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## Appendix A (Blue)

- **Equine Health Update** - Equine Sports Medicine Center Newsletter  
  Vol. 18, Issue No. 1 – 2016

- **Equine Health Update** - Equine Sports Medicine Center Newsletter  
  Vol. 18, Issue No. 2 – 2016

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


- Main RP, Lescun T, Chee Kin Lim, Abigail Durkes. “Assessing Fracture Susceptibility in Horse Limb Bones: A Pre-Clinical Study.”
Appendix C (Green) ~ Research Projects Completed Supported with Pari-Mutual Funds


• Taylor SD, Bianco AW, Moore GE. “Anti-endotoxin Properties of Ketorolac Tromethamine in Horses.”

Appendix D (Purple) ~ Fellowship Supported with Pari-Mutual Funds

• Anthony N. Corsten – MS project: “Evaluation of several pre-clinical tools for identifying characteristics associated with limb bone fracture in thoroughbred racehorses.” Faculty advisor: Professor Russell Main

Appendix E (Tan) ~ Refereed Scientific Articles


Appendix F (Gray) ~ Refereed Scientific Publications


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. 11 client-owned horses were evaluated on the treadmill in 2016. This brings the total number of horses evaluated since the opening of the ESMC in April 1996 to 475. 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 10 treadmill demonstrations were given to groups or dignitaries who visited Purdue’s campus.

Continuing Education and Extension Service:
• Continuing Education presentations:
  • Adams S.B.
    Regional and State
    • Common orthopedic injuries in racing Thoroughbreds. Indiana Thoroughbred Owners and Breeders Association (ITOBA) ownership seminar. Indiana Grand, Shelbyville, IN, July 2016.
  • Buchheit T.
    Regional and State
    • Biosecurity. Purdue Horseman’s Forum, West Lafayette, IN, February 2016.
  • Couëtil L.
    International
    • Effect of a nebulized equine serum product on airway inflammation in horses. The Veterinary Comparative Respiratory Society Symposium, East Lansing, MI, October 2016.
    • Diagnosis of equine asthma with bronchodilator challenge: practical implications. The European College of Equine Internal Medicine Congress, Helsinki, Finland, November 2016.

National
• Aeroallergens involved in RAO pathogenesis. The American College of Veterinary Internal Medicine Forum, Denver, CO. June 2016.
• Diagnostic and medical management of Equine Asthma. The American College of Veterinary Internal Medicine Forum, Denver, CO. June 2016.

Regional and State
• Complementary and alternative medicine. Purdue Horseman’s Forum, West Lafayette, IN, February 2016.
• Boiler Vet Camp. The horse athlete: Treadmill demonstration. Purdue Veterinary Medicine, West Lafayette, IN, June 2016.
• Davern A.
  **Regional and State**
  - Horse Jeopardy – Let’s have fun. *Purdue Horseman’s Forum*, West Lafayette, IN, February 2016.

• Farr A.
  **Regional and State**
  - Horse Jeopardy – Let’s have fun. *Purdue Horseman’s Forum*, West Lafayette, IN, February 2016.

• Gillespie C.
  **Regional and State**

• Hawkins J.
  **Regional and State**

• Hooser S.
  **Regional and State**

• Ivester K.
  **National**

• Lescun T.
  **National**
  - Equine orthopedic surgery, where are we? *American Pre-Veterinary Medical Association Symposium*, Purdue College of Veterinary Medicine, West Lafayette, IN, March 2016.

**Regional and State**
- The Lameness Evaluation. *Indiana Quarter Horse Association continuing education seminar*. Indianapolis, IN, January 2016.

**Local**
- Equine Joint and Nerve Blocks of the Lower Limb. Lecture and Wetlab for the *Student Chapter of the American Association of Equine Practitioners*, Purdue University, West Lafayette, IN, March, 2016.
• Taylor S.
  National
  • Anti-endotoxic properties of ketorolac tromethamine and flunixin meglumine in horses. American College of Veterinary Internal Medicine Forum, Denver, CO, 2016.

• Committee service
  International
  - Townsend W: Research Committee Member, International Equine Ophthalmology Consortium. 2013-present
  National
  - Kritchevsky J: American College of Veterinary Internal Medicine, FAIM Resident Award Committee 2012-2015.
  - Taylor SD. American College of Veterinary Internal Medicine, LAIM Credentials Committee, Chair (2016-17), committee member (2012-2015).
  - Townsend W. Genetics Committee, American College of Veterinary Ophthalmologists, 2012-present

Outreach:

• Purdue’s Equine Web site dedicated to informing horse owners about equine-related activities at Purdue University has undergone a major update. The address of the site is: http://www.vet.purdue.edu/horses/

• Outreach activities
  o Purdue Horsemans Forum, Purdue University College of Veterinary Medicine West Lafayette, IN, February 6th, 2016.
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 2016 (summer and winter) and articles are accessible from our website. The newsletters are included in Appendix A (Blue).

- Buchheit T:

- Lescun T:

- Taylor SD:
  - “Equine Protozoal Myeloencephalitis: To Test or Not to Test, That is the Question.” **Equine Health Update** for Horse Owners and Veterinarians, Vol. 18, Issue No.1, July 2016.
  - “Equine Protozoal Myeloencephalitis: To Test or Not to Test, That is the Question!” **Equine Health Update** for Horse Owners and Veterinarians, Vol. 18, Issue No.2, December 2016 (Editor).

**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).


- Main RP, Lescun T, Chee Kin Lim, Abigail Durkes. “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).


- Taylor SD, Bianco AW, Moore GE. “Anti-endotoxin Properties of Ketorolac Tromethamine in Horses.”
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 race horses 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. This new program was offered for the first time in 2016 and successfully granted to a MA student in the department of Basic Medical Sciences:

Anthony N. Corsten – MS project: “Evaluation of several pre-clinical tools for identifying characteristics associated with limb bone fracture in thoroughbred racehorses.” Appendix D (Purple) – Faculty advisor: Professor Russell Main

Externally funded equine research projects conducted in 2016:


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

Refereed Scientific Articles:


**Abstracts and Proceedings**


Lescun T. “Predicting Fracture Risk in Thoroughbred Racehorses Using a Logistic Regression Model.” *Phi Zeta Research Day,* Purdue University, West Lafayette, IN, 2016 (Poster presentation).


Book Chapters:

Refereed Scientific Publications: Appendix F (Gray)


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# EQUINE RESEARCH ADVISORY BOARD

## Membership

07/2016-07/2017

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Don’t Sweat It: Equine Anhidrosis

By Katie Smith, DVM Student (Class of 2016)
Edited by Dr. Teresa Buchheit, DVM, MS, Dipl. ACVIM,
Purdue Equine Community Practice

Anhidrosis is the term used to describe a condition that results in the inability to sweat effectively in response to heat or exercise. It is most commonly noticed during periods of hot, humid weather or in horses that were recently moved to a hotter environment. Heart rate, respiratory rate and body temperature are used to measure a horse’s physical fitness and ability to recover from exercise. Horses with appropriately functioning sweating mechanisms are able to return to a baseline temperature within 30 minutes of the completion of exercise.

Sweating allows cooling to occur via evaporation and humid environments often don’t allow for the appropriate amount of air exchange to occur in order to efficiently cool a horse. By design, the horse is considered to be at somewhat of a disadvantage when it comes to cooling down. Body temperature is maintained by two mechanisms—sweating and secretions from the respiratory tract. The ability to sweat is responsible for the majority of the cooling process with respiratory secretions accounting for approximately 15% of cooling. If we think of the skin as the major area for heat to dissipate, the whole body (organs, muscle, etc.) of the horse must be cooled down by the surface area of the skin. Horses have a very large body volume in comparison to the area of their skin, meaning that a lot of heat needs to leave the body from a very small area. In addition to patchy and inadequate sweating, affected horses can exhibit any number of clinical signs including exercise intolerance, increased respiratory rate during or after exercise, enlarged blood vessels in the skin and hyperthermia after exercise. Horses that are chronically affected can have a thinner, dry hair coat especially over the face, neck, shoulders, and cannon bones.

(continued on pg. 5)
News & Notes

Centaur Equine Specialty Hospital Takes Shape as Construction Proceeds and Specialist is Hired

Construction is underway with exterior walls now giving shape to the new Centaur Equine Specialty Hospital near Indiana Grand Racing and Casino in Shelbyville, Ind., as the Purdue University College of Veterinary Medicine hires a life-long equine enthusiast and board certified equine surgeon to lead the veterinary medical team that will treat equine patients. Dr. Timm Gudehus will start officially in October as clinical assistant professor of equine surgery. The facility is scheduled to be completed by the end of the year.

As a satellite facility of the College of Veterinary Medicine, the new hospital will provide specialty medical and surgical services for horse owners while also supporting equine research and education of future equine specialists. Its location is just a few miles from the track at Indiana Grand Racing & Casino, and within an hour’s drive from Hoosier Park in Anderson, Ind.

Dr. Gudehus comes to Indiana from Germany where he has served as an equine surgery specialist since 2012. His love of horses and equestrian sports dates back to his early childhood, growing up in a family with a long history of horse riding and breeding. His interest in riding show jumpers turned semi-professional as he finished high school and went on to veterinary school in Munich. After earning the German equivalent of the DVM degree, and completing an internship in Munich, he came to the U.S. for an internship in equine orthopedics in California, followed by a residency in equine surgery at the Louisiana State University School of Veterinary Medicine.

“This additional training in the U.S. exposed me to all the equine disciplines that I hadn’t seen until that point, especially Thoroughbreds, racing Quarter Horses and a little bit of Western performance,” said Dr. Gudehus. “That was followed by a two-year stint as a staff surgeon in Auckland, New Zealand, which added the very last discipline that I hadn’t worked on, which was Standardbreds.”

Dr. Gudehus returned to Germany with his wife, an American citizen and small animal veterinarian, to become the leading surgeon of one of the largest and fastest growing hospitals in Europe, where he worked on Olympic level warmblooded horses. “That adds up to 13 years as a veterinarian, almost ten in my surgical training, and six years as a boarded surgeon, on three continents and back, in every discipline,” he said.

The walls for the Centaur Equine Specialty Hospital are going up in Shelbyville as construction progresses.
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The walls for the Centaur Equine Specialty Hospital are going up in Shelbyville as construction progresses.

Architect’s rendering of the Centaur Equine Specialty Hospital.

Now he looks forward to returning to the U.S. to take-on the challenge of opening the new Centaur Equine Specialty Hospital. “I am excited about the fact that pretty much all these equine disciplines are gathered around the new facility in Shelbyville. I really hope that people will look at this and say ‘cool, here’s somebody who otherwise we would have to fly in,’ to do exactly what I will be providing at this facility,” Dr. Gudehus explained. “I also am really excited to work on racehorses again...my heart beats with the speed horses.”

Dean of the College of Veterinary Medicine Willie Reed said Dr. Gudehus’ experience and expertise, and the state-of-the-art facility, will be great resources for the Indiana equine industry. “I couldn’t be more pleased with the way in which the dream of a world-class equine specialty hospital in proximity to our state’s two racetracks is becoming a reality,” Dean Reed said.

In addition to recruiting Dr. Gudehus, the College of Veterinary Medicine also has hired two equine veterinary technologists who are training in the Purdue Large Animal Hospital in West Lafayette, before moving to the facility in Shelbyville when it opens. The Centaur Equine Specialty Hospital will offer advanced diagnostic imaging, shockwave therapy, nuclear medicine, regenerative medicine, endoscopic laser surgery and specialized equine orthopedic and soft tissue surgery.

Site preparation for the facility began last fall, at the time of the official groundbreaking for the hospital. Actual construction started this spring. The $8.8 million, 18,000 square foot structure is being built on land purchased by the Purdue Research Foundation with $2.3 million in support from Shelby County and the City of Shelbyville. Centaur Gaming, which owns and operates Indiana Grand Racing & Casino and Hoosier Park, pledged $3.1 million to name the facility.
Ask your Vet about Ascarids!
By Stacy H. Tinkler, DVM, MPH, Dipl. ACVIM, Purdue Large Animal Internal Medicine

Parasite control in foals, weanlings and yearlings is different than for the adult horse, and targeted-selective deworming strategies (fecal testing and treatment only of the higher parasite egg shedders) do not apply to young horses. Foals need to be treated more frequently during the first year of life. This is because younger animals are more susceptible to parasitic disease due to an immature and developing immune system and therefore have a need for better protection during this time. A delicate balance needs to be reached between some worm exposure in the gut (necessary for development of the horse’s natural immunity) and prevention of severe parasite infestation leading to illness, poor growth, and possibly death.

What are ascarids?
Ascarids or round worms, are the main parasitic pathogen affecting foals and weanlings, and the most important foal parasite causing poor growth and ill-thrift. Symptoms of ascarid infection in horses include lethargy, inappetence, decreased weight gain, low blood protein, cough and nasal discharge. Most horses become immune to this parasite in the first year of life, and ascarid infection is rarely seen in horses over 2 years of age with well-developed immunity, although this may change in the face of increased parasite resistance to dewormers.

Since 2002, ascarid resistance has been observed in young horses in many countries and has developed in response to an inappropriate and overabundant use of certain dewormers, particularly with ivermectin and moxidectin. Historically, dewormers have been heavily used on breeding farms where many foals receive ivermectin at less than 1 month of age for suspected Strongyloides infection, and they are then often dewormed monthly over the first year, which is far more than is necessary and can actually prevent them from developing a natural immunity to ascarids!

When should I first deworm my foal and with what?
According to recommendations from the American Association of Equine Practitioners (AAEP), during their first year, foals should be dewormed approximately 4 times, and the timing of and type of dewormers used at first deworming are considered of high importance. They recommend first deworming no sooner than 2-3 months of age with a benzimidazole drug (i.e. Panacur, Safeguard, Anthelcide) to ensure efficacy. It is best not to use ivermectin or moxidectin in young foals as there is a significant level of ascarid resistance to these dewormers, they are less effective and at times even dangerous to foals. This is because different classes of dewormers kill parasites differently. Ivermectin and moxidectin, along with pyrantel, (Strongid) kill parasites by paralyzing the adult worms and if a large number are present in the gut of a foal or weanling when the dewormer is given the paralyzed worms can plug up the small intestine causing an impaction, colic and even possible gut rupture.

Most cases of ascarid impactions in foals (70-80%) have been associated with recent deworming with these drugs, and the majority of cases occur in foals 5-8 months of age, coinciding with weaning. Benzimidazoles are not typically associated with ascarid impaction because the worm “kill” mechanism is that it starves the worms and is therefore slower. If you find that you have an older foal that has not yet been dewormed, or looks “wormy” to you (rough hair coat, poor weight gain, abdominal distention) talk to your veterinarian about what to do BEFORE you deworm it. A recent ultrasound scoring system has also been assessed for trans-abdominal monitoring of ascarid worm numbers in foals that may help identify at-risk foals prior to deworming.

How do you treat an ascarid impaction?
It is better to prevent ascarid impactions as they often require surgery, but in some rare cases they resolve with medical management (fluids, pain medication, anti-inflammatories, oral administration of mineral oil) to try and help the foal pass the impaction. The prognosis for ascarid impactions depends on the treatment method and how much gut inflammation and dysfunction the worms have caused. Some surgical success rates have been reported between 50-80%; however, post-surgical long-term survival ranges from 9-60% indicating the seriousness of an ascarid impaction.
Ask your Vet about Ascarsids!
By Stacy H. Tinkler, DVM, MPH, Dipl. ACVIM, Purdue Large Animal Internal Medicine

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Since 2002, ascariate resistance has been observed in young horses in many countries and has developed in response to an inappropriate and overabundant use of certain dewormers, particularly with ivermectin and moxidectin. Historically, dewormers have been heavily used on breeding farms where many foals receive ivermectin at less than 1 month of age for suspected Strongyloides infection, and they are then often dewormed monthly over the first year, which is far more than is necessary and can actually prevent them from developing a natural immunity to ascarids!

When should I first deworm my foal and with what?
According to recommendations from the American Association of Equine Practitioners (AAEP), during their first year, foals should be dewormed approximately 4 times, and the timing and type of dewormers used at first deworming are considered of high importance. They recommend first deworming no sooner than 2-3 months of age with a benzimidazole drug (i.e. Panacur, Safeguard, Anthelcide) to ensure efficacy. It is best not to use ivermectin or moxidectin in young foals as there is a significant level of ascariasis resistance to these dewormers, they are less effective and at times even dangerous to foals. This is because the adult classes of dewormers kill parasites differently, ivermectin and moxidectin, along with pyrantel, (Strongid) kill parasites by paralyzing the adult worms and if a large number are present in the gut of a foal or weanling when the dewormer is given the paralyzed worms can plug up the small intestine causing an impaction, colic and even possible gut rupture.

Most cases of ascarid impactions in foals (70-80%) have been associated with recent deworming with these drugs, and the majority of cases occur in foals 5-8 months of age, coinciding with weaning. Benzimidazoles are not typically associated with ascariasis impaction because the worm “kill” mechanism is that it staves off the worms and is therefore slower. If you find that you have an older foal that has not yet been dewormed, or looks “worry” to you (rough hair coat, poor weight gain, abdominal distention) talk to your veterinarian about what to do BEFORE you deworm with an adulticide as it has been shown that deworming can cause gut impactions. A recent ultrasound scoring system has also been assessed for trans-abdominal monitoring of ascarid worm numbers in foals that may help identify at-risk foals prior to deworming.

How do you treat an ascariasis impaction?
It is better to prevent ascariasis impactions as they often require surgery, but in some rare cases they resolve with medical management (fluids, pain medication, anti-inflammatory, coal administration of mineral oil) to try and help the foal pass the impaction. The prognosis for ascariasis impactions depends on the treatment method and how much gut inflammation and dysfunction the worms have caused. Some surgical success rates have been reported between 50-80%; however, post-surgical long-term survival ranges from 9-60% indicating the seriousness of an ascariasis impaction.

References:

Anhidrosis (continued from cover)
There are several theories as to what causes anhidrosis, but the origin remains unknown. Recent research suggests that the condition more likely stems from the sweat glands not responding to the stimulation to secrete sweat rather than the inability of the body to realize that it should be sweating. One type of test for anhidrosis involves several injections of a drug under the skin which typically causes sweating within five minutes. If a horse is not anhidrotic, sweat will appear at most (if not all) of the injection sites. Horses that are slightly anhidrotic may have sweat appear at a few of the sites, whereas those truly affected will not sweat at all. An alternative test involves hanging your horse for thirty minutes on a hot day. Baseline measurements of heart rate, temperature and respiration are measured prior to hanging to the trot for 30 minutes during hot temperatures. The same measurements are taken post-exercise and the horse is monitored for its ability to sweat during exercise and ability to recover from the work. If the horse does not return to its baseline respiratory rate thirty minutes after exercise, or has delayed recovery of rectal temperature, it indicates that they are having trouble cooling down.

The ultimate solution for anhidrotic horses is moving to a cooler, less humid environment. Many anhidrotic horses once again achieve adequate sweat production upon removal to a cooler climate. If the horse cannot be physically removed to a cooler climate, there are several ways to manage an anhidrotic horse and lessen the risk of heat stress and heat stroke. Once you realize your horse is not sweating appropriately, stop working, immediately move to a cooler location, and decrease your horse’s body temperature via the use of fans, air conditioners, misters and/or cold hosing. Electrolyte supplementation can aid in the recovery from an anhidrotic episode and can be administered under the guidance of your veterinarian. A medication is available (clerbenzol) which will cause a horse to sweat and may be beneficial if used sparingly. Clerbenzol is saved for situations when the horse is in a particularly hot environment and is only a temporary treatment. If it is used too often, it may actually worsen the anhidrosis.

Other management strategies include exploring the possibility of concurrent respiratory disease as affected horses are sometimes found to have inflammatory airway disease. Ensuring that your horse is physically fit prior to warmer weather can also help to control anhidrosis. Avoid heavy training and competition during summer months. For horses maintained in exercise training during the summer, workouts should be conducted during cooler periods of the day. When working with your veterinarian, try to avoid scheduling procedures that require sedation in hot weather as these drugs typically cause a horse to sweat and the inability to do so may result in extremely high body temperatures.

Be sure you’re aware about this condition, the mainstay of treatment is environmental and exercise management. If you suspect that your horse is not sweating appropriately while working or during periods of hot weather please call your veterinarian to discuss the possibilities of anhidrosis and work together to induce management changes to keep your four-legged friend cool, calm, and collected this summer.
Cattle Food is for Cattle, Not Horses!

By Megan Brunn, DVM Student (Class of 2017)
Edited by Stacy H. Tinkler, DVM, MPH, Dipl. ACVIM, Purdue Large Animal Internal Medicine

It is extremely important that horses never eat grain intended for cattle or other food-animal species. Perhaps you’ve heard this before, but didn’t understand why this is so. This article will help clarify the reasons why your horse should only be fed grain or concentrate specifically formulated for horses and should never receive foodstuffs or medicated mineral blocks intended for animals such as cows, goats, sheep, llamas, alpacas, pigs, or chickens.

Different species have different nutritional requirements for growth and maintenance of good health. Each type of feed is specialized for that species to provide adequate nutrition for ONLY that species. So if you feed horse grain to a sheep or vice versa, the animal will receive a feed that is deficient in some nutrients and in excess or even toxic amounts of others. For example, copper requirements vary a great deal between species. Horses require more copper than sheep or camelids, so if a sheep, llama, or alpaca is fed horse feed, over time the copper in their system will accumulate and can increase to a toxic level. The excess copper will then extensively damage the liver and red blood cells and too much can be fatal for the ruminant or camelid.

In addition to different nutrient requirements, feeds intended for food animals or camelids may contain substances that are toxic to horses, even in extremely small amounts. An example of a harmful feed additive is the ionophore antibiotic monensin (Rumensin®), a common additive in ruminant and poultry feeds. Monensin acts to prevent overgrowth of coccidia parasites (i.e., it is a coccidiostat) and is also used as a growth promotant in the species that can tolerate it. Monensin is extremely toxic to horses. It causes severe muscle damage, including the heart muscle, which can lead to heart failure and death. Just a handful of cattle feed containing monensin or one of the other ionophore antibiotics can be fatal to a full-sized horse. If a horse doesn’t die immediately from acute monensin toxicosis, it can suffer from chronic heart problems for the rest of its life. This will severely affect performance and can sometimes result in the horse’s death months to years after ingestion of the toxic feed. Since such a small amount of monensin can be fatal to horses, extreme care must be taken if it is present on a property where horses are housed. Whenever horses and ruminants are at the same location, monensin-containing grain needs to be clearly labeled and separated from horse grain. Additionally, ruminant feed should be kept locked up so that horses cannot accidentally ingest it in the event they get loose and raid the feed room. A grain container that has held medicated ruminant feeds needs to be cleaned out completely before adding horse feed to it. Even the traces of monensin that remain on the walls of uncleaned feed containers have resulted in toxicity when horse grain was later added to these contaminated containers.

Other compounds that are sometimes added to food-animal grains such as antibiotics or the other coccidiostats lasalocid and decoquinate are not as toxic as monensin but can cause problems if given to horses.

Knowledge of the problems that occur when horses ingest food intended for ruminants, poultry, swine, or camelids is the first step towards preventing accidental toxicoses in your animals. If you are ever unsure of what feed to give your animal, stick with hay or forage until you have had a chance to talk to a veterinarian or animal nutritionist for advice on what feed is appropriate.

References:


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Phenytoin, or "bute" is probably the most common pain killer given to horses, but remains one of the most misunderstood drugs in the equine medicine cabinet. Here’s what you need to know when your veterinarian prescribes phenytoin to your horse.

What is it?

Phenytoin belongs to the class of drugs called non-steroidal anti-inflammatory agents, or NSAIDs for short. Other NSAIDs labeled as "Bute" in horses include Banamine and Equinex.

The most famous NSAID is aspirin, and all other NSAIDs work in a similar manner. NSAIDs work by stopping the production of prostaglandins, which are important substances made by the body when there is inflammation, fever, or pain.

Dosing

Imagine if you had a broken bone and your doctor told you to take an aspirin. You would probably think "You have got to be kidding, this isn’t going to help at all." What is more, taking an entire bottle of aspirin wouldn’t take that type of severe pain away either. Phenyltoin is exactly the same, it can relieve a certain degree of pain, but there is a ceiling for how much relief it can provide. Giving more "bute" than the recommended amount just increases the chances of side effects. Additionally, combining "bute" and Banamine or another NSAID (a practice known as "stacking") is not better than giving a higher amount of either drug.

To summarize, it is important to give the appropriate dose of phenyltoin and no more. There are two reasons for this. Most importantly, giving an overdose can quickly lead to toxicity problems. The second is that it won’t provide any more pain relief than the appropriate dose.

Toxicity

Phenytoin is toxic if given at too high a dose. Just like the aspirin some people take for their hearts, however, a horse can have the appropriate amount of "bute" for months or years with no ill-effects. It is important to give no more phenyltoin than is recommended on the label. The label for the 20% injectable solution states "1 to 2 grams of phenyltoin per 100 lb body weight" while the paste formulations are labeled for "1 to 2 grams of phenyltoin per 500 lb body weight, but not to exceed 4 grams daily." If your horse weighs 1,000 pounds, which is the average for an adult light horse, following these instructions is easy. But accurate dosing becomes difficult as the body weight of the patient decreases. The label instructions can be difficult to scale down when dealing with a 250 pound miniature horse or a 100 pound foal. Unless you are giving "bute" to a healthy, adult horse, it is much better to dose in milligrams (mg) of drug per kilogram (kg) of body weight. Phenyltoin can be dosed safely at a 4.4 mg/kg twice a day for the first day and then 2.2 mg/kg twice a day for four days, and then 2.2 mg/kg once a day from then on. A 250 pound miniature horse weights 113 kg, and thus should receive no more than half of a gram on the first day of treatment then down to one quarter of a gram after that. The 100 pound foal should get no more than 99 mg, which is one tenth of a gram. As you can imagine, it’s almost impossible not to overdose smaller horses or foals when trying to give the paste, and it is safest not to try.

Phenyltoin toxicity results in low blood proteins, and mouth, stomach, and intestinal ulceration. Occasionally kidney failure and inflamed colons can occur as well. It is important to note that some horses are more sensitive to the effects of NSAID drugs than others, and may develop ulcers or other issues at lower doses.

What are your alternatives?

The other NSAID drugs on the market have fewer toxic effects than phenyltoin, and may be better tolerated by sensitive horses. Unless one has an accurate scale weight and the means to give it the exact amount needed, "bute" should not be given to foals as they are particularly sensitive to its toxic effects. If a horse is still in a great deal of pain after giving the labeled amount of "bute", one can try other methods instead of or in addition to the NSAID. Call your veterinarian at once if a NSAID does not produce the expected relief, it could mean that the problem is more severe than originally believed.

Bottom Line

Phenytoin is a cost-effective pain killer that can be your first line of defense in horses that are suffering from a wide number of injuries and problems. It is safe to the majority of horses when given at the recommended dosage, even when given for prolonged periods of time; however, phenytoin should never be given at higher doses then are listed on the label in any horse. When treating horses, ponies, and foals less than 500 pounds, the precise mg per kg dose should be calculated and given. If this is not possible, then another form of pain relief should be used.

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/
Equine Protozoal Myeloencephalitis: To test or not to test, that is the question!

By Juli Eaton, DVM Student (Class of 2017)
Edited by Dr. Sandy Taylor, DVM, PhD, Dipl. ACVIM
Purdue Large Animal Internal Medicine

Equine Protozoal Myeloencephalitis, or EPM for short, is primarily caused by the microscopic protozoal organism Sarcocystis neurona. The opossum is the “natural host” for Sarcocystis neurona. They shed sporocysts, or eggs, in their feces on pastures, hay, or occasionally get into feed bins. Horses are a “dead end” host for Sarcocystis neurona meaning they cannot spread it to other animals, but can be clinically affected by the organism themselves. Infection begins with accidental ingestion of sporocyst-containing feces. The sporocysts mature and further develop in the gut, travel to the blood, and then reach the central nervous system. Sarcocystis neurona most commonly sets up shop in the spinal cord where it causes inflammation, resulting in neurologic deficits such as asymmetrical muscle atrophy and ataxia (incoordination). Unfortunately, opossums are very common in the Midwest and Eastern US; keeping them out of barns, out of feed, and eliminating brush close to pasture fence-lines is imperative for EPM prevention.

The most concerning aspect of EPM is the possible permanence of neurologic deficits, even with adequate treatment. Over the last 30 years, researchers have developed tests that enable veterinarians to more accurately diagnose EPM and developed treatments which effectively kill the organism. With new tests and treatments come increased awareness of EPM by veterinarians and horse owners alike. Members of the horse community are asking about screening tests, the most popular being a blood test called an ELISA. This test detects antibodies (“titers”) that the horse’s immune system has made against Sarcocystis neurona. However, antibodies only will tell you if a horse has been exposed to a disease, not if it is actively infected. In fact, EPM usually occurs sporadically, seldom involving more than one horse on a farm. Several studies show that 89% of all horses in the Midwest will test positive on a blood test. This does NOT mean 89% of horses actually have an active infection of Sarcocystis neurona.

(continued on pg. 4)
Over the past year, workers have transformed a farm field in Shelbyville, Ind., into a state-of-the-art facility for advanced medical treatment of equine athletes. A satellite facility of the Purdue University College of Veterinary Medicine, the Centaur Equine Specialty Hospital is set to open early in 2017, with the mission of maximizing the performance of all horses used for sport, competition, or pleasure by preventing, diagnosing, and treating diseases or conditions that keep equine athletes from achieving their full potential.

The $8.8 million structure encompasses 17,000 square feet, and is located just a few miles from the track at Indiana Grand Racing and Casino in Shelbyville, and within an hour’s drive from Hoosier Park in Anderson, Ind. The Centaur Equine Specialty Hospital will offer advanced diagnostic imaging, shockwave therapy, nuclear medicine, regenerative medicine, endoscopic laser surgery and specialized equine orthopedic and soft tissue surgery.

“The goal is to be one of the premier performance horse hospitals in the country,” said Dr. Stephen Adams, Purdue Veterinary Medicine professor of large animal surgery, who helped with planning for the new facility. “To achieve that goal, the hospital will include some of the most advanced diagnostic equipment, including an Equine 4DDI diagnostic imaging system.”

The 4DDI machine contains two robotic arms, allowing a horse to walk in between for more efficient processing. “There are only two other places in the United States that currently offer the 4DDI machine and we will be the third,” said Dr. Mimi Arighi, associate professor of large animal surgery, who is a member of the College’s Department of Veterinary Administration and serves as the lead faculty member on the facility planning committee. The unit can perform all types of diagnostic techniques, including x-rays, fluoroscopy, CT and tomosynthesis. “The big difference with the 4DDI machine is that a horse can stand during the procedure,” explained Dr. Arighi. “With all other systems, a horse has to be under anesthesia for procedures like CT, which is always a risky thing to deal with when treating a horse.”

The new hospital also will offer nuclear imaging, which works with radioactive iodine to pinpoint where the equine patient’s problem might lie and where x-rays should be taken. The system uses a Gamma Camera, just like the kind used for humans, and is capable of a total body bone scan.

“With all other systems, a horse has to be under anesthesia for procedures like CT, which is always a risky thing to deal with when treating a horse.”

The main entrance to the Centaur Equine Specialty Hospital takes shape as construction work nears completion.

“The Centaur Equine Specialty Hospital will accommodate all aspects of equine surgery necessary to optimize the performance of sport horses,” said Timm Gudehus, the hospital’s senior veterinary surgeon. Dr. Gudehus started in October in order to help with preparations for the opening of the facility and to get acquainted with area horse owners and veterinarians in all three breeds of the racing industry as well as other disciplines of horses in the region. “We will treat respiratory, orthopedic and every aspect of fracture repair,” he said.

Other features of the facility include a canopied entrance that leads into two holding stalls for outpatient work. The lobby has hospitality areas for guests and a private consultation room. A separate barn that is connected by a walkway holds six regular stalls and also features two larger mare and foal stalls and one isolation stall. The isolation stall only has access from the outside and is an essential feature for preventing the spread of infection to other animals. A round pen behind the hospital will be utilized for orthopedic and respiratory diagnoses. In addition, a long hallway constructed on the side of the facility will serve as the “lameness hallway”—an area sheltered from the weather where a horse will be able to step up its gait.

“We are committed to several core values that will characterize the treatment provided,” said Dr. Adams. “Those values include excellence in patient care; education of horse owners, trainers, caregivers, veterinarians and veterinary students to optimize the health of horses; improvement of the health and wellbeing of equine athletes through clinical research to advance diagnostics and therapeutics; to act ethically with all clients and exhibit the integrity clients expect and deserve; and to deliver value to each client by providing efficient service and individual care of each patient. The health and welfare of the horse is the highest priority.”

The hospital site was purchased by the Purdue Research Foundation with $2.3 million in support from Shelby County and the City of Shelbyville. Centaur Gaming, which owns and operates Indiana Grand Racing and Casino and Hoosier Park, pledged $3.1 million to name the facility. An official Grand Opening for the new hospital is planned in the spring, at the start of the 2017 racing season.
The Purdue University College of Veterinary Medicine has recruited a team of specialists who will work with horse owners and trainers to provide the most current diagnostic tests and the most effective therapeutics at the Centaur Equine Specialty Hospital. The team is led by Dr. Timm Gudehus, senior veterinary surgeon, who earned his Dr. med. vet. degree from Technische Universität München in Munich, Germany before completing a master’s degree and residency program at Louisiana State University’s School of Veterinary Medicine. With a love of horses and equestrian sports that dates back to his early childhood, Dr. Gudehus has obtained training and experience on three continents and in every equine discipline. He is board certified by the American College of Veterinary Surgeons and the European College of Veterinary Surgeons, and is accredited as an equine surgery specialist by the Bavarian Equine Board Committee in Germany.

Dr. Kayla Le will serve as associate veterinary surgeon. She earned her DVM degree at Kansas State University’s College of Veterinary Medicine in 2014 and then completed an equine medical and surgical rotating internship at the Louisiana State University School of Veterinary Medicine and a large animal surgical internship at the Cornell University College of Veterinary Medicine. Growing up in Omaha, Neb., Dr. Le participated in western horseback riding. She also owned horses and was involved in the rodeo.

Additionally, two veterinary technologists have joined the team. Shelby Harber, RVT, lead diagnostic imaging technologist and surgical nurse, and Cheryl Boyd, LVT, chief anesthesia technologist, came on board earlier in the year and have been training in the College’s Large Animal Hospital during the construction of the facility in Shelbyville.

Dr. Gillian Haanen is originally from Weert, the Netherlands. She studied veterinary medicine—Equine track at Utrecht University and graduated in 2013. After graduating she traveled and visited different equine clinics as an extern. She began an equine internship at Sportpaardenkliniek Wolvega in the Netherlands followed by an equine internship at Moore Equine in Calgary, Canada.

Dr. Haanen started at Purdue University as the large animal medicine intern in February of 2016 and officially began her residency in July of 2016. She will be pursuing her dream to become a large animal internal medicine specialist here at Purdue for the next three years. When she is not working in the clinic she likes out-door activities and exploring the United States!

Dr. Lauren Mundy is originally from Woodbine, Maryland where she grew up riding in Pony Club, 3-day events, steeplechase, and Thoroughbred racehorses. She received her undergraduate degree from the University of Maryland in Animal Science and her DVM from The Ohio State University. After veterinary school, Lauren interned at Rood and Riddle Equine Hospital in Lexington, Kentucky and rotated through surgery, internal medicine, and anesthesia. She is very happy to be at Purdue for a large animal surgical residency and looks forward to working with all large animals. Her interests include emergency and critical care, soft tissue surgery, and performance medicine.

Dr. Kira Tyson is originally from Ridgecrest, California. She received her undergraduate degree from Oklahoma State University in Animal Science and her DVM from Purdue University. Kira first became interested in large animal medicine during her fourth year of veterinary school. She stayed at Purdue University to pursue a large animal internal medicine internship. Her interests are ultrasonography, critical care and neonates. She enjoys camping, traveling, and running with her dog, Rylee.
Similar to how humans can come into contact with an individual who has a cold and not get sick, horses that come into contact with *Sarcocystis neurona* through accidental ingestion of sporocytes rarely get sick; their immune system usually fights it off. For this reason, simply performing a blood titer will only tell you if a horse has come in contact with *Sarcocystis neurona*, and most horses in the Midwest have! It is therefore only recommended to test a horse for EPM if they are having neurologic signs; otherwise, interpretation of the test results is nearly impossible. EPM is an important rule-out for any neurologic horse in geographical areas where opossums are present. The best tests to perform are a combination of a blood titer and a cerebrospinal fluid (CSF) titer. The ratio of blood antibodies to CSF antibodies is arguably one of the best tests with a specificity of up to 97%. If there is an active infection in the central nervous system, antibodies in the CSF will increase compared to antibodies in the blood. A ratio of less than 100 strongly supports a diagnosis of EPM as the cause of clinical signs and in which case immediate treatment is warranted.

So, the answer to the question “to test or not to test” is: test if the horse is showing neurologic signs like ataxia or asymmetrical muscle atrophy, and lives in a geographical area where opossums are common (like the Midwest). If testing, always test both CSF and blood to enable evaluation of the blood serum to CSF ratio for the most accurate results.

Register now to attend the Purdue University College of Veterinary Medicine HORSEMAN’S EDUCATION FORUM

Saturday, February 11, 2017 | 9:00 a.m. - 5:00 p.m. at Indiana Grand Racing and Casino in Shelbyville, Ind.

Attendees will enjoy great educational sessions as well as a sneak peek of Purdue Veterinary Medicine’s new Centaur Equine Specialty Hospital!

REGISTER ONLINE NOW!

$45.00 per person

Discount rates available for active duty military, students, and groups of 5 or more.

Topics this year include:

- Equine Asthma
- Respiratory Infection Prevention
- Balanced Diets
- Pleasure and Trail Horses
- Research Updates
- Race and Performance Horses

Many more great lectures plus tours of Indiana Grand’s racetrack and casino, and the new Centaur Equine Specialty Hospital before its grand opening.

References:


**Calling All Horse Owners in the Tippecanoe County and Surrounding Areas**

Equine Community Practice at Purdue University would like to organize a Facebook page or email list of horse owners who would be willing to haul a horse into Purdue University Veterinary Hospital on emergency—for a fee and with a signed hold-harmless waiver, which would be available for download.

Too often, we are called out to see a horse on emergency that needs referral into the hospital, but the client doesn’t have a trailer, or the truck or trailer is broken or gone at the time. We are then scrambling to find someone to haul the horse into the clinic rather than have to euthanize because we can’t get it in for help.

We are soliciting feedback from horse owners in the area as to interest in involvement and how to best get names on the list and circulated when there’s a need.

Please email us at ecp@purdue.edu with your thoughts, comments, and if you are interested in getting on this list.
Osteoarthritis of the Hock and Injectable Treatment Options
By Jessica Abernathy, DVM Student (Class of 2017)
Edited by Dr. Tim Lescun, BVSc, MS, PhD, Dipl. ACVS, Purdue Large Animal Surgery

If you do any kind of performance riding with your horse, you know that hocks (tarsus) are the work-horses of impulsion and movement, and are a common site of osteoarthritis (inflammation of the joint and associated bones). The hock consists of 5 joints—tibiotarsal (tarsocrural), proximal intertarsal, distal intertarsal (centrodistal), tarsometatarsal, and talocalcaneal. The high motion joint of the hock is the tibiotarsal joint, whereas the proximal intertarsal (PIT), distal intertarsal (DIT), and tarsometatarsal (TMT) joints are important for shock absorption. The DIT and TMT joints are the most common sites of the hock for osteoarthritis to occur.

Common causes of osteoarthritis include overuse or general use over time, poor conformation, injury, and developmental abnormalities such as angular limb deformities. Some examples of poor conformation include cow-hocked, base-wide, base-narrow, or bowlegged (bandy legged).

Osteoarthritis (OA, arthritis, degenerative joint disease) occurs with cartilage deterioration in the joint, followed by bone and soft tissue changes in that area. Cartilaginous damage is often the result of damage to the joint and is exacerbated by inflammatory substances the body naturally produces. OA is a painful condition and thus can decrease the performance level of your horse. There are multiple therapies being utilized and it is important to remember that the treatment that helped your friend’s horse may not work in your horse. The treatment recommended by your veterinarian will depend on several factors. This discussion will focus on the pros and cons of joint injections and some of the therapies that can be used to aide in relieving your horse’s hock pain, decrease the disease progression, and improve performance.

Pros
Veterinarians inject joints in order to medicate them, which decreases active inflammation and pain, improves the synovial (joint) environment and protects what remains of the cartilage (chondroprotective). It is important to understand that this is not a performance-enhancer, but rather an option to allow the horse to have a longer performance career.

Cons
Joint flare (aka reactive synovitis) and joint infections are rare but possible and must be distinguished from one another. Joint flares are less serious than joint infections, but still need to be treated. However, joint flares are not typically career-ending since they are not the result of a bacterial infection but rather an inflammatory reaction to the substance injected. Joint infections can be career-ending and life threatening. Joint infections are due to the introduction and growth of bacteria, usually Staphylococcus aureus, within the joint. Signs of infection include pain, heat, swelling, and non-weight-bearing lameness, usually within 3-5 days after injection, although it may be as long as 2 weeks before signs are present. Joint infections must be treated aggressively to limit the damage and eliminate the bacteria.

Joint injections are costly and are usually charged by the joint. The hock is a complex area containing five joints. Of the 5 joints, the lower two joints (tarsometatarsal and distal intertarsal joints) are injected as these are the most common joints in the hock that get osteoarthritis. Some practitioners inject the tarsometatarsal joint only as there is research to support that drugs can diffuse between the two joints. However, there are some veterinarians that will inject both joints to be sure the medication reaches the desired locations. For more information ask your veterinarian.

(continued on pg. 6)
Joint injections may also not give the desired effect. If your horse’s lameness was not properly diagnosed, or comes from disease in several structures around the joint, the intra-articular (within the joint) block may not have worked because the pain may be coming from tissues surrounding the joint rather than within. If the purpose of the injection was to medicate prior to performance then it is possible that the injection was not done at the proper time before competition, or the damage may be severe enough that joint injections are not sufficient to improve function.

Articular cartilage degeneration/steroid chondropathy can occur if high doses of corticosteroids are used and a suitable rest time after injection is not followed (such as 3-5 days) as this can lead to further destruction of the cartilage. Steroids are beneficial, but if multiple joints are injected multiple times in a short time then this becomes a concern and different treatment strategies may be needed. Repeated injections may eventually decrease the quality of the remaining cartilage.

Another risk is causing corticosteroid-induced laminitis. This is painful and life threatening. The laminae in the hoof become inflamed and the horse subsequently gets laminitis. This is very rare and is only likely to occur if there is a pre-existing susceptibility to laminitis such as metabolic conditions or an illness. However, dosing precautions taken by your veterinarian are aimed at minimizing the risk of this happening to your horse.

**Injectable Therapeutic Options for Hock Pain**

**Corticosteroids:** The most commonly used steroids are methylprednisolone acetate (MPA) and triamcinolone acetonide (TA). What your veterinarian uses depends primarily on their clinical experience, as well as scientific research, the use of your horse, and whether or not the joint in question is a high or low motion joint. The goal of any steroid therapy is to decrease and potentially eliminate the inflammation within the affected joint. This reduces pain and decreases the inflammatory cycle, prohibiting further damage. However, steroids degrade naturally occurring hyaluronic acid (HA), which is an important component of cartilage and joint fluid. For this reason, synthetic HA is commonly injected in conjunction with steroids.

**Hyaluronan:** Examples of this cartilage and joint fluid component include Legend®, Hylartin-V®, and Hyvisc®. Hylartin-V® and Hyvisc® are most commonly used for acute disease of high motion joints, chronic cases with radiographic changes, or chronic maintenance cases with joint soreness.

**Polysulphated glycosaminoglycans (PSGAGs):** Adequan® is most commonly given intramuscularly for preventative treatment, maintenance of chronic arthritis, post-operatively in horses starting back into training, or for acute disease in low-motion joints. It can also be administered directly into the joint.

**Interleukin-1 Receptor Antagonist Protein (IRAP):** Blood is drawn from your horse and incubated so that an anti-inflammatory protein is released from the blood cells which is able to block interleukin-1 (a potent inflammatory mediator in joint disease). The serum containing the IRAP is then injected back into your horse’s affected joint. This is minimally invasive, but it is expensive. However, there are no negative effects on the cartilage and no drug withdrawal times for competition. This treatment is usually reserved for horses that are unresponsive to traditional therapies.

**Tildren:** This drug decreases the amount of bone resorption by blocking one type of bone cell, similar to the effect of anti-osteoporosis drugs used in people. The performing horse puts a lot of stress on the joints which causes the bones to remodel in response to the exercise loads. Some joint diseases involve the rapid remodeling of bone and Tildren is aimed at modulating this process and reducing pain. There is still debate about the use of Tildren for hock OA.

**Polyacrylamide hydrogel:** This has been used in human medicine and a 2015 study supports its use in equine lower limb joints, such as the hock. It is non-toxic, longer lasting, and is non-degradable. The mechanism behind its success is unknown but the same 2015 study showed that one injection can alleviate clinical lameness for up to 2 years in the carpal and fetlock joints. This could be a potential use for hock joints, but further studies need to be done to validate its use in any equine joints.

**Summary**

It can be devastating when your horse is diagnosed with hock osteoarthritis and potentially scary thinking about a needle going into your horse’s joints. There are risks with performing joint injections, but fortunately most of them are rare. Precautions taken by your veterinarian should decrease the odds of your horse getting a joint infection or flare, laminitis, or articular cartilage degeneration. There are many treatment options for OA of the hock and it is important to work closely with your veterinarian to find the one that works best for your horse. For more information about OA, its causes, and treatment options, please talk to your veterinarian.

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**References:**

Sand colic, also known as sand enteropathy or sand-induced colitis, occurs when abnormally large amounts of sand accumulates in a horse’s intestines. Sand usually accumulates gradually over time, and eventually leads to irritation and disruption of the motility of the gastrointestinal tract. This can result in mild signs such as diarrhea, dehydration, and weight loss, but can also cause more serious problems such as impaction of the colon, abdominal pain (colic), and peritonitis (inflammation of the tissue that lines the inner abdominal wall and covers most abdominal organs).

Which horses are at risk?

Any horse that has access to sand is at risk of ingesting sand, which can lead to a sand enteropathy. Areas of particular concern are those with sandy soils such as coastal or desert regions, where sand is ingested when it is torn up with grass roots. Horses housed on sand lots or fed in indoor arenas with sand footing are also at risk. Horses fed directly on the ground in paddocks with no or sparse grass are at increased risk of ingesting sand when it picks its feed off the ground.

How is it diagnosed?

As with any disease, a thorough history and physical exam are important for diagnosis. Occasionally sand can be heard on auscultation of the gastrointestinal tract along the lowest aspect of the abdomen, and is reported to sound like “waves on a beach.” Sand can sometimes be felt on rectal palpation as an impaction in the colon, the inner surface of the rectum may feel “gritty,” or sand may be noted in the feces or on the rectal exam sleeve after palpation.

The two most important tests for diagnosing sand colic are fecal sedimentation and abdominal radiographs (x-rays). Fecal sedimentation is a simple test that can be performed on the farm by both veterinarians and owners. This test is performed by placing 3-4 fresh fecal balls into an exam sleeve or clear plastic bag filled with water. The fecal balls should be manually broken apart. If present, sand will settle to the bottom after a few hours. If no sand is collected after approximately 8 hours, the test is considered negative. More than ½ tsp of sand is considered significant. Unfortunately, only 48% of horses that have sand enteropathy will have sand on the fecal sedimentation, so a negative test cannot definitively rule out this disease. In the hospital setting, sand enteropathy is often diagnosed or confirmed with abdominal radiographs. Sand tends to collect ventrally along the abdomen, which can be seen as a radiopaque (bright white) area in the image.

It is important to note that seeing sand on the fecal sedimentation test or on radiographs does not mean sand is the only possible cause of the clinical signs. Though sand may be one contributor to diarrhea or colic, your veterinarian will rule out other potential causes.

How is it treated?

For horses with sand enteropathy, your veterinarian will most commonly treat with a combination of fluids and laxatives. Fluids may be given orally or intravenously depending on the severity of dehydration and other clinical signs. The most commonly used laxatives for sand are psyllium, mineral oil, or magnesium sulfate (Epsom salts). These may be used separately or in combination. Psyllium is a soluble fiber that mixes with the sand in the gut and forms a gel which promotes evacuation. Evacuation can be further enhanced by the addition of mineral oil. Epsom salts overhydrate the gut to help promote excretion of the sand. New studies have shown that mixing psyllium with a probiotic improves sand clearance. The prognosis for medically managed cases is good. In more severe cases, surgical removal of the sand may be required. Horses requiring surgery have about a 60-65% survival rate. If peritonitis has occurred, antibiotics will also be needed.

How can it be prevented?

Removing the horse from sand is the best way to prevent sand enteropathy, but this is not always possible. To reduce the amount of sand consumed: avoid feeding directly off the ground with the use of feeders that are not easily overturned, use rubber mats under feeders in the paddock/stall to prevent sand ingestion with dropped feed, offer grass hay during the day to give your horse something to do, and allow horses to graze only in pastures with adequate growth so that ingestion of sand is less likely. For those horses which live in a sandy environment, psyllium (either as a powder or flavored pellets) can also be used as a preventative. One recommendation is to feed 8 ounces once daily for 7 consecutive days one week per month as a maintenance dose to prevent sand build-up and is best used in combination with other preventative measures.

References:
Purdue University’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/
APPENDIX B

Research Projects in Progress Supported with Pari-Mutual Funds

• **Kritchevsky J, Croney C, Lescun T.** “Pasture Sound: The Effect of Lameness on Behavior and Other Measures of Welfare in Horses.”

• **Lescun TB, Breur G, Nauman E, Chandrasekar S, Adams S, Jones Y, Main R.** “Finite Element Modeling and Implant Nanosurfacing to Enhance Equine Fracture Treatment.”

• **Main RP, Lescun T, Chee Kin Lim, Abigail Durkes.** “Assessing Fracture Susceptibility in Horse Limb Bones: A Pre-Clinical Study.”
APPENDIX C

Research Projects Completed Supported with Pari-Mutual Funds

- **Hawkins J, Freeman L, Li J, Gillespie C.** “Investigation into the Use of a Topical Application of a Hyperosmolar Nanoemulsion to Wounds of the Distal Extremity in Horses.” The final report consists of a manuscript accepted for publication. Please see appendix E under Gillespie for proof of submission.

- **Taylor SD, Bianco AW, Moore GE.** “Anti-endotoxin Properties of Ketorolac Tromethamine in Horses.”
Title: Anti-endotoxin properties of ketorolac tromethamine in horses

Principal Investigator: Sandra D. Taylor

Co-Investigators: Alex W. Bianco, George E. Moore

The project outlined in the ERAB proposal submitted in December of 2014 is complete. We found equivalent eicosanoid suppression between ketorolac tromethamine (Toradol®) and flunixin meglumine (Banamine®) in an ex vivo model of endotoxemia. Results from this project were presented at the American College of Veterinary Internal Medicine (ACVIM) Annual Forum in June of 2016 [ACVIM Abstract #E-31], proceedings from which can be found in the Journal of Veterinary Internal Medicine, 2016;30(4):1507.

In addition, a manuscript was submitted to the Journal of Veterinary Pharmacology and Therapeutics and is currently under revision.

Since this study found equivocal anti-inflammatory properties of ketorolac and flunixin, the next step is to compare the analgesic efficacy of these drugs. A dose-determination study is underway to identify the most effective yet safe dosage of ketorolac, results of which will be utilized in a larger study that will compare the analgesic efficacy of ketorolac with flunixin and phenylbutazone in a reversible model of foot pain in horses. This is a timely area of research given the current shortage of flunixin due to manufacturing problems. Thus, identification of a novel non-steroidal anti-inflammatory drug such as ketorolac might become a critical need in the near future.

Please see the attached ACVIM proceedings and the submitted manuscript.
Anti-Endotoxic Properties of Ketorolac Tromethamine and Flunixin Meglumine in Horses

Alexandra Bianco, George Moore, Sandra Taylor

Purdue University, West Lafayette, IN, USA

Non-steroidal anti-inflammatory drugs (NSAIDs) play an integral role in equine medicine due to their combined analgesic and anti-inflammatory properties. Despite their widespread use, there are limited NSAIDs available that demonstrate variable adverse effects and safety. While several NSAIDs have been proven to be adequate analgesics, few have undergone rigorous evaluation for anti-inflammatory efficacy. Ketorolac tromethamine (KT) is a non-selective cyclooxygenase inhibitor that has been used in human patients since 1989. The pharmacokinetic properties of KT have recently been determined in the horse, and have been previously determined in several other species. However, there have been only two previous studies examining KT’s anti-inflammatory properties in animals, both of which were in vivo. There have been no published studies evaluating the in vitro anti-inflammatory effects of KT in any veterinary species. The purpose of this study was to evaluate the anti-inflammatory effects of KT compared to flunixin meglumine using an in vitro model of LPS-stimulated equine monocytes.

Equine monocytes were isolated from whole blood from a single horse and incubated with either KT or flunixin meglumine at six concentrations ranging from 2.5 µg/mL to 80 µg/mL for 1 hour. After the initial incubation, E. coli 055:B5 LPS was added to each well at a concentration of 1 µg/mL, which has been shown to consistently activate monocytes. The wells were incubated at 37°C and 5% CO2 for 4, 8, 12, and 24 hours. Samples were collected at each time point and stored at −80°C until analysis. Equine-specific ELISAs were used to measure the eicosanoids PGE2 and TXB2 as well as the cytokines TNFα, IL-6, and IL-8.

Preliminary results demonstrated that flunixin meglumine suppressed PGE2 production up to 12 hours at concentrations ≥5 µg/mL and up to 24 hours at concentrations ≥40 µg/mL. Flunixin meglumine suppressed TXB2 production up to 12 hours at all concentrations and up to 24 hours at concentrations ≥20 µg/mL. Ketorolac tromethamine suppressed PGE2 production up to 12 hours at all concentrations and up to 24 hours at concentrations ≥20 µg/mL. Ketorolac tromethamine also suppressed TXB2 production up to 12 hours at all concentrations but did not suppress production up to 24 hours at any concentration. Peak eicosanoid concentration in the non-treated samples occurred at 4 hours for PGE2 and 12 hours for TXB2.

While in vitro results cannot be directly correlated to in vivo efficacy, the results thus far indicate both drugs effectively suppress eicosanoid production after LPS stimulation, with an effect of both time and drug concentration. Based on the results of this study, a therapeutic dose of 2.5 µg/mL KT would effectively suppress eicosanoid production in cases of endotoxemia. Further research is needed to correlate in vitro results with in vivo efficacy.
Submitted Manuscript: J Vet Pharm Therap

Effects of ketorolac tromethamine and flunixin meglumine administration on eicosanoid inhibition and safety in healthy horses

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Running Head: Eicosanoid suppression by NSAIDs in horses

Keywords: Prostaglandin, thromboxane, constitutive, eicosanoid, horse

Abbreviations:

SIRS Systemic inflammatory response syndrome
LPS Lipopolysaccharide
NSAID Non-steroidal anti-inflammatory drug
COX Cyclooxygenase
FM Flunixin meglumine
KT Keterolac tromethamine
UA Urinalysis
FOBT Fecal occult blood test
TXB₂ Thromboxane B₂
PGE$_2$  Prostaglandin E$_2$
ELISA  Enzyme-linked immunosorbent assay
CBC  Complete blood count
SBA  Serum biochemical analysis
HPLC  High performance liquid chromatography
ESI  Electrospray ionization
CE  Collison energy
AUC  Area under the curve
RAO  Recurrent airway obstruction
PPID  Pituitary pars intermedia dysfunction
TXA$_2$  Thromboxane A$_2$
DIC  Disseminated intravascular coagulopathy

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This work was performed at Purdue University in West Lafayette, IN.

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Results were presented, in part, at the 2016 Forum of the American College of Veterinary Internal Medicine in Denver, CO.

The authors thank Dr. Vanessa Cook, Anna Smith and Anisa Dunham for technical assistance.
Abstract

Background: Ketorolac tromethamine (KT) demonstrates superior efficacy compared to other NSAIDs in humans, but its anti-inflammatory effects have not been investigated in the horse. The safety of repeated dosing of KT has not been evaluated.

Hypothesis/Objectives: To test the hypothesis that KT is more effective than flunixin meglumine (FM) in reducing eicosanoid production in an equine ex vivo model of LPS-induced inflammation, and that repeated dosing is safe.

Animals: 9 healthy horses from Purdue University.

Methods: Following a dose determination study, a randomized crossover study was performed. Following NSAID administration, blood was collected for LPS stimulation, and measurement of eicosanoid and drug concentrations. Each NSAID was administered 6 times, and safety was assessed.

Results: KT did not differ from FM in its effect on production of either TXB\textsubscript{2} (P=0.849) or PGE\textsubscript{2} (P=0.330). Both KT and FM reduced endogenous concentrations of TXB\textsubscript{2} and PGE\textsubscript{2} at 4 hours compared to T=0 (P<0.001). No adverse effects were observed.

Conclusions and clinical importance: KT is as effective as FM in reducing constitutive production of eicosanoids for up to 8 hours, and appears safe in healthy horses. Investigation of analgesic effects of KT in horses is warranted.

Keywords: Prostaglandin, thromboxane, constitutive, eicosanoid, horse
Management of inflammation in horses represents an ongoing challenge in equine medicine. Systemic inflammatory response syndrome (SIRS) is defined as wide-spread and exaggerated inflammation that can be triggered by infectious or non-infectious stimuli.\textsuperscript{1-4} Possible complications of SIRS include coagulopathies and organ dysfunction, which can lead to death.\textsuperscript{1,5,6} Non-steroidal 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.\textsuperscript{7-11} Flunixin meglumine (FM) is currently considered the standard-of-care for LPS-induced inflammation in horses, based on efficacy, affordability and safety.\textsuperscript{11,12} The dosing schedule of 1.1 mg/kg IV q12h is often used and is based on anecdotal perception of clinical efficacy,\textsuperscript{8} but critically ill horses often display clinical signs associated with pain and SIRS despite standard-of-care FM therapy.\textsuperscript{13-15} Identification of another NSAID with more effective anti-inflammatory and analgesic properties could potentially decrease illness and death associated with equine SIRS.

Ketorolac tromethamine (KT) is a non-selective COX inhibitor that has been used in humans to provide potent anti-inflammatory and analgesic therapy since the 1980’s.\textsuperscript{16-18} In several animal models, KT has been shown to have anti-inflammatory and analgesic properties that often exceed the efficacy of other NSAIDs.\textsuperscript{17,19-22} Ketorolac tromethamine is often administered as an IV bolus or as a constant rate infusion for morphine-sparing analgesia in post-operative patients.\textsuperscript{23-26} Although the pharmacokinetics of KT have been evaluated in a variety of veterinary species, including horses,\textsuperscript{27-34} there have been few studies evaluating its analgesic or anti-inflammatory efficacy.\textsuperscript{27,33,35,36} A single veterinary study found that KT was equal to FM in
reducing SIRS parameters in an LPS-induced inflammatory model in calves. To date, this dosage has not been evaluated for anti-inflammatory efficacy in veterinary species. Adverse effects of KT are similar to those caused by other non-selective NSAIDs, but overall incidence in post-operative human patients is low. 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. 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, we hypothesized that KT would be more effective than FM in reducing eicosanoid production in an equine ex vivo model of LPS-induced inflammation. Our second hypothesis was that KT would be as safe as FM following repeated dosing, as assessed by evaluating routine blood work, urinalysis (UA) and fecal occult blood tests (FOBT) over a 3-day drug administration period. A final objective of the study was to assess plasma levels of KT and FM to determine plasma drug concentrations.

**Materials and Methods**

First, a dose determination study was done to verify that KT at a dosage of 0.5 mg/kg could effectively suppress eicosanoid production from LPS-stimulated equine monocytes, and to determine optimal time points for measurement of post-NSAID-exposure eicosanoid concentrations.

*Dose Determination Study*
Approximately 1 L of whole blood was collected aseptically from the jugular vein of a healthy horse and placed in a glass bottle containing 100 mL 40 mM EDTA. The horse had not received any NSAID for over 1 month. Monocyte isolation was performed using a sedimentation-gradient centrifugation protocol as previously described. Total monocyte count was determined and cells were suspended in equine media. A cytospin slide was made to verify that the isolated cells were >75% monocytes. Serial drug dilutions were created from KT and FM to create 6 concentrations of each drug: 80, 40, 20, 10, 5, and 2.5 µg/mL. Monocytes were then plated on 12-well tissue culture plate at a concentration of 1x10^6 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 hour, 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 hours. Thus, there were 4 sets of duplicate wells, with 1 set for each time point (8 wells total for each of the 4 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. Samples were analyzed per the manufacturer’s instructions. Based on these findings, determination was made of KT and FM doses to be used in the subsequent ex vivo study, and time for blood collection for eicosanoid measurements and plasma drug concentrations.

Ex Vivo Study

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, complete blood count (CBC), serum biochemical analysis (SBA), UA and FOBT. 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. All procedures in this study were approved by the Institutional Animal Care and Use Committee at Purdue University.

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. Trial 1 consisted of the even-numbered horses receiving KT and the odd-numbered horses receiving FM. Trial 2, a crossover, consisted of the odd-numbered horses receiving KT and the even-numbered horses receiving FM. A 2-week washout period separated the two trials.

**Drug Administration and Sample Collection**

The day prior to drug administration (Day 0), the horses were transported to the hospital, weighed, and a 14-gauge IV catheter was aseptically placed in each jugular vein. The right IV catheter in all horses and for both trials 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.

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. Immediately prior to drug
administration, blood was collected into lithium-heparin tubes for assessment of endogenous eicosanoid concentrations (T=0). Heparinized blood was again collected at T=5 minutes to assess peak plasma concentration of drug (KT or FM) based on the rapid distribution of drugs after IV administration. Heparinized blood was again collected at T=4, 8, and 12 hours for assessment of both eicosanoid and plasma drug concentration.

Drug Concentrations

Within 1 hour of collection, heparinized blood from each time point (T=5 minutes, and 4, 8, and 12 hours) was centrifuged for 2,000 rpm at 4°C for 10 minutes. 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 validation for KT. 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 to 104.9 and a collision energy (CE) of 18 V. For etodolac, ESI negative mode was used with a transition of 286.1 to 212.1 and a CE of 20 V. For FM, ESI positive mode was used with a transition of 296.8 to 278.8 and a CE of 15 V. For KT, quantitation was based on a 6 point standard curve ranging from 2.5 to 5,000 ng/mL. For FM, quantitation was based on an 8 point standard curve ranging from 150 to 100,000 ng/mL. All standard curves were prepared using unmedicated equine plasma.

LPS Stimulation of Whole Blood and Eicosanoid Measurements

Within 1 hour of collection, heparinized whole blood from each horse was divided into 3 aliquots. Lipopolysaccharide (E. coli 055:B5) was added to 2 of the aliquots at a final
concentration of 1 μg/ml and gently mixed; the remaining aliquot served as the negative control. All 3 aliquots were incubated at 37°C in a 5% CO2 atmosphere for 6 hours, after which they were centrifuged at 2000 RPM for 10 minutes. The incubation period of 6 hours was chosen based on our finding that eicosanoid suppression was equivocal at 4 and 8 hours in NSAID-treated equine monocytes, as well as for convenience of sampling times. The plasma was removed and stored at -80°C until analysis. Concentrations of TXB2 and PGE2 at each time point for each horse were measured using the previously described commercial ELISAs.

**Adverse Effects**

Following the initial dose, horses received KT or FM q 12 hours for 3 days (6 doses total) to assess safety of repeated dosing. The horses were weighed each evening and the drug dose was adjusted accordingly. The horses were continually monitored and complete physical examinations were performed at T=0, 4, 8, and 12 hours on days 1 - 4. A CBC (Abbott Cell-Dyn 3500 Hematology Analyzer, Abbott Park, IL, USA) and SBA (Johnson & Johnson Vitros 5,1 FS Chemistry Analyzer, Holliston, MA, USA) were performed at T=0, 24, 48, and 72 hours. 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.

**Statistical Analysis**

Normality of data was assessed with a Shapiro-Wilk test and data was transformed if necessary to achieve a normal distribution. A 2-way ANOVA with repeated measures was used to assess group differences in eicosanoid production by drug, time and drug-time interactions. Pairwise comparisons of groups within day were performed using the Student-Newman-Keuls’
method of adjustment for multiple comparisons. Areas under the concentration-time curve (AUC) were determined by the composite trapezoid rule, and compared by Welch test. Values of \( P<0.05 \) were considered significant and results were reported as mean ± SD.

**Results**

*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 TXB\(_2\) and PGE\(_2\) production for up to 12 hours. Specifically, KT suppressed TXB\(_2\) and PGE\(_2\) production for up to 12 hours at all concentrations. At 24 hours, KT only suppressed PGE\(_2\) production with drug concentrations ≥ 20 µg/mL and did not suppress TXB\(_2\) production at any KT concentration at this time point. Flunixin meglumine demonstrated slightly superior suppression of TXB\(_2\) than PGE\(_2\). TXB\(_2\) suppression was achieved for up to 12 hours at all concentrations and up to 24 hours at concentrations ≥ 20 µg/mL, while PGE\(_2\) suppression required a FM drug concentration ≥ 5 µg/mL. Peak eicosanoid concentration in non-NSAID-treated samples occurred at 4 hours for PGE\(_2\) and 12 hours for TXB\(_2\).

*Ex Vivo Study*

Four mares and 5 geldings (mean age 15 ± 6 years) were included in the study. Six breeds were represented, including 3 Quarter Horses and 1 each of Saddlebred, Thoroughbred, Standardbred, Warmblood and Appaloosa. The mean starting weight of the horses was 521 ± 54 kg; horses were weighed daily during each drug administration trial to ensure accurate dosing. One horse had also been diagnosed with recurrent airway obstruction (RAO) and pars pituitary
intermedia dysfunction (PPID). At the time of the study, the horse was not exhibiting signs of RAO and was not receiving medication for either RAO or PPID. During the course of the trial, 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.

From the *ex vivo* study, the effect of KT and FM on eicosanoid concentrations over time, with or without LPS stimulation, is demonstrated in Figure 1 (TXB<sub>2</sub>) and Figure 2 (PGE<sub>2</sub>). Based on the dosages utilized in this study, KT did not significantly differ from FM in its effect on production of either TXB<sub>2</sub> (P=0.849) or PGE<sub>2</sub> (P=0.330). LPS significantly increased TXB<sub>2</sub> production (P<0.001) but decreased PGE<sub>2</sub> production (P<0.001). Both KT and FM significantly reduced the endogenous concentrations of TXB<sub>2</sub> and PGE<sub>2</sub> at 4 hours compared to T=0 (P<0.001). By 8 and 12 hours post-administration for either drug, eicosanoid levels were significantly lower compared to baseline (T=0) for PGE<sub>2</sub> (P<0.001) but not TXB<sub>2</sub> (P>0.746).

**Plasma Drug Concentrations**

Mean plasma drug concentrations of KT and FM over time are shown in Figure 3. Both drugs followed a similar pattern, with the highest mean plasma concentrations detected at T=5 minutes 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=0.007). At T=5 minutes, the mean plasma concentration of KT was 1,407 ng/mL and decreased to 3.1 ng/mL at T=12 hours. In contrast, the mean plasma concentration of FM at T=5 minutes was 25,386 ng/mL, and decreased to 164 ng/mL at 12 hours.

**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 Trial 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 Trial 1 (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 hours. The horse did not receive any medications and showed no further signs of colic. The horse was returned to pasture before returning for Trial 2.

Discussion

Based on the results of this ex vivo study, a 0.5 mg/kg dosage IV of KT in healthy horses appears to effectively suppress constitutive TXB2 and PGE2 levels for up to 4 and 12 hours, respectively. An ex vivo model was used in this study primarily because it would spare horses from undergoing the physiologic stress of clinical endotoxemia that results from in vivo LPS exposure, and thus, allowing for the use of a larger sample size.42,43 One disadvantage of an ex vivo model, however, is that successful induction of inflammation cannot be confirmed with clinical signs and leukogram changes as would be observed following IV LPS administration.44,45 Therefore, a limitation of this study was the unexpected failure of LPS to induce a significant increase in TXB2 and PGE2 in the ex vivo model prior to the addition of
NSAIDs (T=0). There are several possible explanations for the lack of LPS-induced inflammation in this study. First, it is possible that the dose of LPS was not high enough to stimulate eicosanoid production of monocytes in vitro, although doses of 1 µg/mL or less have been used previously to stimulate inflammation of whole blood.8,46-48 While there may have been a problem with the LPS product itself, insufficient incubation time may also have been responsible for the failure to induce inflammation with LPS. In our in vitro study, it appeared that an incubation time between 4 and 8 hours was sufficient.49 While some studies evaluating in vitro LPS-induced inflammation have used a similar incubation time,8,50,51 other studies using in vitro or ex vivo LPS stimulation have used 24 to 48-hour incubation times.47,48,52

While LPS did not induce eicosanoid production at T=0, the presence of LPS did result in higher concentrations of TXB2 at T=8 and 12 hours compared to the non-LPS groups. Based on the pharmacokinetic data, the lower concentrations of both NSAIDs at T=8 and 12 hours may be below the therapeutic concentration for inhibition of COX-1 expression, resulting in increased TXB2 production. However, this does not explain the lack of difference between the two groups prior to drug administration at T=0. The presence of LPS also resulted in lower concentrations of PGE2 compared to the non-LPS groups all time points except T=8 hours, regardless of NSAID. The difference between groups is slight and likely not clinically significant.

The results of this study clearly demonstrate the ability of both KT and FM to suppress constitutive COX expression, but it is unclear whether this effect would be significant in the face of inflammatory stimuli. Other similar studies have evaluated the efficacy of NSAIDs in horses without the induction of inflammation and demonstrated significant inhibition of PGE2 and/or TXB2.8,53-59 However, one study that utilized equine synovial explants found no effect of NSAIDs on inhibition of PGE2 in the samples that were not incubated with LPS, likely due to the
low constitutive COX expression of synovial tissue. While the efficacy of FM has been demonstrated in both constitutive and inflammatory models, there are only two other papers examining the anti-inflammatory effects of KT in veterinary species. In dogs, constitutive PGE₂ inhibition was persistent for up to 24 hours after a single IV dose. In calves, KT was equal in efficacy to both FM and ketoprofen at eicosanoid inhibition in an in vivo model of LPS infusion.

The eicosanoids evaluated in this study, TXB₂ and PGE₂, were chosen due to their correlation to COX-1 and COX-2 activity, respectively. Thromboxane B₂ is a stable and inactive metabolite of thromboxane A₂ (TXA₂) and is used as a marker of whole body expression of COX-1, while PGE₂ 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 TXA₂ 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). 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. Therefore, in patients with potential hypercoagulability, treatment with COX-1 specific inhibitors (e.g. aspirin) might be indicated. While their method of inhibition differs, nonselective NSAIDs have been shown to be as effective at reducing TXB₂ as aspirin when compared directly.
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. While the aim of this study was to measure induced COX-2 activity via exposure to LPS, the observed inhibition of PGE₂ likely represents a suppression of endogenous COX-2 expression by selected tissues. In horses, constitutive expression of COX-2 has been demonstrated in the glandular mucosa of the stomach,⁶⁶,⁶⁷ mucosa of the urinary bladder,⁶⁷ jejunum,⁶⁸,⁶⁹ and left dorsal colon.⁷⁰ Higher COX-2 expression in these tissues is likely beneficial as PGE₂ promotes local inflammation and cytotoxic immune responses to prevent pathogen entry.⁷¹

Due to the failure to induce inflammation with LPS, we were unable to determine therapeutic plasma drug concentrations. However, the plasma drug concentration of KT required to produce an equivalent degree of TXB₂ and PGE₂ inhibition was significantly lower than the plasma drug concentration of FM, demonstrating the relatively higher potency of KT. The increased potency of KT is thought to be due to its low distribution in adipose tissue.²⁰,⁷² Most NSAID-related adverse effects are thought to be dose-dependent, but are likely correlated to the degree of constitutive COX inhibition rather than absolute plasma concentration of drug. In this study, KT was found to be more potent than FM as there was no significant difference in eicosanoid inhibition despite the difference in plasma drug concentration; however, an increased potency is unlikely to reduce the risk of adverse effect.

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 5 consecutive treatment days.⁴⁰ 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. 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 research animals or in patients undergoing elective surgery. Here, we demonstrated that 3 days of q12 hour dosing of KT was safe in healthy horses. Additional toxicology studies and evaluation of KT in clinically ill patients is necessary to determine relative risk of adverse effect in comparison to other NSAIDs in horses.

In conclusion, KT was as effective as FM in reducing constitutive production of TXB$_2$ and PGE$_2$ in an *ex vivo* equine model. This data, taken together with the evidence that KT provides superior analgesia compared to other NSAIDs in humans, it is reasonable to investigate the analgesic effects of KT compared to FM in horses in a future study. Finally, a KT dosing schedule of 0.5 mg/kg IV q12 hours for 3 days appeared safe in healthy horses.

**Footnotes**

a Ketorolac tromethamine (30 mg/mL), Hospira, Inc., Lake Forest, Illinois, U.S.A.

b Flunixin meglumine (50 mg/mL), VetOne Prevail, Boise, Idaho, U.S.A.

c Horse Thromboxane B$_2$ Elisa Kit, My BioSource, San Diego, CA, U.S.A.

d Horse Prostaglandin E$_2$ Elisa Kit, My BioSource, San Diego, CA, U.S.A.
References


**Figure 1.** The *ex vivo* effect of ketorolac tromethamine (KT) and flunixin meglumine (FM) on TXB2 concentrations over time, with or without lipopolysaccharide (LPS) stimulation. KT with LPS: dashed and dotted line (---); KT without LPS: dotted line (······); FM with LPS: dashed line (- - - -); FM without LPS: solid line (—). T=0 was immediately prior to drug administration.
Figure 2. The *ex vivo* effect of ketorolac tromethamine (KT) and flunixin meglumine (FM) on PGE2 concentrations over time, with or without lipopolysaccharide (LPS) stimulation. KT with LPS: dashed and dotted line (---··); KT without LPS: dotted line (·····); FM with LPS: dashed line (--- - - -); FM without LPS: solid line (▬). \( T=0 \) was immediately prior to drug administration.

Figure 3. Plasma concentrations of ketorolac tromethamine (- -) and flunixin meglumine (●) after intravenous administration (KT: 0.5 mg/kg; FM: 1.1 mg/kg) in 9 horses.
Competitive Equine Research Fellowship Supported with Pari-Mutual Funds:

- Corsten, Anthony – MS project: “Evaluation of Several Pre-clinical Tools for Identifying Characteristics Associated with Limb Bone Fracture in Thoroughbred Racehorses.” Faculty advisor: Professor Russell Main.
EVALUATION OF SEVERAL PRE-CLINICAL TOOLS FOR IDENTIFYING CHARACTERISTICS ASSOCIATED WITH LIMB BONE FRACTURE IN THOROUGHBRED RACEHORSES

Anthony N. Corsten, MS

Catastrophic racehorse injuries on the track or in training result in euthanasia for the animal and large losses for the owners and trainers. Despite the fact that many injuries resulting in lameness can be recuperated from with rehabilitation, the high costs of treatment make it an unlikely option for most athletes not destined for use as breeding stock. According to the Equine Injury Database, in 2015 0.162% of approximately 300,000 racing starts resulted in a catastrophic injury, amounting to 484 fatalities [1], with many more injuries occurring in training. By far, fractures are the most common racing or training related injury among Thoroughbred racehorses [2] and by one estimate fractures comprise as much as 86% of injuries [3]. Because of the low rate of treatment in favor of euthanasia, it is of great importance for the safety of equine athletes to determine factors related to fracture risk to reduce fracture incidence overall.

Most racehorse related injuries are not considered to be a one-time, random event. It is true that these types of incidents do occur, but they are likely in higher impact races, such as hurdles [4]. It is much more likely that most racehorse fractures are instead pathological and are a result of accumulated bone tissue damage, known as stress fractures [5][6][7]. Stress fractures have a number of characteristics that differentiate them from “bad step” fractures. They occur in high-strain, cyclically loaded environments. Racing and training for equine athletes fall into this category [8]. Further evidence is that catastrophic fractures in horses tend to occur at the same location and plane of the bone consistently between horses [6]. Empirical evidence for stress fractures in horses was shown in early studies with strain-gauges [8], and more recently the pathology has been directly identified using a number of modalities, such as MRI [9], nuclear scintigraphy [10] and scanning electron microscopy [11]. It is important from a prevention standpoint that overt fractures are predicated by stress fractures. A random event cannot be predicted except by probability, but stress fractures are a pathology that should have characteristic factors that can be identified prior to injury.

This study examines four pre-clinical modalities to assess their ability to detect factors related to fracture, being 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 the proximal, midshaft and distal lengths of the diaphysis, with the exception of pQCT which was also performed at either metaphysis. I hypothesize that minimally or non-invasive measures of bone architecture, morphology, mechanical properties and biomolecular composition on cadaveric equine MC3’s from racing populations will correlate with fracture risk in the MC3 and other long bones.

RPI was developed to assess mechanical properties of biological tissues in vivo, including bone, as a complement to traditional mechanical tests, and includes the Biodent [12][13] and Osteoprobe instruments [14][15]. Both the Biodent and Osteoprobe make microindentations into a tissue’s surface using a small needle, making them minimally invasive and viable in a clinical setting. The Biodent is a benchtop device that is intended for ex vivo testing of tissues (though prior to the release of the Osteoprobe it was used for in vivo studies as well) while the Osteoprobe is intended for in vivo clinical use. In this summary, the 1st Indentation Distance (1st ID) and Total Indentation Distance (TID) for the Biodent and Bone Material Strength index (BMSi) for the Osteoprobe will be discussed. It is expected that a greater indentation distance (which results in a lower BMSi) is associated with lower resistance to microfractures.

Traditionally, fracture risk in humans is most commonly assessed by dual-energy x-ray absorptiometry (DEXA) scanning for bone mineral density (BMD) for the diagnosis of diseases like osteoporosis or Paget’s disease [16][17][18][19]. However, it is limited in that it is unable to distinguish between cortical and trabecular contributions to total BMD, and is not able to obtain any volumetric measurements. pQCT is a modern, low-dosage alternative to DEXA. pQCT takes 3D scans of bones and as such can measure parameters related to BMD as well as bone geometry, and can also separate between cortical and trabecular tissues for BMD calculations. We also utilize radiographs in conjunction with pQCT to visualize bone defects or injuries that may have been missed during an autopsy.

Raman spectroscopy is a measurement technique that uses a laser light to detect inelastic scattering effects of an object to identify molecular properties. It has a broad range of usages, though more recently it has been used to assess biological materials, such as bone [20][21].
Among other things, Raman spectroscopy has the capability to assess immature bone deposits [22], determine relative mineralization of bone [23], identify regions of high bone turnover [24] and can be correlated with RPI devices and traditional mechanical tests [25]. Of interest to this summary are the bone mineral to bone mineral matrix ratios, carbonate substitution ratio and bone turnover ratio, where increases in these parameters are related to cortical bone strength, age and remodeling activity, respectively.

A total of 32 Thoroughbred (TB) racehorse MC3s were tested using the aforementioned modalities. This sample included 7 males, 11 geldings and 14 females. The sample was also divided into four statistical 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 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 BMSi was elevated at the midshaft dorsomedial site in the MC3, SSMD and LB fracture groups compared to control. Consistent with this, it was found that 1st ID and TID were decreased at the midshaft dorsal site. These findings were somewhat counterintuitive, as one might anticipate that the Control would be less microfracture susceptible, when the opposite was found. These findings are likely explained by the presence of increased dorsal metacarpal disease (DMD) in the Control horses, a pathology that increases the deposition of immature bone – which is more easily indented – on the dorsal surface of young racing Thoroughbreds. It is possible that these Control horses were still highly fracture susceptible, as DMD is linked to stress fracturing, but they may have died of other causes.

Findings from pQCT included decreased cortical 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 fact that the majority of MC3 fractures occur 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 and 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 would potentially help in fracture propagation of slab fractures, as the bone surface would be stronger but more mechanically brittle. Increased carbonate substitution and decreased remodeling may indicate that these compositional changes occurred sometime in the past (the material is older and not remodeling as rapidly as the other groups) due to some trauma or perhaps just due to genetic differences.

In terms of future work, for RPI it would be very useful to test other bones to see if this dorsal relationship holds, and also to perform histology on the tested bones to quantify DMD on the surface. Regarding pQCT, it may be fruitful to scan sesamoid bones as well as MC3s as the sesamoid group did not separate statistically during this study. It may be that we are unable to determine sesamoid fracture risk by testing long bones alone. Finally, for Raman it could be interesting to analyze the distal metaphysis as a comparison to the pQCT, however it should be noted that this region would not accessible in vivo in the standing horse.

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


APPENDIX E

Refereed Scientific Publications


Refereed Scientific Publications


