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## Appendix A (Blue)

- *Equine Health Update* - Equine Sports Medicine Center Newsletter
  Vol. 16, Issue No. 1 – 2014

- *Equine Health Update* - Equine Sports Medicine Center Newsletter
  Vol. 17, Issue No. 2 – 2014

## Appendix B (Gold) ~ Research Projects In Progress 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.*

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

- **Taylor SD, Bianco AW, Moore GE.** *Anti-endotoxin properties of ketorolac tromethamine in horses.*
Appendix C (Green) ~ Research Projects Completed Supported with Pari-Mutual Funds


- Taylor SD, Bianco AW, Constable PD, Cooper BR. Pharmacokinetics of ketorolac tromethamine, a potent non-steroidal anti-inflammatory drug, in healthy adult horses.

Appendix D (Purple) ~ Publications Supported by the Equine Research Internal Funds


**Appendix E (Tan) ~ 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. Eleven client-owned horses were evaluated on the treadmill in 2014. This brings the total number of horses evaluated since the opening of the ESMC in April 1996 to 454. 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 14 treadmill demonstrations were given to groups or dignitaries who visited Purdue campus.

Continuing Education and Extension Service:
- Continuing Education presentations:
  - Couetil L.
    - International
      - Dorothy R. Havemeyer Foundation Workshop: Inflammatory airway disease; one syndrome, multiple pathways, Cabourg, France, October 2014.
        - Lower airway inflammation and performance
        - Therapeutic options available to the practitioner
        - IAD: Diagnostic methods vs. clinical outcomes
    - National
  - Regional and State
    - Updates on Purdue’s equine research. Purdue Horseman’s Forum, West Lafayette, IN, February 2014.
    - Inflammatory airway disease in racehorses. Presentation to Thoroughbred horse owners and trainers. Indiana Downs, Shelbyville, IN. September 2014.
    - What makes the horse an outstanding athlete? The Elderhostel / Road Scholar group. Purdue Veterinary Medicine, West Lafayette, IN, June 2014
    - Boiler Vet Camp. Purdue Veterinary Medicine, West Lafayette, IN, June 2014
      - A Look Inside a Horse.
      - The horse athlete: Treadmill demonstration
• Hawkins J.
  National
    - Equine field abdominal surgery
    - Equine field male urogenital surgery
    - Equine field surgery of the head and neck
    - Equine standing orthopedic surgical procedures
  Regional and State
  • *Missouri Veterinary Medical Association Annual Meeting*, Lake of the Ozarks, MO, 2014
    - Equine respiratory tract in health and disease
    - What’s new in equine upper respiratory surgery
    - Equine standing surgery
    - Surgical and medical management of sarcoids
  • *Purdue Veterinary Medicine Fall Conference*, West Lafayette, IN, 2014.
    - Standing surgery of horses
    - Alcohol fusion of distal hock joints

• Ivester K.
  Regional and State
  • Barn air quality: minimizing the risk. *Purdue Horseman’s Forum*, West Lafayette, IN, February 2014.
  • Research project on IAD. Presentation to Thoroughbred horse owners and trainers. Indiana Downs, Shelbyville, IN. September 2014.

• Kritchevsky J.
  National

• Lescun T.
  Regional and State
  • Navicular disease – A pain in the heel. *Purdue Horseman’s Forum*, West Lafayette, IN, February 2014.

• Taylor S.
  National
  Regional and State
  • Equine diarrhea. *Purdue Horseman’s Forum*, Purdue University, West Lafayette, IN. 2014.
• Tinkler S.

• Townsend W.  
  **Regional and State**
  • Moon blindness and your horse. *Purdue Horseman’s Forum*, Purdue University, West Lafayette, IN. 2014.

• Waxman S.  
  **Regional and State**
  • Bandaging techniques for emergencies. *Purdue Horseman’s Forum*, Purdue University, West Lafayette, IN. 2014.

• Continuing Education – conference proceedings, articles, other publications:
  
  - Couëtil L.
    - Hawkins J.
        - Equine field abdominal surgery
        - Equine field male urogenital surgery
        - Equine field surgery of the head and neck
        - Equine standing orthopedic surgical procedures
      - Endoscopy of the upper respiratory tract in health and disease
      - What’s new in equine upper respiratory surgery?
      - Standing surgery in the horse
      - Surgical and medical management of sarcoids in the horse

• Committee service  
  **International**
  - Couëtil L: Veterinary Comparative Respiratory Society, 1997-Present, Chair Research Grant Program, 2012-2014.
  - Townsend W: Research Committee Member, International Equine Ophthalmology Consortium. 2013-present
National
- Couetil L:

- Kritchevsky J:
  American College of Veterinary Internal Medicine, FAIM Resident Award Committee 2012-2015.

- Lescun T.:
  American Association of Equine Practitioners, Avenues Task Force, 2012-present
  American College of Veterinary Surgeons, Examination committee, 2014-present

- Taylor SD.
  American College of Veterinary Internal Medicine, LAIM Credentials Committee, 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
  - Couetil L:
    ▪ Treadmill demonstrations. Purdue Horseman’s Forum, West Lafayette, IN, February 2014; Open House, Purdue University College of Veterinary Medicine, April 2014.
  - Ivester K:
    ▪ Treadmill demonstration. Purdue Horseman’s Forum, West Lafayette, IN, February 2014
  - Lescun T:
  - Tinkler S:
    ▪ Clinton County vaccine clinic – spring 2014.
    ▪ Boiler Vet Camp – summer 2014.
    ▪ A2RC Program: Equine Physical Exam 2014
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 2014 (summer and winter) and articles are accessible from our Web site. The newsletters are included in Appendix A (Blue).

Couetil L:
- IAD study. Equine Health Update, Purdue University College of Veterinary Medicine, Summer 2014.

Farr A:
- Avoiding trailering troubles. Equine Health Update, Purdue University College of Veterinary Medicine, Summer 2014.

Tinkler S:

Taylor SD:
- Equine squamous cell carcinoma. Equine Health Update, Purdue University College of Veterinary Medicine, Spring 2014.
- Equine gastric ulcer syndrome. Equine Health Update, Purdue University, College of Veterinary Medicine, In press, Winter 2014.
- Research in equine pleuropneumonia. Equine Health Update, Purdue University, College of Veterinary Medicine, In press, Winter 2014.

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).

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.

Taylor SD, Bianco AW, Moore GE. Anti-endotoxin properties of ketorolac tromethamine in horses.

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

Kritchevsky, J. (PI), Couetil, L. (Co-I). Serum thyroxine concentrations in horses undergoing a standardized exercise test on a high speed treadmill. 2013-2014

Taylor SD, Bianco AW, Constable PD, Cooper BR. Pharmacokinetics of ketorolac tromethamine, a potent non-steroidal anti-inflammatory drug, in healthy adult horses.

Competitive Equine Drug Testing Research Fund:
This initiative started in the fall of 2008 and is funded by the Integrity Fund of the Indiana Horse Racing Commission. The objective of this internal grants initiative is to fund proposals addressing issues related to drug testing in race horses including, but not restricted to, the development of analytical methods for the detection of prohibited substances and the establishment of withdrawal times or threshold for therapeutic substances. The Indiana Horse Racing Commission staff and Racing Commission and Testing Consortium (and their designees) will review the proposals. The ultimate decision to fund a proposal will be made by the Indiana Horse Racing Commission and based on the scientific merit of the proposal and immediate benefit to the racing industry.

Due to lack of funding, there was no call for proposals in 2012-2014.

Externally funded equine research projects conducted in 2014:


Couetil L.L., Ivester K. Microbiome of the equine airway. Quarter Horse Racing Association of Indiana. $3500.


Couetil L. Quantification of cytokines and growth factors in an equine serum product. Central Biomedica, Inc. $46,913.


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

Refereed Scientific Articles:


Abstracts and Proceedings:


Schnur C, Lescun T. What’s the next step? Pedometers as a tool for quantifying pain in horses. Phi Zeta Research Day, Purdue University, West Lafayette, IN, 2013.


Books:
Hawkins JF, ed. Advances in Equine Upper Respiratory Surgery. Wiley Blackwell. In press, 41 chapters. Dr. Hawkins wrote the following 10 chapters:
• Chapter 4: Laser ventriculocordectomy
• Chapter 5: Prosthetic laryngoplasty (co-author)
• Chapter 7: Ablation of the cricoarytenoid joint
• Chapter 9: Evaluation and management of the horse following failed laryngoplasty
• Chapter 10: Evaluation and management of the horse with dysphagia following prosthetic laryngoplasty
• Chapter 19: Sternothyroideus myotenectomy and staphylectomy
• Chapter 21: Laser palatoplasty
• Chapter 27: Choanal atresia
• Chapter 32: Surgical correction of epiglottic entrapment (co-author)
• Chapter 36: Partial arytenoidectomy without mucosal closure

Book Chapters:

Refereed Scientific Publications: [Appendix E (Tan)]


**Research abstracts and posters:**

**Scientific Abstracts:**


**Posters:**

## EQUINE RESEARCH ADVISORY BOARD

### Membership
07/2013-07/2014

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APPENDIX A

- *Equine Health Update* - Equine Sports Medicine Center Newsletter
  Vol. 16, Issue No. 1 – 2014

- *Equine Health Update* - Equine Sports Medicine Center Newsletter
  Vol. 17, Issue No. 2 – 2014
Avoiding Trailering Troubles

By Amanda C. Farr, DVM, Dipl. ABVP Equine Practice,
Purdue Equine Community Practice

Trailering your horse may be something you do on a regular basis or something that hasn’t occurred in years. In both situations it can be a simple low-stress experience or just the opposite for both you and your horse(s). There are many aspects that affect trailering. Understanding some trailering basics can help create a positive experience for you and your horse(s).

It is important to consider the following factors: the trailer itself, the tow vehicle, your horse(s), any paperwork or vaccinations required by your destination (show grounds, fairgrounds, campgrounds) and legal requirements for interstate travel, and the distance or length of time your horse will be on the trailer.

The Trailer:

Your trailer should be carefully inspected before each use. All running lights, brake lights, and turn signals should be checked for proper operation. All horse trailers should be equipped with properly adjusted auxiliary brakes; usually these are electric and require a brake controller in the tow vehicle. The trailer tires should be inspected for abnormal wear, thinning, and damage and should be a matched set made especially for trailers; car tires are not a good substitute. Trailer tires don’t usually wear out due to mileage, but can develop dry rot and other issues just from sitting around. The break-away brake system should be working, with the battery charging when the trailer electrical connector is connected to the tow vehicle. The trailer also must have safety chains attached to the tow vehicle that are rated for the loaded weight of the trailer. In addition to the items mentioned above, the trailer coupler and the ball mount on the tow vehicle must be appropriately rated and of the proper size. Most trailers require a 2-5/16” ball, but there are still some that take a 2” ball.

Ensure the interior is in working order as well—this includes mats, chest and butt bars or slants, and ties. The trailer walls should be inspected for protruding screws, hooks, or bent metal that could cause a laceration. If your trailer has a ramp, that should also be inspected for wear.

Additionally, at least once a year, your trailer should have a full “check-up” by a shop that has expertise in trailer maintenance. This should include brake and tire inspection and repacking of wheels bearings if the trailer has serviceable bearings. Floor mats should be removed, and the flooring should be power washed to remove urine buildup. The trailer floor should be carefully inspected from underneath as well. This is most critical with wood floors, but chronic urine buildup can cause steel and aluminum flooring to corrode, developing weak areas.

(continued on pg. 2)
News & Notes
IAD Study

Dr. Laurent L. Couëtil, director of Purdue University’s Equine Sports Medicine Center and Equine Research Programs, and Dr. Katy Ivester, post-doctoral research assistant, have been awarded funding by the Grayson-Jockey Club Research Foundation to investigate the link between environment, airway inflammation, and performance in racing Thoroughbreds. Airway inflammation is a common cause of poor performance in equine athletes, and the triggers for the inflammation are poorly understood. The proposed study hopes to determine the effect of the different forms of airway inflammation upon racing performance, while establishing the relative importance of environmental exposures, respiratory bacteria, and viruses in airway inflammation. By identifying the various factors contributing to airway inflammation, this study will lay the groundwork for future investigations into the efficacy of management changes, antibiotics, and immune stimulants in restoring a healthy respiratory tract.

Beginning this summer, volunteers for the study will be recruited from horses racing at Indiana Downs in Shelbyville, IN. Horses enrolled in the study will have a thorough evaluation of the airway performed immediately after racing. In addition, the horses will undergo air sampling to estimate individual exposure to dust, endotoxin, and ammonia during the following week, and airway secretions will be collected to assess presence of viruses and the diversity and number of bacteria using DNA analysis. Testing will be at no cost to the owner and test results from individual horses will be provided to owners/trainers as soon as available.

For more information please call Purdue University Large Animal Hospital at 765-494-8548 and ask for Dr. Ivester or Couëtil.

Trailering (continued from cover)

Trailers are available in many sizes and styles. Make sure your trailer is the appropriate height and width for your horses. Although it has been suggested that slant-load trailers, which place horses at a 45-degree angle to the direction of travel, are easier on the horse, in reality many horses prefer to ride facing backwards if left untied in an open-stock trailer.

Proper ventilation is critical for successful trailering and healthy horses upon arrival. Trailers that are improperly ventilated accumulate dust and ammonia, which can lead to respiratory infections (shipping fever) and secondary pneumonia. Consider adding fans or roof vents if your trailer has small windows. Use screens in drop-down windows to protect your horse’s eyes and face from flying debris.

The Tow Vehicle:

When selecting your tow vehicle, make sure it is rated to haul the weight of your trailer, horses, and tack. Tow vehicles that are too small for the weight of your trailer can be deadly in an emergency situation, where the tow vehicle doesn’t have the mass to stabilize the trailer. Consider installing sway bars if you are towing a bumper pull trailer and a weight distribution hitch if the tow vehicle suspension is overloaded by the tongue weight of the trailer. Use a properly installed Class III receiver hitch (e.g., “Reese” hitch) that is mounted to the frame of the tow vehicle (for a bumper pull trailer), rather than mounting a ball to the bumper of the tow vehicle. Make sure your lights and trailer brakes are working, as there are multiple different wiring kits for tow vehicles and trailers. Installing a trailer brake system in the tow vehicle (e.g., Draw-Tite or Reese) will save your vehicle’s brakes and also provide improved safety and braking. Finally, take some time to drive your trailer around prior to hauling your horses in it for the first time. Make sure you can back and turn, without damaging the trailer or your tow vehicle, and get a feel for how wide your turns need to be, and how vehicle stopping distance will change when towing.

The Horse:

One of the biggest causes of stress for horses with trailering is loading. Resistance to loading can cause not only stress to the horse and handler, but also is a common cause of injury to both. It is important to practice loading your horse before an unforeseen emergency or a scheduled trip. Additionally, studies have shown that problem loaders can be trained to load more willingly by breaking the act of loading into separate parts that can be practiced without the trailer (e.g., the Tellington-Touch Equine Awareness Method – TTEAM).
Trailers are available in many sizes and styles. Make sure your trailer is the appropriate height and width for your horses. Although it has been suggested that slant-load trailers, which place horses at a 45-degree angle to the direction of travel, are easier on the horse, in reality many horses prefer to ride facing backwards if left untrained in an open stock trailer.

Proper ventilation is critical for successful trailering and healthy horses upon arrival. Trailers that are improperly ventilated accumulate dust and ammonia, which can lead to respiratory infections (shipping fever) and secondary pneumonia. Consider adding fans or roof vents if your trailer has small windows. Use screens in drop-down windows to protect your horse’s eyes and face from flying debris.

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Use caution when trailering horses in hot, humid conditions as they can easily become dehydrated on the trailer. Check with the State Board of Animal Health for your state’s specific requirements for health certificates. Make sure your horses are healthy and not in need of veterinary care prior to traveling. Horses should not be transported cross-tied or with their heads tied up. Horses need to be able to drop their heads and cough, clearing dust and debris from their airways. When horses cannot move their heads freely in the trailer, their systemic stress response is significantly increased and normal respiratory defense mechanisms are impaired, putting them at risk of developing life-threatening pneumonia.

Vaccination should be performed more than two weeks prior to trailering to prevent overload of the immune system at the time of trailering. Additionally this allows your horse to recover from any adverse vaccine reactions in time for the trip.

Horses should be allowed to move around if possible and be given fresh water to drink every six hours they spend on the trailer.

Taking the time to prepare your trailer, tow vehicle, and horses for a safe trip means preventing trailering disasters, injuries to you and your horse, and help minimize stress for both of you. Additionally, the risk of post-trailering respiratory disease can be decreased with some simple trailering changes.

References:

Acknowledgment: Special thanks to Robin Ridgway for her detailed suggestions regarding tow vehicles and trailers.

Autologous Biologic Therapies: PRP and IRAP
By Jake Jensen, DVM Student, (Class of 2014)
Have you ever heard people using the terms “PRP” and “IRAP” when talking about treatments for their horse and wondered what exactly these products are and what the difference is between the two? This article will help you understand each of these interesting treatment options.
Platelet Rich Plasma (PRP) and IL-1 Receptor Antagonist Protein (IRAP) are both autologous biologic therapies. Autologous means derived from the same individual, so autologous therapies are treatments derived from a horse's own body. In the case of PRP and IRAP, these products are derived from the horse’s blood and then injected into or placed on the horse to treat a disease or injury. Let’s take a look at the differences between PRP and IRAP.
Platelet Rich Plasma
PRP therapy is based on the fact that the alpha granules of platelets contain over 200 different proteins, including many growth factors, which are helpful for tissue healing. PRP is produced by concentrating the platelets in the plasma of a horse’s blood, and then activating the platelets in order to release the content of their granules. PRP is most commonly used following acute injury to muscles, tendons, or ligaments to enhance tissue healing and is typically injected into a lesion.
IL-1 Receptor Antagonist Protein
Interleukin-1 (IL-1) is a cytokine which is a potent mediator of inflammation. IL-1 produces anti-inflammatory effects by opposing the actions of IL-1. IRAP is produced by incubating a horse’s blood with borosilicate glass beads. These beads interact with monocytes, a type of white blood cell, leading to the production of the IL-1 receptor antagonist protein. IRAP is most commonly used as an anti-inflammatory treatment for chronic, progressive osteoarthritis and is typically injected into a joint.
Conclusion
Both PRP and IRAP are exciting therapies that show promise in treating specific conditions in horses. However, because these therapies are highly individualized to each horse and there are many different preparation options available, the final product and results can be highly variable. Your veterinarian can help you determine if either of these treatment options is appropriate for your horse.
What is exertional rhabdomyolysis (ER)?
Rhabdomyolysis is the medical term used for “tying-up”, or what was historically known as “Monday Morning Disease” observed in exercising horses after some days of rest. Horses that tie-up develop a shortened, stiff stride, pain and anxiety and eventually the inability to move forward with light exercise. It is caused by a variety of muscle disorders that can be seen during or after exercise, some of which have a genetic basis.

The severity of each episode varies widely and can be mild to severe and result in recumbency or even death in extreme cases. ER can occur sporadically if it occurs as an isolated or infrequent event in a horse with no history (or family history) of previous performance issues.

Some causes of sporadic ER include: muscle trauma, over-exertion and exhaustion, with dietary and electrolyte imbalances as possible contributing factors. Horses can be of any age, breed or sex and involved in a variety of athletic disciplines.

Horses that have repeat episodes of tying-up from a young age, time of purchase or once put back into training after a long period of rest may have an underlying condition affecting muscle function and a chronic cause of ER should be pursued.

Some commonly observed symptoms are muscle spasms, firm painful muscles, excessive sweating, or reluctance to move. Sometimes the symptoms are confused with colic as the horse seems extremely painful but the pain during an episode of tying-up comes from muscle cells being broken down. Horses with respiratory disease are also more prone to tying-up, so it is not recommended to train your horse when it has signs of respiratory disease such as nasal discharge or cough; this has been observed in horses with EHV-1 (herpes virus) and equine influenza.

What are some of the muscle disorders causing chronic ER in horses?
There are a number of disorders that cause chronic ER in horses. Some of the following conditions can be diagnosed by genetic testing, blood sampling, or muscle biopsy by your primary veterinarian:

- **Recurrent exertional rhabdomyolysis (RER)** - This disorder is most often seen in Thoroughbred and Standardbred race horses. It is more commonly seen in fillies that are of a nervous temperament and is associated with exercise or stress. The specific defect that causes RER is unknown but is thought to be due to defective muscle contraction and relaxation.

- **Polysaccharide storage myopathy (PSSM)** - There are two types of PSSM. Type 1 mainly affects Continental European Draft Horse breeds such as Belgians and Percherons as well as Quarter Horses and Quarter Horse-related breeds such as Paints and Appaloosas. A specific genetic mutation has been identified as the cause of this condition and genetic testing is available. Horses affected by type 2 PSSM show symptoms of tying-up but the cause is currently unknown and no genetic defect has yet been identified. Diagnosis is based on symptoms and specific changes found in muscle tissue on a muscle biopsy. It occurs in Quarter Horses but also in Paint, Appaloosa and Morgan Horses as well as Warmbloods. PSSM results from abnormal sugar metabolism by the muscle cells.

- **Malignant hyperthermia (MH)** - MH is a rare genetic disorder that occurs in Quarter Horses and related breeds as a result of a genetic mutation. Genetic testing is available. Rhabdomyolysis can be caused by exercise or anaesthesia with this genetic mutation. Symptoms of MH are extremely high body temperature, symptoms of rhabdomyolysis, and because symptoms develop rapidly, if they are not treated quickly MH can be fatal. This disorder results from abnormal calcium regulation in the muscle cell resulting in energy depletion and excess heat production. Although this condition is rare, testing for MH is recommended in Quarter Horses and related breeds in case a horse must undergo anaesthesia. Horses known to have the genetic mutation can be given medication prior to anaesthesia to help reduce symptom severity.
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- **Exertional Myopathies in Horses**

**It is worth mentioning that Hyperkalemic Periodic Paralysis (HYPP) is an important muscle disorder in Quarter Horses, Paints, Appaloosa and Quarter Horse-crosses that has a genetic basis and for which genetic testing is available. However, HYPP is not a cause of chronic ER and does not cause rhabdomyolysis (muscle breakdown), but rather is due to a genetic defect in sodium ion channels in skeletal muscles which causes abnormally high potassium levels in the blood and results in a variety of symptoms such as weakness and muscle spasms. In severe cases, this disorder can lead to paralysis, collapse and can be life-threatening.**

**What are the first things that need to be done if my horse starts to “tie-up?”**

Exercise should be stopped immediately and the horse taken to a well bedded stall with fresh water. If the horse produces brown colored urine soon after, treatment with intravenous fluids and anti-inflammatories by a veterinarian is recommended. In severe cases, muscle damage leading to release of muscle pigments (myoglobin) can lead to kidney damage if the horse is not hydrated adequately, and as they say... you can’t make it drink! When you see “coffee-colored urine,” that means that a significant amount of muscle has been broken down and pigments released into the bloodstream. Non-steroidal antiinflammatories such as Banamine can be given after your horse is hydrated to help with pain and inflammation.

Right after your horse recovers from an episode, stall rest him or her for no more than 24-36 hours and then provide turn-out in paddocks of gradually increasing size. Providing horses as much free exercise as possible on pasture is beneficial in the long term. Your horse must return to its normal training schedule gradually, and an initial period of turn-out with no forced exercise is best. Training should not resume until your horse’s muscle enzymes are back to normal, as this could lead to more pain and possibly trigger another episode. Your veterinarian can test for muscle enzyme levels on a blood test.

Are there any management recommendations for horses that “tie-up”?

Specific management recommendations will vary depending on the underlying cause of the ER, and if it is sporadic or chronic, but there are two fundamental components to any management program for horses with ER—diet and exercise. Low starch, higher fat diets are recommended for horses that tie up. Easy access to fresh water, electrolyte supplementation, and vitamin/mineral supplementation are important as well. There are a number of commercial feeds available that are specially designed to provide adequate calories for an equine athlete, but are still lower in the carbohydrates that may trigger an episode; however, the benefits of the low starch, fat supplemented diet only occur if the diet is introduced along with an incremental exercise program. Lastly, vitamin E and selenium supplementation may help to protect muscle fibers, especially in selenium deficient geographic areas like the Midwest.

General principles to keep in mind when exercising horses that have a chronic form of ER include: providing adequate time to adapt to a new diet before starting exercise (2 weeks is usually adequate), introducing gradual and consistent exercise, and minimizing days without exercise. Daily exercise is important in preventing episodes of chronic ER, while stall rest can make it worse.

Identifying any underlying genetic causes for muscle disorders can spare your horse a lot of pain, poor performance, and possibly even avoid a life-threatening situation. Your veterinarian can help you diagnose muscle disorders, find the right diet and help tailor a specific exercise plan for your horse if it is diagnosed with sporadic or chronic ER.

Resources:

Shockwave Therapy: What is it? And what can we use it for?

By Marymir Miranda, DVM Student (Class of 2014)
Edited by Tim Lescun, BVSc, MS, Dipl. ACVS, Purdue Large Animal Surgery

Shockwave therapy has gained vast popularity over the past 10-15 years as a treatment for many disease processes in horses. It is currently being used as an important component of treatment for tendon injuries in many veterinary practices. Multiple veterinarians have reported success in stimulating suspensory desmitis to heal and decrease lameness. But, do we really know what it is, how it works, and what we can use it for?

The effect of shockwaves was first discovered during World War II when the lungs of castaways were noted to be damaged without any superficial evidence of trauma. Shockwaves created by depth charges created the internal injuries. This discovery triggered a lot of interest and research into the biological effects of shockwaves on live tissue. The original use of shockwave therapy was to fragment bladder stones. Interestingly, it was noted that following treatment, the density of a portion of the pelvis within the treatment area had increased. Later, the musculoskeletal applications were noticed when shockwaves were used to stimulate fractured bones to heal. The FDA has approved the use of extracorporeal shockwaves to treat heel spurs and tennis elbow in humans.

A clinical shockwave is a controlled explosion that creates a sonic pulse. Its primary effect is a direct mechanical force. The pressure waves generated are brought to a focal point by lenses or a parabolic reflector. This allows the energy in the wave to aim at a specific point within the tissue. The exact mechanism by which shockwave therapy acts to treat tendon pathology is not known. The leading explanation is based on the inflammatory healing response. The shockwaves cause microscopic trauma to the damaged tissue, which in turn results in inflammation and the release of growth factors, which allows the body to send healing cells and increase the blood flow to the injured area.

Humans that have been treated with extracorporeal shockwaves report an initial decrease in pain in the area treated that lasts up to a week, then some return of the original pain that gradually decreases as the underlying problem heals. A study at Iowa State University found that in the horse, a period of analgesia appears to be present for about 4 days after treatment. There was a significant analgesic effect following therapy from 8 to 48 hours after treatment. Because of this, racing jurisdictions in the US and the FEI have adopted regulations that require a withdrawal period after treatment before horses are allowed to perform. Extracorporeal shockwave therapy is not permitted during competition and for a period of 5 days prior to the first horse inspection. These regulations should not be difficult to follow, since the most important treatment for tendon injuries is strict rest!

Another application for shockwave therapy is the enhancement of wound healing. Where there is chronic inflammation there is a tendency for horses to produce exuberant granulation tissue (proud flesh) which further delays healing. In a study evaluating the effects of shockwave therapy on wounds on the distal (lower) limb in horses, the time for wound healing was shorter following shockwave therapy. It is understood that the healing process seems to be more organized following stimulation with shockwaves, thereby decreasing the formation of proud flesh. Lastly, shockwave significantly decreases the initial inflammatory response in burn wounds and has been shown to promote their healing.

It is important to note that these treatments should be performed by a veterinarian, since different disease processes require different strength and number of shockwaves per treatment, and time intervals between treatments. Typical treatment protocols for musculoskeletal injuries involve 3-4 separate treatments 3-4 weeks apart, combined with appropriate rest periods depending on the injury being treated. It appears shockwave is here to stay as a treatment option for injuries in horses.

References:
Shockwave Therapy: What is it? And what can we use it for?

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It is important to note that these treatments should be performed by a veterinarian, since different disease processes require different strength and number of shockwaves per treatment, and time intervals between treatments. Tissue necrosis has been reported due to the use of very high-energy shockwaves. Typical treatment protocols for musculoskeletal injuries involve 3-4 separate treatments 3-4 weeks apart, combined with appropriate rest periods depending on the injury being treated. It appears shockwave is here to stay as a treatment option for injuries in horses.

Reference:
McClure, Scott R. Extracorporeal shockwave therapy in horses: what we know. 73-75. Print.

Q: Why do horses get SCC?

A: There are several factors that can contribute to a horse developing squamous cell carcinoma. Exposure to sunlight is a major contributor, while inflammation from burns or wounds, and papilloma infection is linked to SCC as well. Carcinomas that develop due to sunlight exposure will first start out as a precancerous area, called actinic keratosis. An actinic keratosis will appear as reddish thickening of the skin with some crusty patches. These are easy to treat in their early stages, but if left untreated can progress into a carcinoma, which can be much more of a challenge to remove.

Recently, a virus called equine calcivirus papillomavirus-2 has been linked to the development of squamous cell carcinoma. Current research leads us to think that some horses that have this virus will develop SCC at some point in their life. Extensive research is being pursued to see how significant this link between the virus and SCC is, and we are also exploring the possibility of a vaccine to prevent the virus, thus preventing SCC.

Q: How do horses get SCC?

A: There are several ways in which we can treat it. We can remove the mass by surgical excision. Another option is cryotherapy, which is used to kill the cells by freezing. Hyperthermia is another alternative; this is the use of extreme heat to “burn” off the mass. Radiotherapy can also be used, which uses radiation to kill the cancer cells. Chemotherapy is the use of chemical compounds that are toxic and result in the death of cancer cells. Finally, photodynamic therapy is another alternative, which utilizes a photosensitizing drug to make the cancer cells sensitive to ultraviolet light; exposure to light then causes damage to the cancer cells. It is important to realize that we often use all of these therapies to treat SCC. Your veterinarian will be able to tell you which of the available treatments, and which one is best for your horse. As stated before, the chances of any of these treatments working are far greater if we can treat a mass early on, because with time, more and more tissue is destroyed by the cancer and surgical excision becomes very difficult.

Q: How is SCC diagnosed?

A: Although SCC has a very characteristic look on a horse, we still have to do something called a histopathology to be certain that SCC is present. Histopathology is the name we give to obtain a sample of the tumor (or even the whole tumor) via surgical excision, and then we send the sample out to a pathologist, where they can take a look at it under a microscope and determine whether or not the cells are consistent with SCC.

Q: How is SCC treated?

A: If your horse does develop SCC, there are several ways in which we can treat it. We can remove the mass by surgical excision. Another option is cryotherapy, which is used to kill the cells by freezing. Hyperthermia is another alternative; this is the use of extreme heat to “burn” off the mass. Radiotherapy can also be used, which uses radiation to kill the cancer cells. Chemotherapy is the use of chemical compounds that are toxic and result in the death of cancer cells. Finally, photodynamic therapy is another alternative, which utilizes a photosensitizing drug to make the cancer cells sensitive to ultraviolet light; exposure to light then causes damage to the cancer cells. It is important to realize that we often use all of these therapies to treat SCC. Your veterinarian will be able to tell you which of the available treatments, and which one is best for your horse. As stated before, the chances of any of these treatments working are far greater if we can treat a mass early on, before the mass involves a lot of tissue and structures and is difficult to treat.

Q: Are there things I can do to prevent my horse from getting SCC?

A: One way to help prevent your horse from developing SCC, especially if you have a lightly-pigmented horse, is to protect him or her from the sun. This can be done by using fly-masks that block UV light, or applying baby sunblock to the lightly pigmented areas, especially on the face. Frequent grooming is a good habit because it makes you aware of abnormal marks on the skin or new bumps that may arise on your horse over time. If you notice an abnormal area on the skin or eye of your horse that persists for an extensive amount of time, contact your veterinarian as soon as possible. It is ideal to try and prevent the development of SCC, but if it does occur, the key to successful treatment of SCC is early recognition and treatment by a veterinarian.

Sunshine and Squamous Cell Carcinoma of the Skin

By Megan McGlothin, DVM Student (Class of 2015)
Edited by Sandra D. Taylor, DVM, PhD, Dipl. ACVIM, Purdue Large Animal Internal Medicine

W ith modern advancements in equine medicine, nutrition, and vaccines, horses are living longer than ever before. Because we are seeing a greater number of horses in advanced stages of life, we are unfortunately diagnosing cancer more often as well. One of the more common cancers in horses is squamous cell carcinoma, or SCC, for short. It is the second most common cancer in the equine, making up about 30% of the cancers that veterinarians diagnose.

Q: What does SCC look like?

A: SCC can occur anywhere on a horse, but it is most commonly found on the non-pigmented areas of horses. These common locations include the eye, eyelid, nose, mouth, ear, genitalia, and around the anus. The light-pigmented breeds, like Appaloosas, American Paints, Pintos, and some draft horses, particularly Belgians, are more prone to developing SCC. SCC can be a slow-growing, benign tumor, or sometimes, it can grow very rapidly and be highly malignant. It can appear on a horse as just a little bump on the skin, a thin layer on the eyeball, or even an ulcerated, dark-colored tumor that has a foul odor. Occasionally, SCC will metastasize, which means that the cancer cells are spread from the original site where the tumor is to another location in the horse, like an internal organ, for example. SCC is a locally invasive type of cancer. So the cancer can invade and destroy the normal tissue that surrounds it. For this reason, it is very important to get these tumors taken care of very early on, because with time, more and more tissue is destroyed by the cancer and surgical excision becomes very difficult.

Q: How is SCC diagnosed?

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www.vet.purdue.edu/esmc/
Pleuropneumonia in Horses

By Dr. Sandra D. Taylor DVM, PhD, Dipl. ACVIM,
Purdue Large Animal Internal Medicine Service

Pleuropneumonia refers to infection of the lungs (pneumonia) that leads to infected fluid accumulating in the space between the lungs and the body wall (pleuritis). This is a very serious disease in horses that can lead to death, and survival rates are much better when the disease is detected early and treated aggressively.

What puts your horse at risk?

There are several known risk factors for development of pleuropneumonia, including:

- **Upper respiratory tract viral infections**
  Viruses such as influenza (flu), herpes virus (EHV-1, EHV-4), rhinovirus and equine viral arteritis virus (EVA) can infect upper respiratory tract epithelial cells that line the nasal passages, throat and upper airways. These infections often cause high fevers, a decreased appetite, and occasionally cough or nasal discharge. These viruses can damage the hair-like projections (cilia) that line the upper airway, which prevents clearance of inhaled organisms and debris. This can allow secondary bacterial pneumonia, since the bacteria is not removed in an appropriate fashion by the cilia.

- **Long trailer rides**
  Even the cleanest trailers have relatively poor ventilation, which means that your horse may inhale a high amount of dust, hay mold and aerosolized components of manure during a trailer ride. Also, many horse’s heads are tied up in the trailer, which prevents drainage of inhaled particles that are normally trapped in mucus that drains out when the horse’s head is down. This allows increased entry of normal bacterial flora (from the throat) and environmental bacteria into the lungs.

- **High-intensity exercise**
  With intense exercise, the immune system may be compromised. Studies have shown that high-intensity exercise can decrease white blood cell function, which can predispose horses to infection. In addition, horses may aspirate more debris during exercise, especially racehorses.

- **Crowded environment**
  Many horses are kept in barns with other horses, and trailer to shows/events with other horses. Just like with people cooped up in small areas, this increases transfer of infectious agents (such as the flu virus) between horses.

- **Stress**
  Immune function can be decreased by stress hormones (e.g. cortisol). Stress can be caused by several things, including transport, exercise, shows/events, pregnancy, lactation, and training.

(continued on pg. 2)
News & Notes

New LA Surgery Fellow:

Dr. Alec Davern is originally from a small town in northern Minnesota, however has spent the past 15 years in the Southeastern US. He obtained a Bachelor’s of Science degree in Animal and Veterinary Sciences from Clemson University prior to pursuing his veterinary education at the University of Georgia. Prior to beginning a residency in Large Animal Surgery at Purdue University he completed an internship in equine medicine, surgery and reproduction at the Equine Medical Center of Ocala in Ocala, Florida. His primary professional interests include lameness and performance horse maintenance as well as emergency surgery. Dr. Davern has a background in western performance horses and Thoroughbred race horses. When away from the clinic, he enjoys fly fishing, woodworking, hiking and camping with his fiancé and dogs.

New LA Surgery Fellow:

Dr. Jesus Hermida is originally from Bogotá the capital of Colombia. Since graduating from veterinary school in 2004, he has held the positions of Veterinary Director of the Escuela de Equitación y Rejoneo La Andaluza and owned and operated Equus Veterinary Services, an ambulatory equine practice for performance horses, both in Colombia, until June of 2011. Through externships and assistantships around the world, he obtained surgical and medical training. Intent on broadening his experience in equine surgery, he took leave from his positions in Colombia to seek an additional internship in equine surgery at the Milton Equine Hospital in Ontario, Canada for 14-months, and the Hagyard Equine Medical Institute in Lexington, Kentucky for 18 months. He is currently completing a fellowship as a graduate student in surgery at Purdue University. He will be the next resident in Large Animal surgery starting next year. His primary professional interests include orthopedic surgery, emergency surgery, lameness, racehorses and sport horses. When away from the clinic he enjoys tennis and equestrian sports like jumping, dressage and polo.

Pleuropneumonia (continued from cover)

How do I know if my horse has pleuropneumonia?

You should be especially vigilant of your horse’s health if the above risk factors are present. You should take your horse’s temperature (if it’s safe!) daily for 1-2 weeks after long-distance trailering (i.e. > 2 hours), or if other horses in a barn develop respiratory disease. The first symptom is typically a fever, followed by reluctance to eat, lethargy, nasal discharge, and cough. Weight loss may also be noticed and can occur quickly. If any of these symptoms are noticed, your veterinarian should be called immediately. Pleuropneumonia is an equine emergency!

Your veterinarian will perform a complete physical examination and routine blood work (complete blood count and serum biochemistry). If these are indicative of respiratory disease, other tests including sampling of the upper airway for a viral infection, sampling of tracheal (windpipe) fluid for bacterial culture, ultrasound of the lungs and sampling of the chest fluid for cell evaluation and culture may be done. Some of these tests can be done in the field, but some may require hospitalization.

How is pleuropneumonia treated?

It is critical that the horse NOT be exercised during treatment of pleuropneumonia. Increased respiratory effort that is required during exercise can make the horse much worse. In severe cases, horses may be treated with continuous flow of oxygen through nasal tubes in order to get an adequate amount of oxygen to the damaged lungs. Many horses are dehydrated when they are diagnosed, because their focus is on breathing (and they often drink when they eat, and many horses are inappetant); thus, many horses require intravenous fluid therapy. Antibiotics are critical to combat pleuropneumonia, but antibiotic use in horses must be done with caution! The equine gut is very sensitive to antibiotics, and normal bacterial flora can be killed by antibiotics, leading to overgrowth of toxin-producing organisms and thus, colitis (inflammation of the colon). Antibiotic-induced colitis can be fatal, so it is very important that you follow your veterinarian’s instructions regarding antibiotic therapy. Although it may be necessary, switching or adding antibiotics must be done judiciously! Anti-inflammatory drugs such as Banamine or Equioxx may be necessary to dampen the inflammation associated with pleuropneumonia. Finally, removal of the infected fluid from the pleural space is critical. This is done by inserting a tube through the body wall into the chest cavity (thoracocentesis), and is typically done on both sides.

What are the potential complications of pleuropneumonia?

One of the most devastating complications of pleuropneumonia is laminitis. Whole-body inflammation that can be induced by pleuropneumonia can lead to laminitis, but the mechanism of this is unknown. Fortunately, the incidence of laminitis in these cases is low, but veterinarians may recommend prophylactic icing of the feet or bedding with deep sand to help prevent this complication.

A second potential complication is thrombophlebitis, which is inflammation and clotting of the vein. This may occur where a catheter is placed if the catheter site becomes infected, but this may also occur simply because systemic inflammation can increase the overall risk of clot formation.
Edema, which is accumulation of fluid in the subcutaneous space, can occur when blood protein (albumin) leaks out of the vessels. This can occur because whole-body inflammation makes blood vessels leaky, and because protein can be drawn into large vats of fluid (such as thoracic cavity fluid) from the blood vessels. Because edema tends to be pulled down by gravity, swelling usually occurs in the lower limbs and along the bottom of the chest/abdomen.

Finally, horses that recover from pleuropneumonia may have scarred lung tissue that prevents them from working at the same level as they did prior to getting sick. However, many horses return to full function after recovery.

**Recent research study at Purdue University**

Researchers at Purdue University recently collaborated with Hagyard Equine Medical Institute (HEMI) in Lexington, KY to compile previous cases of pleuropneumonia to identify factors that may predict survival. The goal was to identify tests that may perform better than others, and to pinpoint the most effective treatments. Because treatment of pleuropneumonia can be expensive, identification of poor prognostic indicators would help owners make decisions based on predicted outcome.

Medical records from 2001 – 2014 were reviewed. A total of 97 horses with pleuropneumonia (40 from Purdue, 57 from HEMI) were included in the study. Over half of the horses (57%) were racehorses, which is not surprising since these horses are often young and therefore more susceptible to viral infections, they are stressed, exercising intensely, live in crowded environments and travel a lot! The average age of affected horses was 4 years. Common initial symptoms included fever (84% of cases), lethargy (78%) and inappetance (77%), and common physical examination findings included increased heart rate (75%), increased respiratory rate (60%) and fever (43%). Blood work was typically consistent with bacterial infection. The most common bacteria isolated from tracheal fluid and pleural cavity fluid was Streptococcus equi subsp zooepidemicus (Strep zoo), which is a common normal inhabitant of the upper airway (this is NOT the “strangles” bacteria). Laminitis occurred in only 8% of cases.

The overall survival rate in treated horses was 67%. Horses with evidence of kidney damage had a worse outcome, and horses with a relatively aggressive treatment called “thoracotomy” had a better outcome. This treatment involves making the thoracocentesis drainage hole bigger by making a 6-8” incision between the ribs; this allows thick pus/fibrin to be removed. Occasionally, a rib needs to be removed to allow adequate drainage. The average length of hospital stay was 10 days, and the average hospital bill was $4,700 (range from $140 for a horse that was immediately euthanized to $15,000 in a very severe case).

**Conclusions**

It is important to recognize risk factors for the development of pleuropneumonia so that you can be watching for early symptoms. The earlier the diagnosis and treatment, the better the outcome. Overall, the prognosis for survival is good if aggressive treatment is initiated and intensive care is provided. Again, if you notice symptoms of respiratory disease in your horse, call your veterinarian immediately.
If there were an outbreak of an infectious or zoonotic equine disease, would you know how to keep you and your horses safe? Would you know what to do if your horse was exposed or became ill? While it is rare for humans to contract a disease from a horse, it is possible. It is therefore very important to know which infectious diseases are zoonotic diseases and understand how to prevent and respond to them.

Our common ancestry with animals was recognized in the nineteenth century by the physician Rudolf Virchow who coined the term zoonoses—diseases that can be transmitted to humans from animals. He said, “Between animal and human medicine, there are no dividing lines—nor should there be.” This common ancestry means we share much of the same biochemistry and therefore, much of the same susceptibilities. There are many bacteria and viruses that are infectious between horses such as rotavirus, Equine Herpesvirus, Equine Influenza, Rhinoviruses, Streptococcus equi, and Rhodococcus equi. However; there are also diseases that can infect horses and are transmissible to either other horses or humans (those we consider to be zoonotic diseases). Three of the most important are salmonellosis, rabies, and leptospirosis.

**Diseases transmitted specifically from horses and humans**

- Salmonellosis is one of the most commonly diagnosed infectious causes of diarrhea in adult horses, and foals are also susceptible. Transmission often occurs by ingesting feed or water contaminated by manure.
- Rabies is a viral disease transmitted by contact between the rabid animal’s saliva into open wounds or mucous membranes. Often the saliva is delivered via the bite of a rabid animal such as a skunk, raccoon, or bat. While the incidence of rabies in horses is relatively low (due to successful vaccination efforts), the disease is invariably fatal and has considerable public health significance.
- Leptospirosis is a potentially fatal bacterial disease that humans and horses can contract through direct or indirect contact with infected urine, as well as ingestion of contaminated water, hay, or grain. Leptospirosis is now classified as a re-emerging infectious disease by the Centers for Disease Control and the World Health Organization. Leptospirosis in horses has a range of symptoms including fever, loss of appetite, lethargy, light sensitivity, abortion, kidney disease, and eye problems such as swelling, redness, discharge, and cloudiness.

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**How to prevent infectious and zoonotic disease on your farm**

Awareness is the first necessary element for prevention. As an owner, not only can you dramatically minimize introduction of and spread of many diseases, you can contribute to a longer and better quality of life for you and your horses by following this simple step-by-step checklist:

1) **Get Informed**
   - Turn to a reliable source such as your veterinarian, your local veterinary teaching hospital, or appropriate state/federal officials for accurate information and advice about infectious and zoonotic diseases, routine vaccinations, and preventive medicine.

2) **Create a Biosecurity Plan/Avoid Exposure**
   - A biosecurity plan is a set of control measures designed to reduce the spread of infectious diseases. Having one in place will decrease the chances that your horse will contract an infectious disease. Consult with your veterinarian to create a biosecurity plan and vaccination program. This should include fly, rodent, bird, and pest control, as well as traffic control on the farm.

   - Don’t take your horse anywhere where horses are known to be sick.

3) **Monitor/Isolate Any Exposed and Sick Horses**
   - If your horse becomes exposed to a contagious disease, isolate him for at least 14 days (30 days is ideal) in a stall/pen at least 30-40 feet away from other horses. Monitor temperature every day. Talk to your veterinarian on what to look for depending on what disease your horse was exposed to.

   - Isolate sick, new, and horses returning from a show or event for a minimum of 14 days. Only mix them with other horses if they have not had a fever and have remained healthy during that time. This will help reduce the risk of introducing any infectious disease to the other resident horses.

   - If your horse becomes sick, work with your veterinarian to provide appropriate care while keeping the sick animal isolated from other horses. A separate barn is ideal, or you can designate a quarantine area at the far end of your barn away from the main traffic area and other horses.

   - Immediately isolate any horse with nasal discharge, cough, fever, or diarrhea.

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4) Prevent Spread
A. Clearly mark the quarantine/isolation section with duct tape on the barn floor or rope along with signs to identify to everyone where the quarantine area is.
B. Reduce the amount of standing water and manure in the environment to limit flies and mosquitoes which can carry disease.
C. Keep feed rooms, tack rooms, and other stable areas tidy and well-swept to help prevent rodents, which can also carry disease.
D. Human traffic carries big potential for disease spread. Therefore, advise staff, trainers, and visitors to disinfect boots and wash hands before entering the barn and handling horses. Keep hand sanitizer available for additional disinfectant and for visitors to use.
E. Wash your hands with soap and warm water thoroughly before and after handling each sick horse or consider using gloves.
F. Always handle sick or exposed horses LAST in your daily routine.
G. Use separate equipment for sick animals.
H. Disinfect all equipment that comes into contact with a sick animal as discussed below.

5) Disinfection
A. Remove all excess dirt/debris from items to be disinfected. This includes nylon halters, bits, lip chains, grooming equipment, buckets, shovels, pitch forks, stall floors and walls, and even shoes and car/truck tires.
B. Wash each item/area first with laundry detergent or dish soap and water.
C. Immerse/soak or thoroughly wet the item/area with an appropriate disinfectant and then rinse thoroughly with water. Consult your veterinarian for guidance as to which is most effective for the surface and pathogen being treated.
D. Create a plan with your staff that includes frequent cleaning routines.

References:
2. Compendium of Measures to Prevent Disease Associated with Animals in a Public Setting. JAVMA, Vol 243, No. 9, November 1, 2013: 243(9) 1270-1288

Lungworms (continued from page 3)

Can donkeys and mules get sick from lungworms?
Here is the interesting thing about this parasite: donkeys and mules can harbor lungworms without showing any signs of infection. In fact, they rarely do. Why? Because they are the parasite’s natural host, so they may have lungworms and you would never know. But this is not the case with horses, which are therefore more susceptible to infection and the disease that results from lungworms.

What are the symptoms of lungworms?
Lungworms cause a parasitic pneumonitis (inflammation of lung tissue) from the larval stages of the worm migrating throughout the lung. This larval migration, which is part of the normal life cycle, doesn’t get completed in the horse like it does in the donkey or mule. This incomplete larval development in the horse causes inflammation and mucus production, and infected horses can have a chronic cough, exercise intolerance and nasal discharge. Pneumonia, pulmonary edema and secondary bacterial infections can be complications of lungworms, and in severe cases heavy worm burdens can lead to death.

How can I prevent lungworms?
Because donkeys and mules can live with these parasites without obvious consequences to their health, they are considered the main source of pasture contamination for horses. For this reason, it is important to be aware of lungworms and what signs to look for in the case of infection of your horse. Ideally, separating donkeys, mules and horses at pasture is the best way to prevent lungworm infection in horses. Routine deworming of your pasture animals as recommended by your veterinarian is helpful as there are few effective dewormers available for treating lungworms and the avermectins work best. If you have donkeys, mules and horses on the same farm, talk to your veterinarian at your next annual visit to make sure you are appropriately deworming for lungworms so that they can continue to live together in good health.
What does EGUS stand for and why do I need to know about it?

EGUS is an acronym for Equine Gastric Ulcer Syndrome. This syndrome is seen in horses of all ages and occurs when the gastric mucosa (stomach lining) becomes damaged. In the horse, there are two parts of the stomach, a glandular and non-glandular portion. These two areas are separated by a delineation called the margo plicatus. Ulcers are most commonly seen in the non-glandular mucosa (lining of the stomach) near the margo plicatus. The disease is very common, with up to 90% of horses reported to be affected yearly.

What causes EGUS?

The development of ulcers is multi-factorial. Associated stressors that can increase the chance of ulcer development include:

- Training and exercise: studies have shown that horses in training or racing are at higher risk of ulceration due to increased production of gastric acid in the stomach and decreased blood flow to the gastrointestinal tract. Additionally, the increased pressure and movement in the stomach that occurs during exercise increases exposure of acids to the more sensitive non-glandular mucosa.

- Feeding schedule: equine gastrointestinal tracts are built to handle frequent, small meals and continuously secrete gastric acids (up to 9 gallons per day!). Therefore, if food is absent for a long period of time, the acid can begin to break down mucosa.

- Type of feed: high concentrate feeds or low-protein, low-calcium hay have been associated with increased ulcer development.

- Environment: travel, competition, limited turnout, and changes in routine can cause stress and increase risk of ulceration.

- Nonsteroidal anti-inflammatory (NSAID) use: chronic and/or high-dose usage of NSAIDs, specifically phenylbutazone (Bute) and flunixin meglumine (Banamine), decreases protective mechanisms of the glandular mucosa.

How can I tell if my horse has EGUS?

Gastric ulcers can be difficult to detect in your horse and signs are often subtle. Signs that may indicate that your horse has ulcers may include:

- Mild signs of colic, such as lying down more frequently, looking at the abdomen, stretching out, pawing, etc. This is more likely to occur after eating.

- Decreased appetite or a change in eating habits, such as disinterest in eating grain.

- Decreased body condition and weight loss.

- Behavioral changes, such as decreased performance or reluctance to train.

- Resistance to grooming and girth application.

- Dull appearance.

It is important to note that foals may show additional signs of discomfort if ulcers are present, including lip curling, bruxism (teeth grinding), or lying directly on their back.

Because NSAID toxicity may also cause colitis (inflammation of the colon) horses with gastric ulcers from NSAID use may also have diarrhea and/or edema (fluid accumulation under the skin, which is typically found on the belly and/or under the jaw).

The only way to definitively diagnose ulcers is through gastroscopy, which is a minimally invasive technique. Your veterinarian will sedate your horse and place a small video camera down into the stomach to look for ulcers. This should be performed after fasting (at least 12 hours) and water deprivation (4 hours) to ensure that the stomach lining can be visualized.

Ulcers can be graded from 0-4, with 4 being the most severe.

What is the treatment for EGUS?

The decision to treat EGUS is generally based upon clinical signs and gastroscopic diagnosis of ulceration. The goals in pursuing treatment of ulcers include pain relief, elimination of clinical signs, promotion of ulcer healing, and prevention. While there are many pharmacologic options for treatment of EGUS, the most commonly used is acid suppressive therapy. This decreases acid production continuously, allowing the mucosa to heal.

The only FDA-approved treatment and preventative medication for EGUS is omeprazole (GastroGard and Ulcer-gard, respectively). While there are less expensive, compounded formulations of omeprazole available, these are not regulated and the formulation, drug concentration, and drug quality are variable and often ineffective. The recommended treatment dose is 4 mg/kg once daily by mouth for 14-28 days. The horse should be re-evaluated 14 days after treatment is initiated to gastroscopically evaluate the response to treatment. Clinical signs typically resolve or improve within a few days to a week of treatment, but treatment for up to 8 weeks may be necessary in severe cases. A dosage of 1-2 mg/kg of omeprazole daily may be used to prevent ulcer formation, but gastroscopy should be used to evaluate efficacy.

"EGUS" Explained

By Kiersten Wiley, DVM Student (Class of 2014)
Edited by Sandra D. Taylor DVM, PhD, Dipl ACVIM, Purdue Large Animal Internal Medicine
Other treatment measures may be considered by your veterinarian if the ulcers are severe or are slow healing and may include:

- **Antacids (cimetidine, ranitidine):** these drugs decrease acid production in the stomach but require frequent dosing (minimum every 8 hours).

- **Coating agents (sucralfate, bismuth salicylate):** these drugs are similar to Pepto-Bismol in humans and function to cover the damaged mucosa, allowing it to heal. Scientific data has not proven these drugs to be effective.

- **Synthetic hormone (misoprostol):** this hormone inhibits acid secretion and increases bicarbonate production in the stomach, which combats the acidity of the stomach. In addition, this drug increases mucus production in the stomach and blood flow to the stomach, both of which protect the gastric mucosa. This medication is contraindicated in pregnant or nursing mares.

**Are there ways to prevent EGUS?**

There are many management changes that might help prevent gastric ulcers from developing:

- Allow constant access to forage. High protein, high calcium forages such as alfalfa hay have been associated with decreased gastric ulcer formation.

- Provide turn-out whenever possible to allow for grazing.

- Use minimal concentrate (grain) feeds.

- Feed smaller, more frequent meals.

- In high risk horses, a treatment dosage of omeprazole can be used for 3 days prior to and after a potentially inciting event.

- A preventative dosage of omeprazole once daily can be used in horses after treatment to prevent recurrence, which commonly occurs if management changes are not instituted.

- Minimize long term use of NSAIDs.
The Equine Sports Medicine Center

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

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

- 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.


- Taylor SD, Bianco AW, Moore GE. Anti-endotoxin properties of ketorolac tromethamine in horses.
December 22, 2014

Project Update: Investigation into the use of a topical application of a hyperosmolar nanoemulsion to wounds of the distal extremity in horses

This document will update you on where we stand with this research project. We have performed a pilot study of two horses. The pilot study allowed us to determine the best way to perform the wounds (location, limb, etc), and perfect banding techniques. Based on the results of the pilot study we decided to place individual wounds on each of the four limbs in the middle of the metacarpus/tarsus. We also decided to change the banding technique. This involved the elimination of a wound pad (which showed to encourage excessive exudate) and simplified the bandaging.

Following the completion of the pilot study, we enrolled 8 horses in the project. Currently, we have 6 horses involved in the project with 2 remaining. Four horses are finishing healing at the veterinary farm and two remain housed in the annex. Bandages have now been removed from the six horses. There are two horses remaining which must be started on the protocol. Those two horses will be started the week of January 5th. Once these two remaining horses have completed the protocol the project will be completed.

We anticipate the study to be completely finished by July 1, 2015.

Please contact me if you have any questions about this project.

Sincerely,

Jan F. Hawkins, DVM, DACVS
Primary Investigator
Significance:

Bone fractures are a significant cause of morbidity and mortality for individual horses and the general horse population. For the individual horse, fractures cause pain and suffering, are challenging and costly to treat, and in some cases result in euthanasia. In the general population, fractures have been estimated to account for approximately 10% of overall equine mortality. Over the last decade, major fractures in high profile equestrian sports have attracted national media attention. Media scrutiny has also linked the issues of rider safety and musculoskeletal injury in horses, and highlighted the animal welfare concern of these injuries, prompting several initiatives by equestrian sports governing bodies to address the safety and welfare of equine athletes. In Thoroughbred racing, the Equine Injury Database was established in 2008 to accurately identify racing injuries at a national level. Currently, the database includes over 1.8 million race starts over 5 years. The database shows an overall fatal injury rate of 1.9/1000 horse starts. As over 80% of fatalities in racing are due to fractures and a similar or greater number of injuries occur during training, it can be estimated that approximately 1,400 horses are euthanized each year in the U.S. due to fractures that occur during racing or training. However, this estimate does not account for non-fatal fractures. Over a 14 year period at 10 Japanese racetracks, the average fracture incidence (fatal and non-fatal) was reported at 1.83% of all race entrants. Applying this fracture incidence to racing starts in the U.S., over 6,500 fractures would be estimated to occur in Thoroughbred racing each year. Similar overall rates of musculoskeletal injury have been reported in Quarter Horse and Standardbred racing, although injury distributions vary between racing breeds.

There is strong evidence that the majority of racing fractures are the result of accumulated bone tissue changes and not a single “bad step” during racing or training. The evidence includes common bone fracture locations and configurations, pre-existing pathology in both fractured and non-fractured bones in the same horse and incomplete fractures identified in the same locations as common complete fractures. The tissue and bone material changes that occur with repetitive high strain loading ultimately decrease fracture resistance of the bone tissue and the entire bone. The cumulative impact of training and racing on the musculoskeletal system, reflected in changes in its resistance to injury, means that it should be possible to reduce the incidence of fractures, when we are able to determine and detect the relevant changes in structural and bone tissue properties in advance of reaching the point of high injury risk. Dr. Tim Parkin, epidemiologist and consultant to the Equine Injury Database, has written previously that “… it is imperative that novel techniques are developed that enable the identification of the at-risk horse. It is only when we can predict with some degree of certainty that an individual is at significantly increased risk that we will see a significant reduction in the number of racehorses that are injured in training or during racing.”

Measurements used in vivo to determine bone toughness, or its resistance to fracture, have primarily been based on bone mineral density (BMD). In humans, the use of dual x-ray absorptiometry to measure BMD has been a benchmark method for the evaluation of patients at risk for osteoporosis. However, other factors such as bone turnover rate, microdamage accumulation, bone matrix properties and bone geometry contribute to skeletal fracture resistance as well.

In the human clinical setting, peripheral quantitative computed tomography (pQCT) is being increasingly utilized to identify individuals at risk for fracture as it provides accurate measures of not only BMD, but also aspects of trabecular and cortical architecture, and estimates of biomechanical properties (stiffness). Recently, pQCT has been used to examine sections of MC3 condyles in horses with condylar fracture and other limb fracture. It was found that the bone volume fraction (bone volume/total volume) of the metacarpal epiphysis tended to be higher in horses with condylar fracture, horses with other limb fracture and horses older than 3 years of age.
Another diagnostic method recently introduced into the human clinical setting to assess bone quality and fracture risk is reference point indentation (RPI).\textsuperscript{22} This novel bone indentation technique has been employed in preliminary studies to assess bone tissue mechanical properties in people,\textsuperscript{23-25} and has been shown to correlate with traditional mechanical measures of bone toughness.\textsuperscript{26} Bone toughness measures using this method have been shown to correlate with whole bone fracture resistance, even at skeletal sites distant to the measurement site\textsuperscript{23-24}. An RPI instrument known as the Osteoprobe, has been developed to allow measurements to be performed directly through the skin in a conscious patient. We have performed preliminary testing using this instrument in standing horses, showing that this technology could be transferred into the equine clinical setting.\textsuperscript{27}

Raman spectroscopy is a pre-clinical technology that has been used to evaluate bone matrix and mineral composition as it relates to aging, disease and injury.\textsuperscript{28} Raman spectroscopy has been successfully applied to equine cortical bone in laboratory conditions and has the potential to be applied in an \textit{in vivo} clinical setting.\textsuperscript{29,30} Raman spectroscopy is uniquely complementary to other diagnostic technologies currently being investigated in the evaluation of bone quality in that it provides information on the mineral and matrix composition of bone.

The common diagnostic imaging modalities currently utilized in equine clinical practice to detect fractures include radiography, computed tomography, nuclear scintigraphy, MRI, and ultrasonography. Radiography, while ubiquitous clinically, has limitations in the detection of subtle early changes associated with bones at risk of fracture. However, it can provide basic structural information (dimensions) as well as identification of gross or macroscopic fractures. Standing low-field MRI or high-field MRI under general anesthesia is becoming more widely available in equine practice. Recently, evidence of edema in the cancellous bone surrounding condylar fissures and fractures in the equine MC3 were reported as a consistent finding associated with these pathologies,\textsuperscript{31} as an indication of stress related bone injury.\textsuperscript{32} We have chosen to utilize radiography in this study, as it is the most common method utilized to detect gross MC3 fracture, and MRI, as it is currently the most promising clinical method for detection of early pathology indicating stress related bone injury and possible impending fracture.

**Specific Aims:**

Aim #1: Validate currently available imaging methods (radiographs, MRI) and \textit{in vivo} approaches for measuring bone tissue material properties (reference point indentation, RPI) for predicting limb bone fracture in the individual horse. Our goal is to establish criteria for specific diagnostic measures from which an accurate assessment of skeletal fracture susceptibility can be determined for an individual horse.

Aim #2: Validate human clinical imaging (peripheral quantitative CT, pQCT) and pre-clinical tools for assessing bone composition (Raman spectroscopy) for improving prediction of skeletal fracture occurrence in horses beyond the methods validated in the 1\textsuperscript{st} specific aim. Our goal is to improve the accuracy in assessing skeletal fracture susceptibility in horses, thereby justifying development of these technologies for use in the clinical setting.

**Progress:**

1. **Summary.**

Our fundamental objective in this project is to validate existing clinical diagnostic tools and to advance pre-clinical tools for characterizing bone structure and tissue properties to form a small battery of non-invasive tests that can be used clinically to assess skeletal fracture susceptibility in the standing horse. To this end, we use the MC3 from cadaveric racehorses to relate fracture likelihood to measures made using imaging modalities and diagnostic tools currently used in veterinary and human clinical settings as well as pre-clinical tools with potential for clinical translation. The MC3 is the focus of these diagnostic tests because it is one of the most
commonly injured bones in racehorses\textsuperscript{8}, and provides the greatest clinical access to the bone’s surface, which is critical for the tissue-level tests we will conduct. While we expect that our tests will detect specific pathologies in the MC3 related to racing, we will also use this bone as a proxy for overall skeletal health. Similar approaches have been taken previously in human clinical assessments of bone health\textsuperscript{23,24}.

To assess the efficacy of the different diagnostic tools for predicting skeletal fracture, the MC3s from six groups of horses are examined:

1. racehorses in race training euthanized due to fracture of the MC3
2. racehorses in race training euthanized due to a fracture of another bone
3. racehorses in race training euthanized for reasons other than fracture
4. untrained horses euthanized due to a MC3 fracture
5. untrained horses euthanized due to a fracture of another bone
6. untrained horses euthanized for reasons other than a fracture

2. Sources for equine skeletal material.

Working in partnership with the Indiana Animal Disease Diagnostic Lab (ADDL), we have been granted access to skeletal material from racehorses euthanized in Indiana. Dr. Lescun has also been able to gain access to a limited number of horses donated through the Purdue Veterinary Teaching Hospital. To date, through two years of the project, we have achieved the following sample numbers for our six experimental groups: (1) 4 (2) 21 (3) 10 (4) 0 (5) 0 (6) 2. We have recently entered into a partnership with the University of Kentucky Veterinary Disease Diagnostic Lab (UKVDL), to gain access to skeletal material from racehorses euthanized in Kentucky and submitted to the UKVDL.

3. Preliminary Data.

Using the assessment techniques outlined in the Significance section, we are able to identify differences in the bone structure, mineral density, and material properties between fractured and non-fractured MC3s in thoroughbred racehorses. Preliminary validation of these assessment techniques (pQCT, reference point indentation, Raman spectroscopy) was conducted on paired limbs from \textit{n}=2 horses that had suffered unilateral MC3 fracture. This sample is composed of a 5yr old gelding and 2yr old female, submitted to the ADDL at Purdue University, following humane euthanasia at an Indiana racetrack. The MC3 fracture in the female was in the distal lateral condyle in the right forelimb (Fig. 1A). The MC3 fracture in the gelding was a comminuted fracture of the midshaft in the left forelimb (Fig. 1B). The contralateral limbs in each were free of noticeable pathology by x-ray. Paired fractured and contralateral intact MC3s were scanned at five anatomic levels by pQCT with the skin intact along the dorsal, medial, and lateral surfaces of the bone (Fig. 2A) and reference point indentation (Biodent & Osteoprobe).
and Raman spectroscopy measures taken at six sites on the bone. In the MC3 with the lateral condylar fracture, the original position of the slab was approximated through reconstruction with elastic bandaging prior to making pQCT measures. Therefore some pQCT measures could still be made at 75% and 90%. Raman and RPI measures at M75% were unaffected by this lateral surface fracture. In the comminuted left MC3 of the gelding, reconstruction of the midshaft was not possible, so pQCT could not be made at 25%, 50%, or 75%. Thus, these data were not included in our analysis here. Raman and RPI measures were still achieved from large dorsal and medial midshaft fragments, since only a small window (2cm²) is required to make these measures. These measures were made on the large fragments at a distance greater than 1cm from the fracture surface. These comminuted fractures are much less common than condylar fractures\textsuperscript{17}, but their presence in our larger sample would prevent some paired measures (fractured vs. non-fractured MC3s) from being made in samples showing this fracture pattern.

Trabecular bone strength index and cortical bone mineral density were reduced in the fractured MC3s, indicating reduced structural properties and bone density in the proximal and distal metaphyses (Fig. 2B). The quality of the bone tissue in the fractured MC3s was also compromised. Mineral crystallinity, as measured by Raman spectroscopy, was decreased at D75% in the fractured MC3 (Fig. 2C) as were the mineral:matrix (CH\textsubscript{2} wag)(D50%) and carbonate:amide I ratios (M50%) (data not shown). Reduced crystallinity, mineral:matrix, and carbonate:amide I all indicate a relatively immature bone tissue at these surfaces\textsuperscript{33}, perhaps consistent with pathological bone formation at these periosteal sites\textsuperscript{34}. Consistent with these observations, the RPI measures on the dorsal surfaces of the fractured MC3s showed decreased bone tissue stiffness (D75%, average unloading slope, Fig. 2E) and reduced fracture resistance (increased IDI at D75%, Fig. 2E). Interestingly, the medial surface (M25%) showed an opposite pattern from the dorsal surface, where both stiffness (unloading slope, Fig. 2E) and

Figure 2: Measures made to assess MC3 structural and bone tissue properties. (A) Position of the pQCT, Raman, and RPI measures on the horse MC3. pQCT measures were made at 10%, 25%, 50%, 75%, and 90% of bone length, relative to the proximal end. Raman and RPI measures were made at 6 locations: 3 on the Dorsal surface [proximal (D25%), midshaft (D50%), and distal (D75%)] and 3 on the Medial surface at the same proximal-distal levels (M25%, M50%, M75%). (B) Trabecular bone strength index (Tb.BSI) and cortical bone mineral density (Ct.BMD) were decreased in fractured MC3s at proximal and distal locations. (C) Trends exist for reduced crystallinity in the fractured MC3s at D50%. (D) Osteomeasure RPI indicates increased bone material strength on the proximal medial surface of the fractured MC3s. (E) Fracture toughness and stiffness, measured by BioDent RPI, were reduced in the fractured MC3s at D75%, indicated by increased IDI and decreased unload slope, respectively, in the fractured MC3s. By contrast, bone tissue stiffness increased (greater unload slope) in the fractured MC3s at M25%. Mean±SD.
bone material strength (Fig. 2D) were greater in the fractured relative to the control limbs, demonstrating that there is clear heterogeneity in the pathological regional response of the MC3, and reinforcing the value of analyzing multiple bone sites in our studies.

Table 1: Bone measures that hold potential for distinguishing between paired (fractured and intact contralateral) MC3s with n=25 samples/group (power analysis based upon paired t-test, p<0.05, 80% power). The dorsal-distal site (D75%) shows particular promise in being able to distinguish at risk MC3s based upon bone structural and material properties. The critical sample size (n=25) is the estimated number of samples we expect to gain in Experimental Group #1 (race-trained with MC3 fracture) over a 3 year period, based upon recent submission numbers to the ADDL and UKVDL.

<table>
<thead>
<tr>
<th>pQCT</th>
<th>Raman Spectroscopy</th>
<th>RPI (Biodent)</th>
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<tr>
<td>ct.BMD ct.BMC</td>
<td>Crystallinity D75%</td>
<td>1st ID D25%, D50%, D75%</td>
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<tr>
<td>ct.BMC</td>
<td>Min:Mat (CH2 wag) D50%</td>
<td>1st ULS D25%, D50%, M50%</td>
</tr>
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<td>tb.BSI</td>
<td>Amide I:Amide III D75%</td>
<td>1st CID D50%, D75%, M50%</td>
</tr>
<tr>
<td></td>
<td>Carb:Amide I M50%</td>
<td>TID D25%, D50%, D75%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>IDI D25%, D75%, M50%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>RPI (Osteoprobe) Avg. CID D75%, M25%, M50%</td>
</tr>
<tr>
<td></td>
<td>RPI (Biodent)</td>
<td>Avg. ULS D25%, D75%, M25%, M50%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Avg. LS D25%, D75%, M25%</td>
</tr>
</tbody>
</table>

Power analyses were conducted for all variables measured in the n=2 paired MC3 samples discussed above. The difference reported between fractured and non-fractured MC3s in our current sample (n=2) were either significant at p<0.05, or showed trends that would likely become significant with the inclusion of the n=25 horses expected to be collected per group. Assuming that the preliminary data measured for the n=2 horses examined here is characteristic of the means and variances of a larger population, Table 1 describes the variables and anatomical sites at which significant differences would be expected between fractured and intact contralateral MC3s based upon power analysis.

Publications arising form this work:
None

Extramural funding stemming from this work:
None in the past year. We do presently have a funded NIFA Hatch Grant for this work: Validation of an in vivo assessment for fracture risk in equine limb bones (2012). In 2014, we applied for funding from (1) Morris Animal Foundation: Large Animal Study (not funded) and (2) Grayson-Jockey Club Research Foundation (pending).

Literature Cited:


Anti-endotoxin properties of ketorolac tromethamine in horses

Principle Investigator: Sandra D. Taylor

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

Specific Aim 1 is currently underway. We have successfully isolated peripheral blood mononuclear cells (PBMC) from healthy adult horses several times, but we have had difficulty in isolating equine monocytes from the PBMC population. Methods described by Cook et al (2011) resulted in monocyte activation and subsequent monocyte adherence to plastic culture plates. After contacting Dr. Cook, we obtained a more specific protocol for equine monocyte isolation that will be attempted in December of 2014. Unique aspects of the protocol that should prevent monocyte activation include a special formulation of Hank’s balanced salt solution (devoid of magnesium or calcium), glass bottles for blood collection, and techniques to handle cells very carefully without introducing bubbles or trauma during isolation and culture.

Minor delays have also been necessary as one of the Co-Investigator’s (Dr. Alex Bianco) is a large animal medicine resident and has limited research time. We are confident that this detailed protocol will be instrumental in successful monocyte isolation. However, in the event that isolation of equine monocytes is not possible, we will contact Dr. Sam Jones and Dr. Sheila Nordone at North Carolina State University, who have extensive experience with white blood cell isolation and culture, to discuss methods for doing the outlined experiment using PBMC instead of equine monocytes.

It is expected that following isolation of equine monocytes (or PBMC if the decision is made to switch cell types), the remainder of Specific Aim 1 will be completed within 1-2 months, with Specific Aim 2 to immediately follow.
APPENDIX C

Research Projects Completed Supported with Pari-Mutual Funds

- Kritchevsky, J. (PI), Couetil, L. (Co-I). Serum thyroxine concentrations in horses undergoing a standardized exercise test on a high speed treadmill. 2013-2014

- Taylor SD, Bianco AW, Constable PD, Cooper BR. Pharmacokinetics of ketorolac tromethamine, a potent non-steroidal anti-inflammatory drug, in healthy adult horses (the manuscript from this study may be found in Appendix D).
Final Report: Serum thyroxine concentrations in horses undergoing standardized exercise testing

College of Veterinary Medicine Competitive Equine Research Funds Grant

Principal Investigator: Janice Kritchevsky

Co-Investigator: Laurent Couetil

Introduction and Statement of the problem:

There is anecdotal evidence that some race horses are given large amounts of thyroid supplement and it has the potential for abuse in a race setting. Veterinarians who are charged with regulation of the racing industry in Ontario, Canada, find extremely elevated post-race thyroxine concentrations in some of the horses they test – in some cases over 10X the upper limit of published normal ranges. The effect of iatrogenic hyperthyroidism on performance and overall health has not been documented in horses. It potentially contributes to the relatively high numbers of otherwise healthy racehorses that develop idiopathic atrial fibrillation. In humans, thyrotoxicosis is the most common reason for the development of atrial fibrillation without identified cardiac disease.

The work that was supported by this grant was aimed at determining thyroid hormone concentrations in fit horses that were undergoing a standardized exercise test that was designed to simulate racing conditions. This was done so we could better understand the dynamics of blood thyroxine concentrations during strenuous exercise. Samples collected in an earlier study were analyzed, greatly reducing the cost of the project.
Hypothesis: There will be little change in blood thyroxine concentrations in Standardbred racehorses before commencement of training and while undergoing a simulated race performance (SRP) on a high-speed treadmill.

Specific Aim: Determine T4 and T3 concentrations in horses before the onset of training, then immediately before and 15, 30, 90, and 120 minutes after the onset of fatigue in horses undergoing a simulated race performance (SRP).

Methods of Approach

Plasma that was collected into lithium heparin and frozen during the course of an earlier experiment designed to study the effect of furosemide administration on blood carbon dioxide concentration. The plasma had been frozen at -80°C shortly after its collection. As part of the original research protocol, 8 Standardbred racehorses were purchased and deemed sound and fit for race training. Each horse underwent a simulated race protocol (SRP) 3 times. Prior to the SRP, they received either saline, or furosemide. Frozen plasma was assayed for thyroxine (T4) and triiodothyronine (T3).

All T4 and T3 determinations were performed at the Cornell University Animal Health Diagnostic Center Endocrinology Laboratory. Because the frozen samples were heparinized plasma and not serum, a chemiluminescent immunoassay would not produce accurate results for thyroid hormone concentrations. Assay via RIA, however, is accurate whether serum or plasma is used. Cornell offers RIA thyroid hormone determination.

Statistical Analysis

Data was analyzed using a statistical computer software package (Statistica; Statsoft, Inc.) using the Wilcoxon matched pairs test to compare repeated measurements within group (pre-training...
vs. post-training; pre-SRP vs. post-SRP). A Mann-Whitney test was used to compare T3 and T4 between furosemide and non-furosemide groups. Significance level will be placed at $p < 0.05$.

**Results**

Because the thyroid hormone concentrations were determined from heparized plasma, one cannot compare them to published reference ranges. However, if one assumes that the values were similar to what would be found in serum, all concentrations were within Cornell’s reference ranges. The blood T4 and T3 concentrations for the horses receiving saline are given in Tables 1 and 2. The values after the horses received 500 mg furosemide are given in Tables 3 and 4.

**Table 1.** Thyroxine Values (T4) in Horses undergoing a standardized exercise test

<table>
<thead>
<tr>
<th>Horse Number</th>
<th>Pre-training</th>
<th>Time of fatigue</th>
<th>+15 min</th>
<th>+30 min</th>
<th>+60</th>
<th>+90</th>
<th>+120</th>
<th>Resting</th>
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Table 2. T3 (ug/dL) Values in Horses undergoing a standardized exercise test

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Table 3. Thyroxine (ug/dL) Values in Horses undergoing a standardized exercise test after receiving 500 mg furosemide

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<td>1.51</td>
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Table 4. T3 (ug/dL) Values in Horses undergoing a standardized exercise test after receiving 500 mg furosemide

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<th>+30 min</th>
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The comparisons of T3 before training and after exercise are given in Figure 1. These are box and whisker plots from Wilcoxon Matched pairs tests. There was no difference between pre- and post-exercise thyroid hormone values in horses that received saline. Figure 2 illustrates the change in T3 after a SRP when 500 mg of furosemide was administered before exercise. The T3 concentrations 15 and 30 minutes after fatigue were significantly lower than other values. Figures 3 and 4 give T4 concentrations after fatigue without (Figure 3) and with (Figure 4) furosemide administration. The T4 values collected 90 minutes after fatigue were significantly lower than the value recorded at the time of fatigue.
Figure 1. Pre-exercise and post-exercise T3 concentrations

Figure 2.
Figure 3.

[Box & Whisker Plot]

Figure 4.

[Box & Whisker Plot]
Discussion

The data generated from this report gives strong indication that extreme exercise does not result in dramatic changes in blood thyroid hormone concentrations, although there may be an interplay between exercise, furosemide administration and T3 concentrations. This, in turn, supports the belief that extremely elevated blood thyroxine concentrations measured in post-racing blood test samples are indicators of excess exogenous administration of thyroid hormone to race horses. It also suggests that there are immediate post-exercise changes in thyroid hormone concentrations that are affected by some consequence of furosemide administration. This may be direct effect of the drug itself, or a consequence of the acid/base and electrolyte shifts that occur secondary to its administration.

This data will be used to strengthen a grant application to the Racing Medication Testing Consortium the next time a call for proposals is issues. Unfortunately, there are several problems with the data in this study that preclude its publication as a standalone paper. There are two major issues. First, as stated above, the samples that were analyzed were heparinized plasma samples. While the laboratory supervisor at Cornell believed that RIA analysis of T3 and T4 would be valid in these circumstances, there is no normal range to which we could compare values. Thus, even though the results correspond to what one might see from the customary serum concentrations, we cannot prove that they are actually in a normal range. Secondly, there were no baseline, pre-exercise samples taken on the day of testing. Rather, an intake sample taken prior to training was used as time 0. As level of fitness has a negative correlation with blood thyroxine concentrations, it is possible that the resting T4 and T3 concentrations in the horses on the day of testing were lower than their intake values and that SRP actually did result in an increase in those values up to the levels present before the horses were fit. Without samples
collected immediately prior to exercise, there is no way to prove whether this was the case or not.

This study highlights the limitations of using materials that were collected for another study with research aims and methods that were not ideal to answer the question at hand. If the goal of measuring thyroid function had been realized prior to the SRPs being performed, samples could have been collected in the proper tubes at the proper times. The research that generated the present values was extremely expensive and time-consuming, and it was not practical to repeat the entire study. Thus, we generated valuable preliminary data which should make securing funding in the future more likely.
APPENDIX D

Publications Supported by the Equine Research Internal Funds


