. Gimble, expanded, and sent back to our lab. Our lab isolated the synoviocytes in collaboration with Dr. Lescun.

Aim 1 progress. Our pilot data indicate that compound C3 and its analog 02-09 promote a partial chondrogenic response in equine MSC pellets. Although there is promise in chondrogenic priming of equine ASC/BM-MSC, with gene expression changes consistent with cartilage differentiation (upregulation of Col2a1, Aggrecan (ACAN), SOX9, and COMP at many timepoints), at other timepoints, C3 and 02-09 also could induce genes that can be related to hypertrophic cartilage differentiation (Coll10a1, Coll1a1; Fig. 1). This pilot data suggests that equine ASC and BM-MSC are both

Fig. 1. Pilot gene expression data of Equine MSC micromass (3D) pellets in response to drugs. In Equine ASC, compound C3 promotes early (day 7) expression of cartilage-specific genes (Sox9, Acan, Comp, Col2a1) by quantitative real time PCR, while its analog 02-09 promotes later expression of Col2a1. Some hypertrophic chondrocyte genes are induced (Col1a1 and Coll10a1). In equine BM-MSC, C3 promotes later gene expression, while analog 02-09 promotes cartilage gene expression at day 7 and day 21, however, hypertrophic genes also are induced (Col1a1, Col10a1). This suggests further optimization of eqMSC chondrogenesis by C3 and analog 02-09 is needed. DMSO (-control), KRT (+control). Color bar, range of up- or down-regulation of gene expression.
promising for promoting partial chondrogenic differentiation in equine cells in these one-cell type MSC micromass (3D pellet) models. Since this preliminary data suggests a partial differentiation capacity for the stem cells, our current approach is that we are repeating the real-time qPCR experiments to confirm the gene expression observed and to have more statistical power to derive more conclusive data that will be publishable at the level of a peer-reviewed journal. Another donor(s) may be added in an independent experiment to examine the efficacy of the drugs in promoting cartilage-specific genes in an independent set of experiments if time allows.

**Aim 2 progress.** Our pilot data indicate that compound C3 and its analog 02-09 promote a partial anti-inflammatory response in equine synoviocytes (Fig. 2). C3 significantly downregulated IL-6 gene expression but not IL-1b, while the 02-09 analog downregulated IL-1b but not IL-6 significantly relative to DMSO control. In combination, our compounds mimic the PEDF peptide mimic P18 (Fig. 2), and represent an improvement over Kartogenin (KRT), a molecule under preclinical translation for OA. We are in the process of repeating this experiment and the real-time PCR analyses and plan to include additional experiments that expand on testing C3 and these compounds also with other physiological stimuli for activating synoviocytes such as IL-1b and TNF. The current goal will be to observe downregulation of both inflammatory genes with an escalating dose range of compounds if time allows.

**Other activities and immediate goals.** Our ultimate goal by the end of the pilot project is to have confirmed through independent experiments that cartilage-specific gene expression has been modulated in both fat and bone marrow stem cells, and that the anti-inflammatory gene expression has been modulated in synoviocytes, with appropriate statistical analyses, and to prepare a manuscript for submission to a peer-reviewed journal in collaboration with the co-investigators (Lescun and Gimble). We have also applied for two grants relating to this project, i.e. an Equine Fellowship for a PhD student, and 2-year CFP ERAB support in order to help enable potential continuation and expansion of the scope into a project that could be strengthen chances at leveraging the research into extramural funding.
ERAB Progress Report

“Recovery of Salmonella bacterial isolates from pooled equine fecal samples”

Dr. Kenitra Hendrix, Dr. Jose Goni and Dr. Janice Kritchevsky

Summary:

Current protocols for equine Salmonella culture include testing a series of five samples, usually collected at 24-hour intervals. The purpose of this study is to evaluate the sensitivity of culturing pools of five fecal samples for Salmonella culture. Testing pooled samples would offer the benefit of decreased cost of diagnostic testing.

Progress:

1. Obtain and propagate a pure culture of Salmonella Group E to serve as the reference bacteria. - complete

Salmonella E1 (ATCC 9270) is maintained in the ADDL Bacteriology section and utilized in subsequent specific aims.

2. Spike equine fecal samples with either 10^2, 10^3, 10^4, or 10^5 CFU Salmonella.

3. Pool 1 spiked and 4 non-spiked fecal samples collected over a 5-day time period into a single container.

4. Perform standard culture to recover the spiked Salmonella species from well-mixed composite fecal samples.

Two iterations of aims 2-4 were performed.

Phase 1 (Table 1): A series of five Salmonella samples from the feces donor horse were cultured and were negative for Salmonella. Feces in 20-gram aliquots from this Salmonella negative horse was initially spiked with either 10^2, 10^3, 10^4, or 10^5 CFU Salmonella. Ten grams were cultured for salmonella to show spiking was successful. The other ten grams were pooled with 40g of salmonella-negative feces, replicating a pool of one positive field samples and four negative field samples. Following both spiking and pooling, feces was homogenized for 1 minute at 230rpm. All spiking was successful based on positive culture results. All pools were positive, with the exception of the pool including the samples spiked with 10^3 CFU. This raised concerns regarding the pooling technique itself, since the pool with less organism was culture positive. All pooled samples were cultured again after 8 days at 4°C, to simulate a field case in which only the first sample was positive for Salmonella. All pools were positive except for the one including the sample spiked with 10^2 CFU.
Table 1:

<table>
<thead>
<tr>
<th></th>
<th>First Culture</th>
<th>Second Culture (8d in fridge)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>20g feces spiked with $10^2$ CFU</td>
<td>Salmonella</td>
</tr>
<tr>
<td>AP</td>
<td>10g A pooled with 40g negative</td>
<td>Salmonella</td>
</tr>
<tr>
<td>B</td>
<td>20g feces spiked with $10^3$ CFU</td>
<td>Salmonella</td>
</tr>
<tr>
<td>BP</td>
<td>10g B pooled with 40g negative</td>
<td>Negative</td>
</tr>
<tr>
<td>C</td>
<td>20g feces spiked with $10^4$ CFU</td>
<td>Salmonella</td>
</tr>
<tr>
<td>CP</td>
<td>10g C pooled with 40g negative</td>
<td>Salmonella</td>
</tr>
<tr>
<td>D</td>
<td>20g feces spiked with $10^5$ CFU</td>
<td>Salmonella</td>
</tr>
<tr>
<td>DP</td>
<td>10g D pooled with 40g negative</td>
<td>Salmonella</td>
</tr>
</tbody>
</table>

Phase 2 (Table 2): In response to the unexpected results in phase 1, variations of homogenization protocols including the duration of homogenization and adding nutrient broth were tested. Samples were spiked with $10^2$ CFU only, and then pooled as described in Phase 1. Triplicates of each set of conditions were tested, and all culture results were positive. This indicated that variables of the homogenization protocol did not affect the final culture results, and that $10^2$ CFU in 50g of feces was consistently detected by culture.

Table 2:

<table>
<thead>
<tr>
<th>Control</th>
<th>Non-spiked feces</th>
<th>Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>1a</td>
<td>1 minute; no broth</td>
<td>Positive</td>
</tr>
<tr>
<td>1b</td>
<td>1 minute; 20mL broth</td>
<td>Positive</td>
</tr>
<tr>
<td>1c</td>
<td>5 minutes; no broth</td>
<td>Positive</td>
</tr>
<tr>
<td>5a</td>
<td>1 minute; 20mL broth</td>
<td>Positive</td>
</tr>
<tr>
<td>5b</td>
<td>5 minutes; 20mL broth</td>
<td>Positive</td>
</tr>
<tr>
<td>5c</td>
<td>1 minute; 20mL broth</td>
<td>Positive</td>
</tr>
<tr>
<td>B1a</td>
<td>1 minute; 20mL broth</td>
<td>Positive</td>
</tr>
<tr>
<td>B1b</td>
<td>5 minutes; 20mL broth</td>
<td>Positive</td>
</tr>
<tr>
<td>B1c</td>
<td>5 minutes; 20mL broth</td>
<td>Positive</td>
</tr>
<tr>
<td>B5a</td>
<td>5 minutes; 20mL broth</td>
<td>Positive</td>
</tr>
<tr>
<td>B5b</td>
<td>5 minutes; 20mL broth</td>
<td>Positive</td>
</tr>
<tr>
<td>B5c</td>
<td>5 minutes; 20mL broth</td>
<td>Positive</td>
</tr>
</tbody>
</table>

Additional plans for culturing pools include decreasing the spiking level and repeating cultures after refrigeration. These studies will be completed in early 2019.
2018 Progress Report for Competitive Equine Research Grants Program

Title: Ultrasonographic morphology of the gastrointestinal tract of healthy horses: in vivo, ex vivo and histological comparison

PI: Dr. Chee Kin Lim

PACUC protocol: 1701001536

Step 1: In vivo sample collection:

14 horses have been examined via ultrasonography from 9/11/2018 – 10/12/2018

<table>
<thead>
<tr>
<th>Horse Name</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>Max Attack</td>
<td>393 142</td>
</tr>
<tr>
<td>Alkabar</td>
<td>393 113</td>
</tr>
<tr>
<td>Mick</td>
<td>393 216</td>
</tr>
<tr>
<td>Freckles</td>
<td>393 329</td>
</tr>
<tr>
<td>Jones</td>
<td>389 740</td>
</tr>
<tr>
<td>Louie</td>
<td>390 433</td>
</tr>
<tr>
<td>Mocha</td>
<td>392 964</td>
</tr>
<tr>
<td>Rangoon Belle</td>
<td>392 962</td>
</tr>
<tr>
<td>Sister Fiona</td>
<td>392 963</td>
</tr>
<tr>
<td>Romantic Reason</td>
<td>392 974</td>
</tr>
<tr>
<td>Shania’s Code</td>
<td>389 457</td>
</tr>
<tr>
<td>Smarty’s Packin</td>
<td>392 736</td>
</tr>
<tr>
<td>Warrior</td>
<td>393 409</td>
</tr>
<tr>
<td>Carly</td>
<td>393 403</td>
</tr>
</tbody>
</table>

Status: Step 1 COMPLETED

Step 2: Ex vivo sample collection and ultrasonography

14 equine gastrointestinal samples have been collected and examined via ultrasonography from 9/11/2018 – 10/12/2018

Status: Step 2 COMPLETED

Step 3: Histopathology slides completed

All histology samples have been prepared, H&E slides prepared, and scanned into Aperio for review.

Status: Step 3 COMPLETED

Step 4: Review of ultrasonography images

To be performed between December 2018 and January 2019 by Dr. Lim and Dr. Heng.

Status: Step 4 TO BE COMPLETED
Step 5: Review of histopathology slides
To be performed between December 2018 and January 2019 by Dr. Jones-Hall

**Status: Step 5 TO BE COMPLETED**

Step 6: Review of data/draft manuscript
Deadline: End of January 2019

**Status: Step 6 TO BE COMPLETED**

Step 7: 1st draft of manuscript with data analysis complete
Deadline: End of February 2019

**Status: Step 7 TO BE COMPLETED**

Prepared by:

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Mechanosensitive Channels in Equine Musculoskeletal Soft Tissues

PI: Dianne Little

Date of Award: 4/13/2018

Hypothesis: Transient receptor potential (TRP) channels and Piezo channel expression is upregulated in the palmar or plantar joint capsule of the fetlock joint with increasing severity of osteoarthritis, and in the flexor tendons and suspensory ligament with increasing evidence of suspensory desmitis or tendinopathy.

Specific Aim 1: Characterize the expression of mechanosensitive calcium TRP and Piezo channels across various sites in the equine fetlock joint and distal limb tendons from cadaveric donors of in different degrees of fetlock, tendon and ligament health.

Specific Aim 2: Characterize the functional role of mechanosensitive calcium channels in tendon and joint capsule extracellular matrix synthesis, matrix organization and contractility under simulated loading conditions.

Personnel:
Dr. Kara Negrini (PVM DVM’18), a candidate for the degree of Master’s of Basic Medical Sciences degree is working on this as her main project towards her thesis requirement, effective May 2018. Her salary is being funded through Dr. Little’s start-up funding from the Department of Basic Medical Sciences, and she is a teaching assistant for the Anatomy courses in the professional DVM program. Thus, she is dedicating approximately 0.2% FTE to this project, providing greater degrees of effort and expertise to this project than originally proposed.

Progress Towards Research Goals:
We are making substantial progress on the research goals, and on the underlying scientific premise, both of which will position us well for clinical relevance, benefit to the horse racing population of Indiana, future funding support and for completion of the proposed studies.

Specific Aim 1: To date, an initial n=11 grossly normal samples from superficial and deep digital flexor tendons, suspensory mid-body and dorsal and palmar joint capsule from research horses euthanized as part of unrelated studies have been obtained. In addition, n=3 samples from these horses had osteoarthritis in one or more joints, or with superficial digital flexor tendinopathies in one or more limbs, allowing for intra-individual
evaluation of normal and diseased tissues. It was not possible to obtain samples from the Indiana Horse Racing Commission Post-Mortem Program, but adequate tissues have been obtained without this resource. Based on our results from our parallel human subjects work (currently in review at the Orthopaedic Journal of Sports Medicine), demonstrating the importance of: 1) accurately characterizing immune cell populations associated with individual TRP channel expression in fibrosis, most notably associated with sub-phenotypes of resolving pro-fibrotic macrophage populations, and 2) accurately characterizing the different fibroblast sub-types present at different disease stages, we incorporated this into our study design for Aim 1. Therefore, we designed an antibody battery to effectively characterize the expression of various TRP and Piezo channels associated with specific cell types in these equine samples. This will generate more useful data with respect to clinical relevance, publication and future funding than the originally proposed focus only on channel expression would have allowed. The upgrades to the CellSens software on the Olympus microscope are finally complete, and protocols for the quantitative evaluation of immunoreactivity in histological sections are now developed.

Refinement to previously proposed approach: The range of antibodies available and likely to work in these equine tissues was far greater for immunohistochemistry of frozen sections, than for formalin fixed paraffin embedded sections. Therefore, tendon sections were frozen for cryosectioning in optimal cutting temperature (OCT) media and sections are currently being cut with the Leica Cryostat housed in the PI’s laboratory. While cryosection of equine tendon is challenging, the CryoJane system affixed to the cryostat is critical for the success of this endeavor. This approach has the additional benefit of saving fees associated with the Histology core, so these funds were used for the purchase of additional antibodies to allow for necessary immune cell and fibroblast sub-phenotype characterization.

Specific Aim 2: For the microphotopatterning component of this work, we have completed the necessary protocol modifications, trouble-shooting, programming, and equipment upgrades in Bindley compared to our previous work on different and substantially better multiphoton microscopy equipment we used at Duke University. This will allow for completion of the microphotopatterning components of Specific Aim 2 in a timely manner. Additionally, we have now validated markers of collagen alignment and of tendon and tendon fibrosis using the microphotopatterns proposed in Specific Aim 2. Other than these critical refinements to our microphotopatterning protocols, the timeline of Specific Aim 2 has been delayed somewhat, for scientific and budgetary reasons:

First, based on our human data the expression of various TRP channels is tightly coordinated with specific macrophage sub-phenotypes, and with specific fibroblast sub-populations. This expression changes across the disease spectrum, at least in joint...
capsule in osteoarthritis (normal, mild end-stage). We need to understand what these populations are, and how they change in normal and diseased equine joint capsule, tendon, and ligament before pursuing functional studies on incompletely characterized cell populations. Once we know the defined cell populations involved, we will be able to sort for these specific cell populations using flow cytometry and know what cell types are represented in our functional assays. This approach will lead to much more robust data and greater likelihood of both clinically relevant information and additional successful funding applications as we move forward.

Second, funding was received at 77% of the original request. This >20% cut in budget has meant that we cannot afford to lease the equipment for cyclic loading and imaging of calcium flux for 15 months, as originally proposed. This lease will now need to be condensed into a 4-6-month period of time once histology results from Specific Aim 1 are known. As a downstream complication of this, in order to avoid expiration or decline in efficacy of expensive purchased calcium channel agonists and antagonists, all work involving isolated cells (microphotopatterning, collagen gel contraction assays, and the cyclic loading work) will now be performed simultaneously once the equipment is leased using an additional set of cells isolated from specific tissues to generate balanced datasets. However, the benefit to these budgetary constraints is that these changes in logistics will allow us to sort for the specific cell populations we know to be involved, to ultimately produce more robust data, and to produce a more accurate timeline of cell/TRP/Piezo channel involvement at different points in the disease process.

**Summary:** Within the constraints identified above, we are close to completion of Specific Aim 1, and once results of this aim are known, are poised for successful completion of Specific Aim 2. Support from this funding application was acknowledged in presentations at the David Van Sickle Musculoskeletal Days in Fall 2018. We have grant submissions for human subjects in review at the Orthopaedic Research and Education Foundation; this combined human-equine clinical approach will strengthen not only the options for improved treatment of horses with joint capsule fibrosis and suspensory desmopathy/flexor tendinopathy but will increase the likelihood of successful federal funding applications to continue this work beyond that which is achievable with the current award.
Bone fractures are a significant cause of morbidity and mortality for individual horses and the general horse population. For the individual horse, fractures cause pain and suffering, are challenging and costly to treat, and in some cases result in euthanasia. In the general population, fractures have been estimated to account for approximately 10% of overall equine mortality.\textsuperscript{1,2} Over the last decade, major fractures in high profile equestrian sports have attracted national media attention.\textsuperscript{3,4} Media scrutiny has also linked the issues of rider safety and musculoskeletal injury in horses, and highlighted the animal welfare concern of these injuries,\textsuperscript{4,5} prompting several initiatives by equestrian sports governing bodies to address the safety and welfare of equine athletes. In Thoroughbred racing, the Equine Injury Database was established in 2008 to accurately identify racing injuries at a national level. Currently, the database includes over 1.8 million race starts over 5 years.\textsuperscript{6} The database shows an overall fatal injury rate of 1.9/1000 horse starts. As over 80% of fatalities in racing are due to fractures and a similar or greater number of injuries occur during training,\textsuperscript{7–10} it can be estimated that approximately 1,400 horses are euthanized each year in the U.S. due to fractures that occur during racing or training. However, this estimate does not account for non-fatal fractures. Over a 14 year period at 10 Japanese racetracks, the average fracture incidence (fatal and non-fatal) was reported at 1.83% of all race entrants.\textsuperscript{11} Applying this fracture incidence to racing starts in the U.S., over 6,500 fractures would be estimated to occur in Thoroughbred racing each year. Similar overall rates of musculoskeletal injury have been reported in Quarter Horse and Standardbred racing, although injury distributions vary between racing breeds.\textsuperscript{8,12,13}

There is strong evidence that the majority of racing fractures are the result of accumulated bone tissue changes and not a single “bad step” during racing or training.\textsuperscript{14} The evidence includes common bone fracture locations and configurations, pre-existing pathology in both fractured and non-fractured bones in the same horse and incomplete fractures identified in the same locations as common complete fractures.\textsuperscript{14,15} The tissue and bone material changes that occur with repetitive high strain loading ultimately decrease fracture resistance of the bone tissue and the entire bone.\textsuperscript{14,16} The cumulative impact of training and racing on the musculoskeletal system, reflected in changes in its resistance to injury, means that it should be possible to reduce the incidence of fractures, when we are able to determine and detect the relevant changes in structural and bone tissue properties in advance of reaching the point of high injury risk.

This ongoing study examines four pre-clinical modalities to assess their ability to detect factors related to fracture: reference point indentation (RPI; Biodent and Osteoprobe), Raman spectroscopy and peripheral quantitative computed tomography (pQCT). Each of these techniques were performed on cadaveric equine third metacarpals (MC3s) on the lateral, dorsal and medial aspects at proximal, midshaft and distal sites of the diaphysis, with the exception of pQCT which was also performed in both the proximal and distal metaphyses. A total of 32 Thoroughbred (TB) racehorse MC3s were tested using these modalities. This sample included 7 males, 11 geldings and 14 females. The sample was also divided into four testing groups based on the type of fracture: third metacarpal (MC3), forelimb proximal sesamoid (SSMD), long bone other than the MC3 (LB) and non-fracture (Control). A mixed-model was used for statistical testing, where the fixed-effect was the individual racehorse identification. The two independent factors tested were bone location (which was the repeated factor) and fracture group.

Results of testing with RPI revealed that the measure of bone material strength (BMS, i.e. resistance to microfracture) was, unexpectedly, elevated at the midshaft dorsal site in the MC3, SSMD and LB fracture groups compared to Control. Consistent with this, it was found that the indentation distances measured by the BioDent instrument were decreased at the midshaft dorsal site. These findings were somewhat counterintuitive, as one might anticipate that the
Control would be less susceptible to propagation of microfracture during these tests. Increased indentation distances and decreased BMS in the Control group could possibly be explained by the increased severity of dorsal metacarpal disease (DMD) in the Control horses. DMD is a pathology that increases the deposition of immature, woven bone – which is more easily indented – on the dorsal surface of young racing Thoroughbreds. It is possible that the ability to form a robust DMD response or the active presence of DMD may be protective against fracture. In the next 12 months, we will examine this set of bones via both high-resolution computed tomography and histology in order to test our hypothesis regarding the presence of a greater DMD response in the Control horses.

Findings from pQCT included decreased cortical bone mineral density (BMD) and increased geometric properties at the distal metaphysis in the MC3 fracture group compared to the LB, SSMD and Control groups. This could imply that in the MC3 group, the distal metaphysis is larger but is comprised of weaker bone. These results are consistent with the majority of MC3 fractures occurring at the distal lateral condyle. Repeated impacts at this site could result in a rapid adaptation response that favored bone geometry over BMD, where the weaker bone material eventually succumbed to stress, ultimately resulting in overt fracture. It should also be noted that decreased cortical BMD was also found at the same site in the LB fracture group compared to the SSMD and Control groups, which could be indicative of a whole-skeletal systemic defect in these long bone fracture groups, though more testing would have to be conducted to determine this relationship.

Raman spectroscopy revealed that the lateral surface of the MC3 group had greater mineral:matrix, carbonate substitution and decreased remodeling rate compared to LB, SSMD and Control groups. These findings are consistent with the pQCT results and the high prevalence of distal lateral condyle fractures. A higher mineral:matrix ratio on the lateral MC3 is potentially indicative of the fracture propagation that occurs in lateral slab fractures, as the bone surface would be stronger but more mechanically brittle.

These four pre-clinical devices undoubtedly have potential in assessing fracture risk in vivo. Their non-invasive nature makes them ideally suited for work in the standing horse, and with further validation the tools could likely be used in human athletes and soldiers for assessing stress fracture risk. This validation must include comparison of in vivo measures in the Osteoprobe, pQCT and Raman spectrometer to ex vivo data presented here. In the longer term, a logistic regression model could be used to not only incorporate factors identified as correlating with fracture here, but also covariates like age, sex and weight could be included to assess their impact.

References


2017-2018 Competitive Equine Research Funds Progress Report:

Title: Analgesic efficacy and safety of ketorolac, phenylbutazone and flunixin in a model of foot lameness in horses

Principal Investigator: Sandra D. Taylor

Co-Investigators: Timothy Lescun, George Moore, Shannon Grady, Alec Davern

Data collection for the project outlined in the CVM Competitive Equine Research Funds proposal submitted in December of 2016 is complete. Statistical analysis is pending, and is expected to be completed by January of 2018. Preliminary results were presented at the Conference for Research Workers in Animal Diseases (CRWAD) in Chicago in December of 2017. Final results will be presented at The ACVIM Annual Forum or the AAEP Convention in 2018 or 2019. A manuscript will be submitted to the Equine Veterinary Journal in 2018.

Abstract: Conference for Research Workers in Animal Diseases (CRWAD), December, 2017

Non-steroidal anti-inflammatory drugs (NSAIDs) are used to provide pain relief in horses. A reversible foot lameness model has been used to demonstrate analgesic efficacy of NSAIDs in horses, but the mechanism by which NSAIDs mitigate pain induced by this model has not been determined. As part of a larger study to compare the analgesic efficacy of ketorolac (KT), phenylbutazone (PBZ), flunixin meglumine (FM) and saline (control) using this lameness model, we aimed to determine whether NSAIDs decrease pain primarily through anti-inflammatory or analgesic effects. Indicators of inflammation included foot temperature (thermography), serum amyloid A (SAA), and cephalic vein PGE2. We hypothesized that foot temperature, SAA and PGE2 would increase following induction of foot pain, indicating the presence of inflammation. We expected that treatment with any NSAID would decrease foot temperature, SAA and PGE2 two hours after NSAID administration (time of peak drug effect) compared to the placebo group. A randomized crossover was done using 5 healthy horses. All horses received each of 3 NSAIDs and saline. Treatment was given 1h after lameness was induced, and lameness was assessed hourly for 12h. For each of the trials, thermography and cephalic blood collection for SAA and PGE2 were done prior to lameness induction (baseline), immediately following lameness induction (0h), immediately prior to treatment (1h), time of peak drug effect (3h) and prior to the next dose (13h). Mean differences between treatment groups and across time were assessed in linear mixed models with horse considered a random effect. Significance was set at p<0.05. For all treatment groups combined, there was an increase in foot temperature from 0h to 1h (p<0.001). There was no difference in mean SAA (p=0.282) or PGE2 (p=0.416) among treatment groups. Time did not affect mean SAA (p=0.272) or PGE2 (p=0.460) in any treatment group. There is no evidence that this model induces inflammation. Although all 3 NSAID treatments improved lameness, the inflammatory markers did not decrease in response to NSAIDs. The increase in foot temperature from 0h to 1h might have been due to increased pooling of blood in the non-weight bearing foot.
APPENDIX C

Research Projects Completed Supported with Pari-Mutual Funds


• Taylor S, Cooper B, Grady S, Lescun T, Moore G, Davern A, Brunner T. Plasma drug concentrations of ketorolac tromethamine, phenylbutazone and flunixin meglumine in horses following single-dose intravenous administration.
Finite element modeling to enhance equine fracture treatment.
Timothy B. Lescun, Gert Breur, Steve Adams, Eric Nauman, Srinivasan Chandrasekar

Final report for Equine Competitive Research Funding

Summary of work
The finite element (FE) models developed in this work were utilized to answer several research questions related to the equine distal limb transfixation cast and specifically the bone-pin interface (BPI). Since bone failure in this location is the underlying mechanism for the most common and clinically significant complications of transfixation casting, the focus of our analysis was predicted stress and strain at the BPI. While not absolute, due to the clinical and biologic factors that always play a role in complications, achieving BPI stress and strain below previously documented cortical bone yield stress and strain values was the underlying assumption used to select preferred bone-pin construct models that would minimize the risk of BPI failure when employed clinically. The long term goal of this area of study is to improve the safety and reliability of transfixation casting in the horse. The central hypothesis was that the safety and reliability of equine distal limb transfixation casting with transcortical pins placed in the third metacarpal bone (MC3) will ultimately be improved through the use of preferred pin configurations, the promotion of pin stability within the cast, and an approach to control the stress environment within the cast. The 4 specific research goals were:

Research goal #1: To utilize FE models of the equine distal limb transfixation cast to determine transcortical pin configurations which result in BPI stress predictions below the expected yield stress of the equine MC3. Examination of a range of pin parameters, including pin diameter, number, type, spacing, orientation and location within the bone, and material properties found that pin spacing and orientation within the bone had only minor effects on BPI stresses; location within the bone, the type of pin used and the pin material used had a moderate influence on BPI stresses, while the pin diameter and number were found to be the dominant influences BPI stresses. These findings were consistent with previously reported studies regarding external fixation pins.
**Research goal #2:** To develop a general approach for determining preferred transcortical pin configurations in anatomic locations other than the MC3 of horses. The unique aspect of transfixation casting compared to external fixation is the manner in which the cast is used to connect all of the transcortical pins into one unit. This prompted an examination of a parameter called total **pin area moment of inertia (PAMi)**. This parameter was found to have a strong relationship with the predicted bone stresses and strains in the FE models developed and it was proposed that this parameter represents the ability of a transfixation cast to resist axial loading. In this way, the total PAMi can be used to compare one transfixation cast construct to another and potentially predict expected bone stress at sites other than MC3 when bone dimensions are considered. A negative power law relationship was found to fit the total PAMi versus maximum bone strain relationship quite well. Taking this relationship further, we used it to help determine preferred bone pin constructs by considering different parameters reflecting the size of the holes required to place the pins, and used these to further refine our selection process.

**Research goal #3:** To determine, using preferred transcortical pin configurations, the effect of **cast pin interface (CPI)** stability on BPI stresses in the equine third metacarpal bone. An examination of the CPI and the manner in which it is modeled in the transfixation cast revealed that it has a clear impact on the predicted BPI stresses. Predictions of BPI stresses based on completely fixed pin ends as a boundary condition are likely to underestimate the BPI stress present within the transfixation cast. The sliding surface contact model appears to be the most likely contact condition to represent the true mechanism of interaction between the pin and the cast material. It was concluded that the CPI is an important consideration in the modeling of the equine transfixation cast.

**Research goal #4:** To determine, using an FE model of the equine distal limb transfixation cast, how changing the loading conditions within the cast distal to the transcortical pins will affect local stresses at the BPI. We used a composite tissue section distal to the transcortical pins to show that increases in tissue stiffness associated with fracture healing decreases the BPI stresses in MC3.
Future Directions

This work was undertaken in an attempt to answer some of the key questions regarding the mechanics of transfixation casting in the horse. While the conclusions will be helpful in guiding current clinical practice, the study also serves as a starting point for further examination of the transfixation cast BPI as well as the CPI. Additional ex vivo validation studies, in vivo testing of promising bone pin constructs and methods to improve the transfixation cast in terms of BPI stresses will be essential to complete the sketch that has been started here.

Specific future work directly related to the present study should determine which of the parameters evaluated or assumed conditions used were most influential on BPI stresses. A sensitivity analysis could be performed from the data generated here and combined with a more complete validation of specific pin configurations. Future work could also investigate a pin surface that may resist the propensity for loosening by promoting osseointegration. The findings of this study could make the potential for success higher in developing an osseointergating pin the horse through reductions in BPI stresses and lower interfacial strains. The advantages of osseointegration of temporary transcortical pins can be questioned, however improved patient comfort and cortical bone density maintenance surrounding the pins rather than its loss, would both offer significant advantages to the horse. In considering the entire fracture healing process that occurs when transfixation casting is employed a future area of investigation may be to examine, using the current FE models, whether fracture dynamization or strain based control of loading is feasible in the clinical patient. While rigid fixation is beneficial early in the healing process, modulation of the strain environment at the fracture site later in the healing process would be desirable. This could be achieved with a better understanding of the transfixation cast mechanics and may be addressed through the use of FE models developed in the present study.
ERAB FUNDING

Final report

Title: Plasma drug concentrations of ketorolac tromethamine, phenylbutazone and flunixin meglumine in horses following single-dose intravenous administration

Principal Investigator: Sandra D. Taylor

Co-Investigators: Bruce Cooper, Timothy Lescun, George Moore, Shannon Grady, Alec Davern, Timothy Brunner

Ketorolac tromethamine (KT) is a non-steroidal anti-inflammatory drug (NSAID) that has recently been evaluated in horses. This drug has been shown to reduce inflammation exhibited by equine white blood cells in the laboratory, and is well-tolerated in horses from a safety standpoint, but its efficacy in controlling pain has not been fully evaluated. As part of a larger project that compared the ability of KT to two commonly used NSAIDs in horses (“Bute” and Banamine®) to decrease lameness in an experimental model of foot pain, blood was collected to measure concentrations of each drug following intravenous (IV) administration. This was done to ensure that variation in blood concentrations of each drug did not differ enough among horses to affect the degree of pain relief conferred by each drug. Blood was collected to measure drug concentration immediately (within 5 minutes) following IV administration of each drug, and again 2, 4, 8 and 12 hours later. Results showed that there was minimal variation among horses in blood drug concentrations for each drug (KT, Bute and Banamine®) at each time point tested. Blood drug concentrations decreased as expected over time in all horses as the drug was metabolized and excreted from the body. Results from this project gave us assurance that the pain relief observed after drug administration in these horses was not affected by variations in the amount of drug found in the blood at various time points.

Manuscript preparation is nearly complete and we expect to submit the manuscript for publication to the Equine Veterinary Journal in January of 2019. These results will also be presented as an oral abstract at a national veterinary meeting in 2019 or 2020.
APPENDIX D

Refereed Scientific Articles:


Abstracts and Proceedings:


In vitro anti-LPS dose determination of ketorolac tromethamine and in vivo safety of repeated dosing in healthy horses

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Flunixin meglumine (FM) is a commonly used Nonsteroidal anti-inflammatory drug (NSAID) in horses, but clinical efficacy is often unsatisfactory. Ketonolac tromethamine (KT) demonstrates superior efficacy compared to other NSAIDs in humans, but its anti-inflammatory effects have not been investigated in the horse. Safety of repeated dosing of KT has not been evaluated. The first objective was to conduct a dose determination study to verify that a previously described dosage of KT would inhibit Lipopolysaccharide (LPS)-induced eicosanoid production in vitro, and to compare KT effects of this inhibition to those of FM. Then, a randomized crossover study was performed using nine healthy horses to evaluate plasma concentrations of KT and FM following IV administration. Administered dosages of KT and FM were 0.5 mg/kg and 1.1 mg/kg, respectively. Safety following six repeated doses of KT was assessed. Ketonolac tromethamine and FM suppressed LPS-induced Thromboxane B$_2$ (TXB$_2$) and Prostaglandin E$_2$ (PGE$_2$) production in vitro for up to 12 hr. Intravenous administration produced plasma concentrations of KT and FM similar to previous reports. No adverse effects were observed. A KT dosage of 0.5 mg/kg IV inhibited LPS-induced eicosanoids in vitro, and repeated dosing for up to 3 days appears safe in healthy horses. Investigation of in vivo anti-inflammatory and analgesic effects of KT is warranted.

1 | INTRODUCTION

Management of inflammation in horses represents an ongoing challenge in equine medicine. Systemic inflammatory response syndrome (SIRS) is defined as widespread and exaggerated inflammation that can be triggered by infectious or noninfectious stimuli (Lewis, Chan, Pinheiro, Armitage-Chan, & Garden, 2012; Moore & Vandenplas, 2014; Palmer, 2014; Roy, 2004). Possible complications of SIRS include coagulopathies and organ dysfunction, which can lead to death (Kelmer, 2009; Kilpatrick et al., 2016; Roy, 2004). Nonsteroidal anti-inflammatory drugs (NSAIDs) are commonly administered to horses with SIRS to reduce eicosanoid production through inhibition of cyclooxygenase (COX) enzymes. Several studies have documented the effectiveness of NSAIDs in reducing lipopolysaccharide (LPS)/endotoxin-induced eicosanoid production in horses, calves and rodents (Dogan, Ataoglu, & Akarsu, 2002; Jackman, Moore, Barton, & Morris, 1994; Moore, Hardee, & Hardee, 1986; Semrad & Dubielzig, 1993; Semrad, Hardee, Hardee, & Moore, 1987). Flunixin meglumine (FM) is currently considered the standard of care for LPS-induced inflammation in horses, based on efficacy, affordability and safety (Hardee, Moore, & Hardee, 1986; Moore et al., 1986). In comparison studies, FM has outperformed phenylbutazone in reducing production of LPS-induced mediators (Bryant, Farnfield, & Janicke, 2003; Hardee et al., 1986; Moore et al., 1986). The dosing schedule for FM of 1.1 mg/kg IV q12 hr is often used and is based on anecdotal perception of clinical efficacy (Jackman et al., 1994), but critically ill horses often display clinical signs associated with pain and SIRS despite standard-of-care FM therapy (Graubner, Gerber, Doherr, & Spadavecchia, 2011; Mair & Smith, 2005a,b). Identification of another
NSAID with anti-inflammatory and analgesic properties superior to those of FM could potentially decrease illness and death associated with equine SIRS.

Ketorolac tromethamine (KT) is a nonselective COX inhibitor that has been used in humans to provide potent anti-inflammatory and analgesic therapy since the 1980s (Littak & McEvoy, 1990; Rooks, Tomoloni, Maloney, Wallach, & Schuler, 1982; Rooks et al., 1985). In several animal models, KT has been shown to have anti-inflammatory and analgesic properties that often exceed the efficacy of other NSAIDs (Dogru, Yesilyurt, Deniz, & Isimer, 1997; Jett et al., 1999; Rooks et al., 1985; Waterbury et al., 2011; Yang et al., 2008). Ketorolac tromethamine is often administered as an IV bolus or as a constant rate infusion for morphine-sparing analgesia in postoperative patients (Beattie et al., 1997; Blackburn, Stevens, Wheatley, Madaj, & Hunter, 1995; Etches et al., 1995; Ready et al., 1994). Although the pharmacokinetics of KT have been evaluated in a variety of veterinary species, including horses (Bianco, Constable, Cooper, & Taylor, 2016; Cagnardi et al., 2013; Nagilla, Deshmukh, Duran, & Ravis, 2007; Nagilla et al., 2009; Pasloske, Renaud, Burger, & Conlon, 1999; Planborg, Bondesson, Fredriksson, Larsson, & Kallings, 1994; Santos et al., 2001; Villa et al., 2015), there have been few studies evaluating its analgesic or anti-inflammatory efficacy in dogs, cats and horses (Cagnardi et al., 2013; Ferraresi et al., 2014; Mathews, Paley, Foster, Valliant, & Young, 1996; Villa et al., 2015). A single veterinary study found that KT was equal to FM in reducing SIRS parameters in an LPS-induced inflammatory model in calves (Semrad, 1993). In veterinary species, a dosage of 0.5 mg/kg, which was originally extrapolated from human literature, has been shown to provide analgesia (Bianco et al., 2016; Cagnardi et al., 2013; Ferraresi et al., 2014; Mathews et al., 1996; Villa et al., 2015). To date, this dosage has not been evaluated for anti-inflammatory efficacy in veterinary species, and few studies have evaluated plasma drug concentrations following administration of this dosage in horses (Bianco et al., 2016; Ferraresi et al., 2014).

Adverse effects of KT are similar to those caused by other nonselective NSAIDs, but overall incidence in postoperative human patients is low (Elia, Lysakowski, & Tramer, 2005; Forrest et al., 2002; Reinhart, 2000). No veterinary study has specifically evaluated KT for safety, but no adverse effects have been reported after single dosing in calves, sheep, goats, dogs, cats or horses (Bianco et al., 2016; Ferraresi et al., 2014; Nagilla et al., 2007, 2009; Pasloske et al., 1999; Santos et al., 2001; Semrad, 1993). Importantly, safety of repeated administration of KT in animals has not been evaluated.

Given that KT provides superior analgesia compared to other NSAIDs in human patients, and that this is highly correlated with its anti-inflammatory potency (Jett et al., 1999), our first objective was to verify that a previously described dosage of KT would inhibit LPS-induced eicosanoid production in vitro, and to evaluate KT effects of LPS-induced eicosanoid suppression compared to those of FM. Our second objective was to assess plasma concentrations of KT and FM following intravenous administration. A final objective was to evaluate the safety of KT following repeated dosing, as assessed by evaluating complete blood counts (CBC), serum biochemical analyses (SBA), urinalysis (UA) and fecal occult blood tests (FOBT) over a 3-day (6-dose) drug administration period.

2 | MATERIALS AND METHODS

All procedures in this study were approved by the Institutional Animal Care and Use Committee at Purdue University. First, a dose determination study was performed to verify that KT at a dosage of 0.5 mg/kg could effectively suppress eicosanoid production from LPS-stimulated equine monocytes, and to evaluate KT effects of LPS-induced eicosanoid suppression relative to those of FM.

2.1 | Dose determination study

Approximately 1 L of whole blood was collected aseptically from the jugular vein of a healthy horse from the Purdue University teaching herd and placed in a glass bottle containing 100 ml 40 mM EDTA.

Monocyte isolation was performed using a sedimentation-gradient centrifugation protocol as previously described (Rooks et al., 1985). Total monocyte count was determined and cells were suspended in equine media. A cytopsin slide was made to verify that the isolated cells were >75% monocytes. Serial drug dilutions were created from KT (Ketorolac tromethamine (30 mg/ml), Hospira, Inc., Lake Forest, Illinois, USA) and FM (Flunixin meglumine (50 mg/ml), VetOne Prevail, Boise, Idaho, USA) to create six concentrations of each drug: 80, 40, 20 (equivalent to 1.1 mg/kg FM), 10, 5, and 2.5 (equivalent to 0.5 mg/kg KT) μg/ml. Monocytes were then plated on 12-well tissue culture plates at a concentration of 1 × 10⁶ cells/well and incubated with serial dilutions of KT or FM. The plates were incubated at 37°C in a 5% CO₂ atmosphere for 1 hr, followed by addition of LPS (E. coli 055:B5) at a final concentration of 1 μg LPS/ml. Positive and negative control wells contained monocytes alone without either drug. The final total volume in each well was 2.5 ml. In order to determine the duration of anti-inflammatory activity, the samples were allowed to incubate for 4, 8, 12, or 24 hr. Thus, there were four sets of duplicate wells, with one set for each time point (eight wells total for each of the four treatment groups). After each incubation period, the entire contents of each well were collected and frozen at −80°C until analysis. Concentrations of thromboxane B₂ (TXB₂) and prostaglandin E₂ (PGE₂) were measured from each sample using commercially available enzyme-linked immunosorbent assay (ELISA) kits validated for use in horses (Horse Elisa Kits, MyBioSource, San Diego, CA, USA). Samples were analyzed per the manufacturer’s instructions.

2.2 | Crossover/pharmacokinetic and safety study

2.2.1 | Animals and experimental design

Nine healthy adult horses from the Purdue University teaching herd were used in this randomized crossover study. The horses were determined to be systemically healthy based on history, physical examination, CBC (Abbott Cell-Dyn 3500 Hematology Analyzer, Abbott Park,
IL, USA). SBA (Abbott Cell-Dyn 3500 Hematology Analyzer, Abbott Park, IL, USA), SBA (Abbott Cell-Dyn 3500 Hematology Analyzer, Abbott Park, IL, USA) and FOBT (Hemoccult, Beckman Coulter, Inc., Brea, CA, USA). All of the horses had been donated for chronic orthopedic diseases at least 2 months prior to use in this study, and their conditions were considered to be static. None of the horses had received any NSAID within 2 weeks prior to the onset of the study. The study was performed over a 5-week period. The horses were randomly assigned a number (1 through 9) and randomly divided into two groups. Phase 1 consisted of the even-numbered horses receiving KT and the odd-numbered horses receiving FM. Phase 2, the crossover, consisted of the odd-numbered horses receiving KT and the even-numbered horses receiving FM. A 2-week washout period separated the two phases.

2.2.2 Drug administration and sample collection

The day prior to drug administration (Day 0) for each of the two phases, the horses were weighed and a 14-gauge IV catheter was aseptically placed in each jugular vein. The right IV catheter in all horses and for both phases was used only for drug administration, and the left IV catheter was used only for blood collection. All blood samples were obtained by first withdrawing and discarding the first 10 ml of blood from the catheter before collecting 10–15 ml of blood for analysis. The catheters were flushed before and after each blood collection or drug administration with heparinized 0.9% saline and were removed following each of the two phases.

On Day 1 at Time zero (T = 0), horses received either KT at 0.5 mg/kg IV or FM at 1.1 mg/kg IV. All drug doses were rounded up to the nearest 0.1 ml. Heparinized blood was collected at T = 5 min (0.08 hr) to assess peak plasma concentration of drug (KT or FM) based on the rapid distribution of drugs after IV administration (Bianco et al., 2016; Semrad, Hardee, Hardee, & Moore, 1985). Heparinized blood was again collected at T = 4, 8, and 12 hr for assessment of plasma drug concentration.

2.2.3 Drug concentrations

Within 1 hr of collection, heparinized blood from each time point (T = 0.08, 4, 8, and 12 hr) was centrifuged at 760 g at 4°C for 10 min. The plasma was harvested and frozen at −80°C until analysis. Quantitation was performed using high-performance liquid chromatography (HPLC) with a triple quadrupole mass spectrometer. Previous work details the sample preparation, instrumental settings, and method of validation for KT (Bianco et al., 2016). For this report, FM was added to the method and validated in a similar fashion. Reversed-phase HPLC was used, with retention times for KT, FM, and etodolac (the internal standard) being 1.8, 2.6, and 4.9 min, respectively. Quantitation was based on multiple reaction monitoring. For KT, electrospray ionization (ESI) positive mode was used with a transition of 256.1–104.9 and a collision energy (CE) of 18 V. For etodolac, ESI negative mode was used with a transition of 286.1–212.1 and a CE of 20 V. For FM, ESI positive mode was used with a transition of 296.8–278.8 and a CE of 15 V. For KT, quantitation was based on a six-point standard curve ranging from 0.0025 to 5 μg/ml. For FM, quantitation was based on an eight-point standard curve ranging from 0.15 to 100 μg/ml. All standard curves were prepared using unmedicated equine plasma. For KT, the limit of quantitation (LOQ) was 0.0019 μg/ml and the limit of detection (LOD) was 0.0006 μg/ml, defined as a peak-to-peak signal-to-noise ratio of 10:1 and 3:1, respectively. For FM, the LOQ was 0.0024 μg/ml and LOD was 0.0007 μg/ml.

2.2.4 Adverse effects

Following the initial dose, horses received KT or FM q 12 hr for 3 days (six doses total) to assess safety of repeated dosing. The horses were weighed each evening prior to each of the two drug administration phases and the drug dose was adjusted accordingly. For each phase, complete physical examinations were performed at T = 0, 4, 8, and 12 hr, and q 12 hr thereafter for up to 72 hr. A CBC and SBA were performed at T = 0, 24, 48, and 72 hr. Urinalysis and FOBT were performed on each horse on Days 1 and 4. Urine was collected via sterile catheterization without sedation in the mares; the geldings could not be catheterized without sedation; therefore, urine was collected via midstream free-catch as early in the day as possible.

2.2.5 Statistical analysis

Areas under the concentration–time curve (AUC) for KT and for FM were determined by the composite trapezoid rule, and compared by Welch test. Values of p < .05 were considered significant, and results were reported as mean ± SEM.

3 RESULTS

3.1 Dose determination study

Ketorolac tromethamine at a concentration equivalent to a 0.5 mg/kg dosage (2.5 μg/ml) and FM at a concentration equivalent to a 1.1 mg/kg dosage (20 μg/ml) suppressed LPS-induced TXB2 and PGE2 production from baseline for up to 12 hr. Specifically, KT suppressed TXB2 >35% from baseline and PGE2 production >30% from baseline for up to 12 hr at all concentrations (80, 40, 20, 10, 5, and 2.5 μg/ml). With drug concentrations ≥20 μg/ml, at 24 hr, KT suppressed PGE2 production >50% from baseline. Flunixin meglumine suppressed TXB2 >40% from baseline for up to 12 hr at all FM concentrations (≥2.5 μg/ml) and >40% from baseline for up to 24 hours at concentrations ≥20 μg/ml, while PGE2 suppression >40% from baseline required a FM drug concentration ≥5 μg/ml. Peak eicosanoid concentration in non-NSAID-treated samples occurred at 4 hr for PGE2 and 12 hr for TXB2.

3.2 Crossover/pharmacokinetic and safety study

Four mares and five geldings (median age 18 years; range 5–23 years) were included in the study. Six breeds were represented, including three Quarter Horses and one each of Saddlebred, Thoroughbred, Standardbred, Warmblood and Appaloosa. The median starting weight of the horses was 515 kg (range 416–616 kg); horses were
weighed daily during each drug administration phase to ensure accurate dosing. One horse had been diagnosed with recurrent airway obstruction (RAO) and pars pituitary intermedia dysfunction (PPID), but had not received any medications during the 6 months prior to the start of the study. At the time of the study, the horse was not exhibiting signs of RAO. During the course of each phase, the horses were individually housed in box stalls. All horses had free access to fresh water. The horse with RAO continued on a complete pelleted feed and soaked alfalfa cubes, while all other horses had ad libitum access to grass and alfalfa hay. During the washout period, all horses were housed on pasture.

### 3.2.1 Plasma drug concentrations

Mean ± SEM plasma drug concentrations of KT and FM over time are shown in Figure 1. Both drugs followed a similar pattern, with the highest mean plasma concentrations detected at T = 5 min (0.08 hr) after drug administration, and then gradually decreasing over time. Drug concentrations of KT were lower at each time point compared to FM, and their AUC significantly differed (p = .007). At T = 5 min (0.08 hr), the mean plasma concentration of KT was 1.52 ± 0.30 μg/ml and decreased to 0.0031 ± 0.0015 μg/ml at T = 12 hr. In contrast, the mean plasma concentration of FM at T = 5 min (0.08 hr) was 25.39 ± 8.82 μg/ml and decreased to 0.16 ± 0.03 μg/ml at 12 hr.

### 3.2.2 Adverse effects

No significant change was noted in the physical or hematological variables of any horse during the course of the study. There were no significant changes in UA values; only one horse had a positive FOBT, which occurred on Day 1 of Phase 2. The subsequent sample (Day 4) from the same horse was negative. None of the horses exhibited signs of RAO. During the course of each phase, the horses were not observed due to their potential for coagulopathy. Critically ill human and animal patients often exhibit signs of coagulopathy, with a hypercoagulable state preceding deterioration into disseminated intravascular coagulopathy (DIC). Several studies have documented that horses with ischemic or inflammatory gastrointestinal disease are at increased risk for coagulopathy that may progress to DIC and death (Cesarini, Cotovio, Rios, Armengou, & Jose-Cunilleras, 2016; Dolente, Wilkins, & Boston, 2002; Epstein, Wilkins, & Boston, 2002; Epstein, Brainard, Giguere, Vrono, & Moore, 2013). Therefore, in patients with potential hypercoagulability, any notable change in attitude, nor displayed colic behavior or diarrhea during the period of drug administration. There was no evidence of thrombophlebitis or a catheter site reaction in any horse. One horse had an episode of colic two days after the completion of Phase 1 (Day 6, during the washout period) when the horse was on pasture. The horse was hospitalized, and the cause of colic was determined to be a mild impaction of the pelvic flexure. One gallon of mineral oil was administered via nasogastric tube, and the horse was hospitalized for observation without feed for 24 hr. The horse did not receive any medications and showed no further signs of colic. The horse was returned to pasture before returning for Phase 2.

### DISCUSSION

Based on the results of the in vitro study, a concentration equivalent to a 0.5 mg/kg dosage of KT suppressed LPS-induced TXB$_2$ and PGE$_2$ production for up to 12 hr. However, it remains to be determined whether this effect would be significant in vivo or in the face of inflammatory stimuli. Other similar studies have evaluated the efficacy of NSAIDs, including FM, in horses without the induction of inflammation and demonstrated significant inhibition of PGE$_2$ and/or TXB$_2$ (Beretta, Garavaglia, & Cavalli, 2005; Brideau, Van Staden, & Chan, 2001; Galbraith & McKellar, 1996; Jackman et al., 1994; Kim et al., 2015; Knyc, Arthur, McMie, & Chapman, 2015; Lees, Ewins, Taylor, & Sedgwick, 1987; Soma, Uboh, Ryder, & Fegely, 1992). Given that FM is currently considered the standard of care for LPS-induced inflammation in horses, it was important to compare the ability of KT and FM in suppressing LPS-induced eicosanoids with the same experimental conditions applied. This not only allowed us to compare endogenous eicosanoid suppression between drugs but also prepares us to perform subsequent experiments evaluating the in vivo anti-inflammatory and analgesic properties of NSAIDs in horses.

The eicosanoids evaluated in this study, TXB$_2$ and PGE$_2$, were chosen due to their correlation to COX-1 and COX-2 activity, respectively. Thromboxane B$_2$ is a stable and inactive metabolite of thromboxane A$_2$ (TXA$_2$) and is used as a marker of whole body expression of COX-1, while PGE$_2$ concentrations are a reflection of COX-2 expression. Given the role of COX-1 expression in maintaining the integrity of the gastrointestinal tract mucosa and protecting blood flow to the stomach and kidney, inhibition of COX-1 activity is typically viewed as an undesirable effect. However, targeted inhibition of COX-1 is utilized in cases where the potential for hypercoagulopathy exists as COX-1 expression by activated platelets leads to production of TXA$_2$ and promotion of platelet aggregation. Critically ill human and animal patients often exhibit signs of coagulopathy, with a hypercoagulable state preceding deterioration into disseminated intravascular coagulopathy (DIC). Cells of any horse during the course of the study. There were no significant changes in UA values; only one horse had a positive FOBT, which occurred on Day 1 of Phase 2. The subsequent sample (Day 4) from the same horse was negative. None of the horses demonstrated any notable change in attitude, nor displayed colic behavior or diarrhea during the period of drug administration. There was no evidence of thrombophlebitis or a catheter site reaction in any horse. One horse had an episode of colic two days after the completion of Phase 1 (Day 6, during the washout period) when the horse was on pasture. The horse was hospitalized, and the cause of colic was determined to be a mild impaction of the pelvic flexure. One gallon of mineral oil was administered via nasogastric tube, and the horse was hospitalized for observation without feed for 24 hr. The horse did not receive any medications and showed no further signs of colic. The horse was returned to pasture before returning for Phase 2.
treatment with COX-1-specific inhibitors (e.g., aspirin) might be indicated (Epstein, 2014). While their method of inhibition differs, nonselective NSAIDs have been shown to be as effective at reducing TXB₂ as aspirin when compared directly (Lees et al., 1987).

Unlike COX-1, which is constitutively expressed throughout the body, COX-2 is primarily an inducible enzyme which increases in response to growth factors and inflammatory stimuli such as LPS. In horses, constitutive expression of COX-2 has been demonstrated in the glandular mucosa of the stomach (Morrissey, Bellenger, Ryan, & Baird, 2010; Nieto, Aleman, Anderson, Fiack, & Snyder, 2012), mucosa of the urinary bladder (Nieto et al., 2012), jejunum (Cook et al., 2009; Hilton et al., 2011), and left dorsal colon (Morton et al., 2009). Higher COX-2 expression in these tissues is likely beneficial as PGE₂ promotes local inflammation and cytotoxic immune responses to prevent pathogen entry (Kaliniski, 2012).

In the current study, mean plasma drug concentrations of KT at all time points were nearly identical to those reported in a pharmacokinetic analysis of KT in healthy adult horses administered the same dosage of 0.5 mg/kg IV (Blanco et al., 2016). A similar study investigating the pharmacokinetics of KT at 0.5 mg/kg IV administered prior to castration in horses reported a relatively high LOQ (0.01 μg/ml) that did not allow comparison to the low (< 0.01 μg/ml) plasma drug concentrations we observed at 4, 8, and 12 hr (Ferraresi et al., 2014). Mean plasma drug concentrations of FM were similar to previous pharmacokinetic studies investigating the same dosage used here (1.1 mg/kg IV) in healthy adult horses (Foreman et al., 2012; Lee & Maxwell, 2014; Semrad et al., 1985; Toutain, Autefage, Legrand, & Alvinerie, 1994). As the therapeutic concentration of KT has not been elucidated in horses, the significance of the observed difference in plasma drug concentrations between FM and KT is unknown.

No adverse effects were associated with either KT or FM in this study. Several studies in human medicine have reported adverse effects following KT administration, which has led to establishment of labeling guidelines that include a limit of five consecutive treatment days (Reinhart, 2000). However, it is important to consider patient population. The primary indication for KT in human medicine is as a postoperative NSAID for abdominal, gynecological, or orthopedic surgery, and these are patients who might inherently be at higher risk of adverse effects (Gillies, Kenny, Bullingham, & McArdle, 1987; Horlocker, Hebl, Kinney, & Cabanela, 2002; Kim et al., 2005). Furthermore, KT is not available without a prescription, limiting its use in treating minor conditions in healthy patients. Lastly, human analgesics are typically administered at a prescribed dose rather than adjusted according to body weight, the latter of which is standard in veterinary medicine. In the veterinary literature, the safety of KT has only been evaluated in healthy dogs, cats, and horses undergoing elective surgery (Cagnardi et al., 2013; Ferraresi et al., 2014; Mathews et al., 1996; Villa et al., 2015). Here, we demonstrated that 3 days of q12 hr dosing of KT was safe in healthy horses. Additional studies designed to evaluate the clinical efficacy of the dosage used in this study (0.5 mg/kg IV q12 hr) as well as the safety of longer courses of KT administration in horses are warranted. In conclusion, given these data, it is reasonable to investigate the anti-inflammatory and analgesic effects of KT compared to FM in horses in a future study. A KT dosing schedule of 0.5 mg/kg IV q12 hr for 3 days appeared safe in healthy horses.

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REFERENCES


RELATIONSHIP BETWEEN TRACHEAL MUCUS, EXERCISE-INDUCED PULMONARY HEMORRHAGE, AND AIRWAY CYTOLOGY IN RACING THOROUGHBREDS

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High endoscopy scores for tracheal mucus and blood following racing are associated with poor performance in racehorses. Visualization of tracheal blood is diagnostic for exercise induced pulmonary hemorrhage (EIPH). Excess mucus accumulation is suggestive of equine asthma but requires confirmation by bronchoalveolar lavage (BAL) fluid cytology. However, whether equine asthma predisposes horses to develop EIPH is controversial. The purpose of the study was to compare post-race BAL fluid cytology and endoscopy scores for tracheal mucus and EIPH in Thoroughbred racehorses.

Thoroughbreds were enrolled prior to racing (2014-2016). Endoscopy was performed one hour after racing to score tracheal blood and mucus followed by BAL. Spearman rank correlations were calculated between mucus and EIPH scores. The effects of BAL cytology on tracheal mucus scores and red blood cell (RBC) counts on EIPH scores were examined using ordinal logistic regression. The effect of EIPH score upon BAL inflammatory cell proportions was evaluated using mixed models.

Ninety-five examinations were performed on 65 horses. The median (range) mucus score was 2 (0-5), and mode was 3 (35/95). The median EIPH score was 1 (0-4), and mode was 0 (44/95). Mucus and EIPH scores were negatively correlated ($r_s = -0.42$, $p < 0.0001$). BAL mast cell proportions were significantly associated with mucus score ($p = 0.042$), while neutrophil and eosinophil proportions were not. EIPH was positively associated with age and BAL RBCs ($r_s = 0.36$, $p = 0.0004$; $r_s = 0.23$, $p = 0.025$), but only age remained significant in the regression model ($p=0.037$). BAL mast cell proportions decreased with increasing EIPH score ($p=0.013$), while neutrophils were not affected.

Excess tracheal mucus after racing is common in this population and is related to BAL mast cell proportions. EIPH score was not related to any measure of airway inflammation.
An observational study of environmental exposures, airway cytology, and performance in racing thoroughbreds

Kathleen M. Ivester | Laurent L. Couëtil | George E. Moore

Background: Mild equine asthma is presumed to arise in response to environmental exposures but the relative impact of differing inflammatory phenotypes upon performance are largely unexplored.

Hypotheses: Airway inflammation negatively affects performance and cytological phenotype varies with environmental exposure.

Animals: Thoroughbred racehorses in active training and racing.

Methods: Thoroughbreds were recruited 24-48 hours before racing. Each horse was eligible for re-enrollment with each race entry. Within one hour of race completion, physical examination, respiratory endoscopy, and BAL were performed. Respirable and inhalable dust, respirable endotoxin, and respirable β-glucan exposures were measured at the breathing zone within one week after racing. Controlling for age, trainer, and pulmonary hemorrhage, the relationship between performance, bronchoalveolar lavage fluid (BALF) cytology, and measures of exposure were modeled.

Results: Performance and BALF data were collected on 64 individual horses from 8 stables for a total of 98 race performances and 79 dust exposure assessments. Evidence of mild equine asthma was found in 80% (78/98) of BALF samples from 52/64 horses. For each percent increase in BALF mast cell and neutrophil proportions, speed figures were reduced by 2.9 (P = .012) and 1.4 (P = .046) points, respectively. Respirable dust concentration was associated with BALF neutrophil proportions (P = .015). Bronchoalveolar lavage fluid mast cell proportions were only associated with respirable β-glucan exposures (P = .030).

Conclusions and Clinical Importance: Mild equine asthma is common in racing horses and negatively impacts performance. The data support that respirable, rather than inhalable, dust exposure measures are pertinent to equine airway health.

KEYWORDS
beta-glucan, bronchoalveolar lavage, dust, endotoxin, horse, parenchymal disease, pulmonary contusion/hemorrhage, respiratory tract

1 INTRODUCTION

Mild to moderate equine asthma, also known as inflammatory airway disease, is commonly observed in poorly performing horses. Because affected horses appear clinically normal at rest except for occasional coughing, diagnosis requires advanced techniques such as endoscopic detection of increased tracheal mucus accumulation or demonstration of increased proportions of inflammatory cells recovered in bronchoalveolar lavage fluid (BALF). While excess tracheal mucus accumulations have been associated with poor race performance, the relationship between performance and BALF cytology remains unclear.
In mature horses with asthma, increased proportions of BALF neutrophils are the most common cytologic finding. However, in young horses, numbers of mast cells and eosinophils in BALF are often increased, suggesting hypersensitivity.1,3–7 Different cytologic manifestations of equine asthma might reflect differing disease mechanisms with different implications for performance. While neutrophilic equine asthma has been associated with cough in the absence of derangement in lung function,5 eosinophilic, and mastocytic airway inflammation are typically accompanied by airway hyperresponsiveness.5,6 Published reports investigating BALF cytology in athletic horses are often limited to horses presented for poor performance.8–10 To our knowledge, no prospective study classifying asthma phenotype by BALF cytology has been performed in racehorses using a nonbiased approach; therefore, the relative impact of neutrophilic versus eosinophilic/mastocytic airway inflammation on performance is unknown.

The heterogeneity of cytologic phenotype also suggests the possibility of heterogeneous etiologies. Exposure to airborne dust and other irritants appears to play an important role in triggering the disease. Upon barn confinement, horses are exposed to higher concentrations of dust11 and endotoxin,11,12 and introduction of otherwise healthy horses to stall confinement is sufficient to induce airway inflammation.13–18 Increased tracheal mucus accumulations are associated with higher particulate concentrations.17 Despite convincing circumstantial evidence, research directly relating BALF cytology to exposure is sparse. In young TB horses entering race-training, respirable dust exposures are correlated with eosinophilic inflammation,7 but the applicability of this data to a more general population is unknown. Accurate assessment of the relationship between airway inflammation and environmental exposures requires measurement in the horse’s breathing zone to capture individual variations in exposure.18

Though asthma can affect horses of any discipline, the racehorse provides an ideal study subject, as the disease is well recognized, the prevalence is high, and objective measures of performance are readily available in this population. Equibase, the official database for TB racing in the US, offers a numeric rating of performance, the “Equibase speed figure” (Equibase, Lexington, Kentucky), that is, a function of racing time adjusted for variations between races such as distance, track conditions, and run-up distance, providing an objective measure of individual horse performance across races of different lengths and conditions.19

Therefore, our study was designed to explore the impact of airway inflammation as determined by BALF cytology upon race outcomes in TB horses racing in Indiana and to investigate the association between environmental exposures to dust and BALF cytology.

2 | MATERIALS AND METHODS

2.1 | Study design

A prospective observational study of TB horses racing in Indiana was performed during three racing meets between September 2014 and October 2016. The study was publicized via meetings with trainers and owners, posting of brochures, and advertisements in the local press. Upon publication of each weekday’s racing program, trainers who had previously expressed an interest in study participation were contacted and permission for the enrollment of each horse was sought 24–48 hours before racing. On the day of racing, an informed consent form was signed before the race. Enrolled horses underwent physical examination, endoscopy of the respiratory tract, and BAL 1 hour after the horse completed the race. Four to 7 days after the race, breathing zone concentrations of respirable and inhalable particulates were measured over the course of 4–6 hours on a single occasion. Horses were eligible for re-enrollment with every race entered. The Purdue University Animal Care and Use Committee and the Indiana Horse Racing Commission approved all procedures.

2.1.1 | Clinical score

Signs of respiratory disease, including cough, nasal discharge, respiratory effort, and auscultation, were scored from 1, representing normal breathing, to 21, representing severe respiratory signs such as dyspnea (Supporting Information Table S1).20

2.1.2 | Endoscopic examination

Horses were restrained with a nasal twitch and a flexible fiber-optic endoscope (7.9 mm outer-diameter, 110 cm long) was passed through the ventral meatus to the level of the pharynx. The degree of pharyngeal lymphoid hyperplasia was scored from 0 (no follicles) to 4 (numerous, large, edematous follicles).21 Any upper respiratory tract abnormality was recorded, and arytenoid cartilage abduction was graded from 1 (synchronous with full abstraction) to 4 (paralysis).22 The endoscope was advanced past the larynx into the trachea until the bifurcation was visible. Scores were assigned to tracheal mucus accumulation from 0 (no mucus) to 4 (large, pool-forming),23 and exercise induced pulmonary hemorrhage (EIPH) from 0 (no blood) to 4 (streams covering more than 90% of tracheal surface).24 To facilitate BAL, the carina and larynx were sprayed with a 0.4% lidocaine solution as the endoscope was removed (20–30 mL at each site).

2.1.3 | BAL

Horses were sedated with xylazine hydrochloride (0.2–0.5 mg/kg IV; AnaSed, Akorn Animal Health, Lake Forest, IL) and butorphanol tartrate (0.02 mg/kg IV; Torbugesic, Zoetis, Parsippany-Troy Hills, New Jersey). A sterile BAL tube (300 cm long; 10 mm outer diameter; Bivona, Gary, Indiana) with inflatable cuff was passed through the nose and wedged into a peripheral bronchus. Two hundred fifty milliliters of sterile 0.9% NaCl were infused and recovered manually with 60–mL sterile syringes. Cytological specimens were prepared by centrifugation and processed with modified Wright stain. Differential cell counts were performed on 400–600 cells.

2.1.4 | Case definition

Equine asthma was diagnosed on the basis of normal breathing effort at rest and BALF differential cytology counts. Horses with >5% neutrophils, >2% mast cells, >1% eosinophils or any combination thereof were considered asthmatics. Informed consent was obtained from each horse’s owner.
2.1.5 | Particulate measurements
Gravimetric filter sampling was conducted as previously described.8 Briefly, particulate samples were collected with personal sampling pumps (AirCheck 2000, SKC, Inc., Eighty Four, PA). The respirable fraction (50% cutoff of 4 μm) was collected using an aluminum cyclone (SKC, Inc, Eighty Four, PA). The inhalable fraction (50% cutoff of 100 μm) was collected using an IOM personal sampler (SKC, Inc, Eighty Four, PA). Pumps were calibrated before and after sampling (Defender Bios, SKC, Inc, Eighty Four, PA). Respirable samples were collected on 37 mm type AE glass fiber filters, while inhalable samples were collected on 25 mm PVC filters (SKC, Inc, Eighty Four, PA). The cyclone and IOM sampler were secured to the noseband of the halter to sample dust at the breathing zone of the horse. The pumps were secured to a surcingle placed around the girth of the horse and connected to the samplers with flexible tubing (Tygon, Saint Gobain, France) secured to the mane and forelock of the horse. The horse was free to move around the stall as usual. Dust measurements were determined gravimetrically by subtracting the average of three weights taken before sampling from the average of three weights obtained after sampling. Filters were placed in a desiccator for 24 hours before weight measurements. Glass fiber filters were stored at −20 C until elution for endotoxin and β-glucan analysis.

2.1.6 | Respirable endotoxin and β-glucan analysis
Endotoxin and β-glucan content of respirable dust was measured using a kinetic chromogenic limulus amebocyte lysate technique (NexGen PTS, Charles River Laboratories, Wilmington, Mississippi). Extraction was conducted in a sterilized laboratory hood using 5 mL nonpyrogenic sterile water for elution and end-over-end agitation for 30 minutes at room temperature. Samples were analyzed immediately after extraction according to the manufacturer’s instructions.

2.1.7 | Statistical analysis
A priori sample size calculations were performed using commercial software (SAS version 9.3, SAS Institute Inc, Cary, North Carolina). We estimated that if BALF cytological variation accounted for 20% of the variation in performance, a sample size of 98 horses would provide 80% power to detect this relationship at an α = 0.05. We set enrollment target of 106 horses to allow for 8% nondiagnostic BALF cytological preparations. Race data was extracted from the official online database for Thoroughbred racing (Equibase, Lexington, Kentucky KY), including horse birthdate, Equibase speed figure, and race place. The Equibase speed figure is an integer that is assigned to each performance based upon speed and adjusted for track and track variants. A speed figure of 100 is assigned to an 8 furlong (1 mile) race run in 1 minute 36 seconds; this speed figure is adjusted up for faster times (80 points per second per furlong) and down for slower times. The relationships between speed figure and cytology variables (BALF neutrophil, mast cell, and eosinophil proportions and absolute numbers) were modeled using generalized estimating equations controlling for trainer, horse age, and EIPH score.9 Mixed logistic regression models were constructed to examine the effect of cytology variables upon the likelihood of winning. A reference population of horses finishing in the top three positions with non-learning trainer, age <5 years, and EIPH score ≤1 were selected. The overall study population, 2015–2016.

3 | RESULTS
Eight trainers participated, with a median of 6.5 horses enrolled per trainer (range 1–52). Seventy-six horses in 118 races were enrolled, with horses enrolled up to four times over a two-year period (2015–2016). Samples were collected between May and October, with 70% obtained between August and October. Of the 118 enrollments, 2 horses were claimed, 7 horses failed to complete the race, BAL was unsuccessful in 2 horses, and 9 BALF cytospin preparations were nondiagnostic because of cell lysis. Thus, 98 BALF samples were available for analysis on 64 individual horses (Figure 1). The mean age of sampled horses was 4.0 ± 1.4 years. Increased inflammatory cell proportions were documented in 78 BALF samples (80%), with increased neutrophil proportions in 33 samples (34%), increased mast cell proportions in 56 samples (57%), and increased eosinophil proportions in only 4 samples. Specifically, inflammation was classified as neutrophilic in 20 samples and mastocytotic in 41, while mixed inflammation was identified in 16 samples (n = 12 neutrophilic and mastocytotic inflammation, n = 3 mixed neutrophilic and eosinophilic inflammation, and n = 3 mixed mastocytotic and eosinophilic inflammation). Exercise induced pulmonary hemorrhage scores ranged from 0 to 4, with a median score of 1. Tracheal mucus scores ranged from 0 to 5, with a median score of 2.

Of the horses with BALF cytology results, only 16 horses were identified as performing well based on placing 1st, 2nd, or 3rd with endoscopic tracheal mucus scores ≤2. The upper limit of 95% confidence intervals of the mean proportions of BAL neutrophils and mast cells were constructed. Mixed logistic regression models were constructed using dichotomous classification variables for inflammation. Models were constructed to evaluate the effect of neutrophilic inflammation when defined as proportions greater than 5%, 10%, or the upper limit of 95% confidence interval calculated for the reference population and the effect of mastocytotic inflammation when defined by cut-offs of 2%, 5%, or using the upper limit of the reference range derived from normal, well-performing horses in the study. The relative effects of exposure measurements (respirable dust, inhalable dust, respirable endotoxin, and respirable β-glucan) upon cytology variables were examined using generalized linear mixed models that included horse as a random effect and controlled for trainer and horse age.7 Assigned link functions and data distributions were evaluated by visual inspection of diagnostic residual plots. Multiplicative over dispersion residual terms were included in those models in which the generalized chi-square to degrees of freedom ratio indicated over-dispersion. Correlations between exposure measures were quantified using Spearman rank correlations. Significance was set at P < .05. Tukey’s post-hoc analysis was used to control for multiple pair-wise comparisons. Descriptive statistics are summarized as mean ± standard deviation. In the case of endoscopic scores, mean and range are reported. All analyses were performed using commercial software (SAS version 9.4, SAS Institute Inc, Cary, North Carolina).
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### FIGURE 1
Flow diagram of sample collection. BALF = Bronchoalveolar lavage fluid

<table>
<thead>
<tr>
<th>Sample Type</th>
<th>Quantity</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>BALF samples</td>
<td>98 (64 horses)</td>
<td>- 79 respirable and inhalable dust samples (52 horses)</td>
</tr>
<tr>
<td></td>
<td>2 BAL procedures aborted</td>
<td>- 3 respirable dust sampling faults</td>
</tr>
<tr>
<td></td>
<td>9 BALF preparations non-diagnostic</td>
<td>- 3 inhalable dust sampling faults</td>
</tr>
<tr>
<td></td>
<td>67 Endotoxin measurements (43 horses)</td>
<td>- 12 endotoxin, 13 β-glucan measurements lost due to machine malfunction</td>
</tr>
<tr>
<td></td>
<td>66 β-glucan measurements (42 horses)</td>
<td></td>
</tr>
</tbody>
</table>

### FIGURE 2
Generalized estimating equation model of performance (Equibase speed figure) versus BALF mast cell proportions. Solid line = predicted mean response fit at age = 3.96 years, EIPH score = 1, trainer = 1, neutrophil proportion = 4.49%. Shaded band = 95% confidence interval of the mean response. Open circles = observations.

### FIGURE 3
Generalized estimating equation model of performance (Equibase speed figure) versus BALF neutrophil proportions. Fit at mast cell proportion = 2.48%. See Figure 2 for full legend.

This population of well-performing horses had a mean age of 4.0 ± 1.6 years.

The mean speed figure of the study population was 53.6 ± 21.3. Modeling demonstrated a statistically significant negative effect of mast cell (Figure 2) and neutrophil (Figure 3) proportions upon performance, with a similar effect seen for absolute cell numbers (Table 1). For each percent increase in BALF mast cell proportions, the speed figure dropped by nearly 3 points, while each percent increase in BALF neutrophils dropped the speed figure by 1.4 points. Age was positively associated with performance (P = .002, Figure 4).

In a mixed logistic regression model controlling for age and EIPH score, with trainer and horse included as random effects, increasing proportions of BALF mast cells were associated with a decreased likelihood of winning (P < .001). However, this model indicated that increasing proportions of neutrophils were not associated with decreased likelihood of winning (P = .77). When the covariates mast cell and neutrophil proportions were replaced by dichotomous class variables, only mast cells ≤ 2%, mast cells ≤ 2.6%, and neutrophils ≤ 6.2% were associated with a greater likelihood of winning (P < .001).

Effect estimates are summarized in Table 2.

Seventy-nine particulate exposure measurements were obtained (Figure 1). All horses were housed on the grounds of the racing facility, were bedded on loose sawdust obtained from various distributors, and fed dry alfalfa hay, also obtained from various distributors. All horses were confined to 12' x 12' box stalls with a hinged, yoked, half-size stall gate that allowed horses to extend head and neck into the barn aisle. Three types of barn construction were present:

### TABLE 1
Generalized estimating equations model of horse performance (Equibase speed figure) versus bronchoalveolar lavage cytology (n = 98), controlling for age, trainer, and EIPH score

<table>
<thead>
<tr>
<th>Variable</th>
<th>Estimate</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mast Cell (%)</td>
<td>-2.92</td>
<td>.012</td>
</tr>
<tr>
<td>Neutrophil (%)</td>
<td>-1.38</td>
<td>.046</td>
</tr>
<tr>
<td>Eosinophil (%)</td>
<td>-0.77</td>
<td>.68</td>
</tr>
<tr>
<td>Mast Cell Number (cells/μL)</td>
<td>-1.05</td>
<td>.58</td>
</tr>
<tr>
<td>Neutrophil Number (cells/μL)</td>
<td>-0.57</td>
<td>.17</td>
</tr>
<tr>
<td>Eosinophil Number (cells/μL)</td>
<td>-0.11</td>
<td>.87</td>
</tr>
</tbody>
</table>
enclosed (with stalls arranged either side of a single aisle, type 1), shed row (with stalls arranged back-to-back, perimeter aisles, and partial fabric walls, type 2), and clear span (with stalls arranged in two shed rows on either side of a central aisle, under a single roof, surrounded by a perimeter aisle with open sidewalls, type 3). Fifty-six (71%) particulate measures were obtained when horses were fed hay from the ground inside the stall, while 23 (29%) were obtained when hay was fed from a hay net hanging outside the stall. Exposures measured at the breathing zone are summarized in Table 3. Respirable dust exposure varied significantly horse-to-horse (\( P < .001 \)), but was not affected by trainer (\( P = .087 \), Supporting Information Figure S1), barn construction (\( P = .68 \), Supporting Information Figure S2), or method of feeding (\( P = .76 \), Supporting Information Figure S3). Conversely, inhalable dust exposure was significantly affected by trainer (\( P < .001 \), Supporting Information Figure S4), barn construction (\( P = .0010 \), Supporting Information Figure S5), and method of hay feeding (\( P = .026 \), Supporting Information Figure S6), and random variation because of horse (\( P < .001 \)). Both endotoxin and \( \beta \)-glucan exposure varied significantly between trainers (\( P < .001 \), caused by significantly higher endotoxin exposure measurements under trainer 2 and higher \( \beta \)-glucan exposures under the management of trainer 3 (Supporting Information Figures S7 and S8, respectively). Endotoxin exposures were not different when horses were fed from a hay net or from the ground (\( P = .061 \), Supporting Information Figure S9).

**TABLE 2** Effect estimates of logistic regression model: likelihood of winning versus bronchoalveolar lavage cytology (n = 98), controlling for age, trainer, and EIPH score

<table>
<thead>
<tr>
<th>Variable</th>
<th>Estimate (Factor Change per unit increase)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mast cell (%)</td>
<td>-0.090 (0.89)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>PMN (%)</td>
<td>0.03 (1.03)</td>
<td>.77</td>
</tr>
<tr>
<td>Mast cell ≤ 2%</td>
<td>0.38 (1.46)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Mast cell ≤ 2.6%</td>
<td>0.57 (1.78)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Mast cell ≤ 5%</td>
<td>-1.01 (0.36)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Neutrophil ≤ 5%</td>
<td>-0.33 (0.72)</td>
<td>.58</td>
</tr>
<tr>
<td>Neutrophil ≤ 6.2%</td>
<td>0.09 (1.09)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Neutrophil ≤ 10%</td>
<td>-0.34 (0.79)</td>
<td>&lt;.02</td>
</tr>
</tbody>
</table>

Bronchoalveolar lavage fluid mast cell proportions were found to increase significantly with \( \beta \)-glucan exposure (\( P = .030 \), Figure 5). Respirable dust and endotoxin exposures did not significantly affect mast cell proportions (\( P = .97 \) and .95 respectively, Table 4). The interaction term between respirable dust and endotoxin exposures was not significant (\( P = .12 \), and did not change interpretation of the model and so was not included. The random effect of horse was statistically significant (\( P < .001 \)).

Neutrophil proportions in BALF were found to be positively associated with increasing respiratory dust exposure (\( P = .0095 \), Figure 6). An interaction term between respirable endotoxin and respirable dust exposure resulted in a significant change in the parameter estimate for respirable dust (1.08 versus 1.3-fold increase in neutrophil proportions with each 0.1 mg/m\(^3\) increase in exposure, Figure 7, Table 4). While the respirable dust effect size increased with the inclusion of the interaction between respirable dust and endotoxin, the effect of the interaction itself resulted in 35% reduction in neutrophil proportions (ie, 0.65% neutrophil %) for each EU/m\(^3\) increase in endotoxin exposure (Figure 8).

Trainer and inhalable dust exposures were found to have no significant effect on either neutrophil (\( P = .60 \) and .34, respectively) or mast cell proportions (\( P = .74 \) and .60, respectively).

**TABLE 3** Summary statistics of breathing zone exposure measurements

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>Mean ± SD</th>
<th>Median</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Respirable Dust (mg/m(^3))</td>
<td>79</td>
<td>0.090 ± 0.24</td>
<td>0.031</td>
<td>&lt;0.02–1.97</td>
</tr>
<tr>
<td>Inhalable Dust (mg/m(^3))</td>
<td>79</td>
<td>1.28 ± 1.79</td>
<td>0.31</td>
<td>&lt;0.02–10.2</td>
</tr>
<tr>
<td>Endotoxin (EU/m(^3))</td>
<td>67</td>
<td>7.35 ± 12.8</td>
<td>2.35</td>
<td>0.18–87.7</td>
</tr>
<tr>
<td>( \beta )-glucan (pg/m(^3))</td>
<td>66</td>
<td>55.5 ± 66.2</td>
<td>32.9</td>
<td>1.44–351</td>
</tr>
</tbody>
</table>

Abbreviation: N, number of samples; SD, standard deviation.

*Gravimetric limit of detection = 0.02 mg/m\(^3\).*
**TABLE 4** Mixed model of inflammatory cell proportions versus exposure parameters (n = 66)

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Variable</th>
<th>Estimate (Factor Change per unit increase)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mast Cell Proportions</td>
<td>Respirable Dust (mg/m³)</td>
<td>-0.032 (97)</td>
<td>.93</td>
</tr>
<tr>
<td></td>
<td>Inhalable Dust (mg/m³)</td>
<td>0.048 (1.05)</td>
<td>.60</td>
</tr>
<tr>
<td></td>
<td>Endotoxin (EU/m³)</td>
<td>0.0011 (1.00)</td>
<td>.93</td>
</tr>
<tr>
<td></td>
<td>β-glucan (100 pg/m³)</td>
<td>0.0028 (1.3)</td>
<td>.30</td>
</tr>
<tr>
<td></td>
<td>Age (year)</td>
<td>-0.13 (0.87)</td>
<td>.099</td>
</tr>
<tr>
<td>Neutrophil Proportions</td>
<td>Respirable Dust (0.1 mg/m³)</td>
<td>6.24 (1.3)</td>
<td>.015</td>
</tr>
<tr>
<td></td>
<td>Inhalable Dust (mg/m³)</td>
<td>-0.17 (0.85)</td>
<td>.34</td>
</tr>
<tr>
<td></td>
<td>Endotoxin (EU/m³)</td>
<td>0.045 (1.05)</td>
<td>.056</td>
</tr>
<tr>
<td></td>
<td>β-glucan (100 pg/m³)</td>
<td>0.0018 (1.2)</td>
<td>.44</td>
</tr>
<tr>
<td></td>
<td>Age (year)</td>
<td>-0.088 (0.92)</td>
<td>.42</td>
</tr>
<tr>
<td></td>
<td>Endotoxin*Respirable Dust</td>
<td>-0.58 (0.56)</td>
<td>.043</td>
</tr>
</tbody>
</table>

4 | DISCUSSION

In otherwise healthy racing TBs, mastocytic airway inflammation significantly impairs performance, reducing both indices of speed and the likelihood of winning in a dose-dependent manner. Neutrophilic airway inflammation also negatively impacts performance, though to a lesser degree. Respirable dust and β-glucan exposures appear to be important determinants in the type and degree of airway inflammation.

Equine asthma appears to be highly prevalent in racing Thoroughbreds. In this study, mast cell inflammation was most common, with over half of the study population exhibiting >2% mast cells in BALF. In contrast to young horses entering training (age <36 months), eosinophilic inflammation was rare in this population (4.0 years ±1.4 years). Such high disease prevalence based on BALF cytology is consistent with a previous study reporting that 129 of 138 clinically healthy racehorses exhibited increased inflammatory cell proportions when using the consensus definition.1,25

As hypothesized, racing performance was negatively impacted by lower airway inflammation, with mast cells having the greatest apparent effect upon the speed of the horse. Similar to the effect on the speed figure, mast cell inflammatory cell proportions were found to influence the likelihood of winning: the likelihood of finishing first dropped 9% with each 1% increase in the mast cell proportion. Neutrophil cell proportions did not significantly affect the likelihood of winning, further corroborating the stronger effect of mast cells upon performance. This prospective field study investigated the association between BALF cytology and performance using airway samples collected 1 hour after racing. Previous studies have compared BALF cytology between good and poor performers categorized retrospectively based on race placings in previous months and collected airway samples days to weeks after the last race.6,26–28 In the current study, the close temporal association between racing and collection of BALF, the dose-response relationship, and the magnitude of the effect suggest strongly that mild equine asthma is deleterious to performance.

The relationships demonstrated between performance and BALF inflammatory cell proportions support a continuous effect of both mast cells and neutrophils upon performance, rather than suggesting any threshold value. However, modeling the probability of winning upon differing case definitions does suggest appropriate cut-offs likely to be pertinent to racing performance in Thoroughbreds. In this population, horses with BALF mast cell ≤ 2% were 1.5 times more likely to win. When this threshold was moved to 2.6%, the upper reference

**FIGURE 6** Generalized linear mixed model of BALF neutrophil proportions versus respirable dust exposure. Solid line = predicted mean response fit at age = 4.02 years, inhalable dust = 1.30 mg/m³, endotoxin = 5.97 EU/m³, β-glucan = 59.02 pg/m³, trainer = 8. Band = 95% confidence interval of the mean response.

**FIGURE 7** Generalized linear mixed model of BALF neutrophil proportions versus respirable dust exposure that includes interaction term between respirable dust and endotoxin exposures and overdispersion component.
performance with a BALF mast cell proportion of 4%, for example, is likely to be reduced by 6 speed figure points when compared to the performance of the same horse with a BALF mast cell proportion of 2%.

In our study, mean breathing zone respirable dust levels (0.089 mg/m³) were generally comparable to those levels reported in conventional equine management systems when horses were fed dry hay (0.064 mg/m³), and lower than those reported under low-dust management when horses were fed haylage (0.22 mg/m³). The range of measurements was large (100-fold difference between lowest and highest readings) but consistent with a previous study in thoroughbred racehorses and likely affected by individual horse behavior. The median respirable dust exposure (0.031 mg/m³) was also similar to that measured in the breathing zone of young TBs fed hay from the ground (0.055 mg/m³). In that study, feeding hay from a net inside the stall increased respirable dust exposures 4-fold, but respirable dust exposure did not differ between those fed hay from the ground and those fed from a net in the current study. This difference is likely because of the fact that hay nets were hung outside the stall rather than inside the stall, so horses in the current study were unable to bury their muzzles directly in the hay. Additionally, hanging the net outside the stall likely resulted in those horses spending more time with their heads outside the stall, though this variable was not measured. Despite relatively low dust exposure levels, results from this study demonstrated a highly significant effect of respirable dust exposure upon BALF neutrophil proportions. For each 0.1 mg/m³ increase in respirable dust exposure, the proportion of neutrophils is predicted to increase by a factor of 1.3. This finding is in agreement with the observation of considerable BALF neutrophilia in otherwise healthy horses exposed to high dust levels during moldy hay challenges, with reported median respirable dust exposures ranging from 0.1 to 0.5 mg/m³. Though inhalable dust concentrations varied with trainer and barn construction, respirable dust exposures did not. Management between trainers was similar, with all horses fed dry hay, bedded on sawdust, and fed the same textured sweet feed (Performance Advantage, Tribune Equine Nutrition, Upper Sandusky, Ohio). These data suggest that exposure to larger dust particles is more easily influenced by barn construction and variations in dry hay than respirable dust exposure. The fact that airway neutrophilia occurs at levels that are relevant to performance even in “low dust” environments highlights the need for better understanding of intrinsic (horse related) and extrinsic factors responsible for such response, as well as the need for appropriate interventions such as lower dust forages and other management practices to reduce exposure.

The apparent protective effect of endotoxin at the levels encountered in the relatively low dust environment of our study is surprising and complex. When the interaction is ignored, the estimated effect size of respirable dust exposure is much smaller than when it is included in the model, but this larger effect size is tempered as endotoxin exposures increase. The median endotoxin exposure (2.2 EU/m³) in our study was lower than those previously reported for horses bedded on sawdust and fed dry hay from the ground (59.2 EU/m³), and were drastically lower than those reported for horses bedded on straw (from 5559 to 7080 EU/m³). Trainer significantly affected endotoxin exposure, with Trainer 2 having higher mean endotoxin levels.
(87.7 EU/m³) than the other trainers (<10 EU/m³). The reason for this difference is not clear, but even this higher exposure level is likely to be significantly less than those reported to augment airway inflammation in the horse, though direct comparison is not possible because of differences in reporting and sample processing. There is a synergistic effect between endotoxin and respirable dust at experimental endotoxin doses of 48 000 EU.23 At an exposure level of 87.7 EU/m³, and assuming a resting minute ventilation of 78 L/min, a dose of 48 000 EU would take nearly 5 days to achieve. In the same report, the response to two environments was compared, and horses developed greater neutrophilic inflammation after confinement to the stable with lower endotoxin concentrations (3.6 ng/m³, ≈36 EU/m³ versus 6.9 ng/m³, ≈69 EU/m³), suggesting that a similar attenuation of inflammation may have occurred. Taken with the pro-inflammatory effects demonstrated upon larger experimental exposures in horses, these data suggest that the relationship between endotoxin exposure and the magnitude of neutrophilic response may not be linear as suggested.33

A statistically significant but modest effect of β-glucan exposure upon BALF mast cell proportions was demonstrated, suggesting a potential hypersensitivity to fungal exposure. If hypersensitivity to specific fungal allergens is responsible for this statistical observation, the small effect size of 1.3-fold increase in the mast cell proportion with each 100 pg/m³ increase in β-glucan exposure might simply reflect that this is a crude measure of overall fungal exposure, and plant and bacterial material can also contribute to β-glucan levels.32 Similar to endotoxin exposures, β-glucan exposures were affected by trainer caused by significantly higher mean concentrations measured under the management of trainer 3 (351 pg/m³) compared to all other trainers (<90 pg/m³), possibly because of poor quality hay based upon visual inspection.

This prospective field study evaluated the effect of mild to moderate equine asthma upon racing performance as defined by BALF cytology. While both cell types had a significant effect on racing performance, the effect of mast cells was found to be roughly twice that of neutrophils. Our findings support the clinical impression that mast cell inflammation is more common in younger horses. Additionally, our data support the hypothesis that differing cytologic phenotypes likely reflect different disease processes with differing etiology, predisposing factors, and consequences. Finally, results of this study suggest that respirable dust exposure is a more relevant measure than inhalable dust when evaluating respiratory health in horses.

ACKNOWLEDGMENTS

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state of Indiana and PVM research account funded by the total wager tax, and the USDA National Institute of Food and Agriculture, Animal Health project INDO20767AH. Portions of the results included in this manuscript were presented at the 2017 World Equine Airways Symposium and the 2017 Veterinary Comparative Respiratory Society Symposium.

CONFLICT OF INTEREST DECLARATION

Dr Moore serves as Consulting Editor for Experimental Design and Statistics for the Journal of Veterinary Internal Medicine. He was not involved in review of this manuscript.

OFF-LABEL ANTIMICROBIAL DECLARATION

No off-label antimicrobial use occurred.

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC) OR OTHER APPROVAL DECLARATION

The Purdue University Animal Care and Use Committee and the Indiana Horse Racing Commission approved all procedures.

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REFERENCES


**SUPPORTING INFORMATION**

Additional Supporting Information may be found online in the supporting information tab for this article.

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EFFECT OF FORAGE TYPE ON PARTICULATE EXPOSURE IN THE BREATHING ZONE OF RACEHORSES.

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Purpose: Particulate exposure have been implicated in the pathogenesis of equine asthma. Feeding dry hay, the most commonly used forage, has been shown to be associated with a 4-fold increase in exposure to dust in the horses’ breathing zone compared with exposure to pasture. Feeding forages with higher moisture content, such as steamed hay and haylage, can reduce respirable dust exposure. The purpose of this study was to compare respirable dust and particle number concentration between hay, steamed hay and haylage in controlled conditions in-vitro and in horses fed those forages.

Methods: Airborne particles released from 3 forages (hay, steamed hay and haylage) were measured in-vitro in a purpose-built agitation chamber. Five measurements were performed on each forage. Respirable dust concentration was measured gravimetrically using PVC filter, a cyclone (225-01-02, SKC, PA, USA) and a sampling pump (3 L/min, model, SPC, PA, USA). Particle number concentration by size was measured with a real time particulate monitor (OPC-N2, Alphasense, UK). Additionally, breathing zone exposures were measured in 30 healthy racing Thoroughbreds using the same instruments. The horses were randomly assigned to be fed one of the 3 forages for 3 weeks and measurements taken on the last day. Respirable dust mass and particles number concentrations were compared between forages using Kruskal-Wallis test. Data were summarized using median and interquartile range [IQR]. P<0.05 was considered significant.

Results: In the agitation chamber, median respirable dust concentration was different between hay (104.3 mg/m³ [29-191.3]) and haylage (5 mg/m³ [2.4-6]); p=0.01), but not steamed hay (33.2 mg/m³ [1.9-35.9]). The mode particle size for hay and steamed hay was 2.5 µm and for haylage was 0.46 µm. The median number concentration of particles 1 µm or smaller was significantly lower for haylage (4.0x10⁶ particles/m³ [3.6x10⁶-5.1x10⁶]) than steamed hay (9.9x10⁷ particles/m³ [2.7x10⁷-1x10⁸]; p=0.02), but not with hay (8.4x10⁶ particles/m³ [8.3x10⁶-1.1x10⁷]). Similar differences were observed for particles between 1 and 3 µm. For particles between 3 and 10 µm number concentrations were lower for haylage (4.0x10⁶ particles/m³ [0-3.7x10⁵]) compared to hay (6 x10⁷ particles/m³ [4.8x10⁷-1.3x10⁸], p=0.02).

The median respirable dust exposure in the breathing zone of horses fed hay 0.07 mg/m³ ([0.05-0.14]; n=10) was higher than for those fed haylage (0.05 mg/m³ [0.05-0.08]; p=0.007; n=8) but not different from steamed hay (0.05 mg/m³ [0.05-0.1]; n=12). The mode particle size for the three forages was 0.46 µm. The median number concentrations for all size ranges were highest in horses fed hay however, it did not reach statistical significance (p=0.2).

Conclusion: Particulate exposure is lowest with haylage both in the agitation chamber and in horses’ breathing zone.
Recommendations for the Diagnosis and Treatment of Equine Metabolic Syndrome (EMS)
Introduction

Equine Metabolic Syndrome (EMS) is characterized by insulin dysregulation, abnormal adipose tissue distribution, and altered adipokine concentrations. The clinical consequence of EMS is an increased risk of laminitis, although other morbidities also occur. This syndrome results from an interaction between genetics and environment, and the risk of laminitis in the individual animal therefore depends on the relative weighting of these influences. We can identify high-genetic risk animals that develop EMS with only mild environmental influences, and early detection is essential in these animals. Other horses have a lower genetic risk but can develop EMS through exposure to improper environments (diets that provide more calories than an animal requires and are high in non-structural carbohydrates [NSC]). It might therefore be assumed that any horse can develop EMS if pushed far enough in the wrong direction by improper management and exposure to environmental factors. Epigenetic influences on gene expression might also promote or advance the development of EMS.

The Equine Endocrinology Group (EEG) is composed of experts in the field of equine endocrinology who provide advice in the form of written guidelines to help veterinary practitioners diagnose and manage equine endocrine disorders. Guidelines are updated every two years or when new information becomes available and can be found on the EEG website: http://sites.tufts.edu/equineendogroup.

Table 1 - Definition of Terms

<table>
<thead>
<tr>
<th>Terms</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Insulin dysregulation (ID)</strong></td>
<td>Any combination of basal (resting) hyperinsulinemia, postprandial hyperinsulinemia (response to oral sugar test or consumed feeds), or insulin resistance. Insulin dysregulation is a central feature of EMS and also occurs in a subset of horses with pituitary pars intermedia dysfunction (PPID) and other conditions such as systemic illness.</td>
</tr>
<tr>
<td><strong>Equine metabolic syndrome (EMS)</strong></td>
<td>A collection of risk factors for endocrinopathic laminitis with ID as the central and consistent feature. Other components of EMS include increased generalized or regional adiposity, weight loss resistance, dyslipidemia, and altered adipokine concentrations. EMS is therefore defined by the presence of ID and commonly one or more of the other risk factors listed above. It is assumed that a horse with endocrinopathic laminitis has increased endocrinological risk factors when more than one additional risk factor is encountered.</td>
</tr>
</tbody>
</table>
Insulin dysregulation is detected in equids with EMS and in some with PPID and this is illustrated in the Venn diagram shown in Figure 1.

Figure 1 - Venn diagram showing the proposed relationships among endocrine disorders discussed in these recommendations. Note that the area of each category within the diagram is purely illustrative and is not intended to be proportionate to the size of the population.

* Note that insulin status is also affected by pregnancy, starvation, and systemic illness.
Figure 2 - Algorithm for the diagnosis and management of EMS (June 2018)

The following algorithm (Figure 2) outlines the recommended diagnostic and management pathways once ID is diagnosed in both groups of animals.

Horse, pony, or donkey
Presented for evaluation of:
- Laminitis
- Generalized obesity
- Infertility
- Pre-purchase examination
- Contemplating intra-articular or systemic steroids
- Divergent hoof rings
- Regional adiposity
- Wellness examination
- Suspicion of endocrine disease

Test for insulin dysregulation
Refer to Tables 3 and 4

NEGATIVE

Manage obesity or PPID as appropriate

Obese EMS

Reduce body fat mass
- Limit caloric intake
- Low NCS diet
- Restricted or zero access to grass
- Exercise (if feet are stable)

Remains obese
- Levothyroxine

Fat loss

POSITIVE

Lean EMS

Maintain body condition
- Low NCS, higher fat, good quality fiber diet
- Restricted or zero access to grass
- Exercise (if feet are stable)

EMS with PPID Obese or Lean

Pergolide and diet appropriate for body fat mass (see boxes to the left)

Re-test insulin status to assess response
Consider other medical treatments for refractory cases
Recommended approach for diagnostic testing

Sample handling & analysis

- Insulin is stable in plasma or serum for at least three days when separated from red blood cells and refrigerated (4°C). Freeze serum or plasma if samples cannot be mailed within this time period. Note that samples may be frozen and thawed once, but multiple freeze-thaw cycles alter insulin concentrations.

- Insulin results vary according to the assay (radioimmunoassay, chemiluminescent assay, or enzyme-linked immunosorbent assay) and analyzer (e.g., Immulite 1000®, Immulite 2000®) used to measure the hormone and cut-off values must be considered accordingly. Contact your laboratory to confirm that the insulin assay in use has been validated for use with equine serum/plasma, and that reference intervals are specific to the assay and analyzer that are being used.

Selection of diagnostic tests

- **Two dynamic tests are recommended:** the oral sugar test (OST) and the insulin tolerance test (ITT). The OST is preferred because insulin concentrations measured reflect a more complete sequence of events including digestion and absorption of sugars, incretin hormone responses, and secretion of insulin from the pancreas, whereas the ITT focuses solely upon tissue insulin sensitivity.

- **Oral sugar test:** Advantages of this test include the ready availability of corn syrup, ease of administering corn syrup, and the test's assessment of insulin responses to ingested sugars. Disadvantages include the requirement for horses to be fasted for 3-12 hours prior to testing and relatively low within- horse repeatability in test results. Variability in results is attributed to multi-factorial influences such as differences in gastric and intestinal transit times, digestion and absorption of sugars, incretin responses and insulin secretion. When monitoring horses over time with this test, binary changes in the positive or negative result and major shifts in insulin concentrations (> 30 μU/mL) are clinically significant. Test performance is improved by administering 0.45 mL corn syrup/kg body weight instead of 0.15 mL corn syrup/kg, and this approach is routinely used in the United Kingdom. Oral glucose powder has been used when corn syrup is not available.

- **Insulin tolerance test:** Advantages of the ITT are that this test does not require pre-test fasting and blood glucose concentrations can be measured with a glucometer so preliminary results are available on the farm. The test is less expensive to perform than the OST. Disadvantages include the cost of purchasing insulin and the risk of clinical hypoglycemia developing (although this is unlikely to occur in horses selected for testing on suspicion of ID). A small amount of grain may be fed to the horse or IV dextrose administered immediately after the 30-minute sample is collected to further mitigate hypoglycemia risk.

- **Resting (basal) insulin concentrations:** A single blood sample is collected with the horse in the fed state (hay or pasture, but not grain), and plasma/serum insulin concentrations are measured to detect resting hyperinsulinemia. This approach may be used to assess the insulinemic effect and laminitis risk of the forage component of the current diet.

- **Two-step approach to diagnosing ID:** Testing can be performed in two steps if the owner raises concerns about dynamic tests inducing laminitis. Note: It has been the collective experience of the EEG that dynamic tests cause only transient alterations in glucose and insulin concentrations and do not induce laminitis. The first step is to measure the resting (basal) insulin concentration to screen the horse for hyperinsulinemia and assess laminitis risk. If the resting insulin concentration is normal, a dynamic test must still be performed as a second step because resting measures have low diagnostic sensitivity. Markedly abnormal OST results may be seen in horses with normal resting insulin concentrations. An OST is also recommended when only mild hyperinsulinemia is detected to estimate insulin responses to grazing on pasture or feeds.

- **Blood glucose concentrations:** Diabetes mellitus occurs occasionally in horses and is likely to be detected with higher frequency in equids affected by EHV or PPID. Resting blood glucose concentrations should be measured to detect diabetes mellitus when any of the above tests for ID are performed.

- **Tests that are no longer recommended:** The glucose:insulin ratio and proxy measures of insulin sensitivity are not recommended as substitutes for the OST or ITT; and the combined glucose-insulin test, frequently-sampled intravenous glucose tolerance test, and euglycemic-hyperinsulinemic clamp procedure are considered too complex and expensive for routine clinical use.
Figure 3 - Algorithm for detection of insulin dysregulation (June 2018)

Assess Insulin Status
Refer to Tables 3 and 4

Recommended tests
To screen for ID if concerns about dynamic testing

Dynamic testing

Assessment of postprandial insulin response
Withhold feed 3-12 hours

Oral Sugar Test (OST)
NEGATIVE* (Insulin dysregulation)
Manage insulin dysregulation (ID)
Refer to Table 6

POSITIVE (Insulin dysregulation)
Perform OST

Insulin Tolerance Test (ITT)
NEGATIVE (insulin resistant)
Manage ID

POSITIVE
Perform dynamic test

Resting insulin concentration
Low diagnostic sensitivity

NEGATIVE
Manage ID

POSITIVE (hyperinsulinemia)

* Consider re-testing and use 0.45 ml/kg dose for OST
# Table 2 - Clinical presentation of Equine Metabolic Syndrome (EMS)

## Equine Metabolic Syndrome

<table>
<thead>
<tr>
<th>Signalment</th>
<th>Clinical Features</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>OBESE (TYPICAL) MANIFESTATION OF EMS</strong></td>
<td><strong>Some or all of the following may be present:</strong></td>
</tr>
<tr>
<td>Genetic risk is implied by certain breeds having higher EMS prevalence</td>
<td>Weight loss resistance (‘Easy keeper’/‘Good Doer’)</td>
</tr>
<tr>
<td>Examples of higher genetic risk breeds: Pony breeds</td>
<td>Laminitis (subclinical or clinical)</td>
</tr>
<tr>
<td>Andalusians</td>
<td>Cresty neck</td>
</tr>
<tr>
<td>Gaited breeds (e.g., Saddlebreds, Paso Finos)</td>
<td>Subcutaneous adipose deposits</td>
</tr>
<tr>
<td>Morgans</td>
<td>Clinical problems may be historical or current</td>
</tr>
<tr>
<td>Miniature horses</td>
<td></td>
</tr>
<tr>
<td>Warmbloods</td>
<td></td>
</tr>
<tr>
<td>Uncertain genetic risk: Donkeys</td>
<td></td>
</tr>
</tbody>
</table>

**LEAN MANIFESTATION OF EMS**

- Genetically at-risk horse kept in controlled environment
- Laminitis (subclinical or clinical) only

**EMS with PITUITARY PARS INTERMEDIA DYSFUNCTION (PPID)**

- EMS may be historical
- Genetically at-risk horse that develops PPID (exacerbates insulin dysregulation)
- Clinical signs of EMS (current problem)
- Regional adiposity and/or obesity
- Laminitis
- No clinical signs of EMS currently (historical problem)
- Lean/thin at present

**OTHER CONDITIONS THAT SHOULD PROMPT TESTING FOR ID**

- Diabetes mellitus, metabolic derangements detected during critical care, equine hyperlipemia, infertility, colic caused by a pedunculated lipoma (associated with obesity), preputial/mammary gland edema, or detection of divergent hoof rings. Testing should also be considered prior to intra-articular or systemic corticosteroid administration, or as part of wellness or prepurchase examinations.

---

* These breeds are overrepresented and there is evidence of a genetic predisposition in Arabian horses.
* Additional studies are currently being conducted to examine the genetic basis of EMS.
* Equine metabolic syndrome is poorly characterized in donkeys because reference intervals for insulin tests are still being determined.
<table>
<thead>
<tr>
<th>Procedure</th>
<th>After hay (no grain)</th>
<th>While on pasture</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Do not feed grain within 4 hours</td>
<td>Used to assess insulin concentrations during grazing (^d)</td>
</tr>
<tr>
<td></td>
<td>Collect into serum or EDTA tube (check with laboratory)</td>
<td>(assessment of current management)</td>
</tr>
</tbody>
</table>

| Assays used\(^a\)          | Results must be interpreted in the context of the insulin assay used                |                                                                                  |
|                            | (chemiluminescent assay, radioimmunoassay, or ELISA)                               |                                                                                  |

<table>
<thead>
<tr>
<th>Results</th>
<th>Interpretation(^c)</th>
<th>Recommendation</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 20 µU/mL(^b)</td>
<td>Non-diagnostic</td>
<td>Dynamic test recommended to better assess</td>
</tr>
<tr>
<td>20-50 µU/mL(^b)</td>
<td>ID suspect</td>
<td>Proceed with ID management</td>
</tr>
<tr>
<td>&gt; 50 µU/mL(^b)</td>
<td>Insulin dysregulation</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) Assay should be validated for use with equine samples

\(^b\) Cut-off values for Immulite 1000; different values may be required for other assays or analyzers. For example, equivalent Immulite 2000xpi values are 31 and 80 uU/mL for 20 and 50 uU/mL, respectively

\(^c\) Quality and NSC content of forages can vary and affect results; cut-off values are only applicable when horses are consuming low-NSC carbohydrate hay

\(^d\) Note that these values reflect the NSC content of the grass being consumed at the time of testing, which can change quickly and significantly over time

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Table 3 - Diagnostic testing: Resting insulin concentrations

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### Table 4 - Dynamic insulin tests

<table>
<thead>
<tr>
<th>Procedure</th>
<th>Oral Sugar Test*&lt;sup&gt;6&lt;/sup&gt;</th>
<th>Insulin Sensitivity</th>
<th>Insulin Tolerance Test&lt;sup&gt;4&lt;/sup&gt;&lt;sup&gt;4&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Postprandial insulin response</strong></td>
<td>Fast 3-12 hours&lt;br&gt;Administer 0.15 mL/kg corn syrup orally via dose syringe&lt;br&gt;Collect blood at 60 and 90 minutes&lt;br Measure insulin and glucose</td>
<td><strong>Insulin sensitivity</strong></td>
<td>Fed (pasture or hay) state. Do not fast&lt;br&gt;Collect blood at time 0 and administer 0.10 IU/kg regular (soluble) insulin&lt;br&gt;Collect blood at 30 minutes&lt;br&gt;Measure glucose&lt;br&gt;Feed meal immediately after last sample</td>
</tr>
<tr>
<td><strong>Interpretation</strong>&lt;sup&gt;C&lt;/sup&gt;</td>
<td>&gt; 45 μU/mL is positive&lt;sup&gt;d&lt;/sup&gt;&lt;br&gt;Assess baseline (fasting) glucose concentration to detect diabetes melitus (rare)</td>
<td><strong>Alternative tests</strong></td>
<td>Combined glucose-insulin test (CGIT)</td>
</tr>
<tr>
<td><strong>Alternative tests</strong></td>
<td>In-feed oral glucose tolerance test (OGTT)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Use of a higher dose of corn syrup (0.45 mL/kg) improves test performance.

United Kingdom/Europe: An insulin cut-off value of 40 μU/mL is used for the 0.45 mL/kg OST when measuring insulin with the chemiluminescent assay and Immulite 1000<sup>©</sup> (http://liphoquequinehospital.co.uk/equine-laboratory/); or 110 μU/mL with the radioimmunoassay.

USA: At the time of writing, cut-off values have not been established for the 0.45 mL/kg OST when using an ELISA to measure insulin.

<sup>C</sup> Note that hypoglycemia is a risk associated with this test. Provide feed after collecting the second blood sample. Administer dextrose solution intravenously if clinical signs of hypoglycemia develop.

<sup>d</sup> Try to minimize stress prior to testing.

<sup>d</sup> When insulin is measured using the radioimmunoassay.
### Table 5 - Additional tests for assessment of horses with Equine Metabolic Syndrome (EMS)

<table>
<thead>
<tr>
<th>Test</th>
<th>Procedure</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Adiponectin</strong>&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Collect blood in serum tube; chill on ice and keep refrigerated</td>
<td>High molecular weight (HMW) adiponectin concentrations &lt; 3.2 ug/mL (ELISA) are consistent with metabolic derangement in adipose tissues and increased risk of laminitis. Total adiponectin concentrations &lt; 2.5 ug/ml (RIA) or &lt; 24 ug/ml (immunoturbidimetric assay) are consistent with EMS.</td>
</tr>
<tr>
<td><strong>Leptin</strong>&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Collect blood in serum or EDTA tube; keep refrigerated</td>
<td>Consult reference interval provided by laboratory. Higher leptin concentrations are associated with increased adiposity and metabolic derangement. Useful for providing evidence of increased internal adiposity. This hormone is more directly associated with obesity than ID.</td>
</tr>
<tr>
<td><strong>Triglyceride concentrations</strong>&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Collect blood in serum tube</td>
<td>Consult reference interval for laboratory. Hypertriglyceridemia associated with ID and obesity, exacerbated by negative energy balance. Hypertriglyceridemia is a predictor of laminitis risk in ponies, with cut-off values of 57 and 94 mg/dL previously reported.&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

#### Potential Future Tests

Glucose-dependent insulino tropic (polypeptide) (GIP) concentrations, glucagon-like peptide-1 and -2 concentrations, C-peptide concentrations, and genetic and metabolomic testing

---

<sup>a</sup> Liphook Equine Hospital (http://liphookequinehospital.co.uk/equine-laboratory/) offers the HMW adiponectin assay (ELISA)

<sup>b</sup> Animal Health Diagnostic Center at Cornell University (https://ahdc.vet.cornell.edu/)
Table 6 - Management recommendations for equine metabolic syndrome

<table>
<thead>
<tr>
<th>Obese (typical) EMS BCS 6–9/9</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Initial diet</strong></td>
</tr>
<tr>
<td>Restrict or eliminate grazing and do not feed grain. For weight loss, feed grass hay with low NSC content in amounts equivalent to 1.5% of current body weight daily. Reassess body weight every 30 days using a weight scale or weight tape and gradually lower to a minimum of 1.2% of body weight as-fed if weight loss resistant. House in a dry-lot or small paddock with a companion. Avoid stress as much as possible. NSC analysis of hay recommended, particularly if severe ID is detected. Select hay with NSC content &lt; 10% as-fed if available. Soak hay in cold water for 60 minutes before feeding to lower the water-soluble carbohydrate content. Incorporate slow feeder or provide frequent, small meals so that prolonged fasting is avoided. Provide a mineral/vitamin/protein ration balancer. Care should be taken to select a ration balancer with low sugar content.</td>
</tr>
<tr>
<td><strong>Maintenance diet</strong></td>
</tr>
<tr>
<td>Restrict grazing and do not feed grain Maintain on initial hay amount until body condition 5/9 is achieved. Improvement in the values obtained from the same test(s) used to diagnose EMS (OST, ITT, HMW adiponectin, and/or leptin concentrations) is expected when re-tested under similar conditions. Soak hay (see above) Provide mineral/vitamin/protein ration balancer Turnout decision based upon follow-up testing of postprandial insulin response and might include use of a grazing muzzle or other methods to reduce grass consumption.</td>
</tr>
<tr>
<td><strong>Exercise</strong></td>
</tr>
<tr>
<td>Exercise is recommended unless laminitis is present. All levels of exercise are likely to be beneficial for accelerating weight loss in obese animals, but more intense exercise programs may be required to improve insulin sensitivity. Exercise levels should be incrementally increased over time until the horse is being worked at a canter to fast canter, ridden or unridden, for &gt; 30 minutes per day for &gt; 5 times per week. If heart rate can be monitored, the intensity of exercise should be sufficient to induce rates of 150 to 170 beats per minute.</td>
</tr>
<tr>
<td><strong>Housing</strong></td>
</tr>
<tr>
<td>Stress should be avoided, and the affected horse should be housed in a small paddock with a companion, instead of being confined to a stall. Take precautions to limit stereotypic behavior by using slow feeders. Turnout on pasture is strongly discouraged until the problems of obesity and ID are successfully addressed.</td>
</tr>
</tbody>
</table>
Management and monitoring of EMS (continued)

**Obese (typical) EMS**

**Medical therapy**

*High-dose levothyroxine*

- **Indications:** For cases with weight loss resistance (no documented response after a minimum of 30 days on weight loss diet) or for accelerated management of obesity in acute laminitis cases
- **Available in the USA, but high cost restricts use in the UK or Europe. Administer levothyroxine at a high dose of 0.1 mg/kg (48 mg or 4 teaspoons of the powdered product for a 500-kg horse) daily in the feed or by mouth while also controlling caloric intake. Gradually reduce the dose and discontinue treatment after weight loss achieved or after 3-6 months of therapy.

*Metformin hydrochloride*

- **Indications:** For animals with persistent hyperinsulinemia, even after management changes have been followed. Metformin is sometimes prescribed for the first two weeks when a horse is transitioned back to pasture, but additional research is required to assess this approach.
- **Administer 30 mg/kg metformin hydrochloride in the feed or by mouth, ideally 30 minutes prior to feeding or turnout, up to 3 times daily. Metformin can also be administered at a higher dose of 50 mg/kg, but oral irritation may occur at this dose. Check insulin concentrations 2 hours post-feeding before and 7 days after initiating metformin treatment because metformin does not improve insulin status in all cases.

*Medical treatments in development*

- Sodium-glucose co-transporter 2 (SGLT2) inhibitors (canagliflozin and velagliflozin) and thiazolidinediones (pioglitazone) are currently being evaluated as drugs for medically managing ID in horses.

*Foot care*

- Routine hoof care is essential in all cases. Laminitis can occur without inducing easily detectable lameness, and radiographs are recommended to identify structural changes.

**Lean EMS**

**Diet**

- Maintain on low-glycemic diet, with severity of restriction dependent on postprandial insulin response.
- **Analyze NSC content of hay if severely affected. Provide diet with low-NSC, high-fat, and add calories in the form of high-quality fiber content such beet pulp or soy hulls.**
- **Provide mineral/vitamin/protein ration balancer**

**Exercise**

- **As above**

**Medical**

- **Levothyroxine not recommended, as weight loss is not required.**

**EMS with diagnosed PPID**

- Follow appropriate recommendations from above, depending upon body condition.

**Medical**

- **Administer pergolide [Prascend® (pergolide tablets); Boehringer-Ingelheim Vetmedica, Inc.]; refer to EEG Recommendations on PPID.**

*Acknowledging that this will not reliably lower the NSC content to <10% in all hays.*

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Table 7 - Consideration of pituitary pars intermedia dysfunction (Cushing’s disease) status

Refer to the most recent Equine Endocrinology Group recommendations on diagnosing and managing PPID in horses: https://sites.tufts.edu/equineendogroup/

<table>
<thead>
<tr>
<th>Consideration</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age</strong></td>
</tr>
<tr>
<td>EMS affects horses across a wide range of ages</td>
</tr>
<tr>
<td>PPID is a common comorbidity in horses above 10 years, and the likelihood of PPID increases as the age of the horse increases.*</td>
</tr>
<tr>
<td><strong>Impact on ID</strong></td>
</tr>
<tr>
<td>PPID is an exacerabating factor for ID speculated to be a consequence of hormone products secreted from the pars intermedia.</td>
</tr>
<tr>
<td>Age alone alters insulin dynamics and responses to different diets, with higher insulin secretion and lower insulin sensitivity detected in aged horses.10,11</td>
</tr>
<tr>
<td><strong>Diagnostic testing</strong></td>
</tr>
<tr>
<td>Early-affected horses should undergo thyrotropin-releasing hormone (TRH) stimulation testing (test results are difficult to interpret in late summer-fall).</td>
</tr>
<tr>
<td>When performed at the same time as diagnostic tests for ID, the TRH stimulation test is performed before, but not during or after the OST.12</td>
</tr>
<tr>
<td>A combined insulin tolerance test/TRH stimulation test has recently been developed and for this test, insulin and TRH are administered together as a single IV injection (personal communication; François-René Bertin).</td>
</tr>
<tr>
<td>Basal plasma ACTH concentrations are measured for more advanced cases. Detection of a high ACTH concentration confirms the diagnosis of PPID, but horses with suggestive clinical signs and negative results should undergo TRH stimulation testing.</td>
</tr>
<tr>
<td><strong>Management</strong></td>
</tr>
<tr>
<td>Diet: Based upon the postprandial insulin response. An OST or oral glucose test is recommended for all horses diagnosed with PPID.</td>
</tr>
<tr>
<td>Exercise: Refer to Table 5.</td>
</tr>
<tr>
<td>Medical: Administer pergolide [Prascend* (pergolide tablets); Boehringer Ingelheim]</td>
</tr>
<tr>
<td>Comorbidities: May require management of other medical problems related to PPID and age, including bacterial infections, dental disease, organ dysfunction, and parasitism.</td>
</tr>
<tr>
<td>Critical illness: Insulin dysregulation and PPID are complicating factors in patients with critical illness and may predispose affected patients to hyperglycemia and hypertriglyceridemia. Endocrine system decompensation may adversely affect treatment outcomes.</td>
</tr>
</tbody>
</table>

*Although a causal relationship between EMS and PPID has been suggested, there are currently no published research studies evaluating their association.

Disclosures

Andy Durham and Lisa Tadros are affiliated with the Liphook Equine Hospital and the Veterinary Diagnostic Laboratory at Michigan State University, respectively and both institutions offer endocrine testing.

Boehringer Ingelheim Vetmedica, Inc. facilitates the development of EEG guidelines by supporting travel expenses for participants but does not influence the recommendations made by the group.

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References


APPENDIX E

Refereed Scientific Publications:


Equine asthma: Integrative biologic relevance of a recently proposed nomenclature

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²Equine Immunity & Inflammation, LABÉO Frank Duncombe, Caen, France
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Email: rleguille@ucalgary.ca

The term "equine asthma" has been proposed as a unifying descriptor of inflammatory airway disease (IAD), recurrent airway obstruction (RAO), and summer pasture-associated obstructive airway disease. Whilst the term will increase comprehensibility for both the lay and scientific communities, its biologic relevance must be compared and contrasted to asthma in human medicine, recognizing the limited availability of peer-reviewed equine-derived data, which are largely restricted to clinical signs, measures of airway obstruction and inflammation and response to therapy. Such limitations constrain meaningful comparisons with human asthma phenotypes. Suggested minimum inclusion criteria supporting the term asthma, as well as similarities and differences between IAD, RAO, and multiple human asthma phenotypes are discussed. Furthermore, differences between phenotype and severity are described, and typical features for equine asthma subcategories are proposed. Based on shared features, we conclude that mild/moderate (IAD) and severe (RAO) equine asthma are biologically appropriate models for both allergic and non-allergic human asthma, with RAO (severe equine asthma) also being an appropriate model for late-onset asthma. With the development of new biologic treatments in humans and the application of more targeted therapeutic approaches in the horse, it would appear appropriate to further investigate the allergic (Th-2) and non-allergic (non-Th-2) phenotypes of equine asthma. Further research is required to more fully determine the potential clinical utility of phenotype classification.

KEYWORDS
animal model, disease severity, horse, IAD, RAO

1 | INTRODUCTION

Numerous terms have been used to describe chronic inflammatory lower airway disease in horses, including heaves, recurrent airway obstruction (RAO), equine chronic obstructive pulmonary disease, inflammatory airway disease (IAD), tracheal IAD, bronchial IAD, small airway disease, chronic bronchitis, summer pasture-associated chronic obstructive pulmonary disease, summer pasture-associated obstructive pulmonary disease, summer pasture-associated obstructive airway disease, summer heaves, and summer RAO. Progressive awareness of various clinical and pathological features of equine inflammatory lower airway disease precipitated the evolution of the above nomenclature; however, this has become unsustainable, resulting in confusion within both the veterinary and lay communities. It has recently been proposed that chronic non-infectious inflammatory lower airway disease in horses be reassigned the designation "equine asthma."¹-³ As highlighted during the 6th World Equine Airway

Abbreviations: AP-1, activator protein-1; ASM, airway smooth muscle; BAL, bronchoalveolar lavage; BALF, bronchoalveolar lavage fluid; CXCL2, chemokine (C-X-C motif) ligand 2; ECM, extracellular matrix; IAD, inflammatory airway disease; IFN, interferon; IL, interleukin; IL4Rα, IL-4 receptor α-chain; LT, leukotriene; NF-κB, nuclear factor-κB; RAO, recurrent airway obstruction; TNF, tumor necrosis factor
Symposium (2017), the biological appropriateness of applying the term “equine asthma” must be considered in light of its current use in human medicine before its widespread adoption in the veterinary literature. Increasing comprehensibility amongst the horse-owning public and the veterinary profession would constitute a clear benefit of the newly proposed terminology; however, the validity and limitations of the proposed change in nomenclature must first be considered and described. Before the proposed use of the term “equine asthma,” RAO/Heaves, and IAD have been widely used and accepted because of their accurate descriptions of the disease processes to which they refer. While a distinction between these 2 phenotypes was initially proposed for research purposes to facilitate comparison between study results, it was not the intent of the workshop participants to suggest that they were 2 separate conditions. However, different names lead clinicians to subsequently consider them to be distinct and both have individually been the subject of expert panels’ workshops and publications. In contrast to, and distinct from IAD, horses with RAO exhibit increased respiratory effort at rest. This distinguishing feature is attributable to the magnitude of bronchoconstriction, increased mucus production and bronchial inflammation associated with this disorder. While IAD and RAO are considered as separate diseases, it is presently unclear whether this distinction reflects a dissimilar pathogenesis, or simply a difference in the clinical severity. There are many factors which potentially differ (ie, clinical signs, pathogenesis, recurrence) among the spectrum of diseases which fall within the proposed new “equine asthma” classification, including severity of clinical signs, pathogenetic pathways, and rates of recurrence. Therefore, further differentiation of the term into mild, moderate, and severe equine asthma has been advocated. Although application of these qualifying terms is currently limited to clinical severity, with mild/moderate and severe equine asthma being analogous to IAD and RAO, respectively, it is hoped that future subclassification efforts might consider additional criteria such as pathogenetic pathways and immunological characteristics. The aims of this review are to: (1) propose minimum inclusion criteria supporting utilization of the term “equine asthma,” (2) compare and contrast features of equine asthma with the most common human asthma phenotypes, (3) propose typical features for subcategories of equine asthma, and (4) provide recommendations for future research directions.

2 | INCLUSION CRITERIA

The biological appropriateness of the term “equine asthma” must be considered relative to its current use in human medicine. It is important to consider both a minimum set of criteria shared by all human and equine asthma phenotypes, as well as additional criteria shared between specific human and equine asthma phenotypes.

3 | MINIMUM INCLUSION CRITERIA FOR APPLICATION OF THE TERM “ASTHMA”

Asthma in humans is a heterogeneous disease characterized by non-septic chronic airway inflammation. Therefore, there is a need for distinct criteria for distinguishing atopic vs. non-atopic asthma.

of respiratory disease (coughing, wheezing, shortness of breath and tightness of the chest) which vary in intensity and over time, combined with airway hyperresponsiveness and expiratory airflow limitation of fluctuating severity. Bronchoconstriction, airway wall thickening, increased mucus secretion, and airway remodeling are accompanying this phenotype. With the exception of shortness of breath and chest tightness, which, as subjective descriptors of a perceived sensation, are not feasibly applicable to the horse, this phenotype is largely shared by both IAD and RAO. Horses with RAO exhibit the same pathophysiologic features as human asthma; namely bronchoconstriction, airway wall thickening, increased mucus production and airway remodeling. This pathophysiology is associated with the increased respiratory effort observed at rest in horses with RAO. Horses with IAD have inflammation of the trachea and bronchi, with an excessive accumulation of mucus in the airways, resulting in a mild increased resistance to airflow. Mild equine asthma decreases racing performance in Thoroughbred racehorses. The pathology exhibited by horses with IAD typically manifests in clinical signs that are subtle at rest, with horses exhibiting chronic (>3 weeks) occasional coughing and normal respiratory effort; and coughing, increased nasal discharge, poor performance, or a combination of these during exercise. Impaired pulmonary gas exchange limits performance, and intensely exercising horses with IAD have worsening of exercise-induced hypoxemia. However, the bronchoconstriction in horses with IAD is sufficiently mild to evade clinical detection via the appreciation of increased respiratory effort at rest without bronchoprovocation. Whilst airway remodeling has not yet been studied in horses with IAD, peribronchial infiltration of inflammatory cells (82/95 horses) and bronchiolar smooth muscle hyperplasia (93/95 horses) are common in racehorses. Although eosinophils or mast cells (or both) are present in the bronchiolar wall of some racehorses, it was not possible for the authors to determine if these findings correspond to a clinical diagnosis of IAD. Notably absent from this list of minimum inclusion criteria is the predominant airway inflammatory cell; this notable omission is further discussed in Section 7.

4 | DIAGNOSIS

A diagnosis of asthma in human patients with signs of respiratory disease is initially based on a detailed clinical history, physical examination (which can be normal at the time of presentation), radiography, and screening questionnaires. Despite the value of context-specific questionnaires in positively screening for high-risk chronic airway disease patients, international guidelines emphasize the diagnostic importance of spirometry. This is especially pertinent considering the shared features common to both asthma and chronic obstructive pulmonary disease. Similarly, a presumptive diagnosis of IAD or RAO is generally based on the horses’ history and clinical presentation, the latter of which has been incorporated into both the independently validated risk-screening questionnaire (RSQ) and horse owner assessed respiratory signs index (HOARS). While these clinical-sign–based screening tools have both excellent sensitivity and negative predictive
to differentiate between healthy horses and those with mild airway inflammation (IAD). Furthermore, in light of the poor diagnostic sensitivity of coughing, mucoid nasal discharge and poor performance, reliance is placed on additional tests, such as tracheal endoscopy, bronchoalveolar lavage fluid (BALF) cytology and lung function evaluation, in an attempt to maximize diagnostic accuracy of both RAO and IAD.

5 | ADDITIONAL INCLUSION CRITERIA BETWEEN SPECIFIC HUMAN ASTHMA AND IAD/RAO PHENOTYPES

Any efforts to advocate equine asthma as an appropriate disease model for the study of human asthma must take into consideration the fact that multiple human asthma phenotypes exist, not all of which will share attributes with RAO and IAD. Similar considerations also relate to the translational application of human asthma-derived scientific findings to the horse, and vice versa. Therefore, the appropriateness of any such cross-species comparisons necessitates the application of additional criteria which specifically distinguish certain human asthma and IAD/RAO phenotypes based on disease-specific key features. It has been proposed that RAO is an ideal equine model for the study of non-allergic, late-onset, and severe asthma phenotypes; however, the biologic appropriateness of IAD for the study of specific human asthma phenotypes has not yet been investigated and is a focus of this review.

6 | PHENOTYPE VERSUS SEVERITY

An "asthma phenotype" is a recognizable cluster of demographic, clinical, pathophysiological, or any combination of these characteristics, however, these do not always have a strong correlation with specific pathologic processes, or even treatment responses. In humans, various asthma management guidelines have described methods to categorize asthma severity; however, there are substantial theoretical and practical differences between recommendations. Asthma severity is differentiated into mild, moderate and severe categories and is predominantly based on the level of treatment required to control symptoms and exacerbation; it is not a static feature of the disease and changes over time. In some instances, it is also used to describe the intensity of symptoms or the magnitude of airflow limitation. However, these approaches do not focus on quantifying markers of airway inflammation, which would assess the severity of the disease process itself. For practical reasons, asthma is only classified after institution of effective treatment and therefore assessment is always subject to treatment effect. To date, there are no treatment-naive predictors of disease severity.

It has been proposed that mild/moderate equine asthma replace IAD, and severe equine asthma replace RAO. Certain criteria have recently been proposed for the subcategorization of equine asthma based on severity. Specific cutoff values or recommendations were proposed for the following methods: clinical presentation, airway endoscopy, airway cytology, bronchoalveolar lavage (BAL) cytology and lung function testing. However, applicability of these criteria to RAO and IAD subcategorization remains arbitrary. A meta-analysis of published studies based on client-owned horses with IAD and RAO would likely offer valuable information on the relative contributions of each of the above criteria to the overall equine asthma subcategorization exercise. Moreover, a poor correlation exists between specific diagnostic results (ie, severe inflammatory bronchoalveolar lavage [BAL] profile) and clinical signs (ie, increased respiratory effort at rest). Although the inclusion of severity of clinical signs as a key criterion in the subcategorization of equine asthma is easy to comprehend (particularly among the horse-owning public), it should not be applied exclusively, particularly in light of the inconsistent correlation between severity of airway inflammation and clinical signs in both human and equine asthma.

7 | PHENOTYPES

There is a need to identify and apply criteria to further subcategorize equine asthma, and it has been suggested that a new classification based on immunological signature data could have greater relevance, particularly in the context of novel, targeted biologic therapeutic approaches. In humans, it is recognized that asthma is a heterogeneous disease, with the underlying pathogenesis differing among phenotypes. There is evidence that RAO has a genetic background with possible locus heterogeneity (discussed in Section 8). In comparison, while genetic susceptibility is suspected in IAD, it has not been investigated. In light of the biologic characteristics common to both equine and human asthma and the marked disease heterogeneity in both, endeavoring to apply currently defined human asthma phenotypes to the horse seems to represent a logical starting point in the process of equine asthma subcategorization. There are multiple human asthma phenotypes, the most common of which are allergic asthma, non-allergic asthma, late-onset asthma, asthma with fixed airflow limitation, and asthma in obese patients. While RAO and IAD do not necessarily share attributes with all phenotypes, similarities and differences between these equine diseases and human asthma are discussed below, and summarized in Table 1. Furthermore, Table 1 also identifies the equine diseases which, at this time, the authors propose to be biologically appropriate models for each human asthma phenotype. The authors acknowledge the requirement for further research to better support these preliminary proposals. Our review aims to focus on the biologic relevance of the proposed nomenclature; however, for an extensive discussion of the advantages and disadvantages of the equine asthma model, the reader is referred to the excellent review article.

8 | ALLERGIC ASTHMA

One of the most common human asthma phenotypes is "allergic asthma," a term which reflects the triggering role of allergens in this process. Allergic asthma is generally associated with a genetic predisposition.
<table>
<thead>
<tr>
<th>Asthma phenotype</th>
<th>Features in humans</th>
<th>Features supporting phenotype model in horses</th>
<th>Equine model appropriate?</th>
<th>Areas identified for future equine research</th>
</tr>
</thead>
</table>
| Allergic asthma  | • Allergic trigger associated with respiratory symptoms/ expiratory airflow limitation  
• Often commences in childhood  
• Past/family history of allergic disease (eczema/ allergic rhinitis/food or drug allergy)  
• Sputum often reveals eosinophilic airway inflammation  
• Usually respond well to ICS treatment  
• Th-2 CD4+ lymphocyte response—IL-5–mediated eosinophil recruitment  
• IL4Rx gene associated with the development of asthma, skin allergies and parasite defense | • IAD  
• Antigenic triggers central to development of lower airway inflammation  
• Stabling exposes horses to high levels of airborne particulates (eg, dust, endotoxin, fungi, molds, ultrafine particles, noxious gases), and is a risk factor for IAD  
• Antigenic triggers (eg, dust, mold spores) associated with increased neutrophil/mast cell% in BALF  
• Antigenic triggers associated with clinical signs (eg, coughing, poor performance)  
• Often occurs in young horses  
• Eosinophilic phenotype associated with dust exposure in young horses  
• Usually respond well to ICS treatment  
• Th-2 response—Increase in IL-4 and IL-5 in BALF linked with mastocytic phenotype | Yes | • Eosinophil involvement in pathogenesis of IAD  
• Effect of BALF phenotype on performance  
• Role of IgE in IAD and RAO  
• Longitudinal and cross-sectional studies investigating an “atopic march” in horses  
• Comprehensive study investigating the effect of various allergenic triggers on both lower airway pathology and clinical signs (ie, investigate causality rather than association) |
| Non-allergic asthma | • Not associated with allergy  
• Sputum can be neutrophilic eosinophilic or paucigranulocytic  
• Often respond less well to ICS  
• Chronically activated mast cells in bronchial mucosa (can be associated with non-allergic stimulus)  
• Th-1 response—cell-mediated immunity and phagocyte-dependent inflammation | • IAD  
• BALF can reveal neutrophilia and/or eosinophilia and/or mast cells accumulation  
• Th-1 response—mRNA encoding TNF-α, IL-1β, and IFN-γ in BALF  
• Th-17 response—Increase in IL-17 and IL-23 linked with increased neutrophil % in BALF  
• Often respond less well to ICS | Yes | • Role of neutrophil/mast cell activation in the development of lower airway inflammation |
| Late-onset asthma | • Initial presentation as adult (particularly women)  
• Less likely to be atopic  
• Decreased baseline pulmonary function  
• Often refractory to ICS/require higher doses for control | • IAD  
• Insufficient evidence  
• RAO  
• Decreased baseline pulmonary function during disease exacerbation  
• Mature/older animals  
• Can require higher doses for control | No | • Disease progression from IAD to RAO over time  
• Correlation between inflamm-aging and development of chronic inflammatory airway disease |
TABLE 1 (Continued)

<table>
<thead>
<tr>
<th>Asthma phenotype</th>
<th>Features in humans</th>
<th>Features supporting phenotype model in horses</th>
<th>Equine model appropriate?</th>
<th>Areas identified for future equine research</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Asthma with fixed airflow limitation</strong></td>
<td>• Chronic asthma patients with fixed airflow limitation; thought to be because of airway wall remodeling</td>
<td>IAD  • Insufficient evidence</td>
<td>No</td>
<td>• Airway remodeling in IAD</td>
</tr>
<tr>
<td></td>
<td>• Increased airway smooth muscle mass and extracellular matrix at all levels of bronchial tree</td>
<td>RAO  • Tissue remodeling is reversible—long-term antigen avoidance strategies and corticosteroid therapy decrease airway smooth muscle mass and subepithelial collagen area</td>
<td>Insufficient evidence</td>
<td>• Reversibility of airway remodeling in human asthmatics/horses with IAD/horses with RAO; there is limited data studying airway remodeling of the peripheral airways of human asthmatics and reversibility in response to therapy, and limited data available in horses with RAO</td>
</tr>
<tr>
<td></td>
<td>• Postbronchodilator FEV₁ &lt; 70% (predicted)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

| **Asthma in obese patients** | • Dyspnea on exertion  • Requires objective measurement of variable airflow limitation—Obesity-associated respiratory symptoms can mimic asthma  • Little eosinophilic airway inflammation | Correlation between body condition score and body fat (%) and increased expression of IL-1 and TNF-α in plasma | Insufficient evidence | • Expression of inflammatory cytokines in BALF or increased pulmonary resistance in obese/equine metabolic syndrome horses |

Abbreviations: BALF, bronchoalveolar lavage fluid; FEV₁, forced expiratory volume in 1 s; IAD, inflammatory airway disease; ICS, inhaled corticosteroid; RAO, recurrent airway obstruction, TNF, tumor necrosis factor.

past/family history of allergic disease (eg, eczema, food allergy) and pretreatment induced sputum from affected patients often reveals eosinophilic airway inflammation; the response to inhaled corticosteroid treatment is generally favorable. Currently, IAD in the horse can be further subcategorized based on the predominant inflammatory cell in BALF: namely, neutrophilic, eosinophilic, mastocytic, or mixed granulocytic. While the pathogenesis of IAD is incompletely defined, it is widely understood to be a multifactorial disease with the relative contribution of etiological influences varying with environment, husbandry, location, season, and preventive medicine strategies. 

Antigenic triggers are central to the development of lower airway inflammation. Horses kept in conventional stables with poor ventilation are exposed to high levels of airborne particulates including dust, endotoxin, fungi, molds, ultrafine particles and noxious gases, and there is strong evidence that stabilizing of horses is a risk factor for IAD. However, the level of respirable particulates in the overall stall air does not necessarily reflect the level of challenge a horse experiences, as the majority of dust exposure occurs in the breathing zone during feeding. Exposure to hay and its accompanying mold spores, such as Aspergillus fumigatus, Saccharopolyspora rectivirgula, and Thermoactinomyces vulgaris, are a risk factor in the development of lower airway inflammation. Furthermore, compared to feeding hay from the ground feeding hay in a net has a 4-fold increase in breathing zone respirable particle concentration. There is little information regarding an association between antigenic triggers (ie, dust, mold spores) and specific IAD phenotypes. A prospective, cross-over study did reveal an association between stabilizing of young horses and an IAD phenotype characterized by increased airway neutrophils. This phenotype has been associated with coughing and poor performance (discussed above in minimum inclusion criteria for application of the term “asthma”), both of which form the basis for the diagnosis of IAD. In contrast with the human allergic asthma phenotype, eosinophils are less commonly detected in equine BALF, with the exception of a subgroup of IAD mainly found in women humans (allergic asthma). If you have trouble accessing this document because of a disability, please contact PVM Web Communications at vetwebteam@purdue.edu.

prevalence lower than other IAD cytological subtypes. In young horses, the recruitment of airway eosinophils appears to be associated with dust exposure and increased BALF eosinophil ratios have been associated with pulmonary hyperresponsiveness. Further studies are clearly warranted to more fully clarify the role of eosinophils in IAD pathogenesis and their effect on respiratory function. Nevertheless, regardless of the BALF cytocrit profile, it appears that antigenic triggers are associated with both the clinical signs and pathology of lower airway inflammation observed in horses with IAD. Similarly, yet more widely reported in the literature, antigenic triggers are strongly associated with both clinical exacerbations and pathologic changes (eg, airway remodeling) in horses with RAO. Of note, however, eosinophils are absent from the airway wall of RAO-affected horses.

In humans, an “atopic march” has been described, whereby the first clinical manifestation of allergic disease, atopic dermatitis, is followed by the subsequent development of food allergy, rhinitis, and asthma. Evidence suggests that 75% of young children that experience severe atopic dermatitis will develop allergic rhinitis, and 50% will develop asthma. In horses, while data supporting the existence of an “atopic march” are lacking, there is genetic, epidemiological and clinical evidence of multiple co-existing manifestations of allergic disease within a single individual. There is a genetic association between RAO and microsatellite markers syntenic with the IL-4 receptor α-chain (IL4Rα) gene on equine chromosome 13. Importantly, the IL4Rx gene is associated with the development of asthma, skin allergies, and parasite defense in humans and is associated with multiple hypersensitivities, including insect bite hypersensitivity and urticaria, as well as increased parasite resistance; specifically, members of a half-sibling family with a high-incidence of RAO shed fewer strongylid eggs compared to genetically unrelated RAO-unaffected pasture mates. Furthermore, RAO-affected offspring within the high-prevalence family had lower strongylid egg counts...
appears associated phagocyte-dependent rapid exist IAD, 6 eral Although other role cells the other as non-allergenic cytokine Th-2 cytokine signature has been detected in BAL cells derived from mastocytic forms of IAD, characterized by increased expression of IL-4 and IL-5 mRNA.69,70 Whilst further data, derived from longitudinal studies, are required to support the existence of an “atopic march” in the horse, an “allergic equine asthma” phenotype currently appears biologically appropriate.

9 | NON-ALLERGIC ASTHMA

A common asthma phenotype in human adults is “non-allergic asthma,” where there is no apparent association with allergy. Analysis of pretreatment patient-derived sputum reveals neutrophilic, eosinophilic, or paucigranulocytic inflammation. Paucigranulocytic asthma is associated with normal or near-normal levels of eosinophils and neutrophils. Human asthma, particularly the allergic phenotype, displays an IL-5–mediated eosinophil recruitment predominantly driven by a Th-2 CD4+ lymphocyte response. However, the role of a Th-1 immune response and its ability to evoke cell-mediated immunity and phagocyte-dependent inflammation is exhibited both in chronic severe asthma and acute asthma exacerbations, the latter being associated with airway neutrophil recruitment as early as 4 hours after allergen exposure. Furthermore, in chronic asthma in humans, there are persistently activated mast cells in the bronchial mucosa, evident as elevated cytokine expression and synthesis.71–73 Although mast cell activation is often assumed to be allergen induced, there are multiple non-allergic stimuli which can cause this activation, including proteases,74 cytokines,75 and Toll-like receptor ligands.75 These and other mechanistic pathways are described in detail in a review article.76 In addition to the varied mechanisms (both allergic and non-allergic) which underpin mast cell degranulation, differences also exist with respect to the kinetics of degranulation. In contrast to the rapid mast cell degranulation observed after allergen challenge, the ultrastructural appearance of some asthmatic airway mast cells appears consistent with a slower degranulation process.73 Whilst mast cells are well known for their role in allergic and anaphylactic reactions (where rapid degranulation is observed as part of a Th-2-mediated response), increasing evidence supports an alternative role of mast cells in inflammation, whereby they exhibit “differential” or “selective” secretion of mediators without degranulation.77 Similarly, there is evidence that both the Th-1 and Th-2 immune responses are involved in the pathogenesis of IAD and HAO.67,78,83,84,85 Furthermore, T-cells gene expression data derived from horses with IAD, it is important to consider whether the diagnosis was based on a generalized increase in airway inflammatory cells or an increase in a specific inflammatory cell (neutrophilic, mastocytic, eosinophilic). Evidence of a Th-1 response in the lower respiratory tract, characterized by upregulation of IFN-γ mRNA in BALF-derived cells, has repeatedly been reported in association with a generalized increase in BAL inflammatory cells, both in the presence and absence of clinical signs.53,70,78 Additionally, a Th-17 response has been implicated in neutrophilic IAD, with an association between the BALF neutrophil ratio and increased IL-17 and IL-23 mRNA expression.53,69 It is important to consider that these responses might reflect sequential phases of the chronic inflammatory process in the respiratory tract; consequently, it might not be appropriate to consider them as mutually exclusive.79 Such considerations remain speculative, particularly in naturally occurring cases, and additional studies are required for clarification.

Chronic innate immune activation is a feature of both neutrophilic human asthma, as well as RAO, which persists during disease remission.80,81 The chronic activation of peripheral blood neutrophils reported in RAO could, in part, contribute to the greater disease severity compared with IAD, whereby exposure to an inhaled stimulus (eg, dust, mold spores) could result in an exaggerated and inappropriate inflammatory response. Although such exposures can induce mild neutrophilic pulmonary inflammation in both healthy horses and humans, the degree of cellular activation decreases in hours/days, even if the inciting stimulus is maintained.82,83 In contrast, if exposure to an antigenic stimulus is maintained in horses with IAD, pulmonary inflammation persists for up to 3 months.84 Whilst further research into the innate immune response in IAD and RAO is required to fully understand the role of neutrophil activation in the development of lower airway inflammation, given that a non–Th-2 immune response has also been associated with both IAD and RAO, the proposed existence of a “non-allergic equine asthma” phenotype currently appears biologically appropriate.

10 | LATE-ONSET ASTHMA

Some patients (particularly women) present with asthma for the first time as adults. These patients are less likely to be atopic, as “age of onset” is significantly lower in patients with allergic asthma, compared with those with non-allergic asthma.85 They also have decreased baseline pulmonary function and are either refractory to inhaled corticosteroid therapy or require higher doses of inhaled corticosteroids to achieve asthma control.23 Horses with RAO exhibit decreased baseline pulmonary function during disease exacerbation, and tend to be mature to older animals.1 “Inflamm-aging” describes a reduction in the capacity of the aging body to cope with a variety of stressors together with a progressively increasing chronic low-grade inflammatory status, associated with aging and provoked by a continuous antigenic load.85 There are age-related increases in pro-inflammatory cytokines in both humans and horses, with aged healthy horses having increased expression of IL-6, IL-8, IFN-γ, and peripheral blood mononuclear cell-derived TNF-α mRNA concentration in plasma.86 Furthermore, T-cells...
than those of younger animals, and peripheral blood lymphocytes and monocytes derived from this cohort exhibit an increased basal expression of IFN-γ and TNF-α mRNA, respectively. However, age-related changes appear to be more tightly regulated in the lungs than in the systemic circulation. Inflammatory cell populations in the lung represent a balance between cellular recruitment, via airway epithelial cell and macrophage-derived chemotactic cytokines, and removal, via apoptosis and phagocyte-mediated clearance. Lung granulocytes (neutrophils and macrophages) in horses with RAO exhibit altered apoptosis, which together with increased activity of transcription factors such as nuclear factor-κB (NF-κB) and activator protein-1 (AP-1) might contribute to the maintenance of neutrophilic inflammation in horses treated with glucocorticoids and maintained in an allergenic environment. Whilst no age-related trends in BALF cytological profiles in horses with IAD or RAO have been reported, there is an age-associated increase in mRNA expression of IFN-γ producing lymphocytes in stimulated BAL cells. Whilst there is a paucity of definitive data on the progression of IAD to RAO over time, there is anecdotal evidence suggesting the progression from IAD in younger age to RAO in some horses. Although potentially influenced by the high prevalence of IAD, such a phenomenon of disease progression does warrant further study. There is no current correlation between inflamm-aging and the development of chronic inflammatory airways diseases. However, based on the human phenotype, we believe it is biologically appropriate to use RAO as an equine model for late-onset asthma, as recently reviewed.

11 | ASTHMA WITH FIXED AIRFLOW LIMITATION

Patients with fixed airflow obstruction are often grouped under the heading of chronic obstructive pulmonary disease (COPD), with distinct pathological and functional characteristics compared to those with a history of asthma; for example, asthmatic patients do not exhibit a loss of airways as observed in COPD. It is thought that fixed airflow limitation in asthmatic patients is because of airway wall remodeling, with both airway smooth muscle (ASM) mass and extracellular matrix (ECM) deposition being increased at all levels of the bronchial tree, with the increased ASM mass being the functionally dominant alteration. Consequently, in addition to the clinical similarities between RAO and human asthma, both diseases also share certain structural features. The structural alterations seen in human patients with fixed airflow limitation are currently thought to be irreversible; however, appropriate studies are lacking to verify if indeed this is correct. In contrast, tissue remodeling in RAO is partially reversible under certain circumstances. In horses with RAO, long-term corticosteroid therapy (fluticasone) and antigen avoidance strategies have been shown to significantly decrease both smooth muscle mass (30% decrease over 3 months, but remained twice that of healthy controls) and subepithelial collagen area. Corticosteroid administration increased the rate of decline in smooth muscle mass, although antigen avoidance was better at controlling airway inflammation. Airway remodeling in horses with IAD has not yet been investigated. In light of the paucity of data, this is an area requiring further investigation.

12 | ASTHMA WITH OBESITY

In humans, obese patients with asthma can have moderate to severe respiratory symptoms, with little eosinophilic airway inflammation; there is no evidence for an increase in sputum inflammatory cells. Whilst it is unknown whether obesity per se contributes to asthma, there are marked alterations to respiratory physiology including an increased demand for ventilation and work of breathing. Breathing at low lung volumes enhances airway responsiveness which improves after bariatric surgery. The altered mechanics of breathing that favor airway narrowing and airway hyperresponsiveness can result in a more severe clinical presentation than that predicted upon consideration of the underlying inflammatory cytologic profile. Whilst there is evidence that obesity increases the risk of developing asthma in people, some studies suggest that insulin resistance, systemic IL-6 inflammation and clinical features of metabolic dysfunction have a stronger association with more severe asthma than body mass index (BMI) or body mass. Whilst there is a positive correlation between both body condition score and body fat (%) and IL-1 and TNF-α in equine plasma, there is currently no report of increased expression of inflammatory cytokines in BAL fluid or increased pulmonary resistance in horses with obesity. Furthermore, to the best of authors’ knowledge there are no reports of a link between equine metabolic syndrome and the presence of chronic lower airway inflammation in horses. Therefore, there is currently insufficient evidence to consider equine asthma a suitable model for human asthma associated with obesity.

13 | PHENOTYPE VERSUS ENDOTYPE

Our inability to identify consistent genetic and environmental correlations with IAD and RAO can potentially be attributed to our limited understanding of the various pathophysiologic mechanisms underlying these diseases. In human medicine, “asthma endotypes” are disease subtypes defined by their distinct, underlying pathophysiology. The broad syndrome of asthma can therefore be divided into distinct disease entities, or subtypes, on the basis of 7 variables; these include clinical characteristics, biomarkers, lung physiology, genetics, histopathology, epidemiology, and response to treatment. Recently, several groups have used transcriptomic data derived from stimulated peripheral blood mononuclear cells (ex vivo) and bronchial epithelium (in vivo) to identify differentially expressed genes and pathways between RAO and non-RAO horses. Stimulation with hay dust extract resulted in the greatest differential gene expression, the most dominant among the upregulated genes being those involved in immune cell trafficking, neutrophil chemotaxis, immune and inflammatory responses, and cell cycle regulation and apoptosis. The presence of obesity is an equal access/equal opportunity university. If you have trouble accessing this document because of a disability, please contact PVM Web Communications at vetwebteam@purdue.edu.
CXCL13, a B cell chemoattractant predominantly produced by Th17, but not Th1 or Th2, cells. Rather than indicating a primary gene dysregulation, this might represent an abnormal response to allergens in horses with RAO. Interestingly, levels of CXCL13 have been shown to be upregulated 8-fold in BALF from human asthmatics compared to controls. Furthermore, treatment of a sensitized murine asthma model with an anti-CXCL13 antibody reduces inflammatory cell recruitment, bronchial-associated lymphoid tissue formation, and airway inflammation, potentially supporting CXCL13 as a novel treatment target. Another potential mechanistic pathway which could underpin the inflammatory cascade in RAO is the activation of neutrophils by the bronchial epithelium, leading to epithelial injury and impaired repair and differentiation. With the development of new biologic treatments in human asthma and the application of more targeted therapeutic approaches in the horse, it is appropriate to further investigate and clarify the clinical characteristics, biomarkers, lung physiology, genetics, histopathology, epidemiology, and response to treatment to better elucidate the pathophysiologic mechanisms in RAO, thus enabling the description of the allergic (Th-2), non-allergic (non-Th-2) and late-onset endotypes of equine asthma.

14 | RESPONSE TO TREATMENT

Human asthma control is assessed in terms of both symptom control and risk of future adverse outcomes. The level of control is the extent to which symptoms are experienced by the patient, and is determined by interactions between the patient’s genetics, underlying disease processes, treatment, environment, and psychosocial factors. In comparison, there are multiple challenges associated with assessing the control of signs of respiratory disease in equine asthma; therefore, the majority of peer-reviewed studies are short-term therapeutic efficacy clinical trials. As maintaining appropriate air hygiene, through a reduction in antigen and airborne dust exposure, constitutes the most important therapeutic and prophylactic approach to both IAD and RAO, one of the greatest challenges in the design of clinical trials is maintaining a degree of control over environmental exposures. Currently, there is a need for a long-term longitudinal study assessing the relative and combined beneficial effects of both drug therapy and environmental management on the control of IAD clinical signs. Indeed, even clinical research on the efficacy of treatments on airway hypersensitivity and hyperreactivity in IAD cases is limited with treatment decisions typically based on clinical experience, data derived from horses with RAO, or both. Initially, therapeutic trials in RAO focused primarily on the beneficial effects of bronchodilators, in light of the lower airway obstruction and increased respiratory effort at rest exhibited by these cases. Recently, however, the therapeutic research focus in equine asthma has partly shifted towards the control of airway inflammation.

Airway inflammation is due in part to the increased activity of transcription factors that in turn lead to an increased production of inflammatory mediators and recruitment of inflammatory cells. Therefore, the efficacy of anti-inflammatory drugs, such as corticosteroids, in RAO has partly been evaluated via their influence on the expression of selected inflammatory genes. As many of these factors are associated with asthma, it is reasonable to assume that treatment of RAO, and bronchial asthma, with corticosteroids would be effective.

Additional factors, such as allergen exposure, may also contribute to the pathogenesis of equine asthma. Asthma in horses has been associated with exposure to dust and mold, which may stimulate the immune system and contribute to the development of airway inflammation. Moreover, the role of Th17 cells in the pathogenesis of asthma has been highlighted, with Th17 cells playing a key role in the immune response to allergens and other environmental triggers. The role of Th17 cells in equine asthma is currently under investigation, and therapeutic strategies targeting Th17 cells hold promise for the treatment of equine asthma.

15 | CONCLUSIONS

Upon consideration of the shared factors between human asthma, IAD and RAO, we conclude that adoption of the term equine asthma is appropriate, whilst acknowledging that important heterogeneity exists within this broad disease category. We therefore support the proposal that the term mild/moderate equine asthma replace IAD and severe equine asthma replace RAO in the literature from this point onwards, whilst recognizing the need to preserve the spectrum of diseases which fall within the proposed new “equine asthma” classification. Furthermore, in addition to the subcategorization of equine asthma based on severity, we propose that equine equivalents to specific human asthma phenotypes exist, based on shared clinical and pathophysiologic characteristics. Finally, with the development of novel therapeutic approaches and targeted treatments for equine asthma, the future of asthma management in horses holds promise.

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targeted therapeutic approaches in the horse, it might be appropriate to further investigate and clarify the allergic (Th-2), non-allergic (non-Th-2) and late-onset phenotypes of equine asthma; however, further research is required to more fully determine the potential clinical utility of such a phenotypic classification exercise. Currently, there is insufficient evidence to recommend an equine model for asthma with fixed airflow limitation, and asthma in obese patients.

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Authors declare no conflict of interest.

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Authors declare no off-label use of antimicrobials.

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Effect of valacyclovir on EHV-5 viral kinetics in horses with equine multinodular pulmonary fibrosis

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Background: Equine herpesvirus-5 is commonly isolated from the lungs of horses with EMPF, suggesting an etiological link. Valacyclovir is used empirically to treat EMPF; however, no data is available concerning its impact on EHV-5 viral kinetics.

Objectives: To determine the effect of oral administration of valacyclovir on EHV-5 viral load measured by qPCR in blood, nasal secretions (NS) and BALF in horses with EMPF.

Animals: Six horses diagnosed with EMPF.

Methods: A prospective clinical trial was performed. Horses received 10 days of PO administered valacyclovir (loading dose 30 mg/kg, maintenance dose 20 mg/kg). Blood, NS, and BALF were collected for EHV-5 viral kinetics analyses during treatment. Blood and NS were collected every other day, BALF was collected on day 0 and day 10.

Results: There was no statistical difference in median EHV-5 viral load between day 0 and day 10 for all samples tested. In blood median EHV-5 viral load was 7676 (range 575-39 781) on day 0 and 6822 (range 1136-18 635) glycoprotein B (gB) gene copies per million cells on day 10. For NS median EHV-5 viral load was 2.944 × 106 (range 184 691-3.394 × 106) on day 0 and 8.803 × 106 (range 251 186-9.868 × 106) gB gene copies per million cells on day 10. For BALF median EHV-5 viral load was 59 842 (range 61 315 655) on day 0 and 185 083 (range 3562 542 417) gB gene copies per million cells on day 10.

Conclusions and Clinical Importance: Valacyclovir might not be an effective short-term antiviral treatment but efficacy in treatment of EMPF is unknown.

KEYWORDS
anti-viral drugs, herpesvirus, interstitial pneumonia, qPCR

1 | INTRODUCTION

Equine multinodular pulmonary fibrosis (EMPF) is a chronic, progressive, interstitial lung disease of adult horses. The disease is characterized histologically by marked interstitial fibrosis and mixed inflammatory cell infiltration of the lungs. The exact pathogenesis and predisposing factors currently remain elusive; however, there is increasing evidence that equine herpesvirus-5 (EHV-5) is associated with EMPF. Equine herpesvirus-5 has been detected from the lungs of the majority of EMPF cases described in the literature, suggesting an etiological link. Furthermore, pulmonary fibrosis has recently been experimentally induced with EHV-5 isolated from the lungs of horses with EMPF and inoculated endoscopically into the accessory lung lobe of clinically normal horses. Nodular pulmonary fibrosis and myofibroblast induction occurred in the EHV-5 inoculated horses. Parallels have been drawn between human idiopathic pulmonary fibrosis (IPF) and EMPF. In IPF, gammaherpesviruses such as human herpesvirus-4 (HHV-4; Epstein Barr Virus [EBV]) are believed to be important cofactors in development of the condition. Current theories suggest that EHV-5 might be an

Abbreviations: BALF, bronchoalveolar lavage fluid; EBV, Epstein Barr virus; EHV-1, equine herpesvirus-1; EHV-5, equine herpesvirus-5; EMPF, equine multinodular pulmonary fibrosis; HHV-4, human herpesvirus-4; HPLC, high performance liquid chromatography; IPF, idiopathic pulmonary fibrosis; NSAIDs, non-steroidal anti-inflammatory drugs; qPCR, quantitative polymerase chain reaction

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inciting cause of epithelial injury in EMPF cases, with the development of an unrestrained, disproportionate healing response resulting in pulmonary fibrosis. Current treatment of EMPF cases is focused on attempting to control the ongoing pulmonary inflammation and prevent further fibrosis. Corticosteroids and nonsteroidal anti-inflammatory drugs (NSAIDs) are frequently used to reduce pulmonary inflammation and control pyrexia. Tetracycline antimicrobial drugs, including minocycline and doxycycline, can attenuate the activity of metalloproteinases and are beneficial in murine models of pulmonary disease. Tetracycline antimicrobial drugs are often employed for their antibiotic and anticollegenase effects. Use of the antiviral prodrug valacyclovir has been described in 1 horse with EMPF that was reported to be clinically healthy 2 years after treatment. Valacyclovir is produced by ester linking of acyclovir with the amino acid L-valine, increasing the drug’s oral bioavailability. After oral administration, the prodrug valacyclovir is promptly hydrolysed to acyclovir, an inhibitor of viral DNA polymerase. Valacyclovir has been used both prophylactically and therapeutically in equine herpesvirus-1 (EHV-1) outbreaks to decrease viremia and virus shedding. Treatment of horses with PO administered valacyclovir is relatively expensive and to the authors’ knowledge, there are currently no studies investigating the effect of valacyclovir on EHV-5 viral kinetics in horses with EMPF. We hypothesised that treatment with valacyclovir would decrease the EHV-5 viral load as measured by real-time quantitative polymerase chain reaction (qPCR) of blood, nasal secretions (NS), and bronchoalveolar lavage fluid (BALF) in horses with EMPF. The objective of our study was to determine the effect of oral administration of valacyclovir on qPCR EHV-5 viral kinetics on blood, NS and BALF in horses with EMPF. Plasma samples collected during the maintenance phase of treatment were analyzed by high performance liquid chromatography (HPLC) to ensure the acyclovir concentrations obtained were comparable to previous studies.

2 | MATERIALS AND METHODS

2.1 | Study population

The study population consisted of 6 adult horses with a median age of 19 years (range 11–22 years) and examined between 2015 and 2017. Four horses were seen at the William R. Pritchard Veterinary Medical Teaching Hospital, School of Veterinary Medicine, University of California at Davis. One horse was seen at the Veterinary Teaching Hospital, College of Veterinary Medicine, Purdue University and 1 horse was examined at Stillwater Equine Veterinary Clinic in Minnesota. The horses belonged to the following breeds: Quarter Horse (n = 2), Warmblood (n = 1), Thoroughbred (n = 1), Paint (n = 1), and Saddlebred (n = 1). The study population included 3 geldings and 3 mares. All horses presented with tachypnea, increased respiratory effort, fever and weight loss. Five horses were diagnosed with EMPF based on consistent histopathological results and qPCR-positive EHV-5 testing of lung tissue or BALF. The remaining horse had consistent clinicopathological and diagnostic imaging findings in lungs consistent with EMPF, with no qPCR-positive results on BALF or lung tissue. For EHV-5 on testing of BALF but the owners declined confirmation by histopathology and EHV-5 qPCR testing of lung tissue.

2.2 | Sample analysis

The study was designed as a prospective clinical trial during which horses diagnosed with EMPF would undergo 10 days of treatment with PO administered valacyclovir. This duration of valacyclovir treatment was selected based on previous evidence that 1–2 weeks of PO administered valacyclovir treatment was sufficient to decrease viremia and virus shedding of EHV-1 in horses. Throughout treatment, the viral load of EHV-5 was measured via qPCR of blood, NS and BALF. The horses in our study were treated with a loading dose of 30mg/kg valacyclovir (Valtrex, GlaxoSmithKline, Research Triangle Park, North Carolina) PO q8h for the first 48 hours. The dose was then decreased to 20 mg/kg valacyclovir PO q12h for a further 192 hours. This protocol was chosen based on pharmacokinetic studies demonstrating that these dosages achieved appropriate therapeutic concentrations of acyclovir in the blood for treatment of EHV-1. Four horses required administration of flunixin meglumine (Banamine, Merck Animal Health, Madison, New Jersey) (0.5mg/kg PO q12h) to control fevers over the 10-day treatment course. After 10 days of valacyclovir treatment, these 4 horses were administered minocycline (minocycline hydrochloride, Actavis Pharma Inc, Parsippany, New Jersey; 4 mg/kg PO q12h) and dexamethasone (Dexamethasone, Bimeda-MTC Animal Health Inc, Cambridge, Ontario, Canada; 0.1 mg/kg IV or IM q24h). The remaining 2 horses presented later in the course of the disease process based on severity of diagnostic imaging findings and clinical signs. Therefore, these 2 horses received combination treatment with valacyclovir, dexamethasone (0.1 mg/kg IV or IM q24h), flunixin meglumine (0.5 mg/kg PO q12h), and minocycline (4 mg/kg PO q12h).

Blood and NS for EHV-5 qPCR were collected every other day during treatment (days 0, 2, 4, 6, 8, and 10). For each horse, whole blood was collected into a 3-mL evacuated glass vial containing EDTA and refrigerated before processing for nucleic acid extraction. Two 15-cm rayon-tipped swabs (Puritan Sterile Rayon Tipped Applicators) were used to collect NS. The swabs were placed into the ventral meatus of either the right or left nostril and allowed to absorb the secretions for approximately 5 seconds while gently turning the swab. After collection of the NS, both swabs were immediately placed into a red-top tube (no anticoagulant) and refrigerated until nucleic acid processing. Bronchoalveolar lavage fluid was collected on day 0 (before treatment) and day 10 (after valacyclovir treatment was completed). The horses were sedated with a combination of detomidine hydrochloride (Dormosedan, Zoetis, Parsippany, New Jersey; 0.01 mg/kg IV) and butorphanol tartrate (Torbugecic, Zoetis, Parsippany, New Jersey; 0.01 mg/kg IV) for BALF collection. A 240-cm BAL tube was passed into the ventral meatus of either the left or right nostril and into the trachea. To reduce coughing and irritation during passing of the BAL tube, 50 mL of 1% lidocaine (Xylocaine, Fresenius Kabi USA LLC, Lake Zurich, Illinois IL) was instilled in the distal trachea and bronchi. The tube continued to be passed until it was wedged in the most distal bronchus. The tube was held in position while 240 mL of warm sterile 0.9% saline was injected into the BAL tube. The BALF was then

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until processing for nucleic acid extraction. If multiple syringes of BALF were retrieved, a pooled sample of BALF (equal amounts from each syringe) was submitted for analysis. Pretreatment and post-treatment BALF qPCR results were available for 5 horses. The 6th horse had the pretreatment BALF analyzed and the owner declined the post-treatment BALF.

Nucleic acid extraction from whole blood, NS and BALF were performed as previously described. Briefly, an automated nucleic acid extraction system (CAS-1820 X-tractor Gene) was used. Blood, NS, and BALF were assayed for the presence of the glycoprotein B (gB) gene of EHV-5. All samples were assayed for the presence of the housekeeping gene eGAPDH as previously described to ensure sample quality and effectiveness of nucleic acid extraction. Standard curves for EHV-5 and eGAPDH, expressed as EHV-5 gB gene copies per million cells, were performed to allow complete quantification of EHV-5 target molecules.

2.3 Acyclovir assay

Serum concentrations of acyclovir were determined by HPLC. Blood samples were collected from all horses during the predicted steady-state phase of treatment (day 4) to determine steady-state peak and trough plasma acyclovir concentrations. Peak samples were collected 45 minutes after oral administration of valacyclovir. Trough samples were collected 12 hours after administration of the previous valacyclovir dose, just before the next dose. Ten milliliter of whole blood was collected into a vacuum-sealed tube containing lithium heparin. The tube was centrifuged within an hour of collection at 2800g for 5 minutes. Plasma was separated and frozen at -80°C until analysis. Plasma acyclovir concentrations were determined by HPLC, as previously described.

2.4 Data analysis

A Mann Whitney test was used to compare the differences in EHV-5 gB genes per million cells measured by qPCR between day 0 and day 10 for each of the 3 sample types (whole blood, NS, and BALF). P values <.05 were considered statistically significant.

3 RESULTS

There was no statistical difference in median EHV-5 viral load between day 0 and day 10 as measured by qPCR in whole blood, NS, and BALF (Figure 1). The median and range EHV-5 viral loads in gB gene copies per million cells for each sample type are included in Figure 1. The peak acyclovir concentrations obtained were (mean ± s.d. = 1.48 ± 0.6 μg/mL) and the trough acyclovir concentrations obtained were (mean ± s.d. = 0.53 ±0.26 μg/mL; Figure 2); 5/6 (83%) of the horses were euthanized because of progression of clinical signs. These signs included continued weight loss, fevers uncontrolled by administration of NSAIDs and development of respiratory distress.

Median time from diagnosis to euthanasia was 34 days (range 18–63 days). One horse is currently alive at 1 year after diagnosis and is maintained on fluticasone and oral PO with good quality of life.

4 DISCUSSION

In our study, valacyclovir was not effective at decreasing the viral load of EHV-5 in horses with EMPF. There was no significant change in EHV-5 viral load in any of the 3 sample types measured. Individual variation in EHV-5 viral load was evident in the samples tested over the 10 days of treatment but median values were consistent. In all horses tested, EHV-5 viral load in BALF increased after the treatment, indicating ongoing viral replication in the lungs. Acyclovir administered PO has poor bioavailability and therefore the use of the prodrug valacyclovir is necessary to ensure adequate acyclovir plasma concentrations. Acyclovir has a time-dependent pharmacokinetic-pharmacodynamic pattern with trough acyclovir plasma concentrations most predictive of acyclovir efficacy. Mean trough acyclovir concentration achieved was slightly lower but comparable to the mean trough concentration achieved in the previous Maxwell et al. study. The mean peak acyclovir concentrations attained were considerably lower than those achieved previously. These differences were likely attributable to the delivery method of the valacyclovir. In our study, the valacyclovir tablets were administered PO via a syringe in contrast to the intragastric delivery utilized in the previous study. Oral administration of valacyclovir allowed ease of delivery of the drug but might have resulted in a partial loss
Our study confirmed that a loading dose of 30 mg/kg PO q8h for 2 days followed by a maintenance dose of 20 mg/kg PO q12h maintains therapeutic serum acyclovir concentrations for the alphaherpesvirus EHV-1. In comparison, EHV-5 is a slow-growing gammaherpesvirus that is challenging to culture. As a result, establishing anti-viral sensitivity testing for EHV-5 is difficult and the IC50 for EHV-5 is currently unknown. The dose or duration of treatment of valacyclovir in our study might therefore have been inadequate to alter EHV-5 viral loads. Human studies using valacyclovir in the treatment of the gammaherpesvirus EBV have decreased viral load with prolonged treatment of up to 12 months. Oral administration of valacyclovir (500 mg/day) reduced the number of EBV infected B cells in blood but not the number of EBV DNA copies per B cell. The cost of valacyclovir treatment in horses (~$25/day) makes such prolonged treatment with valacyclovir unpractical for most owners.

Consistent with previous case reports, horses with EMPF had a poor outcome in our study, with 5/6 horses euthanized. Interestingly, the single surviving horse was the youngest horse in the study and appeared to present earlier in the disease course as evidenced by clinical presentation, less marked radiographic changes and notably lower BALF EHV-5 viral load on presentation compared with the other horses in the study. Given the irreversibility of pulmonary fibrosis, it appears logical that identifying and treating horses earlier in the disease course to prevent further fibrosis would be more likely to produce a favorable outcome. A similar correlation has been noted in human cases of IPF with improved response to treatment generally noted in younger patients with less marked disease. The combination of strains or genotypes of EHV-5 present in a horse with EMPF might have clinical importance; however, further work is necessary to determine whether strain variability is an important component of pathogenicity. In human IPF cases, clinical phenotypes with discrete comorbidities and survival times have been defined. A subset of patients have a rapidly progressive disease course with a shorter survival time known as accelerated IPF. The differences in survival times noted in our study and previous studies might be indicative of varying phenotypes with different rates of progression. Whether this difference is dependent on genetic, environmental, viral or a combination of factors is currently unclear.

The main limitations of our study were the small sample size and nonhomogenous study population because of the sporadic nature of EMPF. In the interests of ensuring the comfort of the horses during the study, treatment with additional medications was necessary. Four cases received NSAIDs and in the remaining 2 cases, delaying conventional treatment with anti-fibrotic drugs and corticosteroids was considered unethical. Ideally, the effects of valacyclovir would have been considered in isolation with no concurrent treatments. One horse in the study had consistent clinicopathological and diagnostic imaging findings and was qPCR positive for EHV-5 testing of BALF but EMPF was not confirmed on lung biopsy specimen or on postmortem examination. Recent studies have displayed that detection of EHV-5 in BALF is very strongly associated with EMPF. The detection of EHV-5 in BALF combined with consistent diagnostic imaging findings warranted inclusion of this horse as a presumptive case of EMPF.

In conclusion, our study showed that 10 days of oral administration of valacyclovir treatment at the chosen dosage was insufficient to alter EHV-5 viral kinetics in horses with EMPF. The study did not seek to determine the efficacy of valacyclovir treatment of horses with EMPF. Given the associated costs of treatment, it is recommended that conventional treatment with anti-fibrotic medications and anti-inflammatories (steroidal and nonsteroidal) continues to be the mainstay of treatment. There is currently limited knowledge concerning the susceptibility of equine gammaherpesviruses, such as EHV-5, to antiviral medications. Further research is needed to determine the potential antiviral options in the effective treatment of equine viral pneumonia.

### Table 1

<table>
<thead>
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<th>Blood</th>
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<td>Min</td>
<td>Max</td>
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<td>575</td>
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<td>Day 6</td>
<td>2119</td>
<td>1382</td>
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<tr>
<td>Day 8</td>
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<td>400</td>
</tr>
<tr>
<td>Day 10</td>
<td>6822</td>
<td>1136</td>
</tr>
</tbody>
</table>

### Figure 2

Mean peak and trough plasma concentrations of acyclovir (µg/mL) obtained during the predicted steady-state phase treatment with PO administered valacyclovir in 6 horses with equine multinodular pulmonary fibrosis. The mean peak and trough acyclovir concentrations obtained in a previous study with matched horses and sample size. If you have trouble accessing this document because of a disability, please contact PVM Web Communications at vetwebteam@purdue.edu.
ACKNOWLEDGMENTS

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CONFLICT OF INTEREST DECLARATION

Authors declare no conflict of interest.

OFF-LABEL ANTIMICROBIAL DECLARATION

The use of valacyclovir in horses in our study was off-label.

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC) OR OTHER APPROVAL DECLARATION

Sample collection and animal use was approved by the IACUC at the University of California at Davis. Owner consent was obtained for animals used in our study.

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Ultrasonographic and computed tomographic features of rice bodies in an Arabian horse with atlantal bursitis

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Abstract
A 19-year-old castrated Arabian male horse presented for evaluation of a firm mass at the dorsal cervical region. Ultrasonography and computed tomography revealed multiple well-defined fusiform structures within the atlantal bursa. Multiple glossy smooth, white to yellowish, flattened fusiform structures were removed surgically. These structures were composed of dense fibrin with some leukocytes and red blood cells. The imaging and histopathological features of these structures were similar to chronic 'rice bodies' reported in humans with bursitis or tenosynovitis. This is the first veterinary report describing the imaging features of 'rice bodies' in a horse with atlantal bursitis.

KEYWORDS
bursa, cervical, equine, tenosynovitis

1 SIGNALMENT, HISTORY, AND CLINICAL FINDINGS

A 19-year-old castrated Arabian male horse was presented to the Purdue University Veterinary Teaching Hospital for evaluation of a firm soft tissue mass at the dorsal aspect of the cranial cervical region. According to the owner, the mass had gradually grown in size over the past 3 years and has doubled in size over the past 2 months. Upon physical examination, a large and firm soft tissue mass was present extending over both sides of the poll immediately behind the occiput with greater swelling on the right side of the poll. No pain was elicited on palpation and no heat was noted in the area. The horse was reluctant to flex the poll and to be tied.

2 IMAGING, DIAGNOSIS, AND OUTCOME

Standing laterolateral radiographs of the cranial cervical spine were taken (Canon iXCD XI-80C, Tokyo, Japan; Vet Rocket LLC reader, California, USA; 80 kVp and 15 mAs). There was a focal soft tissue swelling dorsal to the atlas (C1), with an oval area (7.4 x 19 mm) of circumscripted stippled mineralization within it. The atlantal crest of the bicipital process showed a focal area of palisading periosteal reaction (Figure 1). Radiological findings were suggestive of chronic, severe atlantal bursitis with dystrophic mineralization and bicipital periostitis, likely a large chronic mineralized hematoma.

Ultrasonography (MyLab 70 XVG, Biosound Esaote, Italy; LA523 linear transducer with 4–13 MHz bandwidth) was performed for evaluation and sampling of the content within the atlantal bursa. The bursa contained a moderate volume of anechoic fluid and multiple stacked, elliptical structures (Figure 2). Histopathological examination of the structures revealed dense fibrin with some leukocytes and red blood cells, consistent with chronic reactions.
fusiform structures that were mildly hyperechoic to the cervical musculature. The wall of the bursa was thickened up to 1.7 cm. The outer wall of the bursa was hyperechoic with few short curvilinear hyperechoic mineral specks within. The inner wall of the bursa was mildly hypo- to isoechoic to the cervical musculature (Figure 2). The serology test for Brucellosis was negative. Cytology of synovial fluid from the atlantal bursa showed a chronic inflammatory process with negative bacterial culture.

For presurgical planning, noncontrast helical computed tomography (CT) (GE LightSpeed VCT 64-Slice, Milwaukee, Wisconsin; 140 kV, 198 mA, 2.5mm slice thickness in detail algorithm with reconstruction interval of 1.25mm, tube rotation time 13s, and pitch of 1.0) of the cranial cervical region (from caudal occiput to mid-axis) was performed under general anesthesia with the horse in right lateral recumbency. Dorsal and sagittal multiplanar reconstruction with soft tissue and bone windows was performed to further assess extent of involvement. The caudal and cranial aspects of the atlantal bursa contained large numbers of stacked fusiform, free-floating soft tissue attenuating structures (Hounsfield unit (HU) 30–50) of variable sizes interposed with small amounts of fluid attenuating material (HU 10–20). The wall of the bursa was irregularly mineralized (Figure 3).

Numerous glossy smooth, white to yellowish flattened fusiform structures (up to 4 x 2 cm each) with yellowish fibrinous material were removed via atlantal bursotomy (Figure 4A). A small section of the wall of the bursa was submitted for culture and sensitivity. No aerobic bacterial growth was yielded. The fusiform structures together with the yellowish fibrinous material within the bursa were submitted for histopathological analysis. On histopathology, the fusiform structures were composed of cellular necrotic and basophilic tissue admixed with fibrin, red blood cells, and rare fibroblasts (Figure 4B–D). The presence and formation of these fusiform structures were likely due to chronic inflammatory process of unknown cause. The ultrasonographic, CT, and gross appearance of the numerous fusiform structures within the atlantal bursa as well as the histopathological findings were similar to intra-articular rice bodies reported in human literature and hence the diagnosis of rice bodies atlantal bursitis was made.1–3

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**DISCUSSION**

This paper is the first in veterinary literature that describes the radiographic, ultrasonographic, and CT findings for histologically confirmed “rice bodies” in a horse with atlantal bursitis. One case of rice bodies has been reported in the stifle of a draft stallion without any imaging description. Intra-articular rice bodies have been reported extensively in human literature. Morphologically, human rice bodies are free-floating oblong structures with a glossy surface within the joint capsule and bursae.

The fusiform structures identified within the atlantal bursa were white to yellowish in color, smooth, glossy, and free-floating within the fibrinous fluid, grossly similar to rice bodies described in human literature. These were also histopathologically similar to human rice bodies, comprising of tightly packed fibrin and collagen with a mixture of intermittent fibroblasts and red blood cells.

Rice bodies bursitis has been described as multiple stacking oblong-shaped filling defects by positive bursography in the human. Although positive bursography was not performed in our case, we expect to yield similar fusiform shaped filling defects if we have done so. The oval-shaped stippled mineralization seen on bursa radiographs corresponds with dystrophic mineralization of the thickened wall of the atlantal bursa seen on CT. Mineralization of the synovium or joint capsule was not observed in human. The chronicity of the atlantal bursitis (over the duration of 3 years) may have led to thickening and synovial proliferation of the bursal wall, with subsequent fibrosis and dystrophic mineralization.

The horse has been used for pleasure and therapeutic riding for the past 3 years since recovering from surgery, with no complications or regrowth of the mass.

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Ultrasonography was found to be useful in depicting the appearance and shape of the rice bodies in this case with minimal invasion and also facilitated the sampling of the synovial fluid. While the ultrasonographic appearance of the rice bodies within the atlantal bursa were similar in shape (fusiform) and echogenicity (isoechoic to the muscle) to the intra-articular rice bodies described in human literature, the rice bodies in our case were relatively much larger in size (up to 4 cm in diameter vs. 1 cm diameter in humans). This may be attributable to the relatively larger size and volume of the atlantal bursa of a horse compared to those bursae found in the human radius and ulna. The size of these rice bodies measured on ultrasound also corresponded well with the CT and direct measurements of the gross samples acquired from the bursotomy.

The previous CT case reports on humans with chronic bicipito-radial and subacromial bursitis failed to detect these individual rice bodies. Instead, CT of these rice bodies and bursae only showed distended bursa with homogeneous fluid to soft tissue attenuation within, with a mild rim of enhancement of the wall of the bursa. We were unable to assess for rim enhancement of the wall of the atlantal bursa as no contrast agent was administered. The well-defined, blbob-like rice bodies in human cases were only visible on magnetic resonance imaging, with the rice bodies being almost isointense with the adjacent musculature on T1W and T2W sequences. On the contrary, we were able to visualize these rice bodies within the atlantal bursa and use this to estimate the true extent and dimensions of the affected atlantal bursa as well as for surgical planning.

The pathophysiology of rice bodies formation has been theorized to include synovial chondromatosis, chronic inflammation, and secondary to trauma of the joint space, rheumatoid arthritis, and tuberculosis infection. Regardless of the underlying cause of human rice bodies, they have a similar fusiform appearance. For rheumatoid arthritis, it has been hypothesized that the cells that made up the rice bodies were initially cells from synovial lining that had sloughed from the synovium from microinfarction to bursal hydrops. These cells subsequently developed into free-moving bodies within the joint. Continuous immune-mediated response and synovitis perpetuates arthritis and further tissue hydrops in the joint, making this a self-perpetuating cycle of rice bodies formation. Presence of larger rice bodies has been reported in humans with tuberculosis arthritis compared to those with rheumatoid arthritis. We attribute the larger sized rice bodies to the greater size of the equine atlantal bursa.

We speculate that the most likely cause of the rice bodies formation in this horse was due to chronic inflammation of the fragmentation of part of the synovial surface that served a nidus for formation of these structures. This would be consistent with the prolonged duration and gradual enlargement of the atlantal bursal mass over time in our case. Although mycobacterial tenosynovitis and arthritis with rice bodies have been reported in humans, it was unlikely in our case as the bursal samples of the horse yielded a negative result for mycobacterial culture. Removal of the rice bodies with debridement of the thickened bursal wall via surgical bursotomy appears to have good outcome as indicated in the present case, with no evidence of recurrence.

We conclude that the rice bodies in this horse with atlantal bursitis had similar ultrasonographic and CT characteristics to those reported in humans. The findings of multiple fusiform rice bodies within a bursa on ultrasonography and CT should prompt the clinician to include chronic bursitis as a differential diagnosis in horses.

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Category 1

(a) Conception and Design: Hohu KK, Lim CK
(b) Acquisition of Data: Lim CK, Adams SB, Ramos-Vara JA
(c) Analysis and Interpretation of Data: Lim CK

Category 2

(a) Drafting the Article: Hohu KK, Lim CK
(b) Revising Article for Intellectual Content: Lim CK, Adams SB, Heng HG, Ramos-Vara JA

Category 3

(a) Final Approval of the Completed Article: Hohu KK, Lim CK, Adams SB, Heng HG, Ramos-Vara JA

REFERENCES


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Clinical Commentary

Orthopaedic case management: A balancing act

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Summary
Balancing the activities of a foal with an orthopaedic injury can be challenging. Physeal fractures also provide additional complexities to consider. This commentary examines both the juxtaposition of confinement and reduced activity, and the complex features of physeal injuries.

The case report by Valk and Schumacher (2018) describes the diagnosis and conservative management of an intra-articular, distal physeal femoral fracture in a 10-day-old foal. The fracture healed without radiographically detectable osteoarthritis and the Standardbred filly ultimately raced. The report highlights some challenges encountered when managing physeal fractures in the foal and the balancing act that often needs to be struck. The successful outcome illustrates that juvenile fractures, particularly those of the physeal, heal rapidly, and in an occasional horse, overcomes a degree of imperfect fracture alignment in the process. The unique feature of this case report, however, was the way in which the challenge of controlling the activity level of the foal was approached. The authors describe the filly cantering the length of the stall and pivoting on the hindlimbs while ‘confined’. The foal was ultimately managed in a small paddock (0.4 hectares; approximately 1 acre) rather than in a double sized box stall. A judgement was made with the farm manager that the foal may be less active in a paddock area. Upon follow-up, the foal was sound at the walk within six weeks (although still carrying the limb when moving at faster gaits), considered grade 3 lame at the trot at 3 months, and judged to be sound at 7 months following the initial injury. This commentary will explore two interesting aspects of the case: confinement versus reduced activity, and the complexity of physeal fractures.

Confinement versus reduced activity
Enforcing confinement does not always result in reduced activity. While the majority of horses, including foals, adjust to being confined to a box stall, there are exceptions. A study measuring limb activity during box stall confinement in six horses found that one horse displayed stereotypic locomotor activity and shifted weight on its forelimbs four times more, and on its hindlimbs 10 times more, than the average of the rest of the study group (McDuffee et al. 2000). These exceptions are challenging to manage in the fracture patient and result in greater cycling of orthopaedic implants and potential complications when managing external coaptation. The reported prevalence of stereotypic behaviour varies widely but may be observed in up to 33% of horses (Roberts et al. 2017). Efforts to reduce stereotypic behaviour include ad libitum access to forage, slow-feeding strategies such as small weave hay bags, reduced concentrate feeding and increasing social interactions for confined horses (Roberts et al. 2017). Providing either contact with other horses or the ability to observe other horses can reduce locomotor stereotypic behaviours. Mirrors or large posters of other horses have been used to reduce stall weaving and stall walking successfully. The ultimate remedy is increased turnout, grazing and exercise as most horses with locomotor stereotypies in the box stall do not display these behaviours when turned out to pasture. Of course, the orthopaedic patient may be at increased risk of repair failure or re-injury if turned out to pasture too early in the healing phase. However, gradually increasing levels of controlled activity must be a part of the rehabilitation programme for all orthopaedic patients.

Foals with orthopaedic injuries can present additional challenges for activity management. A study of the locomotor activities of warmblood foals on pasture during the first 4 months of life found that the percentage of time spent cantering and in other high impact activities was greatest in the first 4 weeks of life (Kurvers et al. 2006). However, the percentage of time observed cantering and trotting was only 1.4% of the total time observed on pasture in these foals, or approximately 20 min per day. This may be an overestimation of total time per day since the study observations were performed during daylight hours only. Regardless, the remainder of the time (98.6%) was spent grazing, walking, lying down or standing, all activities that are also performed by a foal confined to a box stall. The time spent cantering or trotting reduced to 0.5-0.6% of time in older foals, or approximately 7 min per day. Interestingly, the same study also found that foals kept in a box stall for part of each day were more active during their time on pasture, spending more time cantering, trotting, walking and grazing, when compared to foals that were on pasture 24 h a day, perhaps in some way compensating for their time in confinement. It was proposed that greater locomotor activities during the early weeks of life may be important for healthy musculoskeletal development in the foal (Kurvers et al. 2006). That said, just 1 min of cantering or high impact activity may be all that is needed for a foal to convert a minimally displaced fracture or a minor injury into a career or life-threatening one. Another challenge in managing the foal’s activity as an orthopaedic patient is the mare. Some mares are going to make their foal move more on pasture than others, and some mares are going to be difficult to manage in a box stall for a prolonged period of time. In a box stall environment, both the reproductive health of the mare and the orthopaedic health of the foal are potentially compromised. Recommending pasture turnout for the initial
management of significant orthopaedic injuries cannot be considered under most circumstances, however it is illustrative to consider that the foal in the case report by Valk and Schumacher (2018) may have been less active in the small paddock based on the description of its stall behaviour and the information known about normal activity levels of foals on pasture.

**The complexity of physeal fractures**

The initial description of physeal (epiphyseal plate) injuries by Salter and Harris aimed to classify the observed fracture configurations according to their prognosis. This was based on the likelihood of growth disturbance and articular involvement of the injury (Salter and Harris 1963). Physeal fracture configurations are dependent upon many factors, including the size and age of the animal, the magnitude and direction of force that causes the injury, the particular physeal involved, its shape, any pre-existing local physeal abnormalities and the associated soft tissues of the region. The uncommon configuration of the physeal fracture reported by Valk and Schumacher (complex type 3 Salter-Harris fracture of the distal femur) is also uncommon in other species, including children (Berg et al. 1984; Arkader et al. 2007; Little and Milewski 2014; Pennock et al. 2017). This type of fracture in adolescent children is recognised to have a delay of diagnosis in almost 40% of cases, particularly when the fracture is minimally displaced (Pennock et al. 2017). That presentation bears similarity to the foal in the report by Valk and Schumacher, where initial evaluation by the attending veterinarian, and presumably the relative comfort of the foal, did not raise fracture sufficiently as a likely diagnosis to perform radiographs of the stifle. Despite the delay in diagnosis, the foal was able to perform athletically following fracture healing. This type of fracture also carries an excellent prognosis in children for future athletic endeavours (Pennock et al. 2017).

Physeal fractures are known to heal and stabilise rapidly (Salter and Harris 1963; Chung and Xian 2014; Levine and Aitken 2017). The reasons for this are two-fold. First, most physeal fractures occur through the relatively weak hypertrophic zone (with its high cell to matrix ratio), or the junction of the mineralised cartilage and the hypertrophic zone (Ogden et al. 1993). This region of the physeal is already fully engaged in bone formation, with the creation of the primary spongiosa and their active remodelling into metaphyseal trabeculae. Re-establishing physeal alignment and contact can allow this process to continue and healing to occur rapidly providing local blood supply and cellular viability are intact. The proliferative cells of the physeal continue to divide following injury as they are typically away from the damaged region and the processes of matrix mineralisation and endochondral ossification resume within 7–10 days (Salter and Harris 1963; Chung and Xian 2014). Healing can occur in as little as 2–3 weeks. Second, the physeal itself is typically loaded in compression, transferring joint forces from the epiphysis through to the thicker cortex of the diaphysis. Bending forces in the region of the physeal are generally small and counteracted by soft tissue attachments. The thick perichondrium, periarticular soft tissues and muscle, ligament and tendinous attachments all play a role in stabilising the physeal. As a result, soft tissue injury and subsequent healing, which invariably accompany physeal fracture, also contribute to the rapid return of stability in this region of the bone. The degree of soft tissue support surrounding the fracture in the report by Valk and Schumacher (2018) was likely one reason that the fracture remained minimally displaced and healed without long-term complications.

While it is known that physeal fractures can heal rapidly, for any particular case it is unknown whether the injured physeal will resume symmetrical longitudinal growth, grow asymmetrically and result in an angular deformity, or close prematurely due to significant damage or blood supply compromise. Follow-up radiographs of the foal in the report by Valk and Schumacher (2018) performed at 13 months of age showed that the distal femoral physeal was closed and the proximal tibial growth plate was open but thin. Radiographic closure time for the distal femoral physeal is reported as 19–27 months of age, while the proximal tibial physeal closure occurs from 23 to 32 months of age (Strand et al. 2007). While that particular study was performed in Icelandic horses, overall closure times correlated well with reports from other breeds at other physes. The radiographs suggest that the fractured physeal in this case closed prematurely and that the femur of the foal was likely to be shortened (Levine and Aitken 2017). This is consistent with studies in the dog and children (Shapiro 1982; Berg et al. 1984; Arkader et al. 2007). However, as noted in previous reports, this presumed shortening was not appreciated grossly (Emberson et al. 1986). Mechanisms for compensation of shortened long bones due to early physeal closure include compensatory overgrowth of adjacent physis and an alteration of the joint angles within the limb. Interestingly, in both children and dogs, the evidence for compensatory overgrowth by the proximal tibial physeal as a result of early physeal closure of the distal femoral physeal is weak. In fact, it has been suggested not to occur to any significant degree by some (Shapiro 1982; Berg et al. 1984). In contrast, in cases of femoral diaphyseal fracture, physeal overgrowth of both the distal femoral physeal and the proximal tibial physeal, is a well-recognised phenomenon in children, which occurs even when anatomic bone alignment is achieved (Shapiro 1982; Stilli et al. 2008). This has also been reported in dogs (Schoefer et al. 1995). Altered joint angles have been shown to compensate for up to a 20% shortening of the femur in dogs without a significant effect on gait. The stifle angle was reduced on the shortened limb and increased on the unaffected limb, while the angle at the tarsus compensated minimally (Franzuszkii et al. 1987). While it is pure speculation that the horse would follow these same mechanisms for compensation, it is clear from this case report and clinical experiences that the young animal has a tremendous capacity to adjust to various injuries over time, or at least obscure the facts sufficiently from our visual recognition.

The specific set of circumstances in the case report by Valk and Schumacher (uncommon fracture configuration, excessive stall activity by the foal, presumably substantial soft tissue stabilisation of the fracture site) were able to result in a favourable outcome. While the authors concluded ‘Conservative management consisting of turnout into a small level paddock should be considered an option …’, the option of box stall confinement, had the foal been amenable to this approach, would have also been appropriate. The take home message from this statement should not be that stall confinement is necessarily ‘bad’ or ‘good’, but rather
that under specific circumstances an alternative to the normally recommended course of action may be appropriate or necessary. It is often a balancing act to keep a foal confined and reduce its activity when necessary.

Author’s declaration of interests
No conflicts of interest have been declared.

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References
The influence of hay steaming on clinical signs and airway immune response in severe asthmatic horses

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Abstract

Background: Avoidance of antigenic stimuli was found to significantly reverse airway obstruction of horses with severe equine asthma (sEA). To date, no published study investigated the influence of steaming hay on lower airway condition of sEA-affected horses. The objectives were to determine the clinical, cytological and cytokine respiratory responses of both sEA and control (CTL) horses experimentally exposed to steamed or dry hay.

Results: A cohort of 6 sEA horses and 6 CTL horses was involved in this field study. On day 0, both groups were fed with steamed hay for 5 consecutive days, followed by a wash-out period of 26 days prior to be fed with dry hay for 5 consecutive days. Investigations performed 2 days prior to and 5 days after each challenge included clinical score, tracheal mucus accumulation, and bronchoalveolar lavage fluid (BALF) cytology and cytokine mRNA expression. Feeding steamed hay significantly decreased its mould content (P < 0.001). Mucus score significantly increased when feeding dry hay (P = 0.01). No significant influence of challenge type was found on clinical score. Percentages of neutrophils (P < 0.001) as well as mRNA expression of IL-1β (P = 0.024), IL-6R (P = 0.021), IL-18 (P = 0.009) and IL-23 (P = 0.036) in BALF of sEA affected horses were significantly increased after both (steamed and dry hay) challenges. Relative mRNA expression of IL-1β, IL-6R and IL-23 in BALF were also significantly correlated to neutrophil percentages and both clinical and tracheal mucus score.

Conclusions: Steaming significantly decreased mould content but inconsistently influenced the respiratory response of sEA affected horses when fed hay. Based on BALF cytology and cytokine profiles, its relevance might be controversial as a non-medicinal therapy for sEA-affected horses.

Keywords: Equine asthma, Hay steaming, Inflammation, Cytology, Cytokines

Background

Severe equine asthma (sEA; previously known as recurrent airway obstruction) is a chronic disease of adult horses characterised by frequent coughing and increased respiratory effort at rest, as well as marked lower airway inflammation and reversible airway obstruction [1]. While neutrophils involvement in the pathophysiology of this disease is well defined, controversies still persist in terms of T-helper (Th)-1 and/or Th-2 polarisation, as determined by cytokines mRNA expression in BALF-derived cells [2, 3]. Involvement of Th-17 pathway [4, 5] and regulatory T cells (Treg; [6]) have also been suggested. Exposure to airborne environmental antigens is central in both initiation and maintenance of the disease, especially through stabling and exposure to hay and straw [7]. Feeding mouldy hay indeed represents a long-established model for sEA exacerbation in susceptible horses [2], and also allows differentiation from mild/moderate equine asthma (mEA; previously known as inflammatory airway disease) [3]. Inhalation challenges with aqueous mould extracts were previously found to induce neutrophilic inflammation in sEA affected horses but not in controls [8]. Results from experimental exposure of sEA affected...
horses to hay dust suspensions highlighted the synergistic effect of endotoxins and other dust components [9].

Environmental change, especially pasture turnout, with or without anti-inflammatory medication was found to significantly reverse airway obstruction of sEA affected horses [10]. On the other hand, corticosteroid therapy while maintaining the horse in a dusty environment leads to improved clinical signs and lung function but persistent airway inflammation [11, 12]. Feeding haylage and bedding wood shavings have been associated with significantly lower levels of both respirable dust (< 5 μm diameter) and endotoxins, within the horse’s breathing zone, when compared to hay/straw environment [13, 14]. Alternatively, soaking and high-temperature steaming of hay for 16 h and 50 min respectively, significantly reduced respirable particles exposure; however endotoxin levels were not investigated [15, 16]. Steaming was also found to be effective at reducing mould content in hay, unlike soaking hay for 9 h [17]. Steaming hay indeed aims to reach and uniformly reduce endotoxins within the horse’s breathing zone, when compared to hay/straw environment [13, 14]. Alternatively, soaking and high-temperature steaming of hay for 16 h and 50 min respectively, significantly reduced respirable dust exposure; however endotoxin levels were not investigated [15, 16]. Steaming was also found to be effective at reducing mould content in hay, unlike soaking hay for 9 h [17]. Steaming hay indeed aims to reach and uniformly diffuse a temperature of 100 °C within the hay bale. To date, there is no published study that has investigated the potential influence of steaming hay on lower airway inflammation of sEA-affected horses.

The aim of the study was to determine clinical, cytological and cytokine respiratory responses of both sEA and control (CTL) horses experimentally exposed to steamed or dry hay. Our hypothesis was that feeding sEA affected horses with dry hay will result in higher respirable dust exposure, airway inflammation and clinical signs, as compared to feeding high-temperature steamed hay. Steaming hay indeed significantly decreased mould content but inconsistently influenced the respiratory response of sEA-affected horses. Both cytological and cytokine profiles actually revealed a significant airway inflammation when feeding hay (steamed or not).

**Results**

One sEA horse exhibited fever during the wash-out period and was subsequently excluded from the study. In the end, 5 sEA horses and 6 CTL horses completed both challenges (Additional file 1).

**Hay and air sampling**

Both bacterial and mould content were significantly lower ($P < 0.001$) in steamed hay (195 280 ± 465 270 and 1 507 ± 1 864 CFU/g, respectively), when compared to dry hay (4 042 000 ± 6 748 619 and 118 150 ± 187 624 CFU/g, respectively; Additional file 2). *Aspergillus* family (principally *A. glaucus* and *A. nidulens*) was identified in 9/10 samples of dry hay and 10/10 samples of steamed hay; *Aspergillus fumigatus* has been identified in one sample of dry hay only. Similarly, *Penicillium* spp. was identified in 3/10 samples of dry hay and 1/10 samples of steamed hay. Concentrations of respirable dust collected within the breathing zones (CTL and sEA horse) were 0.0015 and 0.0034 mg/m$^3$, respectively, during challenge #1 (steamed hay) vs. 0.0024 and 0.0057 mg/m$^3$ during challenge #2 (dry hay). Similarly, the inhalable fractions collected were 0.0045 and 0.0082 mg/m$^3$, respectively, during challenge #1 vs. 0.31 and 0.26 mg/m$^3$ during challenge #2. Endotoxin activities from respirable fraction were 0.08 and 0.98 EU/mL, respectively, during challenge #1, vs. 2.19 and 3.88 EU/mL during challenge #2. Levels of β-D-glucan were 340 and 1067 pg/mL, respectively, during challenge #1, vs. 895 and 2367 pg/mL during challenge #2.

**Clinical score and tracheal mucus**

Overall, the mean clinical scores were not significantly different between sEA and CTL horses (‘disease status’; $P = 0.071$) and no significant influence on clinical scores was found for either ‘challenge’ or ‘time’ (Fig. 1), controlling for age. A significant interaction ($P = 0.049$) was found for clinical score between ‘challenge’ (steamed vs. dry hay) and ‘time’ (d + 5 vs. d-2); the post-hoc tests were however not significant for any further comparison. Mean mucus score was significantly higher ($P = 0.005$) for sEA horses when compared to CTL horses and overall significantly higher ($P = 0.024$) at d + 5 when compared to d-2 (Fig. 1). A significant interaction ($P = 0.022$) was also found for tracheal mucus between ‘challenge’ and ‘time’; the score being significantly higher for dry hay at d + 5 when compared to d-2 ($P = 0.01$), but not significantly different between d + 5 and d-2 for steamed hay ($P = 0.98$).

**BALF cytology**

After controlling for age, no significant influence on the proportions of BALF volume recovered was found for either ‘challenge’, ‘time’ or ‘disease status’, nor any interaction between categories. Total cell counts were overall significantly higher ($P = 0.037$) for sEA compared to CTL horses (Additional file 3). The mean percentage of BALF neutrophils was significantly higher ($P = 0.011$) for sEA horses when compared to CTL horses and was overall significantly higher ($P < 0.001$) at d + 5 when compared to d-2 (Fig. 2), while no significant interaction was found between ‘challenge’ and ‘time’ ($P = 0.197$). The mean percentage of BALF lymphocytes was significantly lower ($P = 0.001$) at d + 5 when compared to d-2. A significant interaction ($P = 0.009$) was found between ‘disease status’ and ‘time’ for the percentage of BALF macrophages; the post-hoc tests being however not significant. The mean percentage of BALF metachromatic cells were significantly lower ($P = 0.001$) for sEA horses when compared to CTL horses (Additional file 3). No overall influence of the ‘challenge’ type (steamed vs. dry hay), or any interaction between ‘challenge’ and either
Clinical score and tracheal mucus score, before and after the initiation of each challenge. sEA, severe equine asthma; CTL, control; d-2, 2 days before challenge; d + 5, 5 days after challenge. *, ** significant difference between groups (P < 0.05 and < 0.01), based on ANOVA investigation. Significant overall interactions (P < 0.05) were also found between 'challenge' (steamed, dry) and 'time' (d-2, d + 5); the p-value correspond to the subsequent Tukey-Kramer's post-hoc test.

**BALF cytokines**

After controlling for age, mean mRNA expression was significantly lower for IL-4 (P = 0.022) and significantly higher for IL-17 (P < 0.001) and TNF-α (P = 0.006) in BALF of sEA affected horses, when compared to CTL horses (Fig. 3). Overall mRNA expression of IL-10 was significantly higher (P < 0.001) at d + 5 when compared to d-2, irrespective of the horse disease status (Fig. 3). Significant interactions were found between ‘disease status’ and ‘time’ (higher at d + 5 than d-2 for sEA horses only) for relative mRNA expression of IL-1β (P = 0.024), IL-6R (P = 0.021), IL-18 (P = 0.009) and IL-23 (P = 0.036) in BALF (Fig. 4). No significant variation was found throughout challenges for relative mRNA expression of IL-2, IL-5, IL-6, IL-8, IL-13, IFN-γ and TGF-β (Additional file 4). No overall influence of the ‘challenge’ type (steamed vs. dry hay), or any interaction between ‘challenge’ and either ‘time’ or ‘disease status’ was found for any cytokine relative expression in BALF.

**Correlations**

Relative mRNA expression of IL-1β, IL-6R, IL-8, IL-10 and IL-23 were significantly associated with BALF neutrophil percentages of sEA affected horses (Fig. 5), and mRNA expression of IL-1β, IL-6R, IL-8, IL-13, IL-23 and TGF-β in BALF were also significantly correlated with tracheal mucus score (Additional file 5). Relative mRNA expression of IL-6R was negatively correlated with the percentage of macrophages in BALF of sEA affected horses, and IL-4 was significantly correlated with metachromatic cell percentages (Additional file 5).

**Discussion**

This is, to our knowledge, the first study in which the inflammatory and immune airway responses of both sEA and CTL horses have been investigated through a controlled exposition challenge with steamed and dry hay. Horses with sEA had been on pasture for at least 6 weeks prior to the challenge initiation. Unsurprisingly and due to the persistent nature of this syndrome, some level of clinical and/or cytological abnormalities at the time of enrolment was observed for 2 out of the 5 sEA-affected horses. Interestingly, the initial clinical scores were indeed 12/21 for both horses, and decreased to 6/21 at the end of the first exposure period (to steamed hay). Despite a significant interaction between ‘challenge’ (steamed vs. dry hay) and ‘time’ (d + 5 vs.
Fig. 3 Relative mRNA expression of cytokines in bronchoalveolar lavage fluid (BALF), before and after the initiation of each challenge: a tumour necrosis factor (TNF)-α; b interleukin (IL)-17; c IL-4; d IL-10. sEA, severe equine asthma; CTL, control; d-2, 2 days before challenge; d + 5, 5 days after challenge. *, **, *** significant difference between groups or timepoints (P < 0.05, 0.01 and 0.001, respectively), based on ANOVA investigation.

Based on the inflammatory responses measured after both challenges, it might be hypothesised that other non-evaluated stimuli might still be present within the hay even after steaming (e.g. mould and bacterial wall fragments). Horses were kept in paddocks during exposure periods, in order to avoid the influence of environmental confinement [20] and associated management of stables ventilation. Alternatively, steaming hay may not have been sufficient to prevent the synergistic inflammatory activity of various hay dust components, especially including endotoxins, which has previously been demonstrated experimentally [9]. When considering air sampling within the breathing zone during the present study, the inhalable fraction was 100 times lower when feeding steamed hay compared to dry hay. The influence of steaming hay on the respirable particles was however more limited in the current study, while this fraction was found to be significantly associated with lower airway inflammation of horses [21]. Respirable dust, endotoxin and β-D-glucan levels may also have been affected by individual horse’s behaviour when eating hay, as previously demonstrated for particulate concentrations [22]. The mean values measured for endotoxin activities within the breathing zones were 5.7 times lower with steamed hay, when compared to dry hay.

d-2), the individual variability of clinical scores may have confounded the results. However, 4 out of the 5 sEA horses showed significantly (P = 0.01) elevated tracheal mucus score (≥ 3) after being fed dry hay, while the score remained < 3 for a majority of them (3 out of 5) after being fed steamed hay.

Percentages of neutrophils in BALF were overall higher at d + 5 when compared to d-2, irrespective of ‘challenge’ (steamed and dry hay) and ‘disease status’ (sEA and CTL). Induction of a significant neutrophilic airway inflammation has been previously reported in CTL horses through environmental challenges [18, 19]. In the present study, all BALF neutrophil percentages measured from CTL horses systematically remained < 10%, while the same “good quality” (non-mouldy) hay was used in both challenges. Steaming hay significantly decreased mould content by a hundred fold, as previously described [17]. The mould content in hay was investigated in the present study by mycological culture only; the presence of spores, antigenic fragments or mycotoxins has not been evaluated. Targeted and non-targeted methodologies, such as qPCR and Maldi-Tof mass spectrometry, respectively, would considerably increase both the sensitivity and broadness of aeroallergen detection.
while a large variability was observed among horses. Interestingly, values for endotoxin activities within the breathing zone when feeding steamed hay were also comparable with previously published data for haylage/wood shavings combination [14].

No significant influence of the ‘challenge’ type (steamed vs. dry hay) was found for any cytokine mRNA expression in BALF. Significant interactions were found between ‘disease status’ (sEA vs. CTL) and ‘time’ (d + 5 vs. d - 2) for mRNA expression of IL-1β and IL-18; relative mRNA expression of TNF-α was also significantly higher in BALF from sEA affected horses, when compared with CTL horses. Such observations are coherent with the measurable inflammatory response of sEA affected horses to both (steamed and dry) hay challenges. Interestingly, mRNA expression of IL-6R in BALF was also increased post-challenges for sEA affected horses only. To date, the relative expression of this receptor has not been investigated in BALF of horses. Interestingly, a genome-wide association study identified a significant association between a single nucleotide polymorphism in the IL-6R gene and the risk of asthma in humans [23]. Further investigations are warranted to characterize the IL-6 signaling pathway involvement in equine asthma, especially in terms of subsequent T-helper (Th) polarisation as recently demonstrated in humans [24].

The previously suggested involvement of Th17 pathway in the airways of horses with severe asthma [4, 5], is supported by the present study. Overall IL-17 mRNA expression was indeed significantly higher in BALF of sEA affected when compared with CTL horses. The IL-23 mRNA expression in BALF was also increased post-challenges for sEA affected horses only, and was furthermore significantly correlated with both tracheal mucus score and BALF neutrophil percentages. Surprisingly, IL-8 mRNA expression was neither significantly different among groups (P = 0.11; controlling for age of the horses) nor significantly influenced by the challenge types. However, a significant correlation (R = 0.688) was found with neutrophil percentages in BALF. While over-expression has been associated with sEA chronicity [4, 5], it is likely that IL-8 contributes to neutrophil chemotaxis in the earlier stages of equine asthma [2]. The short duration of each challenge period in the current study might partly explain the limited amplitude of IL-8 mRNA expression in BALF.

The lower mRNA expression of IL-4 in BALF of sEA versus CTL horses was unexpected, mainly based on the
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Fig. 5 Correlations (95% confidence interval) between relative mRNA expression of cytokines and neutrophil proportions in bronchoalveolar lavage fluid (BALF) of sEA affected horses, before and after the initiation of each challenge: a interleukin (IL)-1β; b IL-8; c IL-6R; d IL-10; e IL-23. Straight line, linear regression; dotted lines, 95% confidence

previously demonstrated Th2 polarisation for this syndrome [25]. Interestingly, mRNA expression of IL-4 in BALF of both sEA and CTL horses was also not significantly influenced by either challenge. The sEA group exhibited significantly lower percentages of metachromatic cells in BALF, when compared to CTL horses; and IL-4 mRNA expression was significantly correlated with both eosinophil and metachromatic cell percentages. When considering a possible Treg involvement in sEA, mRNA expression of TGF-β was interestingly not significantly modified throughout the study. On the other hand, IL-10 was the only cytokine which mRNA expression in BALF was significantly higher post-challenges and correlated to BALF neutrophil proportions, with however no significant difference between sEA and CTL horses. Surprisingly, mRNA expression of IL-10 in BALF from sEA affected horses was previously found not to be significantly influenced by various environmental challenges [26]. Alternatively, percentages of different Treg subpopulations, as characterised by flow cytometry, were found to be significantly higher in BALF of sEA horses during crisis exacerbation when compared to remission [6].

Study limitations

All 12 horses involved in this field trial were client-owned, with differences in terms of geographic
origins prior to enrolment in the study. The limited number of horses enrolled in this study was consistent with similar works previously published [4, 8]. Based on the current results, the retrospective statistical power analyses highlights the variability of sample size required in relation to the multiple outcomes investigated. Values of power indeed ranged from 30% for clinical score to 82 and 98% for respectively mucus score and BALF neutrophil percentages. Unlike mucus and cytology (for which estimated sample size = 4–6), a minimum of 20 horses would have been required for clinical signs to reach a 80% power with a α-level of 0.05. The average age was significantly different between groups; the CTL horses being significantly younger than the sEA affected horses. This confounding factor was then taken into account in all further statistical analyses. In terms of design, both challenges (steamed and dry hay) were not performed as a cross-over study, since horses were initially fed with steamed hay (challenge #1) and subsequently fed dry hay (challenge #2). For ethical reasons, the design (i.e. steamed then dry hay) aimed at limiting the risk of horse exclusion (due to crisis exacerbation and subsequent treatment) at the end of the first exposure period, which would have precluded the horse to be involved in the second period. No significant difference was found for each group at d-2 (i.e. initiation of each exposure period) for all investigated parameters; suggesting that horses returned to “baseline level” prior to challenge #2. Also, clinical and tracheal mucus scoring were performed by clinicians not blinded to either challenge type (steamed vs. dry) or horse disease status (sEA vs. CTL), unlike all subsequent laboratory analyses, and lung function tests were not available for this study. Finally, the immune responses in BALF were investigated for the various cytokines through relative mRNA expressions only. Measuring BALF cytokine concentrations using commercially available ELISA kits however lacks sensitivity, since many BALF samples previously showed undetectable cytokine concentrations [27, 28].

Conclusions
Steaming hay significantly decreased mould content and induced less tracheal mucus in horses. However, both types of hay (steamed or not) induced BALF neutrophilia, and none induced respiratory clinical signs. The relevance of steaming hay however warrants further investigations both in the context of prevention/therapy for mEA horses at training and for long-term preclusion of lately developing sEA.

Methods
Horses
A cohort of 12 privately owned leisure horses were used in the study, and was subdivided into 2 separate groups. The control (CTL) group included 6 young horses (4 mares and 2 geldings, aged 2–5 years). These horses had no history of coughing or nasal discharge, exhibited no tracheal mucus upon endoscopy, and had less than 10% neutrophils in bronchoalveolar lavage fluid (BALF) from each lung when investigated 10 days prior to the trial. The other group included 6 adult horses (5 mares and 1 gelding, aged 9–17 years) that had all previously been diagnosed by the same clinician as suffering from sEA, based on clinical investigations, BALF cytology and reversible airway obstruction after medical/environmental change. Only horses kept in pasture and without any medical treatment or access to hay for at least 1 month prior to the trial were enrolled in the study. Recruitment for the study was made on a volunteer basis from private training centres (CTL horses) and from the caseload previously referred to the hospital (sEA horses; Oniris, Nantes). All owners signed an informed consent, and all procedures were performed by veterinarians and complied with relevant guidelines (Directive 2010/63/EU). The study was also approved by the Institutional Ethic Committee for Clinical Research (CERVO-2017-8-V).

Study design
This study was conducted during spring 2017. At the initiation of the trial, all horses were gathered and kept in a 4 ha pasture without access to hay for a duration of 2 weeks (Fig. 6). Since stabling was previously found to be associated with increased airway inflammation [29], horses were kept in 1 000 m² paddocks by subgroups of three individuals during both exposure periods. On day 0 of the trial, both groups (sEA and CTL) were fed on the ground with about 5 kg per horse of steamed hay (challenge #1), twice daily for 5 consecutive days (i.e. exposure period #1); followed by a wash-out period of 26 days in the 4 ha pasture without access to hay, prior to a second challenge with similar distribution of dry hay (challenge #2) for 5 consecutive days, (exposure period #2). Horses had access to water ad libitum and were also fed flaked cereals and dry pellets (Twenty Horse®) twice daily. Good quality (non-mouldy) grass hay was harvested as square bales (12–15 kg). For the challenge #1, the hay was steamed using a commercial device (HG-600²), according to manufacturer’s recommendations, and immediately distributed to horses. Briefly, the hay bale was placed onto the steamer and pushed down firmly until the manifold spikes pierce the hay to their full length; the steam generator (containing water) was then activated and steam diffused in hay through the spikes. At the end of exposure period #2, asthmatic horses received dexamethasone (0.1 mg/kg IV for 2 days, then progressively decreasing doses over 1 week) and all horses (sEA and CTL) were returned to their owners. Two days before (d-2) and 5 days
after (d + 5) the initiation of each challenge, all horses underwent a full clinical evaluation, as well as a tracheal endoscopy and BALF sampling. The 2010 CONSORT guidelines for reporting clinical trial were used for this report (Additional files 1 and 6) [30, 31].

**Hay characterisation**

Hay samples (random sampling by hand for a total of 1 kg) were collected from 10 different square bales before and after the steaming procedure, and stored in dedicated bags. All square bales originated from the same batch of hay (one single pasture), and all analyses were performed immediately after sampling. For bacteriology, 20 g of crushed hay were mixed with 180 ml of 0.1% peptone water and diluted 1/10 up to $10^{-6}$. One ml of each dilution was plated onto a Plate Count Agar (PCA) and incubated at 37 °C. One ml of each dilution was also cultured in oxytetracycline-glucose-yeast Extract Agar (OGA) and incubated at 25 °C for fungal growth. Bacterial and fungal growths were expressed as colony forming units (CFUs) after 5 days of incubation. The fungal colonies were then isolated on Sabouraud agar in order to be identified.

**Air quality**

Air sampling within the breathing zone was performed continuously for 2-3 h during hay feeding, on d + 1 (one CTL horse) and d + 4 (one sEA horse) of each exposure period. Respirable and inhalable particulates were collected according to previously published procedures [21].

**Particulates**

Horses were equipped with two samplers. The respirable fraction was collected with a flow rate of 2.5 L/min (AirCheck XR5000) onto 37 mm type AE glass fibre filters using aluminium cyclone. The inhalable fraction was collected with a flow rate of 2.0 L/min (AirCheck 3000) onto 25 mm PVC filters using Institute of Occupational Medicine (IOM) personal sampler. Sampling pumps were calibrated (Defender Bios calibrator) before and after sampling. Measurements of respirable and inhalable dust were assessed by gravimetric method, in triplicate measurements. The weight of particulates was divided by volume of air sampled to obtain airborne concentrations. Filters were placed in a desiccator for 24 h prior to any weight measurement. Negative controls were similarly prepared and only transported to and from the site of trial.

**Endotoxins**

Endotoxins were measured from the 37 mm type AE glass fibre filters, which were kept frozen (−20 °C) after particulates measurements. Filters were eluted in 10 mL of 0.05% Tween 20 within pyrogen-free water. Samples were then shaken during 1 h (MS-NRK-30). After elution, the suspension was centrifuged (1000 g; 15 min). The supernatant was then kept frozen (−20 °C) until being analysed. The eluates provided from sample filters were diluted at 1/10 in pyrogen-free water. Endotoxin activities were assayed using Endosafe – PTS2005F, according to manufacturer's recommendations.

**β-D-glucan**

Following endotoxin elution, heat extraction was performed in order to measure (1–3)-β-D-glucan content. Samples were then respectively diluted 10-fold for blank filters and 100-fold for sample filters in pyrogen-free water. Measures were carried out in duplicates, using a Glucatell test, according to manufacturer’s recommendations. The range of quantification was 5–40 pg/mL.
Clinical score, tracheal mucus and bronchoalveolar lavage fluid cytology
On d-2 and d + 5 for each exposure period, a clinical score, based on a 0–21 scale (Additional file 7) [32], was assigned to each horse. Detomidine hydrochloride (0.01 mg/kg IV) and butorphanol tartrate (0.01 mg/kg IV) was given prior to endoscopy. Airway endoscopy and BAL collection were performed using a flexible 3.2 m long, 12.8 mm tip diameter videendoscope. The amount of tracheal mucus was scored (grade 0–5) according to the previously published scale [33]. Left and right lungs were then sampled according to previously published procedures [34], with minor modifications. Briefly, a total of 500 mL of warmed isotonic saline solution (2 boluses of 250 mL each) was instilled and aspirated for each lung. Samples were conditioned (+ 4 °C in EDTA tubes) and processed (cytocentrifugation, May-Grünwald-Giemsa staining) as described previously [35]. Differential cell counts were performed on 300 leukocytes. Unlike clinical and mucus scores, cytological investigations of BALF from both lungs were conducted by investigators blinded to challenge type and horse status.

RNA isolation and reverse transcription
Each BALF sample obtained from the right lung was conditioned in order to determine cytokine relative expressions. Expression of 15 cytokines (representative of the different pathways previously associated with sEA) and one receptor (described in human allergic asthma) have been targeted in this study: interferon (IFN)-γ, interleukin (IL)-1β, IL-2, IL-4, IL-5, IL-6, IL-6R, IL-8, IL-10, IL-12, IL-13, IL-17, IL-18, IL-23, transforming growth factor (TGF)-β and tumour necrosis factor (TNF)-α. Twenty milliliters of BALF was centrifuged and the cell pellets resuspended in RNA protect Cell Reagent. Total RNA was extracted from cell pellets using the RNasy Plus Mini Kit. Concentration and purity of total extracted RNA was assessed using a NanoDrop 2000c Spectrophotometer; and RNA integrity was evaluated using a 2100 Bioanalyzer according to the manufacturer’s recommendations. All cDNA samples were stored at −20 °C until further use. Apart from the cytokines of interest, three reference genes, namely β-actin (ACTB), glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and β-glucuronidase (β-GUS), were also assessed. For each reaction, cDNA was amplified in a 25 μL standard reaction (Taqtman Universal PCR Master Mix). Thermal cycling profile used was: 10 min at 95 °C, followed by 50 cycles of 15 s at 95 °C and 1 min at 60 °C. The relative amount of mRNA was calculated using the relative standard curve method (Genex 6.1), and results were expressed as mean fold difference (target / reference genes). Using Normfinder, GAPDH and β-GUS were both selected for normalization (Additional file 8). Molecular investigations were also conducted blinded, in terms of horse ‘disease status’ and ‘challenge’ type.

Statistical analyses
Continuous data distributions which were not normally distributed, as assessed by Shapiro-Wilk W test, were log-10 transformed. For hay-related parameters, paired t tests (steamed vs. dry) were performed for the bacterial/mould content. For all horse-related parameters, effects of challenge (steamed vs. dry), time (d + 5 vs. d-2) and disease status (sEA vs. CTL) were investigated by 3-way analysis of variance (ANOVA, General Linear Model) and Tukey-Kramer’s post-hoc tests, with age as covariate. Correlations among sEA affected horses were determined by Spearman’s correlation coefficient. Absolute values of $R < 0.5$ were arbitrarily not reported. The different analyses were conducted using Prism 7 and NCSS12, and values of $P < 0.05$ were considered significant.

Endnotes
1Tromelin & Cie, Ploeren, France
2Haygain, Lambourn, Berkshire, UK
3SCK Inc., Eighty Four, PA, USA
4Merck-Millipore, Molsheim, France
5Major Science, Saratoga, CA, USA
6Charles River Laboratories, Saint-Germain-Nuelles, France
7Associates of Cape Cod Inc., East Falmouth, MA, USA
8Optomed, Les Ulis, France
9Quiagen, Courtabeuf, France
10Thermoscientific, Villebon-sur-Yvette, France
11Agilent Technologies, Les Ulis, France
12Life Technologies, Saint-Aubin, France
13bioMMC, Freising, Germany
14GraphPad, La Jolla, CA, USA
15NCSS – LLC, Kaysville, UT, USA

Additional files
Additional file 1: CONSORT flow diagram for the clinical trial involving 6 control (CTL) horses and 6 horses with severe equine asthma (sEA). (PDF 46 kb)
Additional file 2: Microbiological content in hay (n = 10), before and after steaming: a) bacterial content; b) mould content. *** significantly different ($P < 0.001$), based on paired t test. (TIF 917 kb)
Additional file 3: Cytology of bronchoalveolar lavage fluid (BALF), before and after the initiation of each challenge: sEA, severe equine asthma; CTL control; d-2, 2 days before challenge; d + 5, 5 days after

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Additional file 4: Relative mRNA expression of cytokines in bronchoalveolar lavage fluid (BALF), before and after the initiation of each challenge: a) interleukin (IL)-2; b) IL-5; c) IL-13; d) TGF-β; e) IL-6; f) IL-8; g) Interferon (IFN)-γ. sEA, severe equine asthma; CTL, control; d-2, 2 days before challenge; d + 5, 5 days after challenge. (TIF 2119 kb)

Additional file 5: Correlations (95% confidence interval) between relative mRNA expression of cytokines and clinical/cytological parameters, before and after the initiation of each challenge: IL, interleukin; (95% CI), non-significant and/or absolute value of R < 0.5. (DOCX 11 kb)

Additional file 6: CONSORT-check list for the clinical trial involving 6 control (CTL) horses and 6 horses with severe equine asthma (sEA). (PDF 134 kb)

Additional file 7: Clinical scoring system for respiratory conditions, adapted from Tesarowski et al. (32). (DOCX 18 kb)

Additional file 8: Nucleotide sequences of equine-specific primers used in real-time PCR assays. (DOCX 22 kb)

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Availability of data and materials
All data generated or analysed during this study are included in this published article and its supplementary information files.

Authors’ contributions
MO contributed to data collection and study execution, data analysis/interpretation, and preparation of the manuscript. EH contributed to data collection, analysis and interpretation. AC contributed to study design, data collection and study execution. MPT contributed to data collection and study execution. MMC contributed to study design and preparation of the manuscript. SP contributed to data analysis and interpretation. RP contributed to data interpretation and preparation of the manuscript. MD contributed to study execution and preparation of the manuscript. EAR contributed to study design, data interpretation, and preparation of the manuscript. All authors provided final approval of the manuscript.

Ethics approval and consent to participate
All owners signed an informed consent, and the study was approved by the Institutional Ethic Committee for Clinical Research (Comité d’éthique en recherche clinique et épidémiologique vétérinaire d’Oniris; CERVO-2017-8-V).

Consent for publication
Not applicable.

Competing interests
MMC is Scientific Consultant for Haygain. The authors declare that they have no competing interests.

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References
— Stratégies thérapeutiques

Les brûlures chez le cheval : abécédaire de la prise en charge locale et systémique

Une évaluation attentive de l’étendue et de la profondeur des brûlures est primordiale. Les traitements locaux et systémiques débutent dans l’urgence, le pronostic vital étant parfois engagé, et des soins de longue durée sont à envisager.

Les brûlures, bien qu’assez rares en médecine vétérinaire, sont des lésions difficiles à gérer, surtout si elles sont profondes et étendues. En pratique, la majorité de celles rencontrées sont superficielles. Néanmoins, les brûlures plus sévères sont un réel défi, en raison de l’atteinte cardiovasculaire et/ou respiratoire, de l’état de choc et de l’atteinte éventuelle de zones difficiles à soigner, tels que les yeux ou les articulations. N’étant jamais à l’abri des feux de forêts, d’écuries, des produits chimiques ou de la foudre, tout vétérinaire peut être amené à soigner le cheval brûlé d’un propriétaire motivé.

La gestion d’un grand brûlé nécessite des connaissances et de l’engagement, car le praticien doit non seulement maîtriser les conséquences systémiques d’une brûlure étendue, mais également appréhender les lésions locales et la douleur de l’animal. Les principes de la gestion thérapeutique ont été calqués sur la médecine humaine et adaptés à la médecine vétérinaire.

—Les brûlures chez le cheval : circonstances d’apparition et évaluation de la sévérité

Circonstances des accidents
Les chevaux brûlés dans les feux d’écuries sont majoritairement touchés au niveau du dos, de la face et des voies respiratoires (chute de débris, fumée toxique) [23]. Quant aux chevaux brûlés lors d’incendies de forêt, ils vont plutôt présenter des lésions au niveau du ventre et des membres, ainsi que de la fourbure (choc thermique podal), en raison des pas effectués sur le brasier [7].

Évaluer l’étendue des brûlures
Une bonne estimation de la surface corporelle brûlée et de la profondeur des lésions est indispensable pour adapter la prise en charge en urgence et établir un pronostic. La surface totale de la brûlure (total burned surface area [TBSA]) est calculée afin de décider d’une fluidothérapie adaptée (figure 1). La chaleur met du temps à se dissiper dans les tissus et il est très difficile d’évaluer rapidement, après la blessure, la TBSA exacte – en particulier chez les animaux, en raison de la présence de poils qui retarde l’apparition des lésions jusqu’à 92 heures post-brûlure [14].

En cas de TBSA supérieure à 25 % de la surface totale, il est possible d’observer en général des manifestations systémiques graves, telles qu’une hypovolémie, une anémie (due à une hémolyse intravasculaire) et parfois même un état de choc, d’où la nécessité impérative de perfuser.

Les brûlures étendues sont difficiles à gérer chez les chevaux : lorsque la TBSA dépasse les 50 %, l’issue est souvent fatale, sauf si les lésions ne sont que très superficielles [23]. Il est quasiment impossible de prévenir les surinfections chez les chevaux, car ils ne peuvent pas être maintenus dans un environnement stérile et l’automutilation est fréquente [22].

Évaluer la profondeur des brûlures
Chez le cheval, l’estimation de la profondeur des brûlures est rendue délicate par la présence des poils et des débris cutanés présents secondairement à une brûlure (tableau). La difficulté principale concerne surtout les brûlures du 2e degré qui peuvent être :

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Éléments à retenir

✦ Une brûlure se refroidit avec de l’eau tiède à 15 °C pendant 20 minutes au minimum.
✦ Dès 25 % de surface corporelle atteinte (total burned surface area, TBSA), il est indispensable de mettre en place une fluidothérapie (cristalloïdes hypertoniques puis isotoniques) selon la formule de Parkland : 4 ml/kg x % TBSA.
✦ Le topique à privilégier est la sulfadiazine d’argent pour ses propriétés antiseptiques, antifongiques, hydratantes et procicatrisantes.

Les chiffres correspondent aux pourcentages pour chaque partie du corps chez l’adulte et le poulain. D’après [7, 9].

- superficielles (degré IIa) : la totalité de l’épiderme et le derme superficial sont concernés. Les bulbes pileux contenant les cellules souches sont intacts. Ces brûlures vont guérir spontanément en 14 jours, avec peu de cicatrices. Très douloureuses, elles ne laissent pas de lésion neurovasculaires ;
- profondes (degré IIb) : une atteinte de la totalité de l’épiderme et jusqu’au derme profond, comprenant les bulbes pileux, est observée. Ces brûlures vont nécessiter une prise en charge chirurgicale, afin qu’aucune cicatrice ni dommage vasculaire sévère ne se produise.

En cas de doute, une biopsie punch des tissus brûlés peut être transmise pour analyse histologique pour confirmer la profondeur de l’atteinte.

Les conséquences locales d’une brûlure

Les lésions cutanées

Lors de lésion thermique, en général, plusieurs zones transitoires dites intermédiaires se trouvent entre les tissus lésés et les tissus sains (figure 2) [27].

✦ La zone centrale au plus près de la source de chaleur est typiquement totalement nécrosée en raison de la dénaturation des protéines cellulaires (qui a lieu dès que la température dépasse 45 °C) et de la coagulation intravasculaire (zone de coagulation). Elle est composée de tissus morts dont les vaisseaux sont thrombosés.
✦ La zone intermédiaire est caractérisée par une diminution du flux sanguin et des formations d’agrégats d’hématies (zone de stase). Le thromboxane A2, puissant vasoconstricteur et agrégant plaquettaire libéré par les tissus brûlés, est responsable d’une ischémie dermique progressive dans les 24 à 48 heures (photos 1 et figure 3). Les lésions sont potentiellement réversibles selon la qualité des soins.
✦ Enfin, la zone de la plaie la plus éloignée de la source au moment de la brûlure est caractérisée par une augmentation du flux sanguin et des lésions tissulaires minimales (zone d’hyperhémie) [11, 14, 26, 30].
# TABLEAU : BILAN SELON LA PROFONDEUR DES BRÛLURES

<table>
<thead>
<tr>
<th>CLASSIFICATION DES BRÛLURES</th>
<th>STRUCTURES LÉSÉES</th>
<th>SIGNES CLINIQUES</th>
<th>PRONOSTIC DE CICATRISATION</th>
</tr>
</thead>
</table>
| 1er DEGRÉ                   | Couches supérieures de l’épiderme | Couleur : rouge  
Cloques : absence  
TRC : rapide  
Douleur : +++  
Piqûre aiguille : saigne  
Autres : chaleur, œdème, desquamation | Cicatrisation aisée |
| 2e DEGRÉ SUPERFICIEL (IIA)  | Toutes les couches de l’épiderme | Couleur : rouge à rose  
Cloques : présence  
TRC : rapide  
Douleur : +++  
Piqûre aiguille : saigne  
Autres : chaleur, œdème, perte des poils | Cicatrisation aisée avec soins locaux (7 à 14 jours) |
| 2e DEGRÉ PROFOND (IIB)      | Épiderme + une partie des couches du derme | Couleur : rose pâle ou rouge tacheté  
Cloques : parfois  
TRC : absent  
Douleur : 0  
Piqûre aiguille : ne saigne pas  
Autres : œdème, nécrose, escarres, perte des poils | Cicatrisation plus lente, avec soins locaux |
| 3e DEGRÉ                    | Épiderme et derme en entier | Couleur : noir violacé/blanc  
Cloques : absence  
TRC : absence  
Douleur : 0  
Piqûre aiguille : ne saigne pas  
Autres : œdème, nécrose, ulcérations, escarres, perte de sensibilité sauf en périphérie des lésions, perte des poils | Cicatrisation lente, perte des follicules pileux, glandes sébacées et sudoripares, complexe, nombreuses surinfections |
| 4e DEGRÉ                    | Épiderme, derme, muscles, tendons, os | Couleur : noir violacé/blanc  
Cloques : absence  
TRC : absence  
Douleur : 0  
Piqûre aiguille : ne saigne pas  
Autres : œdème, nécrose, ulcérations, escarres, perte de sensibilité, destruction des tissus sous jacentes | Cicatrisation lente, complexe, nombreuses surinfections, perte de fonction possible |

TRC : temps de remplissage capillaire ; +++ : intensité très élevée.
D’après [4, 8, 16, 19, 24].

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**Les lésions oculaires et faciales**

Le film lacrymal constitue une protection efficace contre la chaleur, même si ce n’est pas toujours suffisant. L’atteinte de la cornée, des paupières et des annexes est généralement très préoccupante quand elle survient.

L’examen ophtalmologique réalisé dès l’admission doit donc être méticuleux, car le développement d’un œdème cornéen et palpébral peut rapidement empêcher l’examen oculaire [5].

Enfin, la principale lésion liée à l’atteinte des oreilles est une chondrite, c’est-à-dire une inflammation du cartilage qui entraîne une déformation définitive de celui-ci.

**Les lésions pulmonaires**

En cas de brûlure au niveau de la face, il convient de s’assurer de la présence ou non de brûlure d’inhalation en regardant notamment si les poils des naseaux ont été brûlés ou non [31]. Si c’est le...
Les brûlures chez le cheval : abécédaire de la prise en charge

Les brûlures peuvent être sévères ou graves (encadré 1 complémentaire sur www.lepointveterinaire.fr) [26].

Les conséquences systémiques
La destruction des lipides membranaires des zones brûlées entraîne la perte de leur rôle de barrière naturelle protectrice. Ainsi, plus la surface brûlée est étendue, plus le potentiel de pertes de fluides, d’électrolytes et de calories augmente. Le choc hypovolémique, l’immunodépression et le choc septique vont engendrer une vulnérabilité vis-à-vis des agents pathogènes.

—Prise en charge des brûlés en urgence
- À l’arrivée sur un lieu d’incendie, il convient de suivre plusieurs recommandations :
  - libérer les chevaux des boxes sans se mettre en danger ;
  - effectuer un triage en cas de multiples victimes entre les chevaux au bon pronostic vital, ceux au pronostic vital sombre (TBSA supérieure à 80 %) et ceux au pronostic vital engagé, mais dont les soins vont augmenter les chances de survie [10]. C’est de ces deux dernières catégories que le praticien doit s’occuper en priorité.

Il est recommandé de procéder selon un moyen mnémotechnique alphabétique utilisé en médecine humaine (figure 4).

Clichés : L. Coutelé, PUVTH

Figure 3 : Répartition et profondeur des brûlures estimée lors de l’admission du cheval des photos 1

Gauche

| 3e et 4e degrés |
| 2e degré |
| 1er degré |

Droite

| 3e et 4e degrés |
| 2e degré |
| 1er degré |

cas, des lésions de l’appareil respiratoire peuvent être suspectées. Les lésions pulmonaires peuvent être dues à l’inhalation directe de fumée, mais également secondaires au syndrome de réponse inflammatoire systémique lors de brûlures très sévères (encadré 1 complémentaire sur www.lepointveterinaire.fr) [26].

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02. Cheval ayant potentiellement inhalé de la fumée car les naseaux sont brûlés.
Cliché : L. Couëtil, PUVTH

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VERS UNE DERMATOLOGIE ÉQUINE RAISONNÉE

02. Cheval ayant potentiellement inhalé de la fumée car les naseaux sont brûlés.
Cliché : L. Couëtil, PUVTH

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A et B : *airways and breathing* (voies respiratoires et ventilation)
Le praticien doit tenter de savoir si un cheval a inhalé de la fumée par la détection de :
- détresse respiratoire ;
- dyspnée, toux ;
- changement de la voix, cornage ;
- poils des naseaux brûlés ou suie dans la bouche (photo 2) ;
- auscultation thoracique anormale (œdème pulmonaire).

Des aspirations fréquentes des sécrétions doivent alors être réalisées et une oxygénothérapie est mise en place dans la mesure du possible. En cas de détresse respiratoire, qui peut intervenir aussi dans un second temps, le cheval est intubé ou une trachéostomie peut se révéler nécessaire [8].

Dans une structure hospitalière, il est impératif d’oxygéner à 15 à 20 l/min pour un cheval adulte, soit par l’emploi en parallèle de trois extracteurs d’oxygène (Oxybox*), soit en utilisant de l’oxygène en bouteille avec détendeur et régulateur de débit aérien qui peut apporter jusqu’à 15 l/min (générant une fraction inspirée en oxygène [FiO2] d’environ 50 % à 15 l/min).

Une deuxième sonde nasale peut être appliquée pour administrer des débits supérieurs [29]. Pour des débits élevés, le diamètre interne des sondes doit être au minimum de 7 mm.

Lors d’œdème pulmonaire (à soupçonner en cas de tachypnée, de crépitements à l’auscultation pulmonaire ou de “mousse” [jetage spumeux] au niveau des naseaux dans les cas les plus sévères), le traitement inclut aussi l’utilisation précoce de furosemide, surtout pendant les 24 premières heures. Cependant, cette utilisation est controversée lors d’atteinte rénale précoce dans les cas sévères de réponse systémique aux brûlures. Il est donc nécessaire d’évaluer le rapport bénéfices/risques [17].
Si l’œdème pulmonaire est lié à un œdème généralisé à la suite de pertes protéiques très importantes, une plasmaphérèse est alors indispensable.

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C : *cooling* (refroidir)
Les brûlures ayant reçu des soins adéquats en premier secours voient leur chance de cicatrisation augmenter nettement, même sans intervention chirurgicale [10].

Il est indispensable de réagir très rapidement et d’utiliser de l’eau propre et tiède (15 °C) pendant au moins 20 minutes. Ceci est à réaliser le plus tôt possible (bénéfique jusqu’à 3 heures après l’accident).

Cette mesure permet de soulager la douleur, de dissiper la chaleur, de stopper la progression de la brûlure et de limiter les dommages tissulaires, ainsi que les œdèmes.

L’eau froide doit être évitée, car elle réduit le flux sanguin nécessaire à la réaction tissulaire et entraîne une hypothermie.

C : *circulation* (fluidothérapie d’urgence)
La mise en place rapide de la fluidothérapie est utile dès 15 % de TBSA et indispensable dès 25 %. En général, dans les cas graves, deux cathéters sont mis en place et des cristalloïdes hypertoniques (3 à 5 ml/kg/15 min sans jamais dépasser 1 ml/kg/min), puis isotoniques (Ringer Lactate) sont administrés en bolus.

En médecine humaine, où la plasmathérapie est très peu pratiquée sur les brûlés pour des raisons de risques de transmission d’infection, l’administration des colloïdes (gélatines) est controversée : à cause de l’augmentation de la perméabilité vasculaire présente lors de brûlure, l’extravasation des colloïdes risquerait d’aggraver les œdèmes tissulaire et pulmonaire. Cela représente plutôt un risque théorique que réel : si la pression oncotique sanguine baisse beaucoup, entraînant ainsi un risque élevé de développer...
Les brûlures chez le cheval : abécédaire de la prise en charge

ENCADRÉ 2 : LE TRAITEMENT LOCAL SELON LA SÉVÉRITÉ DES BRÛLURES

Les brûlures mineures, la lésion est rincée avec de l’eau à 15 °C pour enlever les débris, la refroidir et retirer les corps étrangers. Des bandages sont posés et des anti-inflammatoires non stéroïdiens (AINS) administrés pour soulager la douleur. Une crème de type sulfadiazine d’argent (SSD) est largement en place, agissant comme un bandage naturel, jusqu’à la cicatrisation de la brûlure [14, 21]. La plaie est nettoyée deux à trois fois par jour et un antibiotique topique est appliqué systématiquement pour réduire la charge bactérienne sur la brûlure [14, 21].

Les brûlures du 3e degré menacent potentiellement la vie du cheval. Le traitement du choc et/ou de la détresse respiratoire est donc la priorité face à ce cas de figure. Les soins de plaies commencent une fois l’animal stabilisé. Les plaies sont refroidies par hydrothérapie (bains, douches ou compresses humides). Ensuite, elles sont nettoyées du mieux possible avec une solution stérile de chlorhexidine 0,05 % [21].

03. Exemple de cas où l’examen ophtalmologique est indispensable en première intention. Le cheval présente un blefarospasme, un œdème cornéen et un œdème palpebral, malgré l’absence d’ulcère cornéen. En cas d’œdème de la paupière, une pomme de antibiothérapie peut être appliquée de façon préventive toutes les 6 heures et la présence d’ulcère vérifiée deux fois par jour.

Les corticoides sont proscrits. Si la cornée est nécrotique, un débridement sous tranquillisation et anesthésie locale est nécessaire.

Cliché : L. Couëtil, PUVTH

Les oedèmes, le recours à la plasmathérapie est prioritaire [13].

D : disability (douleur)
La présence de traumatismes concomitants (fractures, par exemple) doit être recherchée. Dans l’urgence, l’administration de sédatifs (détomidine, notamment) et de morphiniques est indispensable pour maîtriser la douleur d’intensité très élevée d’un grand brûlé.

E : exposition (environnement)
Il est important de mettre le cheval dans un environnement (box) propre et de garder une hygiène irréprochable pour minimiser le risque de surinfection cutanée. Une attention particulière doit être portée aux mouches et à l’automatulation. En urgence, il est souhaitable de tondre et de nettoyer localement avec de la chlorhexidine (le choc thermique est suivi de la production de cytokines pro-inflammatoires et médiateurs extravasculaires, des œdèmes et une hypovolémie). Une attention particulière est nécessaire pour maîtriser la douleur d’intensité très élevée d’un grand brûlé.

F : fluidotherapy (fluidothérapie)
Une zone brûlée perd quatre fois plus d’eau par évaporation. Il est donc évident que la déshydratation est la priorité face à ce cas de figure. Une crème anti-inflammatoire (SSD) est appliquée et l’escarre peut être protégée avec un hydrogel qui peut absorber 30 fois son poids en exsudat, vaporisé et utilisé pour protéger l’escarre, prévenir la dessiccation et éviter les surinfections. Si un pansement semi-occlusif est réalisé (par exemple, sous les membres), il est possible d’utiliser un pansement à l’alginate de calcium contenant un milieu favorable à la cicatrisation et leur présence est moins douloureuse qu’une plaie. Passé cet intervalle, la bulle est partiellement excisée. Une crème antibactérienne (SSD) est appliquée et l’escarre peut se former. Les débris de peau mortes qui veulent tomber sont retirés progressivement, alors que l’escarre est laissée en place, agissant comme un bandage naturel, jusqu’à ce qu’elle tombe d’elle-même. La plaie est nettoyée deux à trois fois par jour et un antibiotique topique est appliqué systématiquement pour réduire la charge bactérienne sur la brûlure [14, 21].

Médicament à usage humain.

Médicaments à usage humain.
Pour calculer les besoins en fluides à la suite des pertes, la formule de Parkland, calquée sur la médecine humaine, peut être utilisée :

\[ 4 \text{ ml/kg} \times \% \text{TBSA} \]

La moitié du montant obtenu est administrée en 8 heures, puis l’autre moitié sur les 16 heures suivantes. Le deuxième jour, le calcul est :

\[ 2 \text{ ml/kg} \times \% \text{TBSA} \]

Par exemple, pour un cheval de 500 kg brûlé à 50 % de TBSA, l’apport de fluides doit être de 100 l pour les premières 24 heures (4 ml x 500 x 50 = 100 l). Dans la mesure du possible, il est recommandé de surveiller la diurèse (0,5-1 ml/kg/h).

Les pertes électrolytiques doivent également être compensées. S’il est recommandé de doser les électrolytes, la tendance est à l’hyperkaliémie/hyponatrémie pendant 24 heures (ceci résulte de : la destruction cellulaire, la perturbation de la pompe sodium-potassium [Na/K], l’hémolyse, l’Altération de l’excérétion rénale). Puis, entre J3 et J5, la tendance s’inverse avec une hypokaliémie/hypernatrémie (résultant de la diurèse, de la production d’hormones minéralcorticoïdes et de la résorption des œdèmes).

La présence d’une hypoprotéinémie chez les grands brûlés entraîne une tendance à la formation d’œdèmes graves. Les pertes de protéines sont maximales 8 à 12 heures après la brûlure à la suite de divers mécanismes :
- augmentation de la perméabilité vasculaire, donc fuite des protéines plasmatiques vers le milieu extracellulaire ;
- dénaturation thermique de protéines ;
- exsudation : une brûlure du 2° degré exsude un liquide contenant 4 à 8 g de protéines/100 ml.

Lors de brûlures, une anémie est également notée, qui est souvent grave à partir d’une TBSA supérieure à 40 % [12, 14, 15]. Elle fait suite à :
- des pertes sanguines lors des interventions chirurgicales ;
- une diminution de l’érythropoïèse en raison de l’inflammation qui entraîne la séquestration du fer par les macrophages ;
- une hémolyse due à trois phénomènes : effet immédiat de la chaleur (T° supérieure à 65 °C), avec une lyse érythrocytaire proportionnelle à la TBSA (jusqu’à perdre 5 à 12 % de la masse érythrocytaire globale par jour) ; effet des radiocaux libres oxygénés ; des facteurs plasmatiques.

La transfusion plasmatique ou sanguine doit être considérée si l’hématocrite descend en dessous de 17 %, souvent entre 2 et 5 jours après l’accident. L’anémie peut persister 50 à 60 jours. Il s’agit donc de minimiser le risque d’anémie inflammatoire en prévenant les surinfections, et de compléter en fer et en vitamine B12 pour encourager l’érythropoïèse et compenser les carences probables à la suite des pertes sanguines.

**Prise en charge de la douleur**

Lors de brûlures, plusieurs composantes douloreuses existent :
- la nociception initiale (T° supérieure à 45 °C) : sensation douloureuse provenant d’une stimulation des terminaisons des fibres nerveuses Aδ et C ;
- l’hyperalgésie, liée aux stimuli des médiateurs de l’inflammation, neurotransmetteurs, etc. Un stimulus non douloureux devient douloureux pour une peau lésée. Ainsi, tous les soins sont extrêmement douloureux. La douleur liée aux actes thérapeutiques, très fréquents, est décrite comme extrême ;
- la douleur neuropathique due à la régénération des structures nerveuses au cours de la cicatrisation ;
- la douleur chronique, liée à la répétition de stimulation nociceptive qui entraîne une sensibilisation persistant après la guérison de la lésion initiale.

La douleur “sourde/de fond” peut être traitée avec des anti-inflammatoires non stéroïdiens (AINS) (flunixine méglumine à 0,5 à 1,1 mg/kg, une à deux fois par jour) ou de la morphine (0,1 à 0,2 mg/kg toutes les 4 heures), qu’il convient d’associer à des sédatifs (détomidine, par exemple), afin de diminuer les effets excitateurs centraux, ainsi que le stress du cheval lié à la douleur. De même, des perfusions continues de lidocaïne peuvent être envisagées, avec un bolus de 0,65 mg/kg sur 15 minutes, puis une perfusion de 0,025 mg/kg/min [3].

La douleur due aux procédures (soins) peut être maîtrisée par la sédation (détomidine à 6 µg/kg et butorphanol à 0,04 mg/kg), voire l’administration d’anesthésiques comme la kétamine, avec des doses inférieures à 0,2 mg/kg ou encore de la lidocaïne par voie topique ou locorégionale. La douleur postopératoire nécessite également l’utilisation d’opioïdes (morphine, patchs de fentanyl).

(2) Médicaments à usage humain.
Hypermétabolisme et prise en charge des besoins nutritionnels

Lors de brûlures dépassant 30 % de TBSA, l’inflammation et la réponse “hypermétabolique” commencent immédiatement et peuvent durer très longtemps (jusqu’à 3 ans en médecine humaine).

En effet, à la suite du stress, l’organisme répond par la production de glucocorticoïdes. L’augmentation du métabolisme entraîne une hausse des dépenses énergétiques, multipliées par 1,8 en moyenne.

L’augmentation du cortisol sanguin inhibe l’action de l’insuline, donc accroît la néoglucogenèse, ce qui favorise une hyperglycémie. Ainsi, chez le cheval, le développement d’un état d’insulinorésistance doit être envisagé avec en conséquence un risque de fourbure.

Le traitement possible, décrit en médecine humaine, est une combinaison d’insuline (augmente la synthèse protéique, prévient la fonte musculaire et la résorption osseuse, diminue la quantité de cytokines pro-inflammatoires) et de metformine (diminue la néoglucogenèse, augmente la sensibilité à l’insuline). L’état catabolique induit par l’hypercortisolémie conduit en plus à de fortes pertes de poids, à une amyotrophie, et à une baisse de la croissance chez le jeune.

La gestion de l’analgésie est capitale pour que le cheval continue à s’alimenter. L’hypermétabolisme combiné au processus de cicatrisation consomme énormément d’énergie.

Des aliments de qualité doivent être apportés au cheval, et l’apport énergétique est multiplié par 2 à 2,5 par rapport à la ration d’entretien, pendant plusieurs mois.

Il est possible d’envisager des rations ménagères complémentées (dextrose, foin de luzerne, caséine, électrolytes et minéraux [zinc et méthionine]), riches en protéines de qualité.

Les surinfections

La peau, qui joue habituellement le rôle de barrière naturelle, devient en cas de brûlure un excellent milieu de culture en raison de l’œdème, des sécrétions et de la nécrose.

Les premiers signes d’infection sont très délicats à détecter chez les grands brûlés. Ils sont à rechercher souvent 2 à 6 jours après l’événement accidentel et peuvent impliquer différentes populations bacteriennes :
- Gram+ (G+), provenant de la flore cutanée : elles résistent au choc thermique. La surinfection commence généralement dès 48 heures après l’accident ;
- Gram- (G-), par contamination gastro-intestinale, respiratoire, cutanée, environnementale, etc. La surinfection a lieu le plus souvent 5 à 7 jours après l’accident ;
- levures et champignons.

La meilleure prévention est d’avoir une hygiène irréprochable, que ce soit pour les actes thérapeutiques ou l’environnement, ce qui est difficile à obtenir avec les chevaux.

L’administration d’antibiotiques est envisagée en cas d’atteinte cutanée profonde, respiratoire ou de signes avérés d’infection locale ou généralisée. Avant de choisir un antimicrobien, il est indiqué de réaliser un prélèvement (selon la zone affectée, cutanée, respiratoire, etc.) afin de cibler l’antibiothérapie. Dans la mesure du possible, et pour les infections cutanées superficielles, les antibiotiques topiques sont préférés.

Il a été notamment rapporté que l’administration d’antibiotiques systémiques n’influence pas favorablement la cicatrisation des plaies, la présence de fièvre ou la mortalité [21].

Le statut antitétanique doit aussi être vérifié et, si besoin, un sérum antitétanique est administré.

De l’immunosuppression à un syndrome de défaillance multi-organique

L’immunosuppression peut apparaître dès 20 % de TBSA [14]. Peuvent alors suivre un syndrome de réponse inflammatoire systémique (SIRS), un sepis, un choc septique, voire un syndrome de défaillance multi-organique (MODS), le plus souvent dans la deuxième semaine après la brûlure.

Le traitement du choc hors fluidothérapie inclut :
- de la flunixine méglumine : le protocole consiste à alterner entre la dose anti-endotoxémique à 0,25 mg/kg, deux fois par jour, et la dose
VERS UNE DERMATOLOGIE ÉQUINE RAISONNÉE

**FIGURE 5 : CHRONOLOGIE DES PHASES DE LA CICATRISATION CUTANÉE**

<table>
<thead>
<tr>
<th>Phase inflammatoire aiguë</th>
<th>Phase proliférative</th>
<th>Phase de remodelage</th>
<th>Résistance à la tension</th>
</tr>
</thead>
<tbody>
<tr>
<td>Synthèse de collagène</td>
<td>Réorganisation des fibres de collagène</td>
<td>80 % de la résistance initiale</td>
<td></td>
</tr>
<tr>
<td>Blessure J3</td>
<td>J3</td>
<td>J14</td>
<td>J21 1 an</td>
</tr>
</tbody>
</table>

D’après [25].

---

anti-inflammatoire et analgésique à 1,1 mg/kg, deux fois par jour ;
- de l’héparine : elle peut être employée en prévention ou en traitement des troubles de la coagulation liés au SIRS et à l’endotoxémie (50 à 100 UI/kg par voie sous-cutanée, une fois par jour) ;
- de la lidocaïne, qui permet de diminuer l’activation leucocytaire liée à l’endotoxémie, de prévenir l’apparition de fourbure, a des effets analgésiques et diminue la phagocytose par les polynucléaires neutrophiles (1,3 mg/kg par voie intraveineuse lente).

En soutien de la pression artérielle, il est parfois possible d’utiliser de la dobutamine à 2 à 15 µg/kg, de la dopamine à 2 à 15 µg/kg, de la norépinephrine à 1,5 µg/kg/min ou encore la vasopressine en dernier recours. Les corticoïdes sont à éviter dans la mesure du possible, le cheval étant déjà en état d’immunosuppression.

Défauts organiques
Lors de MODS, les organes les plus demandeurs en oxygène sont les plus atteints.

- Les poumons sont donc les plus touchés en raison du MODS, mais aussi des lésions d’inhalation souvent présentes : il est nécessaire de contrôler l’inflammation au moyen d’un AINS le moins néphrotoxique possible [14, 21]. La bronchoconstriction réactionnelle, l’accumulation de mucus et le risque de surinfection doivent être maîtrisés par administration systémique ou par la nébulisation de bronchodilatateurs, de mucolytiques et d’antimicrobiens. Les bronchodilatateurs β2-agonistes ont l’avantage de stimuler la clairance du mucus par la muqueuse ciliée. L’emploi de molécules antitussives est contre-indiqué, car la toux permet au cheval d’évacuer les débris cellulaires et le mucus obstruant les voies respiratoires [8, 20]. En cas de signes évocateurs de pneumonie, il est fortement recommandé d’effectuer un lavage trachéal pour cytologie et culture/antibiogramme.

- Les reins peuvent également subir des atteintes aiguës résultant essentiellement de la toxicité glo-merulaire de l’hémoglobine et de la myoglobine libérées secondairement aux lésions tissulaires subies, parallèlement à l’hypotension souvent rencontrée [6, 28].

Le risque de fourbure
La fourbure aiguë est un motif important d’euthanasie chez les chevaux brûlés. Elle peut survenir selon différents procédés :
- métabolique : les brûlures extensives conduisent à une déshydratation et à une augmentation des glucocorticoides endogènes, entraînant des modifications vasculaires pouvant parfois provoquer une fourbure ;
- inflammatoire : l’état d’inflammation sévère présent chez le brûlé (SIRS) peut entraîner de la fourbure ;
- mécanique : si le cheval a galopé sur de longues distances et s’est déplacé sur un sol en braises, les lésions thermiques et mécaniques (vasculaires et cellulaires) peuvent amener à la désolidarisation podophylle-kéraphylle, donc à la fourbure.

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Prise en charge locale après l’urgence
Bien connaître les phases de la cicatrisation est d’une importance primordiale dans la gestion des brûlures (encadré 3 complémentaire sur [www.lepointveterinaire.fr](http://www.lepointveterinaire.fr) et figure 5).

Désinfection
Il est impératif de continuer à utilisation de la chlorhexidine à 0,05 % une fois par jour, ou bien une fois tous les 2 jours en l’absence de surinfections.

Parage de la plaie
Il est recommandé de retirer les débris cutanés et de réaliser une hydrothérapie quotidiennement...
Les greffes cutanées permettent de regagner un aspect plus esthétique pour la surface brûlée.

- Punch grafts : ce sont de petits îlots de greffons de peau épaissie qui sont prélevés via des biopsies punch (tous les 1 cm) et implantés dans du tissu de granulation. Cette technique est aisément réalisable sur le terrain en médecine équine.

D’après [2, 18].

Topiques

Les topiques cutanés doivent être appliqués deux à trois fois par jour :
- SSD(2) (Flammazine®), bon spectre G+, G- et Candida albicans, propriétés anti-inflammatoires et hydratantes. C’est la crème de référence en médecine humaine ;
- acétate de mafénide(3) (Sulfamylon®) lors de suspicion de surinfection par Pseudomonas ;
- aloe vera (pour les brûlures moins profondes), actif sur Pseudomonas et Candida, a des vertus anti-oxydantes, anti-inflammatoires et procicatrisantes ;
- miel de manuka (dans l'idéal), topique le plus économe, si les moyens financiers sont très limités : antimicrobien, cicatrisant et anti-inflammatoire. Durant l’été, il convient de faire attention aux mouches.

Les stratégies chirurgicales locales

Le recours à la chirurgie est indiqué dans certains cas de brûlures de degrés IIb, III ou IV, de brûlures circonférentielles ou surinfectées. La chirurgie est contre-indiquée en cas d’hypothermie (T° inférieure à 34 °C) ou d’instabilité cardiovasculaire et respiratoire. La destruction du derme laisse une structure collagénique appelée escarre qui fournit une protection naturelle à la plaie de brûlure jusqu’à sa chute. C’est une barrière sèche composée d’exsudat, de collagène, de couches de peau morte qui constituent un obstacle pour les bactéries. L’excision de cette escarre est réalisée en médecine humaine, mais chez les chevaux cette pratique est peu courante, étant donné les risques très élevés de contaminations externes et les difficultés à réaliser des bandages sur certaines parties du corps telles que le dos, les flancs et la croupe (technique fermée). Dans la mesure du possible, la plaie est débarrassée des tissus nécrotiques.

Les risques de cette technique sont les surinfections bactériennes, très fréquentes, les pertes d’eau par évaporation et de chaleur. De plus, la profondeur des destructions tissulaires peut être augmentée pendant le processus d’assèchement. L’escarre est donc recouverte, en prévention d’agents antibactériens, deux fois par jour après l’avoir désinfectée (technique semi-ouverte). Ces crèmes antibiotiques permettent de prévenir les pertes de chaleur et d’eau, de protéger la plaie ou l’escarre, de prévenir les contaminations bactériennes, ainsi que d’humidifier et de faciliter le départ des débris et d’autres tissus nécrotiques. La contraction de la plaie n’a pas lieu tant que l’escarre est présente. Celle-ci se retire en général au bout de 4 semaines. Le lit sous-jacent peut alors être greffé ou va se contracter (encadré 4).

Un fin voile éventuellement imbibé de solution antiseptique peut être mis comme une couverture sur le dos du cheval pour protéger des zones sensibles qui ne peuvent pas être mises sous bande (dos, flancs, croupe). Cela permet d’éviter le contact direct avec des nuisibles (mouches) et des poussières extérieures [14, 21]. Lorsque les escarres d’aspect cartonneux ne semblent pas infectées, il est parfois utile de les conserver dans les zones difficiles à protéger chez les chevaux (technique ouverte). Cependant, il convient de les surveiller attentivement et de les retirer au moindre signe d’infection [17].

Traitements novateurs

En faisant le parallèle avec la médecine humaine, il est possible d’envisager les greffes de cellules souches, la thérapie laser ou la thérapie par ondes de choc pour favoriser la cicatrisation. Des greffes réalisées avec de la sous-muqueuse intestinale d’origine porcine, employées en médecine humaine pour stimuler la cicatrisation dans différents organes, ont été utilisées dans le cas du cheval traité par Couëtil [Couëtil L., cas clinique du PUVTH, 1999, communication personnelle].
Conclusion
Une bonne évaluation de la sévérité des brûlures est le premier pas indispensable à une prise en charge qui doit toujours être urgente, car les effets locaux et systémiques sont nombreux et parfois difficiles à déterminer.

Lors d’une prise en charge tardive, les répercussions complexes et retardées de ce processus inflammatoire grave (surinfections, fourbure, œdème pulmonaire, insuffisance rénale, dénutrition, cicatrisation délabrante) peuvent être extrêmement sévères. Néanmoins, un cheval brûlé ne devrait pas être condamné trop rapidement, à moins que les moyens financiers des propriétaires ne soient limités, car le rétablissement demande de longs et nombreux soins.

**RÉSUMÉ/SUMMARY**

Les brûlures étendues nécessitent une prise en charge rapide et adaptée. Sur le plan systémique une fluidothérapie adéquate doit être mise en place, palliant la déshydratation, l’hyperprotéinémie, l’anémie, les désordres électrolytiques et le choc. Il convient de porter une attention particulière à la douleur, aux surinfections et aux organes tels que les poumons, les reins et le foie. Du fait de l’hypermétabolisme engendré par les brûlures, la nutrition d’un grand brûlé doit être adaptée et suivie attentivement.

Les soins des plaies de brûlures représentent également un réel défi. En fonction de leur profondeur, une combinaison de chirurgie et de traitement conservateur à l’aide de topiques et de pansements est nécessaire.

Mots clés : brûlures, cheval, urgence, traitement local, traitement systémique.

**BI_RESUME_TITRE_ANGLAIS**

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**Keywords:** BI_RESUME_Keywords
Viral testing of 18 consecutive cases of equine serum hepatitis: A prospective study (2014-2018)


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Background: Three flaviviruses (equine pegivirus [EPgV]; Theiler’s disease–associated virus [TDAV]; non-primate hepacivirus [NPHV]) and equine parvovirus (EqPV-H) are present in equine blood products; the TDAV, NPHV, and EqPV-H have been suggested as potential causes of serum hepatitis.

Objective: To determine the prevalence of these viruses in horses with equine serum hepatitis.

Animals: Eighteen horses diagnosed with serum hepatitis, enrolled from US referral hospitals.

Methods: In the prospective case study, liver, serum, or both samples were tested for EPgV, TDAV, NPHV, and EqPV-H by PCR.

Abbreviations: ACVIM, American College of Veterinary Internal Medicine; EqPV-H, equine parvovirus-hepatitis; EPgV, equine pegivirus; NPHV, non-primate hepacivirus; TAT, tetanus antitoxin; TDAV, Theiler’s disease–associated virus.
1 | INTRODUCTION

Equine serum hepatitis, also known as Theiler's disease or idiopathic acute hepatitis, is a serious and often life-threatening disease of adult horses that was 1st described in 1918 in South Africa by Sir Arnold Theiler. The 1st cases of serum hepatitis in horses reported in the United States occurred during the pandemic of western equine encephalomyelitis in the 1930s. The incidence of fulminant hepatitis among horses receiving antiserum in these outbreaks was between 1.4% and 18%. Serum hepatitis has since been described in horses worldwide after treatment with a variety of equine serum products, including tetanus antitoxin (TAT), botulinum antitoxin, Streptococcus equi antiserum, pregnant mare’s serum, and equine plasma. Of these equine biologic products, the disease has been most commonly associated with TAT, possibly because this is the most frequently administered equine blood origin biologic product. The incubation period for clinical disease usually ranges between 4 and 10 weeks after administration of an equine origin biologic, although it can be as long as 14 weeks. In the majority of cases, the association of equine serum hepatitis with the parenteral injection of antiserum or plasma suggests an infectious blood-borne cause, and the history, incubation period, and histopathologic findings appear most similar to serum hepatitis (hepatitis B) in human beings.

Recently, 3 novel flaviviruses were identified in horses, of which 2, non-primate hepaviruses (NPHV) and Theiler’s disease–associated virus (TDAV) were proposed to be associated with liver disease. NPHV, also called equine hepavirivirus, is a member of the genus Hepacivirus, and its hepatotropism and production of hepatic disease after experimental and natural infection in horses is well documented. TDAV is a member of the genus Pegivirus and was identified during an outbreak of acute clinical hepatitis in horses, 6 weeks after prophylactic administration of botulinum antitoxin of equine origin. TDAV's close relative, equine pegivirus (EPgV), commonly infects horses in the United States, Western Europe, and China, although this virus is not hepatotropic and has not been associated with hepatic disease. NPHV and EPgV are frequently detected in commercial horse food products and has not been detected in commercial horse feeds or nontarget animal species. More recently, a novel equine parvovirus (equine parvovirus-hepatitis, EqPV-H) was discovered in the liver and serum of a horse that died of Theiler’s disease. EqPV-H nucleic acids were also found in the TAT administered to this horse 9 weeks before onset of hepatitis. Experimental administration of EqPV-H–positive TAT samples to 2 horses resulted in EqPV-H viremia 6.4 weeks later, followed by marked biochemical evidence of liver disease in both horses and clinical disease in one of the horses.

After the discovery of these viruses, a prospective study on field cases of Theiler’s disease involving American College of Veterinary Internal Medicine (ACVIM) Diplomates was initiated to investigate the association of these viral infections with naturally occurring cases of serum hepatitis. This report details case information and virus testing of 18 consecutive cases of serum hepatitis.

2 | MATERIALS AND METHODS

2.1 | Prospective clinical case study

In collaboration with North American academic and private referral equine hospitals, we initiated a prospective clinical case study to assess the possible role of the newly identified viruses in the etiology of acute serum hepatitis via a letter sent to ACVIM Large Animal Diplomates at teaching and large referral hospitals. Case definition included (1) acute onset of clinical signs of hepatic failure with laboratory or liver histopathologic findings characteristic of serum hepatitis (Theiler’s disease) and (2) a history of receiving an equine biologic product 4-14 weeks earlier. For each case, a diagnosis of Theiler’s disease or serum hepatitis was made at the referral practice before submitting samples to the New York State Animal Health Diagnostic Center for viral testing. Cases were enrolled between January 2014 and February 2018, and all submitted (consecutive) cases were included in the study.

2.2 | Sample collection for prospective study

Serum samples collected from horses in the prospective clinical case study were shipped from the clinic or referred equine hospital to the New York State Animal Health Diagnostic Center. If you have trouble accessing this document because of a disability, please contact PVM Web Communications at vetwebteam@purdue.edu.
Abbreviations: EPgV, equine pegivirus; EqPV-H, equine parvovirus-hepatitis; NPHV, non-primate hepacivirus; TDAV, Theiler’s disease-associated virus; qRT-PCR, real-time PCR.

2.3 | Polymerase chain reaction

Viral nucleic acids were extracted from serum or liver with QiaGen Viral RNA Mini kit (catalog no. 52906) according to the manufacturer’s instructions. No DNase treatment was applied. All PCR mixtures used the Path ID multiplex RT-qPCR kit (catalog no. 4442137; Thermo Fisher Sci, Waltham, MA, USA) and 4 μL of extracted nucleic acids in a 25 μL reaction volume. The primers are listed in Table 1. Two primer pairs were used for EPgV; a positive result in either pair was considered positive. Primers were used at 0.4 μM concentration and probes at 0.12 μM. All PCR reactions were run on the ABI Step-One-Plus Real-Time System and analyzed with StepOne software (Thermo Fisher Sci, Waltham, MA, USA). Real-time PCR conditions included an initial incubation at 48°C for 10 minutes, then 95°C for 10 minutes followed by 40 cycles at 95°C for 15 seconds and 60°C for 60 seconds. PCR methods were validated using the AAVLD accreditation guidelines (data not shown).

2.4 | Statistics

Descriptive statistics of demographic data are provided. Continuous variables are reported as median and range because of non-Gaussian distribution of some variables, as assessed by the examination of skewness, kurtosis, and Q-Q plots.

3 | RESULTS

3.1 | Signalment and biologic product history

Eighteen cases of serum hepatitis were enrolled between December 2014 and February 2018. Demographic data and virologic testing are summarized in Table 2, and greater individual case details are included in Supporting Information Supplemental Table 1. Multiple breeds of horses were affected. There were 6 mares, 1 stallion, and 11 geldings. The ages ranged from 2 to 18 (median 12) years. Of the 18 cases, 12 horses received commercial TAT 4-13 weeks (median 8 weeks) before acute onset of signs of liver failure. The antitoxin (same vial or lot number) was available for testing in 11 cases. In 4 cases, the TAT lot number was narrowed to 2 possibilities and both were tested (Supporting Information Supplemental Table 1). In 2 of these cases, PCR results were the same for both lots. In the other 2 cases, there was a discrepancy between the 2 lots in either NPHV (1 case) or EqPV-H (1 case) status. Therefore, we are confident that EqPV-H-positive TAT was administered to at least 10 of the 12 cases. Of the remaining 6 horses, 3 had received allogenic stem cells as a treatment for soft tissue orthopedic injuries 6.4, 6.7, and 7.6 weeks earlier, and 3 horses received equine plasma 6, 6.4, and 8.6 weeks earlier as colloid treatment, after abdominal surgery in 1 horse and for diarrhea in 2 other horses. Stem cell inoculum (frozen) was available for virus testing from 1 case (case 17) only, and the inoculum was EqPV-H positive. Sera from the donor horses of the remaining 2 cases were tested 20 weeks after inoculation (case 8) and 15 weeks before inoculation (case 12), and both donor horses were PCR negative for EqPV-H. Samples from the commercial plasma given to the other 3 horses were not available for virus (PCR) testing. This commercial plasma was from 2 separate vendors, although 2 cases (cases 13 and 14) received plasma with an identical
TABLE 2  Demographic data and virologic testing results for 18 cases with equine biologic-product associated serum hepatitis

<table>
<thead>
<tr>
<th>Biologic administered (number of horses)</th>
<th>TAT (12)</th>
<th>Plasma (3)</th>
<th>Allogenic stem cells (3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y) (median [range])</td>
<td>11 (2-17)</td>
<td>15 (12-16)</td>
<td>13 (9-18)</td>
</tr>
<tr>
<td>Breed</td>
<td>AQH, 6;  WB, 2; others, 4</td>
<td>WB, 2; UNK, 1</td>
<td>AOH, TB, WB</td>
</tr>
<tr>
<td>Sex</td>
<td>Mare, 6; Stallion, 1; Gelding, 3</td>
<td>Gelding, 3</td>
<td>Gelding, 3</td>
</tr>
<tr>
<td>Incubation period (wk) (median [range])</td>
<td>8 (4-13)</td>
<td>7 (6-8)</td>
<td>6 (5-8)</td>
</tr>
<tr>
<td>Survival</td>
<td>4/12</td>
<td>2/3</td>
<td>0/3</td>
</tr>
<tr>
<td>Serum qRT-PCR*</td>
<td>EqPV-H 9/9; NPHV 2/9; TDAV 0/9</td>
<td>EPGV 2/9</td>
<td>EqPV-H 6/6; NPHV 0/6; TDAV 0/6; EPGV 0/6</td>
</tr>
<tr>
<td>Liver qRT-PCR*</td>
<td>EqPV-H 9/9</td>
<td>NA</td>
<td>1/1</td>
</tr>
</tbody>
</table>

Virology testing (in rows indicated by *) is shown as the number of positive samples out of the number of samples tested. Biologic products tested were mainly aliquots of the same lot administered to the actual cases. Four horses had the TAT lot narrowed to 1 of 2 lots; 2 sets had identical virology results and are included in this table; 2 sets had discrepant results reported in Supporting Information Supplemental Table 1 and are not included in this table. Abbreviations: AOH, American Quarter Horse; EPGV, equine pegivirus; EqPV-H, equine parvovirus-hepatitis; NA, not available; NPHV, non-primate hepatitis; qRT-PCR, real-time PCR; TAT, tetanus antitoxin; TB, Thoroughbred; TDAV, Theiler's disease-associated virus; WB, Warm-blood; UNK, unknown.

3.2 | Virology

All 18 horses were positive for EqPV-H infection (Table 2). Serum and liver samples were available for testing in 6 of the 18 cases; serum only was available in 8 cases and liver only in 4 cases; all these samples were positive for EqPV-H. Of the 14 serum samples, 5 were also positive for EPGV, 2 were positive for NPHV, but none of the 14 samples were positive for TDAV. All 10 liver samples were only positive for EqPV-H. Twelve of the 18 horses died (n = 6) or were euthanized (n = 6) because of the severity of liver failure. One of the horses (case 7) that survived acute fulminant hepatitis was tested 13 months later and still had detectable EqPV-H viremia without any biochemical evidence of hepatic disease.

3.3 | In-contact horses

The newborn foal of case 4 received the same TAT as her dam at foaling. Serum from the foal was not available for virology, and was therefore less likely the source of liver damage. If you have trouble accessing this document because of a disability, please contact PVM Web Communications at vetwebteam@purdue.edu.

3.4 | Clinical data

Although not a primary aim of the study, information on clinical signs, biochemical findings, and necropsy findings was available for many of the cases and are reported here for clinical interest. The clinical findings in horses that necessitated the initial veterinary examination were reported to be acute onset of neurologic signs in 12 of 16 cases where the initial clinical findings were available. Ten of the 12 horses were reported as having predominantly cerebral signs, including blindness, head pressing, and obtundation, whereas 2 horses had severe ataxia that preceded the cortical signs. Other initial clinical findings noted in the case records that were provided included icterus (n = 9), discolored urine (n = 5), colic signs with gastric reflux (n = 2), and recumbency in 1 horse that was severely hypoglycemic. Pyrexia was only reported in 2 cases. In 7 cases, the owners reported decreased appetite and dullness for 1-2 days before the onset of neurologic signs. Serum biochemistry findings at referral admission are summarized in Table 3. Median percentage direct to total bilirubin was 17% (13%-27%, n = 7). Glucose values were reported for 9 horses. Two values were moderately low (60 and 52 mg/dL), and 2 values were severely low (<20 mg/dL) in recumbent horses. Duration of clinical signs before death or euthanasia (median 3 days, range 1-7 days) was available in all 12 horses that died. Information regarding time to clinical improvement after hospitalization was available for 4 of 5 surviving horses, and number of days after hospitalization to clinical improvement was 3, 3, 4, and 7 days. One horse (case 16) was being treated for a chronic respiratory disease and had a complete blood chemistry panel (which was normal) 8 days before onset of liver failure. This was the only horse in the study receiving medical treatment for another condition at the time of onset of liver failure.

Gross findings of the liver were noted on 7 of the necropsy reports, and in all but 1, the liver was reported to be small (normal >1.5% of body weight) and friable. A reticular pattern was noted in 3 cases. Reports on the microscopic findings in the liver were available in 15 cases that had either biopsy (4 cases) or necropsy reports (11) submitted; 3 horses that survived did not have liver biopsies performed. Histopathologic examination of liver could be visualized on 100-fold higher than the dam) when tested at 8 weeks of age; the foal was clinically normal; however, no biochemical analysis was performed. After case 5 recovered and returned to the farm, an in-contact horse developed clinical and biochemical findings of liver failure 6 weeks later. This horse was also sent to the university (Missouri) referral hospital, diagnosed with acute hepatic failure, successfully treated, and returned to the farm 1 week later. The serum of this in-contact horse also tested positive for EqPV-H but no biologic product had been administered to this horse, suggesting the possibility of horse-to-horse transmission (from case 5) as has sporadically been observed in serum hepatitis outbreaks. In case 6, the field veterinarian reported that 2 horses had been inoculated with the same lot of TAT after castration and both developed signs of liver failure, although only 1 of the horses (case 6) was referred for hospitalization and included in our study. Case 6 died, although the other horse recovered on the farm and a blood sample from that horse was positive for EqPV-H.
ultrasonographic examination. Microscopic findings reported in affected horses consistently included acute centrilobular to massive necrosis, collapse of the lobular architecture, and replacement with cellular debris and sometimes hemorrhage. Lesser affected periportal hepatocytes were often described as degenerate, swollen, and containing cytoplasmic vacuoles. In all but 1 case, a mild to moderate lymphocytic/plasmacytic periportal infiltration was noted. Bile stasis and biliary proliferation were noted less commonly. Alzheimer type 2 cells in the brain, consistent with hyperammonemia, were present in 4 of 5 reports that included microscopic examination of the brain. All necropsy and biopsy reports summarized the histologic features as being most suggestive of, presumptive for, or compatible with serum hepatitis.1,8,11,12

4 | DISCUSSION

The 18 cases in our study were (1) clinically and clinicopathologically consistent with previous descriptions of serum hepatitis in horses, (2) all infected with EqPV-H, and (3) rarely infected with the equine flaviviruses that have recently been suspected of causing the disease.13,19 When samples of the biologic product or their same lot number were available for virus testing, EqPV-H was found in the products administered to the horses before onset of hepatitis. Despite the limitation of a lack of controls, the 100% EqPV-H prevalence among these 18 cases compared to the low prevalence of 13% EqPV-H viremia among normal horses30 is highly suggestive that this association is significant. These findings are indicative that EqPV-H can be transmitted by administration of equine biologic products and is the likely cause of equine serum hepatitis.

The clinical and histopathologic findings in these cases, along with the knowledge of administration of an equine origin blood product 4-12 weeks earlier, were considered diagnostic for serum hepatitis.6,9,11,12,15 Therefore, additional testing (eg, heavy metals and other hepatotoxins) was limited. All except 1 horse in our study were in the "typical" 4- to 10-week incubation period for serum hepatitis8,10-12,15 and the longest incubation period was 12.7 weeks. One of 2 adult horses inoculated with TAT containing EqPV-H had clinical signs of liver failure and abnormal liver function test results 12.7 weeks after inoculation, supporting the possibility that some cases of serum hepatitis can result in chronic sequelae. If proven, the virus may represent a potential hazard for veterinary professionals.6

Some potential explanations for differences in incubation time for disease after administration of virus-laden blood products could include (1) different viral loads or specific antibody titers in the biologic products, (2) individual horse differences in the immune responses to infection, (3) partial protective immunity from previous exposure, or (4) concurrent liver injury of another etiology.

Although the overall incidence of clinically recognized serum hepatitis in adult horses receiving TAT is low, TAT has been the most common blood product associated with the disease in the United States for the past 50 years.6,7,9,11,12 Our findings concur with those reports as 12 of the 18 cases with serum hepatitis received TAT. Serum hepatitis could be more commonly associated with TAT administration than with administration of equine plasma because of the more frequent administration of TAT to horses or because TAT is produced as a pooled donor product. The latter could increase the risk of virus contamination of TAT compared to plasma, whole-blood products, and allogenic stem cell inoculations, which are more commonly single donor products. Commercial TAT is usually heat treated (60°C for 1 hour) for the purpose of virus inactivation, and both phenol and thimerosal are added as preservatives. If effective in sterilizing the product, such treatments could leave detectable viral nucleic acids in TAT that are no longer infectious. However, although this form of heat treatment of blood products is known to inactivate heat-labile viruses such as lentiviruses,32 the paraviruses (and especially animal paroviruses) are resistant to both heat inactivation and solvent detergent treatments.32-35 Indeed, EqPV-H can be successfully transmitted using heat-treated, commercially available TAT.30 In contrast to EqPV-H, it appears that transmission of the flaviviruses NPHV and EPGV might have been effectively reduced or eliminated by heat and chemical treatment of equine TAT used for the cases in the present study. This is supported by the fact that among 9 cases that had serum and the administered lot number of TAT tested, TAT was positive for EPGV in all 9 lot inocula and NPHV was positive in 6 horses, but only 2 horses were positive for EPGV and 1 positive for NPHV in serum samples. Those flavivirus-positive cases might have been infected either by receiving contaminated TAT that was not properly heat-inactivated or by exposure to these viruses via another source before antitoxin administration. Because the virus prevalence rate of both NPHV and EPGV in the adult horse population is approximately 15%, the reported incidence of serum hepatitis in this study is not unexpected.
The association of equine plasma administration and serum hepatitis is also well documented. The time between plasma administration and development of hepatic failure in the 3 cases in our study is typical of previous plasma-associated cases of serum hepatitis. The 2 plasma products (2 horses received the same lot number) administered to these horses were not available for virus testing; therefore, the spread of infection by commercial plasma in these 3 plasma-related cases remains presumed.

An association between the allogenic stem cell treatment and serum hepatitis, as occurred in 3 horses in our study, has not been previously reported. The incubation period between the stem cell inoculation and the onset of disease in these 3 horses was typical for serum hepatitis. In only 1 horse (case 17) was the stem cell inoculum available for testing, and although this sample was EqPV-H positive, transmission via this method remains supposition. If the stem cell inoculation was responsible for EqPV-H transmission, the contamination might have occurred from either EqPV-H infection of the stem cells themselves or carryover of donor serum used to culture the stem cells. Viral testing results of stem cell donor horses, albeit more than 14 weeks distant from the inoculation of cases 8 and 12, did not support transmission of EqPV-H by this route. Although the incubation time in these 2 cases was typical for serum hepatitis, EqPV-H infection might have occurred by another method.

The virologic testing in our study clearly links EqPV-H, but not the flaviviruses, with serum hepatitis. We found no evidence that infection or coinfection with the other known hepatotropic virus, NPHV, was associated with clinical disease. Although an original study by 2 of the current authors (T.J.D., B.C.T.) and others found an association between TDAV and plasma-associated hepatitis in a group of horses, the prospective study described here could not find TDAV in any of these field cases. Importantly, retrospective analysis of the commercial plasma botulinum antitoxin and experimental pony infection samples from the 2011 outbreak of Theiler’s disease in which TDAV was discovered showed that EqPV-H was also present in the antitoxin, in diseased horses on the farm, and in the 4 experimental horses inoculated with the same plasma antitoxin lot. Parvovirus was likely not detected in the original investigation because sequencing in that study focused on RNA viruses with proximity to hepatitis C virus, and so a DNAse treatment was performed on the RNA pellet before sequencing. Although TDAV and EPGV nucleic acids have been found in commercial plasma and serum products, pegivirus are neither believed to be hepatotropic nor have they been documented to cause liver disease in any mammalian species. Our findings also suggest that they are rarely transmitted via TAT administration.

Epidemiologic data regarding Theiler’s disease and virologic testing for EqPV-H are both consistent with the theory that subclinical or silent infection is likely common. This is supported by the low incidence of clinical disease after inoculation with the same biologic product. In addition, subclinical disease has been documented in multiple horses in 2 studies of biologic-associated serum hepatitis outbreaks. Similarly, 2 horses experimentally inoculated with EqPV-H developed only mild clinical or subclinical disease. Finally, horses inoculated with EqPV-H do not develop clinical disease. Taken together, these findings suggest that many horses infected with EqPV-H could have a short period of subclinical disease followed by complete recovery. Why some horses develop severe and often fatal disease after EqPV-H infection and others do not is unknown. Hepatic cell damage related to the high level of viremia and direct cytopathic effects is one possibility. Alternatively, injury might result as an indirect consequence of the immune response directed against the virus or injured hepatocytes, as occurs with hepatitis B virus in people. The lymphocytic infiltration seen in many of these cases and in the experimentally EqPV-H–infected horses could be consistent with an immune-mediated mechanism of liver damage.

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CONFLICT OF INTEREST DECLARATION

Melissa Laverack, Randall Renshaw, and Edward Dubovi are employees of the New York Animal Health Diagnostic Center where equine hepatitis panel PCR testing is offered as fee-for-service. These authors were instrumental in viral testing development, validation, and performance but did not contribute to the specific analysis of results. Joy E. Tomlinson received speaker honoraria for presenting parts of this data at the 2018 ACVIM Forum, Seattle, Washington.

OFF-LABEL ANTIMICROBIAL DESTRUCTION

Authors declare no off-label use of antimicrobials.

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC) OR OTHER APPROVAL DECLARATION

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HUMAN ETHICS APPROVAL DECLARATION

Authors declare human ethics approval was not needed for this study.

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SUPPORTING INFORMATION

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

**Clinical Commentary**

**Use of transabdominal ultrasonography in the acute abdomen: Has it really revolutionised our colic work-ups?**

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Colic is one of the most economically important diseases affecting the horse industry and one of the most common reasons why horses present to a veterinarian as an emergency. It is the leading cause of death in equids between the ages of 1 and 20 years (National Animal Health Monitoring System (NAHMS) 2017). The equine practitioner is familiar with the routine colic work-up, which generally at minimum includes a physical exam, rectal exam and nasogastric intubation. While the majority of horses with signs of colic will respond to medical management, it is extremely important for the primary veterinarian to determine whether the horse can be treated medically, or if a recommendation for referral to a surgical facility should be made.

The decision for surgery is primarily based on the degree of abdominal pain, its ability to be controlled by medication and the results of examination and diagnostic tests. While uncontrollable pain alone can be reason enough to elect for exploratory laparotomy, if a horse’s comfort can be maintained then gastrointestinal specific tests should be performed and interpreted in an attempt to formulate an appropriate treatment plan. Does the horse have distended small intestine or significant gas distention of the large intestine on rectal exam? Does the horse have excessive gastric fluid? Does abdominocentesis yield fluid with abnormal parameters? The equine surgeon may be presented with a clear-cut case for surgery but it is not always that simple. The patient may be too small to safely undergo a rectal exam or the rectal exam may be unremarkable; no gastric reflux may be present; an abdominocentesis may not yield any fluid to evaluate or could be within normal reference ranges. When presented with a scenario such as this, the surgeon must look to ancillary diagnostic tests to determine the best course of action for the patient.

One of the earliest articles reporting the use of ultrasound in equine internal medicine was by Byars and Halley (1986). Their list of gastrointestinal diseases that could be evaluated included mostly those of the liver and spleen, but also included peritoneal effusions, ileus, and bowel displacements and distention, however specific conditions were not described in detail.

Klohnen et al. (1996) first reported on the evaluation of the use of transabdominal ultrasound in the equine colic patient. They determined that it was accurate for detecting small intestinal abnormalities and allowed quicker surgical intervention when other diagnostics were unremarkable. These authors did however state that abdominal ultrasound cannot identify the primary small-intestinal lesion. Although not the aim of the study, the authors did observe that horses having primary large-intestinal lesions did have large colon walls that were subjectively more distended and hyperechoic as compared with clinically normal horses; however, at that time they stated that other than for nephrosplenic entrapments, abdominal ultrasonography was non-diagnostic for primary large colon lesions.

Since then, the quality of the ultrasound machine has drastically improved and clinicians have become more experienced. Numerous articles have since been written on the subject, including review articles describing the complete abdominal ultrasound procedure to date (Jeune and Whitcomb 2014), the development of the fast localised abdominal sonography of horses (FLASH) protocol (Busoni et al. 2006), articles covering specific ultrasonographic findings relating to specific diseases (Bernard et al. 1989; Santschi et al. 1993; Taintor et al. 2004; Abutarbush 2006; Buchanan et al. 2006; Grenager and Durham 2011; Ness et al. 2012; Nielsen et al. 2016; Manso-Diaz et al. 2018), and articles analysing the accuracy of ultrasonography in the colic patient (Beccatii et al. 2011; Naylor 2015; Cribb and Arroyo 2018).

Jeune and Whitcomb (2014) presented a nice review of how to perform a thorough abdominal ultrasound examination in the acute colic patient and provided very high quality images of common gastrointestinal abnormalities that could be present. Busoni et al. (2006) described the FLASH protocol. The goal of this protocol is to quickly evaluate a colic patient by evaluating seven different topographical locations. The mean time to complete the evaluation was 10.7 min and clinicians could successfully complete the protocol even without extensive ultrasound experience. The FLASH protocol was able to detect free abdominal fluid and abnormal intestinal loops. When dilated turgid small intestinal loops were found using this protocol, it had positive and negative predictive values of requirement for surgery at 88.89% and 81.48% respectively. Fairburn (2017) reviewed the literature to determine whether a fast scan (the FLASH protocol) has comparable sensitivity and specificity to a detailed exam for finding small and large intestinal lesions requiring surgery. It was concluded that the FLASH examination was indeed comparable, although some limitations in data precluded the ability to fully assess large intestinal conditions. The one concern was whether the FLASH technique would miss some cases of right dorsal displacement of the large colon due to the viewing window being more dorsal than the location of the vessels, which are often near the costochondral junction of the right body wall.

Very specific ultrasonographic findings and how they relate to a definitive diagnosis have been described throughout the literature including left dorsal displacement of the large colon (Santschi et al. 1993), small intestinal...
intussusception (Bernard *et al.* 1989), ceco-colic intussusception (Taintor *et al.* 2004), pyloric-duodenal intussusception (Buchanan *et al.* 2006), large colon displacement (Gnegner and Durham 2011; Ness *et al.* 2012), large colon volvulus (Abutarbush 2006), ascarid burden (Nielsen *et al.* 2016), pedunculated lipomas (Manso-Diaz *et al.* 2018), and described in this issue, the visualisation of a small intestine adenomatous polyp causing intestinal obstruction (Younkin *et al.* 2018). Due to the depth of the adult equine abdomen there are obviously some limitations to the true completeness of the exam. Some of these specific findings are reliably assessed (such as large colon wall thickness and ability to visualise the left kidney). Encountering a specific finding such as an intussusception or the exact cause of a strangulation or luminal obstruction (i.e. lipoma or polyp), while exciting and can obviously support a decision for surgery (or euthanasia), in my experience, is usually rare.

The ultimate question, of course, is whether or not an ultrasound examination can truly help the clinician make a sound decision regarding the need for surgery. As an equine surgeon I can say yes, there have been times that my standard colic work-up is relatively unremarkable (e.g. occasional mild discomfort, no significant findings on rectal exam, no net reflux obtained, bloodwork within reference interval, no fluid yielded on abdominocentesis) and then an abnormal ultrasound examination (e.g. a single loop of thickened, partially distended, amolitile small intestine) is what led me to recommend surgery for the patient, which had an early strangulating lesion that was correctable. A review of the literature finds studies that have attempted to answer this question scientifically. Beccati *et al.* (2011) performed a retrospective analysis of numerous colic cases that received ultrasound exams prior to surgery. They concluded that ultrasonography can help to distinguish between small and large intestinal lesions: finding small intestinal loops that are completely distended (round shape) and lacking motility is highly related to a definitive diagnosis of strangulating obstruction, while finding partially distended small intestinal loops (square shape) with reduced or normal contractility is related to a large intestinal lesion. Increased peritoneal free fluid, reduced duodenal motility, and a completely distended appearance of the small intestinal loop with absent motility was significantly associated with a small intestinal strangulating lesion. Failure to visualise the left kidney was significantly associated with renosplenic entrapment of the large colon. A thickened appearance (>5 mm) and absent motility of the large intestine were found to be significantly associated with a strangulating volvulus of the large colon while absent motility of the large colon and failure to visualise small intestine was shown to be significantly associated with a non-strangulating volvulus of the large colon. Naylor (2015) and Cilibb and Arroyo (2018) each reviewed the literature to report the accuracy of ultrasonography for predicting various gastrointestinal diseases by analysing statistics, including sensitivity, specificity, positive predictive values, and negative predictive values. Their findings were very similar to the retrospective analysis of Beccati *et al.* (2011).

To conclude, I wholeheartedly believe that transabdominal ultrasound has completely changed the colic work-up over the last few decades. It has made it more complete, allows for quicker recognition of small intestinal distention and wall thickening, can find a pocket of peritoneal fluid for successful abdominocentesis, and can assess motility of small and large intestine. This information can help expedite the decision for surgical intervention for the benefit of the patient. There are still cases of surgical colic that I do not believe require an ultrasound examination, especially if the horse is so violently painful that it would be dangerous. I also believe that the ‘routine field colic’ does not generally require an ultrasound examination, unless there are findings that need further evaluation, in which case it is no longer ‘routine’. In regards to finding a specific diagnosis via ultrasound, I think that it is very interesting and extremely helpful to find a surgical lesion pre-operatively, however other than leading to faster progression to euthanasia in horses without a surgical option, it will not likely change the clinical course in those that do. This does not mean that a clinician should not be on the lookout for such a finding, especially as the literature has continued to be expanded by them, however during the emergency ultrasound evaluation I think that the main focus should be on evaluating the horse for surgical need. If a definitive diagnosis can be quickly found it will help direct the clinical course but I do not think a prolonged amount of time should be spent trying to locate one of these specific findings if the horse has a surgical option and the colic work-up and/or general ultrasound findings are directing towards that recommendation. I commend the authors of this accompanying case report (Younkin *et al.* 2018) for discovering a rare lesion ultrasonographically and reporting it for the benefit of expanding the literature.

**Author’s declaration of interests**

No conflicts of interest have been declared.

**Ethical animal research**

Not applicable.

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