

RESEARCH REVIEW 2015

New and Potential Discoveries in Equine Medicine



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October 2015

Center for Equine Health

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Published by the Center for Equine Health

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Director's Message

VETERINARIANS ARE NATURAL PROBLEM SOLVERS.

They rely on critical thinking and analytical skills to decipher the problem at hand in a silent patient. The investigation that ensues uses all tools that veterinary science has to offer. As research advances veterinary medicine, new tools are developed that improve the veterinarian's ability to diagnose disease and determine how it will be resolved. It is not a coincidence that many of the great scientific discoveries benefitting both animals and humans have come at the hands of a veterinarian.

For the past 42 years, the Center for Equine Health has worked to fund research that has advanced veterinary medicine and provided new knowledge. This effort has been successful largely because the horse racing industry in California has stepped up to support research to advance equine welfare. And it has also been successful because of the financial support from many donors and foundations who value horses and their enduring bond with humans. This investment in scientific discovery has provided practicing veterinarians with new, evidence-based tools and knowledge for preventing, diagnosing and treating injury and disease.

Fifteen years ago, there was no West Nile Virus in this country nor a vaccine to prevent it. There were no laboratory tests generally available to distinguish respiratory or enteric viruses in sick horses, or evidence-based options for prevention or treatment. Equine dentistry was in its infancy, and the understanding of the connection between oral health, performance and longevity was just emerging. The decoding of the equine genome was far off on the horizon, and the relationship between genetics and immune competence was unexplored. Our teams of veterinary scientists have dedicated their work to improving the quality of the care we deliver, and their productivity is visible in this collection of research.

Why does UC Davis lead the world in advancing veterinary medicine? The combination of exceptional people leading research teams, advanced infrastructure to support the cutting-edge programs, and research funding to enable the work have culminated in a fertile field of opportunity. UC Davis has the unique strength

of housing a School of Veterinary Medicine, a School of Medicine and a College of Engineering with programs in Biomedical Engineering, all on the same campus. This landscape allows us to attract the best and brightest faculty, researchers and students. Teams of scientists are able to approach veterinary issues from a variety of fields and perspectives seeking answers to vital questions. This collaboration reflects a sophisticated One Health approach to medicine that has been shown to have far-reaching impact.

Horses are of particular interest in veterinary medicine and research as they are second only to humans as world travelers. Many horses are athletes and the research focused on preserving athleticism is shared between humans and horses, whether it be with joint health, tendon and ligament injury, or injury prevention and surface engineering. Equine veterinary medicine has informed human medicine about fracture repair, wound healing, regenerative medicine, diagnostic imaging, and neonatal brain development, to name a few.

So what do these advancements mean for you and your horse?

- New biological screening and treatment options for horses with musculoskeletal injuries.
- Advanced imaging tools to differentiate chronic from acute lesions within the equine foot.
- New diagnostic tests and treatments for emerging infectious diseases.
- Improved anesthetic protocols and safety for patients requiring general anesthesia.
- New options for treatment of foals with neonatal health issues.

These are a few highlights of updates you will read about in this Research Review, which has been made possible through the generosity of many donors who love horses and support research that ultimately allows us to better care for them. Thank you to all who invest in the future of the horse.

**Claudia Sonder, DVM, Director
Center for Equine Health**



CEH Scientific Review Committee

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The Scientific Review Committee evaluates all research proposals submitted for funding, critiquing and scoring the grants based on overall relevance and scientific merit. Grants scoring within the accepted range are funded.

CEH Focused Research Initiatives

These initiatives were founded by the generous contributions of private individuals and/or organizations concerned with the health and well-being of animals, especially horses.

The Center for Equine Health has established several focused research initiatives to concentrate resources, expertise, cutting-edge technology and state-of-the-art equipment in certain areas of scientific research. These initiatives are conducted under the auspices of the CEH and were founded by the generous contributions of private individuals and organizations concerned with the health and well-being of animals, especially horses.

Established in 1988, the **J. D. Wheat Veterinary Orthopedic Research Laboratory** focuses on equine musculoskeletal diseases. In 1997, the Dolly Green Research Foundation of Southern California provided a \$1 million endowment in Dr. J. D. Wheat's name. Dr. Wheat was a professor emeritus and a founding faculty member of the UC Davis School of Veterinary Medicine whose visionary leadership helped develop this orthopedic research laboratory. The Dolly Green Foundation, having recognized the orthopedic laboratory's contribution to the welfare and protection of the equine athlete, wanted to protect the work of ensuing generations of scientists and to honor one of its founding scientists.

Performance horses incur a wide variety of athletic injuries that are unique to their particular athletic pursuit. Scientists are working hard to discover risk factors, preventive measures and effective treatments for each. The orthopedic laboratory has expanded its scope to include companion animals, livestock, and wildlife species. Under the direction of Dr. Susan Stover, the orthopedic laboratory's vision is to (1) improve sport horse and companion animal welfare, (2) understand causes of injury and disease, (3) develop better methods for diagnosing, treating, and preventing injury and disease, and (4) provide education to ensure that equestrian sports, pleasure riding and companion animals may be safely enjoyed.

Over the past few years, researchers in the J. D. Wheat Veterinary Orthopedic Research Laboratory have achieved some major accomplishments that will significantly benefit horseracing, performance and/or pleasure horses:

- Discovered the specific sites of stress remodeling that precede and precipitate proximal sesamoid bone fractures in racehorses.
- Found that cushion depth had a much greater effect on race surface properties than the composition of the surface.
- Demonstrated that the type of race surface material affects the interaction of the hoof with the surface, specifically hoof slide.
- Demonstrated that nailing the horseshoe to the hoof further back restricted normal hoof heel expansion in racehorses.
- Discovered the large variability in the mechanical performance of different arena surface properties, and that the mechanical performance did not align in the expected direction with the type of surface material.

- Demonstrated that dressage arena surface properties affect fetlock motion, and thus are likely to affect risk for fetlock suspensory apparatus injuries.
- Demonstrated that a technique for measuring bone toughness may be useful in detecting horses with bone fragility syndrome (silicate-associated osteoporosis).
- Developed a method that could be useful for assessing the efficacy of regenerative therapies on bone healing.
- Determined differences in fetlock extension and how the hoof interacts with the surface between dirt and synthetic surfaces.

Great strides have been made in discovering the causes of catastrophic injury in racehorses. We have an even better understanding of the events leading to bone fracture and better techniques to detect stress fractures in live horses. We can identify race surface and exercise factors that place horses at increased risk for catastrophic injury and provide horseshoe recommendations to prevent suspensory apparatus injury. But the challenge continues in order to make equestrian sports and horseracing safer for horses and people. Additional support of the J.D. Wheat Endowment is crucial to the laboratory's continued productivity.

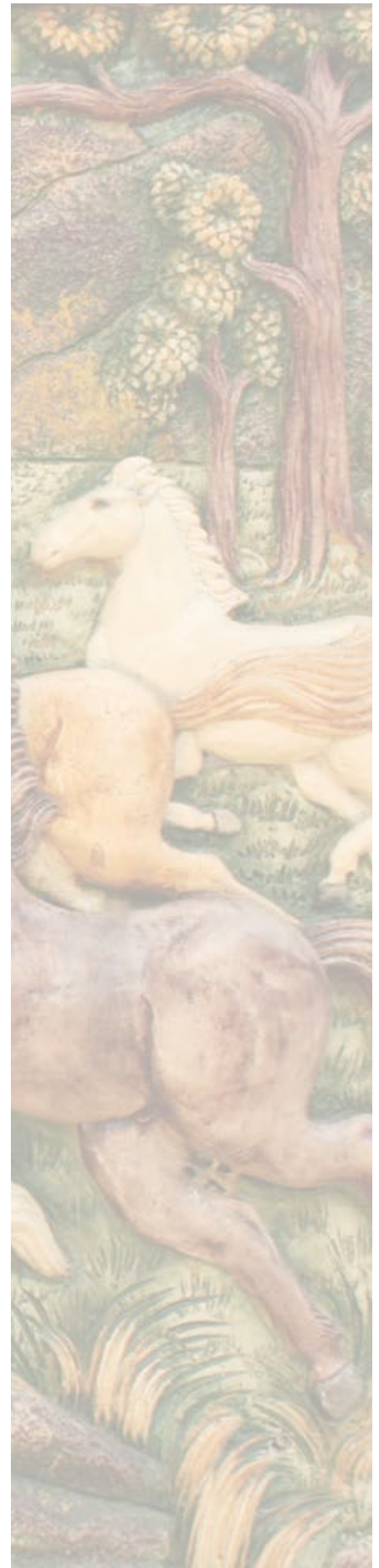


The Center for Equine Health's **Stem Cell Regenerative Medicine Group** was established in 2007, fueled by the high level of veterinary intervention required to re-establish soundness in performance horses. The program has continued to grow and has made remarkable progress in basic translational* and clinical research as well as the therapeutic use of these cells for a wide variety of equine disorders and injuries. The group has maintained collaborative partnerships with other stem cell scientists from the university's **College of Biological Sciences, Department of Biomedical Engineering, and the School of Medicine's Institute for Regenerative Cures.**

The initial emphasis of the program was to target the potential of mesenchymal stem cells (MSCs) for orthopedic repair, including bone healing and tendon and ligament repair. We continue to expand our use of MSCs to treat equine patients with these disorders. We are also developing a panel of "biomarkers" that we can detect in blood and tissue samples from horses and other species to help us understand how stem cells are working to heal tissues and moderate inflammation as well as help predict what patients and diseases are most likely to respond to stem cell therapy.

The regenerative medicine team has developed additional projects focused on research and clinical work for both equine and small animal medicine. We now have strong scientific disease teams in the following areas:

* Translational research applies findings from basic science to clinical practice and meaningful health outcomes. In human medicine, translational research is described as "from bench to bedside" or from laboratory experiments through clinical trials to point-of-care patient applications.





- Tissue engineering – heart valve replacement; small vessel repair; cartilage repair
- Stem cell imaging – tracking stem cells to the liver, foot and hoof, spinal cord and intestines.
- Oral disease – mandibular repair in dogs with bone loss due to cancer or trauma and the treatment of chronic, severe oral inflammatory diseases in cats
- Spinal cord injury – a unique “neural” stem cell derived from canine skin is isolated, expanded and will be injected into dogs with spinal cord injury
- Ophthalmic disease – inflammatory and immune-mediated eye diseases in dogs and horses, including dry eye and recurrent uveitis, diseases with significant morbidity in people and animals
- Gastrointestinal disease – inflammatory bowel disease in dogs
- Wound healing – MSCs embedded in matrix to augment wound healing in horses
- Cardiovascular repair – using small molecules secreted by MSCs to help heal diseases of the heart
- Neurologic disease in horses – wobblers (cervical vertebral stenotic myelopathy) and chronic inflammation associated with equine protozoal myeloencephalitis (EPM).

Each of these areas of research will have a major impact on the health and medical care of animals and humans in the near future.

Areas of Future Funding Needs in Equine Regenerative Medicine

Infectious, Inflammatory and Immune-Mediated Diseases in Horses

Currently, MSCs are being investigated for the treatment of sepsis and pulmonary diseases in humans, including chronic obstructive pulmonary disease. MSCs alter inflammatory cell function and they also respond to bacterial pathogens. Studies will be needed to determine how equine MSCs interact with the cells that respond to bacterial infection (neutrophils) and how MSCs function in inflammatory airway diseases in horses.

Equine Exercise-Induced Pulmonary Hemorrhage

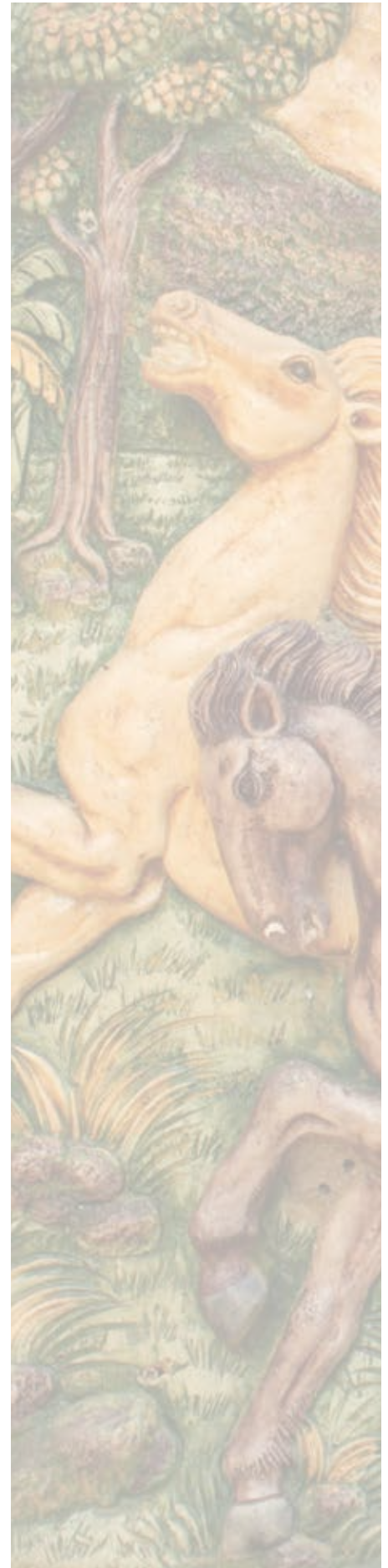
MSCs may be a very potent therapy to prevent or treat exercise-induced pulmonary hemorrhage either alone or in combination with other therapeutics. Research is needed to identify biomarkers that are associated with a risk of pathologic bleeding and to determine if MSCs moderate underlying inflammation and coagulation changes that may lead to severe bleeding.

Laminitis

MSCs increase blood flow and decrease inflammation. It is these qualities that make MSCs such a promising therapy for inflammatory and vascular diseases like laminitis. Although we have successfully used MSCs to treat laminitis, it is unclear how the cells function to help heal this condition. Studies are needed to determine what kind of stem cells are best for the treatment of laminitis, how best to administer the cells, and how they heal this unique tissue.

Recurrent Uveitis

MSCs have been shown to be effective in treating many immune-mediated and inflammatory diseases. Initial research suggests that MSCs are safe and may also help reduce inflammatory flares in horses with recurrent uveitis. Research is needed to support a clinical trial in patients with recurrent uveitis.



CEH Awards

The CEH has adopted an active role in the recruitment and development of the next generation of veterinary scientists. The equine industry will need many highly skilled and talented individuals to advance the medical management and care of horses. The CEH has stepped forward to meet this challenge by developing programs that will attract and support individuals who have demonstrated their affinity and dedication to equine medicine.

2013 JAMES M. WILSON AWARD

The 2013 James M. Wilson Award was presented to Dr. Jacob Setterbo for his work on testing the dynamic properties of equine racetrack surfaces. The Wilson Award is given each year to an outstanding equine research publication authored by a graduate academic student or resident in the UC Davis School of Veterinary Medicine. Dr. Setterbo's publication, *Validation of a Laboratory Method for Evaluating Dynamic Properties of Reconstructed Equine Racetrack Surfaces*, was honored with the Wilson Award. Dr. Setterbo received his PhD in Biomedical Engineering from UC Davis in 2011.

Track-testing device (TTD) impact tests were conducted to simulate equine hoof impact on dirt and synthetic race surfaces. Tests were performed both at the racetrack and using laboratory reconstructions of harvested surface materials. A Clegg Hammer (a portable drop hammer that measures decelerations) was used to take racetrack measurements and to guide surface reconstruction in the laboratory. Most dynamic surface property setting differences were small relative to surface material type differences. Overall, laboratory reconstruction of racetrack surfaces guided by Clegg Hammer measurements yielded TTD impact measurements similar to racetrack values. This validation of laboratory analysis of footing holds great promise for future investigation of the role of arena footing on limb load and strain.



Dr. Jacob Setterbo

2014 JAMES M. WILSON AWARD

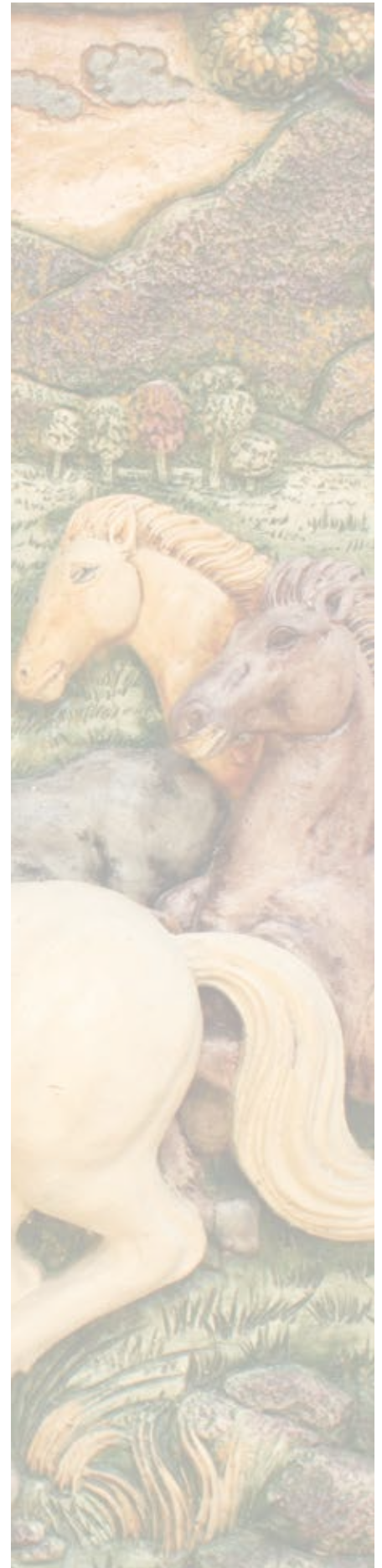
The 2014 James M. Wilson Award was presented to Dr. Isabelle Kilcoyne for her work on *Corynebacterium pseudotuberculosis* infection (Pigeon Fever) in horses. The Wilson Award is given each year to an outstanding equine research publication authored by a graduate academic student or resident in the UC Davis School of Veterinary Medicine. Dr. Kilcoyne's publication, *Frequency of Corynebacterium pseudotuberculosis Infection in Horses Across the United States During a 10-Year Period*, was honored with the Wilson Award.

Infection caused by *Corynebacterium pseudotuberculosis* in horses assumes many forms, the most common often called *pigeon fever* due to the swelling of the horse's pectoral region resembling a pigeon's breast. This study documented that the number of cases of infection has risen dramatically over the past ten years and has spread beyond the western United States to affect equine populations across the country. Although further research is needed, the study results suggest that changing environmental factors might play a role in the spread of pigeon fever. The prevalence of infection in Texas, which had 70% of the cases over the period of study, occurred during a time of extreme drought. These environmental changes likely had a significant effect on the life cycles of organisms that transmit the infection or on other unknown factors that facilitated the spread of the bacteria.



Dr. Isabelle Kilcoyne

Dr. Kilcoyne graduated from the University College of Dublin, where she spent a year as an equine surgical intern at the University Veterinary Hospital. She subsequently joined the UC Davis Equine Field Service for two years, after which she completed a residency in equine surgery at the William R. Pritchard Veterinary Medical Teaching Hospital. She is currently working as an Emergency Surgery and Equine Field Service clinician at the William R. Pritchard Veterinary Medical Teaching Hospital. Her main clinical and research interests are in emergency surgery and medicine, particularly gastrointestinal surgery.



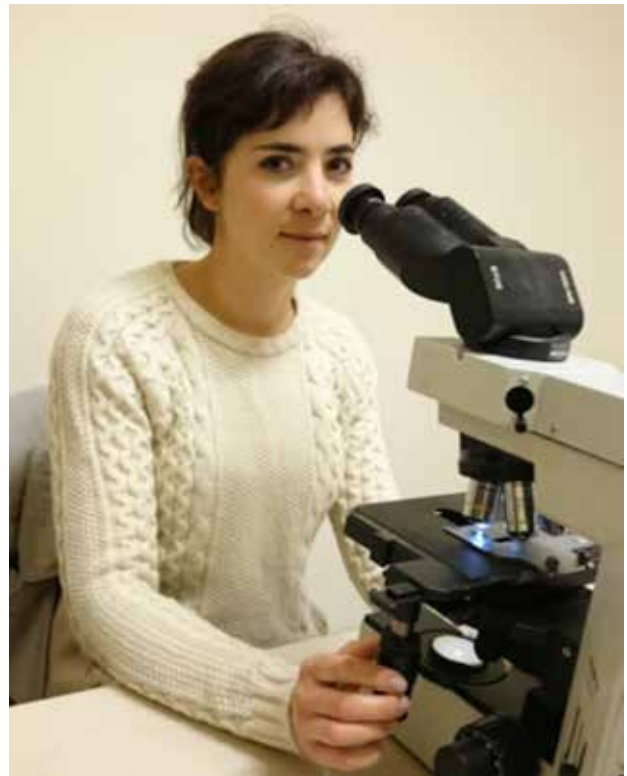
CEH Awards

The Louis R. Rowan Fellowship is funded by donations from the Oak Tree Racing Association and was established in memory of Lou Rowan, noted racehorse owner and breeder who was active in many areas that benefited people and horses in the Thoroughbred world.

2013 LOUIS R. ROWAN FELLOWSHIP

Dr. Regina Zavodovskaya was awarded the 2013 Louis R. Rowan Fellowship by the California Thoroughbred Foundation.

Dr. Zavodovskaya received her DVM from the UC Davis School of Veterinary Medicine, where she subsequently completed a three-year residency in anatomical pathology and was senior resident in her third year. She is board-certified in Veterinary Pathology. Prior to receiving her DVM, she completed a Master's program in Comparative Pathology and has authored two publications from accomplishments and experience gained during that time. Since then, she has been a contributing author on seven additional publications. Her current research interests are focused on the pathogenesis of silicate-associated osteoporosis in horses with the goal of identifying the molecular signals that link inflammation in the lung and dysregulation of cells in bone.



Dr. Regina Zavodovskaya

The Louis R. Rowan Fellowship, which is funded by donations from the Oak Tree Racing Association, was established in memory of one of the California Thoroughbred Foundation's founders. In addition to being a noted racehorse owner and breeder, Lou Rowan was active in many areas that benefited people and horses in the Thoroughbred world.

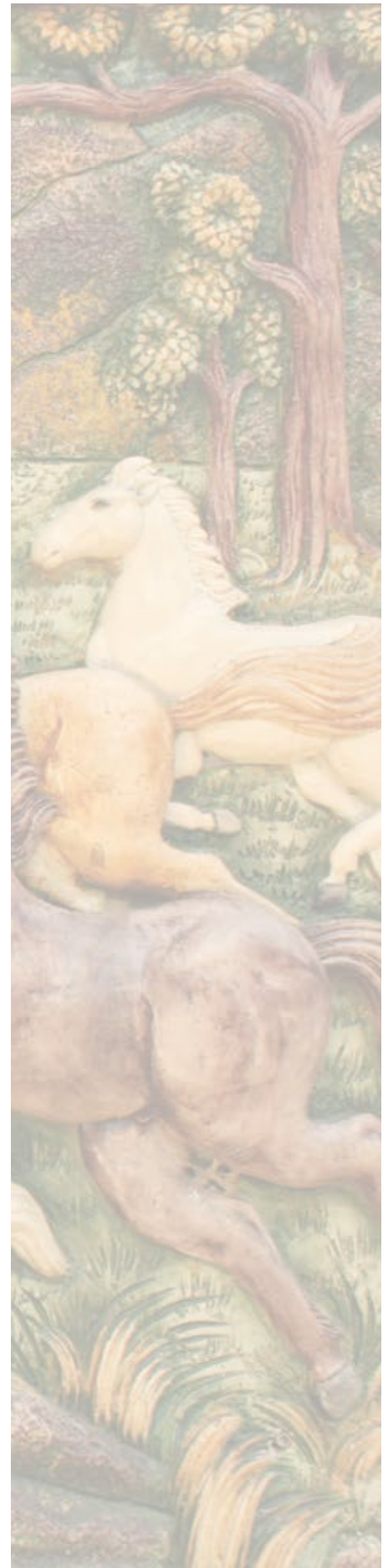
2014 LOUIS R. ROWAN FELLOWSHIP

Dr. Rana Bozorgmanesh, a resident in large animal internal medicine at the William R. Pritchard Veterinary Medical Teaching Hospital, was awarded the Louis R. Rowan Fellowship by the California Thoroughbred Foundation.

Dr. Bozorgmanesh is a graduate of the Royal Veterinary College in London. After completing an internship in internal medicine and reproduction in the UK, she accepted a fellowship in internal medicine at Hagyard Equine Medical Institute in Lexington, KY. She is currently completing a residency in large animal internal medicine at UC Davis. Her interests include gastrointestinal disease, neonatal medicine, and medical management of the critical broodmare.



Dr. Rana Bozorgmanesh (left)



CEH Focused Research Endowments

*Perpetual funding sources
for specific areas of
equine research are
essential to the current
and long-term success
of the CEH.*

Through the University of California's managed endowment system, the Center for Equine Health has established perpetual funding sources for specific areas of equine research. These endowments are essential to the CEH's current and long-term success. So far, 17 such endowments have been established, ranging from \$10,000 to more than \$1 million. Individuals interested in supporting the CEH may contribute to one or more of these endowments or work toward creating a new one in an area of equine medicine that is of interest to them personally. For more information regarding the endowment programs, contact Dr. Claudia Sonder at **(530) 752-6433** or send an e-mail to **csonder@ucdavis.edu**.

Director's Endowment

Provides general funding for CEH research, educational or welfare activities most critical to the needs of the horse in any given year. This endowment also provides the foundation for all future CEH endeavors.

Performance Horse Endowment

Focuses on the medical problems of the mature show and event horse. Also funds long-term, in-depth studies of problems that preclude horses from performing athletically as they age. Areas of study include colic, nutrition, cardiopulmonary health, degenerative orthopedic processes and infectious disease.

Equine Athletic Performance Laboratory

Provides for the development of analytical methods for accurately evaluating the athletic conditioning and performance capability of individual horses. Once these analytical techniques are fully developed, the goal of the program will be to provide an objective evaluation of the ability of drug agents and training methods to enhance performance and decrease the risk of injury in competitive horses.

J. D. Wheat Equine Orthopedic Research Laboratory

Provides for investigation of the underlying causes of bone fractures, their prevention, and new methods of fracture repair. (Originally established by the Southern California Equine Foundation, Inc., with funds provided by the Dolly Green Research Foundation.)

Bernard and Gloria Salick Equine Viral Disease Laboratory

This endowment supports a program dedicated to international scientific investigations of emerging equine viral diseases. Its goal is to identify and control viral diseases of the horse that can affect the international movement, commerce and health of competitive equine athletes.

Animal Rescue and Disaster Medicine Endowment

Focuses on developing improved techniques for the rescue of large animals during natural disasters. The fund also supports research into various medical conditions of the animals and the development of improved treatment regimens.

Lucy G. Whittier Endowment for Equine Perinatal and Infectious Disease

Dedicated to improving the health and medical treatment of newborn foals and their dams and to conduct research on infectious diseases associated with foals.

Polly and Bill Swinerton Director's Endowment

This fund supports the activities of the CEH Director to advance the facility's teaching, research and service missions.

Peray Memorial Endowment

Provides funding for resident house officers of the UC Davis Veterinary Medical Teaching Hospital (VMTH) to conduct equine respiratory disease research.

John P. Hughes Memorial Endowment

Provides funding for VMTH resident house officers to conduct clinical research in any area of equine medicine or surgery.

Dan Evans Memorial Endowment

This endowment provides funding for VMTH resident house officers to conduct research in any area of equine medicine and surgery that is relevant to the development of their specialty board certification.

Enduring Legacy Endowment for the Advancement of Clinical Equine Medicine and Surgery

This endowment was established by a generous donor to provide for the administration of experimental or high-risk therapies to severely ill or injured horses with unique veterinary conditions for which there is a high degree of learning value associated with their condition. The fund also supports the clinical trials program within the School of Veterinary Medicine.

Marcia MacDonald Rivas Research Endowments

These funds are available to teaching and research personnel, including all faculty at the Assistant Professor level, Lecturers, and MSP Professionals with less than five years of employment in the School of Veterinary Medicine. New and junior faculty members are preferred, as are equine-related projects.

Juliette Weston Suhr Fellowship Fund

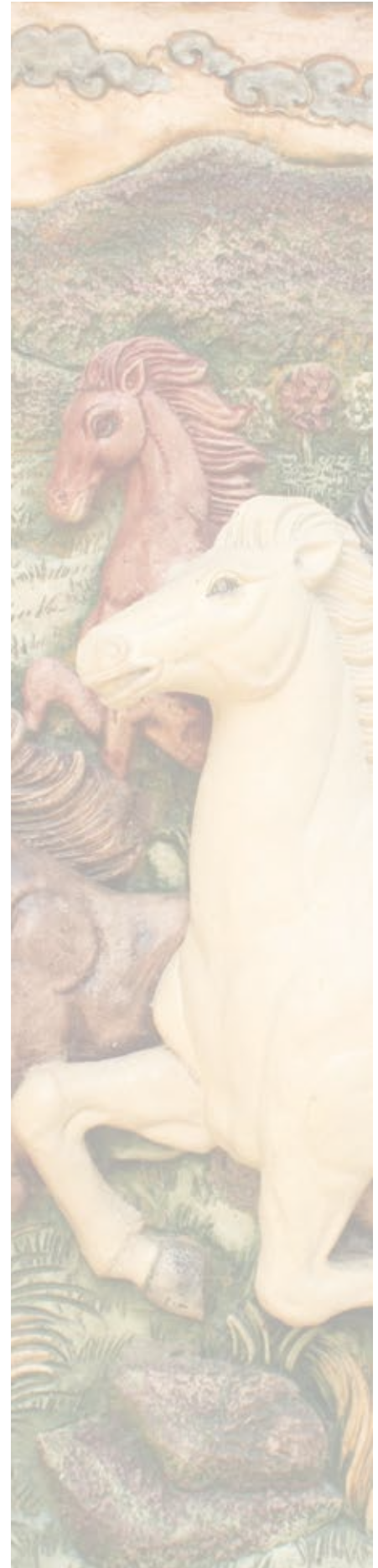
This fund provides annual fellowships for postgraduate veterinary students who are interested in conducting research in the areas of exercise-related cardiopulmonary and metabolic disorders.

William and Inez Mabie Family Foundation Endowment

This is a permanent endowed fund to support the Center for Equine Health in its operational, educational and research efforts. Endowment is distributed at the discretion of the CEH Director for advancing the health, well being, performance, and veterinary care of horses through research and/or education.

Sundance Ranch Endowment

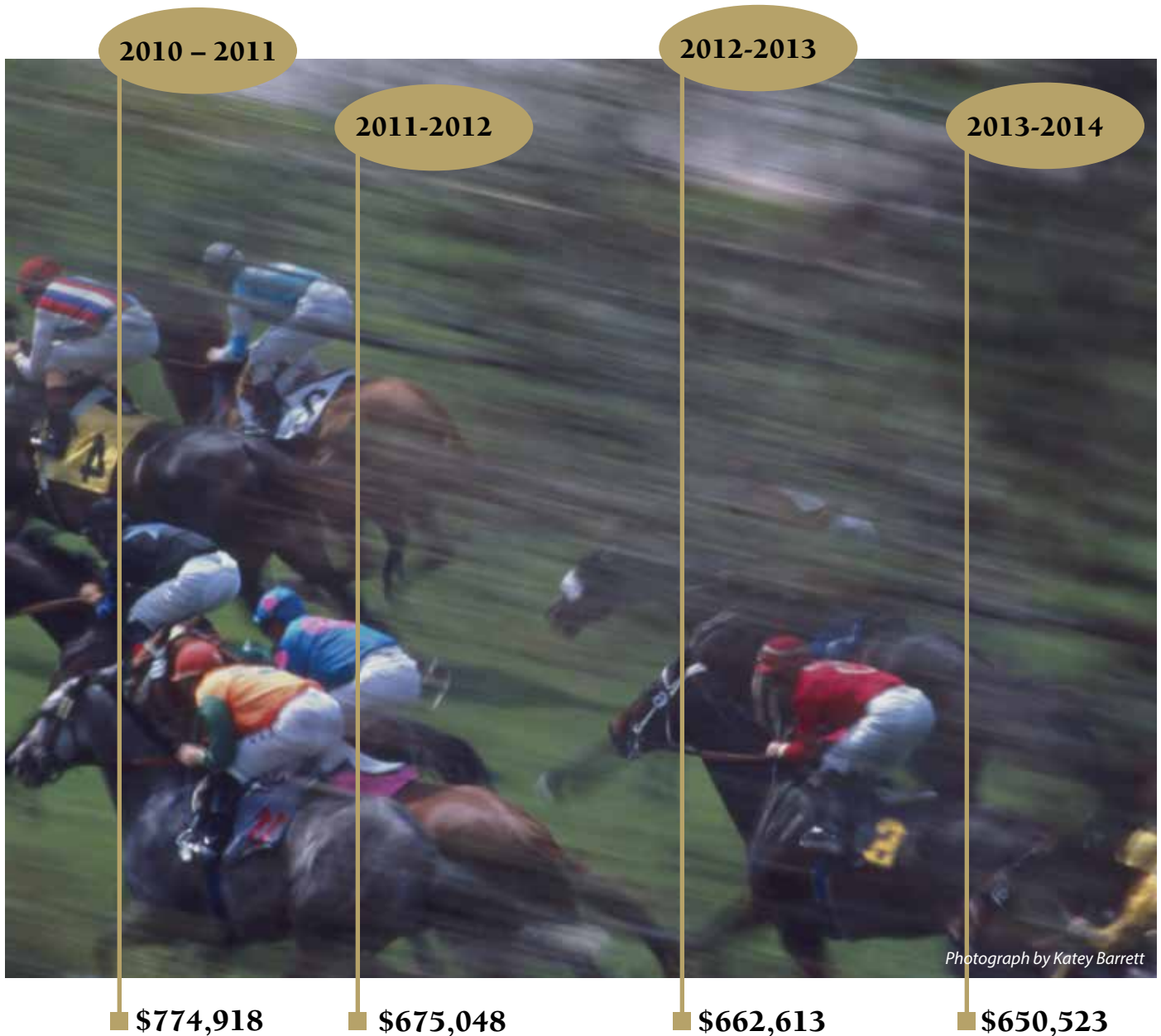
This fund was established by the late Carol Green to provide funding support for research in biological and translational research in the pursuit of effective treatments and cures for systemic diseases of the horse. Ms. Green had particular interest in medical conditions related to the development of laminitis.





Photograph by Katey Barrett

Simulcast Racing Contributions



In 1987, the Satellite Wagering Act (Senate Bill 14) designated one-tenth of one percent of California's simulcast racing handle to be used for equine research. In 1994, Senate Bill 518 was passed, designating the redistribution of the simulcast racing percentage. One-third of the simulcast money is now designated for research, while the other two-thirds is designated for the Equine Analytical Chemistry Program which has three components: (1) a full-service, routine drug testing program, (2) a forensic toxicology program,

and (3) a pharmacology research and methods development program. The latter includes the development of new tests and documentation of drug testing effects on racehorse performance. In 2001, the Account Wagering Bill (AB 471) was passed, directing simulcast contributions made through televised wagering to UC Davis equine research and drug testing programs.



Completed
RESEARCH STUDIES



Regenerative Medicine



The new Veterinary Institute for Regenerative Cures in the School of Veterinary Medicine began as a small group of research and clinical faculty who led the way in stem cell research for treating disease and injury in animals. Studies have been funded by grants from Dick and Carolyn Randall, the Harriet Pflieger Foundation, and other generous donors.

Effects of Multiple Intravenous Injections of Allogenic (Non-Self) Equine Mesenchymal Stem Cells

Mesenchymal stem cells (MSCs) are increasingly used in equine medicine to treat a variety of diseases and injuries. These stem cells can be expanded from the patient's own tissue to produce autologous cells or from a donor horse as allogenic cells. Both autologous and allogenic MSCs are being tested in human and equine clinical trials.

Allogenic MSCs offer many advantages compared with autologous MSCs because they do not require patient-specific tissue harvest, they are available for immediate application, and cell batches can be well characterized, providing a consistent source of multiple cell doses. The disadvantage of allogenic cells is that the patient may have an immune reaction against the MSCs.

Both autologous and allogenic MSCs have been safely administered to horses via different administration routes: regionally (perfusion) and locally (for example, into the joint or directly into a tendon lesion). In human clinical trials, intravenous (IV) injection is used in patients with systemic inflammatory or immune-mediated diseases (for example, heart, lung and gastrointestinal disease). In horses, IV injection may be used increasingly as we move toward cell-based therapies for systemic inflammatory diseases such as lung disease or colic.

In this study, our goal was to determine whether intravenous administration of allogenic MSCs would be safe in healthy horses as determined by measuring the systemic immune and inflammatory response to the MSCs after multiple injections. Allogenic MSCs were derived from bone marrow and fat tissue. We found no adverse reactions or changes in any clinical parameters measured (e.g., heart rate, temperature) and that, on the whole, multiple IV injections of allogenic MSCs were well tolerated in healthy horses.

Interestingly, allogenic MSCs derived from bone marrow, but not fat tissue, induced a mild, detectable immune response in peripheral blood, but the clinical significance of this response is unknown. This study also helped us understand how MSCs may interact with immune cells in a healthy horse.

How does this research benefit horses?

Intravenous administration of MSCs will potentially allow for treatment of a greater variety of lesions that involve multiple systems or cells of the immune system, including laminitis, recurrent uveitis, inflammatory skin diseases, intestinal inflammation, and neurologic disease. These results have informed ongoing

clinical trials sponsored by the Center for Equine Health and indicate that further development of novel therapies using IV administered allogenic MSCs for equine systemic diseases should be pursued.

Investigators: Joshua Wood, Danielle Carrade Holt, Jessica Gillette, Laurie Bohannon-Worsley, Sarah Puchalski, Naomi Walker, Kaitlin Clark, Johanna Watson, and Dori Borjesson



Equine Bone Marrow Volume Reduction, Red Blood Cell Depletion, and Mononuclear Cell Recovery Using the PrepaCyte-CB Processing System

Mesenchymal stem cells (MSCs) have been used extensively for treating soft tissue and orthopedic injuries in horses. While many tissues in adult horses as well as other animals can be used to create a stem cell product, bone marrow is perhaps the most common tissue that is collected from a patient and expanded in the laboratory for treatment with autologous cells—cells derived from the patient’s own body.

Because bone marrow samples contain MSCs along with many other cell types, and high numbers of MSCs are needed for patient treatment, a variety of isolation, concentration and enrichment techniques must be used to prepare the cells. The clinical Regenerative Medicine Laboratory at the School of Veterinary Medicine has developed protocols and techniques to optimize MSC isolation for clinical use.

In this study, we tested a proprietary kit that allows rapid and specific isolation of living cells that are not contaminated with bacteria. The kit is known as the PrepaCyte-CB system. In human studies, the PrepaCyte-CB system provides fast and reliable separation of human cells with less equipment and technical expertise than the automated processing systems currently used in veterinary medicine to optimize stem cell isolation from blood and bone marrow samples.

Over the past seven years, faculty have committed significant time and effort into transforming the School of Veterinary Medicine into a national leader of veterinary regenerative medicine.



Researchers in the Veterinary Institute for Regenerative Cures have established laboratory techniques and animal models in which to study regenerative therapies in veterinary and human medicine. They have characterized equine, canine and feline stem cells isolated from different tissues with a focus on adult-derived mesenchymal stem cells.



The goal of this study was to determine whether the PrepaCyte-CB system could be used to effectively and reliably concentrate equine bone marrow samples to promote isolation of MSCs.

When we compared processing with the PrepaCyte-CB system against the automated bone marrow processing method we used previously, we found that the PrepaCyte-CB system resulted in higher recovery of the bone marrow cell fraction that contains MSCs. In addition, the PrepaCyte-CB system reduced blood contamination of the sample and substantially reduced the sample volume to facilitate long-term sample storage. Smaller sample sizes reduce cost and decrease the need for preservatives, which can decrease stem cell quality.

This study showed that the PrepaCyte-CB processing system provides a superior method for the recovery of bone marrow cells that are critical for MSC expansion while also reducing sample volume and blood contamination in a sterile system. The processing system is relatively inexpensive and is technically easy to use and thus can be used in many settings.

How does this research benefit horses?

The PrepaCyte-CB system has now become a standard tool in our clinical Regenerative Medicine Laboratory. Equine bone marrow samples collected from client-owned horses with naturally occurring diseases and from horses enrolled in clinical trials can now be reliably processed with minimal risk of contamination and cell loss. Samples processed using the PrepaCyte-CB system can also be stored for later therapeutic use in long-term storage units with little risk to cell quality and function.

Investigators: Andrew Burton, Kaitlin Clark, Dori Borjesson, Danielle Carrade, Julie Burges, and Sean Owens

Equine Mesenchymal Stem Cells Inhibit T Cell Proliferation through Different Mechanisms Depending on Tissue Source

Equine mesenchymal stem cells (MSCs) are primarily used for tissue regeneration and repair. However, approximately 11% of human MSC-based clinical trials are for the treatment of autoimmune diseases and disorders, including graft-versus-host disease, Crohn's disease and colitis. MSCs function in this context by decreasing the cells and products of inflammation, including inhibiting lymphocyte proliferation.

We previously determined that equine MSCs secrete mediators that decrease lymphocyte proliferation and function, regardless of whether the cells were derived from fat, bone marrow or umbilical cord tissue. This is also the case with human MSCs. The goal of this study was to investigate how equine MSCs interact with and inhibit lymphocyte proliferation.

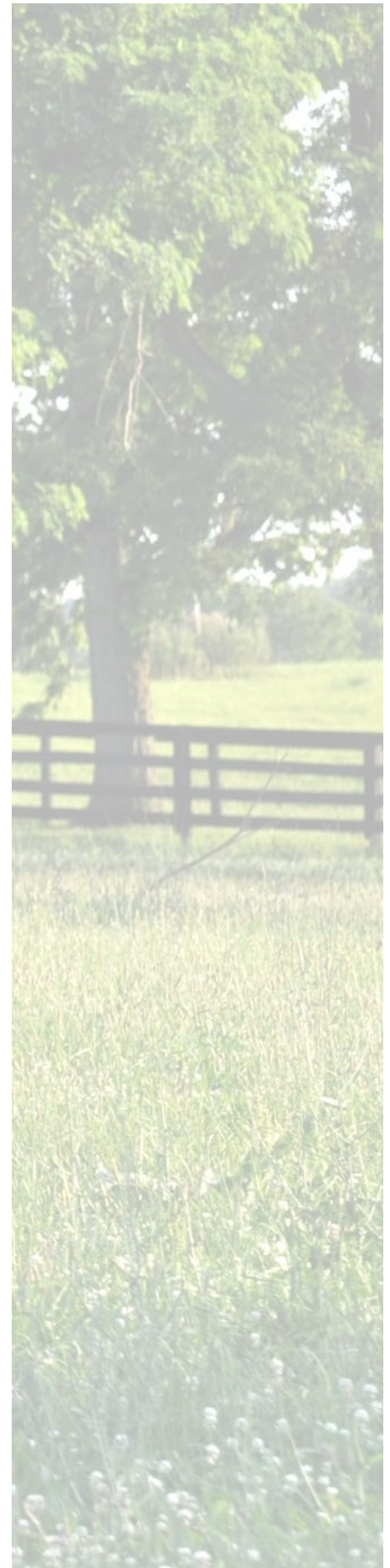
We discovered that equine MSCs all secreted high concentrations of prostaglandin E2 and that this mediator was primarily responsible for inhibiting lymphocyte proliferation and production of inflammatory proteins. Interestingly, MSCs derived from the different tissue sources altered lymphocyte function in both similar and unique ways. For example, only equine MSCs derived from bone marrow or cord blood produced nitric oxide in culture. Nitric oxide is a key mediator that helps dilate blood vessels and could be important for lesions where blood flow is a problem.

We also discovered that MSCs derived from fat and umbilical cord tissue induced lymphocyte death, while MSCs derived from bone marrow and umbilical cord blood stopped lymphocytes from dividing. This work expands what we know about how MSCs function in the context of activated lymphocytes and may have very practical implications for tailoring MSC therapy for specific diseases.

How does this research benefit horses?

Through a deeper understanding of how MSCs function in the context of inflammation and activated immune cells, we will be able to design better stem-cell-based therapeutic options for inflammatory and immune-mediated diseases in horses and other species.

Investigators: Danielle Carrade Holt, Joshua Wood, Jennifer Granick, Naomi Walker, Kaitlin Clark, and Dori Borjesson



Autologous Point-of-Care Cellular Therapies Variably Induce Equine Mesenchymal Stem Cell Migration, Proliferation and Cytokine Expression

Mesenchymal stem cells (MSCs) derived from bone marrow, fat or umbilical cord blood are being used increasingly for bone, tendon, ligament, and cartilage repair in horses and other animal species. However, MSCs that are created from the patient's own bone marrow or fat, known as autologous cells, cannot be used for treating the acute phase of disease because the cells must be cultured and expanded in a process that requires 2 to 3 weeks. At the same time, there is evidence that early treatment (within 3 to 10 days of injury) facilitates healing.

Early treatment options include allogenic (non-self) MSCs, which are cells obtained from a donor horse, or a mixture of regenerative cell products obtained from the patient that includes adipose-derived stromal vascular fraction, bone marrow mononuclear cells, cord blood mononuclear cells, and platelet rich plasma (PRP). All of these products are options for treating acute orthopedic lesions. These products likely contribute to healing by decreasing inflammation and secreting growth factors.

These cell products have a number of advantages in that they are minimally manipulated, they can be produced near patient-side for same-day treatment, they pose no risk of an immune response because they are from the patient's own body, and they will be less regulated by the FDA. These cell products are often mixed (co-injected) with allogenic or autologous MSCs, presumably to facilitate MSC activation and secretion. However, we do not completely understand how these products interact.

For this study, we developed an *in vitro* model (in a test tube/culture dish) to determine how these cell therapy products may alter MSC functions including MSC migration, proliferation (increase) and secretion. We found that all cell therapy products increased MSC proliferation, but fat-derived stromal vascular fraction induced significantly more proliferation than any other product. This fat-derived product also promoted MSC movement (migration) through a barrier of proteins. This type of movement is critical for MSC function as MSCs need to travel through blood vessels and tissues to reach their target of inflammation or disease. Cord blood mononuclear cells stimulated MSCs to produce high concentrations of important factors that control inflammation including interleukin-6, transforming growth factor-b1, and prostaglandin E2.

Although stromal vascular fraction and PRP did not directly stimulate MSCs, these products themselves variably contained high concentrations of prostaglandin E2 and interleukin-6 (SVF) and transforming growth factor-b1 (PRP). Overall, we found that these mixed autologous cell products stimulated MSC functions with two primary patterns apparent: products either contained preformed mediators that may have intrinsic healing function, or products stimulated MSCs to secrete mediators. We did not find any contra-indications for mixing MSCs and other cell products in terms of cell function.



How does this research benefit horses?

This research showed that there may be specific clinical indications for administering these non-cultured, mixed-cell therapy products including adipose-derived stromal vascular fraction, bone marrow mononuclear cells, cord blood mononuclear cells, and platelet rich plasma. Therapy indications may include administration as a sole treatment modality prior to MSC injection, as these products are rich in some mediators, or administration concurrently with MSCs to activate MSCs for treatment of chronic lesions.

Investigators: Amir Kol, Naomi Walker, Larry Galuppo, Kaitlin Clark, Sabine Muerchler, Alyssa Bernanke, and Dori Borjesson



Effects of Therapeutic Concentrations of Gentamicin, Amikacin and Hyaluronic Acid on Cultured Bone Marrow-Derived Equine Mesenchymal Stem Cells

It is common practice in veterinary medicine to combine pharmaceuticals for local or regional treatment of injured tendons, ligaments and joints. For example, acute and chronic osteoarthritis are frequently treated with intra-articular injections of single or multiple pharmacological agents. The increasing demand for a more efficacious treatment option for osteoarthritis has led equine clinicians and researchers to investigate bone marrow-derived mesenchymal stem cells (BM-MSCs) for these types of acute and chronic conditions. MSCs facilitate tissue healing by proliferating (multiplying) and producing growth factors and other substances that decrease the cells and proteins associated with inflammation, all of which promotes tissue repair and reduces inflammation.

Joint inflammation and septic arthritis are both potential complications of intra-articular injections of BM-MSCs. As BM-MSCs gain mainstream approval for clinical use, veterinarians have been combining MSCs with other medications, including antibiotics and hyaluronic acid (used in humans to improve joint function), for soft tissue and joint therapies. It is not known if this practice compromises the health and survival of MSCs. A recent study reported that 46% of equine practitioners surveyed admix an antimicrobial agent, such as amikacin, with any intra-articular therapy in an attempt to avoid joint infection after intra-articular injection. Previous reports detail toxic effects of gentamicin on human BM-MSCs; however, the effects of these pharmacological agents on equine BM-MSC function and viability are not fully known.

The goal of this study was to determine the effects of therapeutic levels of gentamicin, amikacin and hyaluronic acid on cultured equine BM-MSCs *in vitro*. BM-MSCs from healthy horses were co-incubated with clinically relevant doses of either gentamicin, amikacin, or hyaluronic acid and the effect of these drugs on BM-MSC viability, proliferation and mediator secretion was measured. We found that incubating BM-MSCs with gentamicin resulted in >95% MSC death after 45 minutes, and that incubating BM-MSCs with amikacin resulted in >95% MSC death

Researchers have also developed interdisciplinary "disease teams" that focus on "bench to bedside" translation of stem cell therapies and have built strong collaborations with researchers in the School of Medicine, College of Engineering (Biomedical Engineering), and College of Agricultural and Environmental Sciences.

Our program has continued to grow and has made remarkable progress in the therapeutic use of stem cells for a wide variety of equine disorders and injuries.

after 2 hours. Incubation of BM-MSCs with hyaluronic acid did not change BM-MSC viability, proliferation or mediator secretion.

These results indicate that therapeutic concentrations of aminoglycoside antimicrobials (amikacin and gentamicin) are toxic to equine BM-MSCs, but the effects of hyaluronic acid on MSC viability, proliferation and mediator secretion are minimal.

How does this research benefit horses?

The findings of this study directly inform clinical practice and strongly suggest that the addition of clinical-grade aminoglycosides into a cultured BM-MSC product or BM-MSC-aminoglycoside co-injection into a joint should be avoided. These antimicrobials have a strong toxic effect on the cells and will result in cell death, thus eliminating any potential benefit of cell therapy. Our findings also suggest that co-injection of hyaluronic acid and BM-MSCs does not have any overt, negative consequences for cell function and viability.

Investigators: Laurie Bohannon-Worsley, Sean Owens, Naomi Walker, Danielle Carrade Holt, Larry Galuppo, and Dori Borjesson



Comparative Analysis of the Immunomodulatory Properties of Equine Adult-Derived Mesenchymal Stem Cells

Mesenchymal stem cells (MSCs) are being used to treat a variety of inflammatory and degenerative orthopedic injuries in horses with variable success. One potential problem is that we have little understanding of how MSCs respond to inflammation. This is critical because once MSCs are injected, they are exposed to an environment that contains cells and proteins associated with inflammation. In order to understand how MSCs heal tissues and how to optimize cellular therapy, we must determine what MSCs do when exposed to injured and/or inflamed tissues.

MSCs derived from equine bone marrow, adipose tissue, umbilical cord blood, and umbilical cord tissue are currently used by different hospitals and clinicians to treat equine disease. We know that MSCs, in general, modulate the immune system in part by producing proteins and growth factors that interact directly with blood cells and tissues to reduce inflammation, but we do not fully understand how MSCs function to heal tissues. MSCs have a distinct profile of bioactive products that work to inhibit scar formation, inhibit cell death, increase blood flow, and stimulate local cells to regenerate function.

In humans and rodents, it is clear that animal species and tissue origin are both important determinants of MSC function. To improve our understanding of MSC functioning in horses, we compared the ability of MSCs derived from different tissue sources (bone marrow, fat, cord blood, cord tissue) for their ability to inhibit lymphocyte proliferation and secrete mediators in response to activation. We found that regardless of tissue of origin, in the absence of inflammatory activation,

MSCs did not alter lymphocyte proliferation or secrete anti-inflammatory mediators except for transforming growth factor- β (TGF- β 1). When stimulated with inflammation, MSCs of all tissue types decreased lymphocyte proliferation, increased prostaglandin (PGE2) and interleukin-6 (IL-6) secretion, and decreased production of the highly pro-inflammatory mediators, tumor necrosis factor- α (TNF- α) and interferon- γ (IFN- γ). This profile of changes indicates a shift from a pro-inflammatory environment to an anti-inflammatory and pro-regenerative environment.

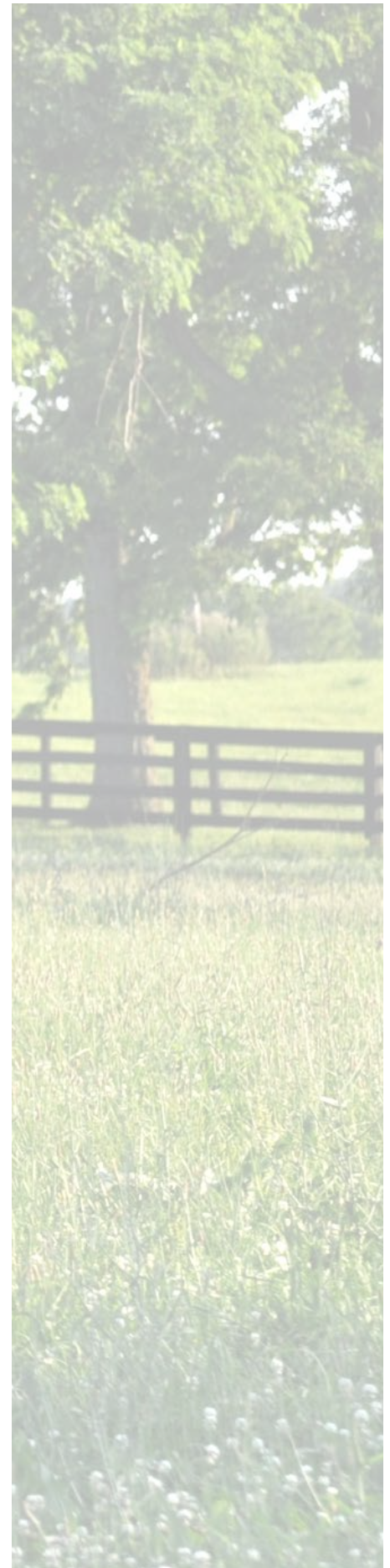
Unlike human MSCs, equine MSCs did not produce indoleamine 2,3-dioxygenase (IDO). IDO is an important enzyme that helps decrease bacterial growth and limit immune cell activation. In humans, the production of IDO by MSCs is considered one important way that MSCs decrease immune cell activity. Taken together, these data suggest that activated equine MSCs derived from bone marrow, adipose tissue, umbilical cord blood, and umbilical cord tissue secrete high concentrations of mediators and are mostly similar to MSCs from rodents and humans in how they function to decrease inflammation and promote tissue repair. Ultimately, we hope that these specific equine MSC secretion and function profiles can be used to tailor cellular therapies and better predict their ability to repair a variety of lesions.

How does this research benefit horses?

A more thorough understanding of the factors used by equine mesenchymal stem cells to help limit inflammation and heal tissues could expand the uses of these cells to include an array of equine inflammatory lesions. Among these are laminitis, inflammatory skin diseases, immune-mediated diseases and wounds, intestinal inflammation, neurologic disease, and infection. Regardless of the disease, the key to understanding MSC efficacy for tissue repair or inflammatory disease is to understand how MSCs modulate or modify inflammation.

The findings of this study will be helpful in the treatment of inflammatory lesions dominated by activated lymphocytes and the pro-inflammatory mediators, TNF- α and IFN- γ . MSCs with potent anti-inflammatory effects may be specifically selected and grown for patient use. Studies in other species, focused on immune-mediated and inflammatory diseases, suggest that MSCs may have efficacy for a variety of equine diseases including laminitis, colitis, immune-mediated dermatologic disease, intraperitoneal inflammation, and sepsis.

Investigators: Danielle Carrade Holt, Michael Lame, Michael Kent, Kaitlin Clark, Naomi Walker, and Dori Borjesson



Medicine
Orthopedics
Genetics
Immunology
Reproduction

Study of Equine and Swine Isolates of *Lawsonia intracellularis*, an Agent of Equine Proliferative Enteropathy (EPE) in Weanling Foals

Equine proliferative enteropathy (EPE) is an emerging intestinal disease in horses that primarily affects weanling foals 4 to 7 months of age. Affected foals develop a variety of nonspecific clinical signs, including decreased appetite, weight loss, fever, colic, diarrhea, intestinal thickening, and swelling.

Horses that have presented at UC Davis have received supportive treatment via intravenous fluid, plasma and antibiotics to treat the infection, with good outcomes. Diagnosis can be challenging because the clinical signs are vague. As a result, animals with EPE are often not presented until the disease has advanced and treatment is less effective. Infection can progress rapidly in weanling foals.

EPE is caused by a bacterial pathogen, *Lawsonia intracellularis*, that affects a wide range of animals besides horses, including pigs, hamsters, rabbits, fox, deer, ferrets, ostriches, and nonhuman primates. The clinical features among the different hosts are very similar. *L. intracellularis* bacteria isolated from lesions from a variety of animal species and analyzed through molecular testing showed 98% similarity of the 16S-rDNA gene. A preliminary investigation into the relationship between bacteria isolated from pigs and horses suggests that they represent different strains and therefore may be species-specific.



This Spanish filly is healthy, but at age 7 months she was brought to the William R. Pritchard Veterinary Medical Teaching Hospital where she was diagnosed with EPE. The foal was successfully treated and made a full recovery.

Although EPE infections in foals have been linked to exposure to swine feces, recent work suggests that *L. intracellularis* is species-specific and that further study of this is warranted. The objective of our study was to determine whether species-specific *L. intracellularis* isolates would cause differences in disease severity in weanling foals infected with either an equine or swine isolate of *L. intracellularis*. Clinical observation, weight, serum concentration of total protein, ultrasonographic evaluation of the small intestine, fecal excretion of *L. intracellularis*, and seroconversion (the level of antibodies in blood) were measured for each foal. Diseased foals were treated promptly with appropriate antimicrobials and supportive care.

Our study results showed that foals infected with the equine isolate developed moderate to severe clinical signs and had a lower average weight gain and serum concentration of total protein compared with control foals (those not infected). These foals also had higher IgG titer (antibodies) against *L. intracellularis* compared with the porcine-infected foals. Foals infected with the porcine isolate had no clinical signs and maintained weight gain and serum total protein concentrations similar to the control foals.

How does this research benefit horses?

This study has served to deepen our understanding of the bacterial pathogen that causes EPE in foals. Our finding that host susceptibility to EPE is driven by the origin of the *L. intracellularis* isolate will form the basis for further studies using comparative genomic analysis to characterize species-specific strains and potentially novel bacterial subspecies or genotypes.

Investigators: Nicola Pusterla, Connie Gebhart, and Fabio Vannucci

Study ID: 11-01

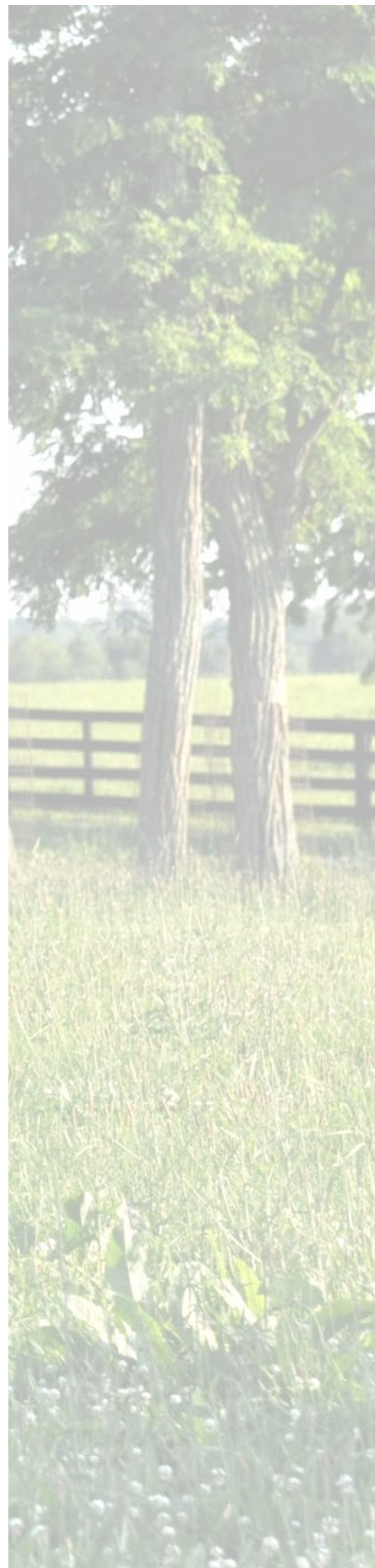


Effect of Antiprotozoal Treatment on Antibodies Against *Sarcocystis neurona*, an Agent of Equine Protozoal Myeloencephalitis (EPM)

Protozoal diseases comprise one of the most difficult areas of study for medical researchers because their modes of transmission, life cycles for reproduction, and modes of infection are not always known. Many of these diseases are impossible to reproduce experimentally, making controlled laboratory research extremely challenging.

The diagnosis of protozoal diseases also can be difficult because diagnostic tests often rely on the detection of antibodies to the parasite produced by the host. And while the presence of these antibodies demonstrates exposure, they do not prove either the active presence of the protozoa within the body or that they are the true cause of the clinical signs of disease observed.

Equine protozoal myeloencephalitis (EPM) is a disease of the central nervous system of horses caused by infection with the protozoan *Sarcocystis neurona* and typifies these difficulties and challenges. It is a progressively debilitating disease



Protozoal diseases comprise one of the most difficult areas of study for medical researchers because their modes of transmission, life cycles for reproduction, and modes of infection are not always known. Many of these diseases are impossible to reproduce experimentally, making controlled laboratory research extremely challenging.

that can affect any part of the central nervous system, from the brain to the end of the spinal cord. Clinical signs depend on the area of the central nervous system that is parasitized. Spinal cord involvement can cause gait abnormalities, incoordination, ataxia (inability to control voluntary muscle movement), spasticity in all four limbs, and muscle atrophy. Brain stem involvement can cause depression, behavioral changes and cranial nerve paralysis (facial nerve paralysis, tongue paralysis, difficulty swallowing).

A recent study by researchers at UC Davis revealed that EPM is widespread throughout the United States. The single-celled protozoal parasite *Sarcocystis neurona*, which is shed in the feces of opossums, is the most commonly recognized cause of this neurologic disease in horses. However, this study found evidence that *Neospora hughesi*, another EPM-causing parasite that was first identified in California, is now being identified in horses across the United States as well.

While the epidemiology of *S. neurona* has been well characterized, the diagnostic tests, long-term implications, and response to treatment are not well understood. Diagnosis is usually based on the presence of antibodies against *S. neurona* in serum and/or cerebrospinal fluid. Recently, a quantitative indirect fluorescent antibody test (IFAT) was developed to detect specific antibodies against both *S. neurona* and *N. hughesi*. These tests provide a quantitative indication of EPM infection as well as greater sensitivity and specificity than previous tests, enabling veterinarians to better determine the likelihood of infection in a neurologic horse.

One of the key barriers to successful treatment of EPM is the lack of understanding of how EPM titers change with treatment of the disease and whether the titers can be used to indicate a successful cure. The temporal variation of specific antibodies to *S. neurona* following treatment with antiprotozoal drugs has not yet been studied.

Previous work has shown that up to 35% of neurologically normal adult horses have serum antibodies to *S. neurona*, which reflects either recent exposure or latent infection. The objective of the present study was to determine if neurologically normal adult horses with high serum titers against *S. neurona* would display a temporal decline in such antibodies following a 60-day treatment course with ponazuril paste. The antibody response following treatment was expected to be similar between clinically infected neurologic and non-neurologic, healthy, seropositive horses.



The single-celled protozoal parasite Sarcocystis neurona is shed in the feces of opossums.

From this study, we found that seropositive titers to *S. neurona* were detectable for 60 to 120 days for horses treated with ponazuril paste and for 90 to 210 days for the seropositive nontreated horses. There was no statistically significant difference in antibody decline between the two seropositive horse groups.

The results of this study suggest that administration of ponazuril paste has little direct effect on antibody measurements in seropositive horses as long as re-exposure does not occur, and that discontinuation of antiprotozoal treatment should be based on clinical improvement and not on antibody response.

How does this research benefit horses?

The information gained from this study will further our understanding of antibody production specific to *S. neurona* in healthy, non-neurologic horses. The authors expect to extrapolate the results to horses with clinical EPM. Our findings will also increase the accuracy of the IFAT in predicting the probability of disease.

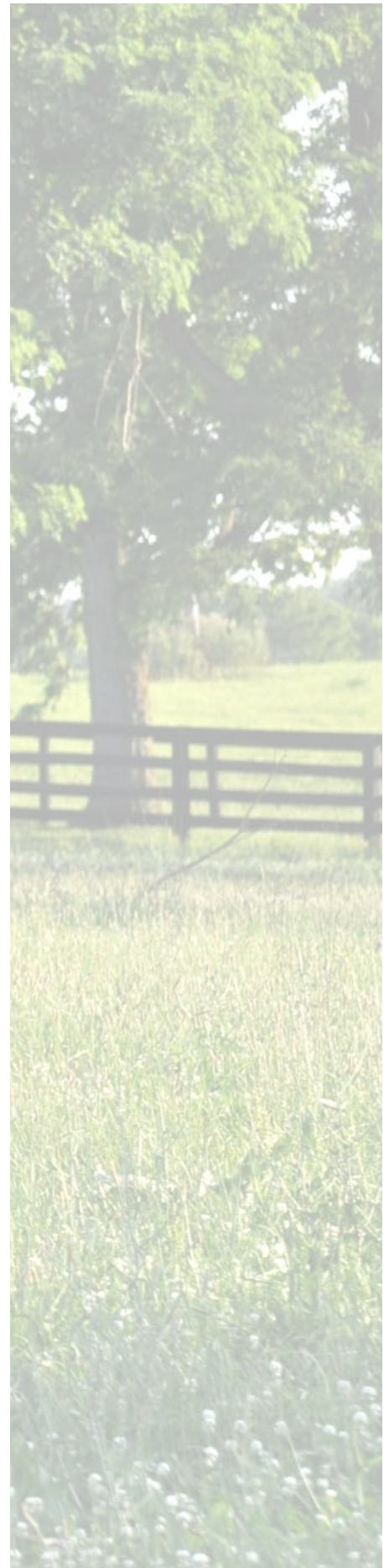
Investigators: Nicola Pusterla, W. David Wilson, Patricia Conrad, Anna Renier, and Andrea Packham

Study ID: 11-02



Heat Shock Protein 70 and 90 Modulate the Sperm Stress Response During Cryopreservation

Given the high level of global movement of sport horses, the use of frozen semen is on the rise and research aimed at improving fertility associated with frozen semen is necessary to facilitate this industry trend. The fertility of frozen semen is poor compared with that of freshly collected or cooled, shipped semen. Heat





shock proteins (HSP) protect sperm from adverse conditions, but we do not know if these proteins play any role in the survival of sperm after cryopreservation (freezing).

In this study, we aimed to identify the mechanism by which these proteins become activated during the various stages of cryopreservation and hypothesized that HSP 70 and 90 activation is necessary for sperm cell survival after cryopreservation and oxidative insult of equine sperm.

The results of this study showed that HSP 90 expression by equine sperm is increased in response to an artificially induced oxidative stress. We also found that inhibition of HSP 90 activity by the antibiotic geldanamycin, specifically targeting HSP 90, results in improved sperm motility compared with controls.

This study suggests that overexpression of HSP 90, which offers some protection for sperm exposed to oxidative byproducts, contributes to depressed sperm function and may accelerate sperm death. We suggest that treatment of stallion sperm with geldanamycin would allow the sperm to recover from the oxidative stress that occurs during freezing and improve the cryopreservation of sperm from a greater number of stallions.

How does this research benefit horses?

Insight into the ability of sperm to cope with stress is critical for developing improved sperm cryopreservation techniques. This study will benefit a variety of breed registries in the United States and abroad, as improvement in preservation of stallion genetics will allow more stallions within breeds to contribute to breeding programs and progeny production.

Investigators: Stuart Meyers and Katie Klooster

Study ID: 11-05



Genetic Study of a Neurologic Disorder in Foals with Neuroaxonal Dystrophy/ Equine Degenerative Myeloencephalopathy

During the first year of life, genetically predisposed foals may develop a neurologic disorder known as neuroaxonal dystrophy/equine degenerative myeloencephalopathy (NAD/EDM). NAD/EDM is a degenerative and irreversible disease of young horses characterized by the voluntary movement of limbs beyond the intended goal (called hypermetria). Horses suffering from NAD/EDM typically display a symmetric (left to right) incoordination that may be more severe in the hind limbs than in the forelimbs. Affected horses also display proprioceptive deficits (they do not know where their limbs are as they walk) and may also be abnormally quiet and dull.

At the present time, the only way to diagnose NAD/EDM is by examination of the spinal cord and brainstem once the horse has died. It appears that the disease is



Pictured here is a 5-year-old Quarter Horse mare with the abnormal base-wide stance of the forelimbs characteristic of NAD/EDM.

inherited and this has been supported by breeding studies in Morgans, Appaloosas, Quarter Horses, and some other breeds.

Because the entire horse genome has been sequenced, we have the ability to identify a potential genetic mutation associated with NAD/EDM. Preliminary studies have identified four candidate regions that could potentially contain a genetic mutation for NAD/EDM. The objective of the present study was to prioritize one of the four regions for further investigation. To this end, additional markers were evaluated in the four regions to determine which region was highly associated with NAD/EDM. Then the genetic makeup (genotype) was determined at each of these markers for 94 Quarter Horses (33 NAD/EDM affected and 61 unaffected).

We found that the region on chromosome ECA8 was the most significant because there were two candidate genes (PIK3C3 and RIT2) involved in transmitting signals between cells of the nervous system.

Variants were detected within both of these genes, but when they were tested to determine if they could be associated with NAD/EDM, no such association was found. Efforts are now focused on evaluating the region between these two genes.

How does this research benefit horses?

At the present time, the only way to diagnose NAD/EDM is after the horse has died or been euthanized. The disease appears to occur frequently. In evaluating horses that were euthanized due to neurologic disease, NAD/EDM was diagnosed in 23 of 96 (24%) of the cases at Cornell University. The discovery of a genetic mutation for NAD/EDM would allow veterinarians to test for the disease in neurologically abnormal horses in order to diagnose the condition. A genetic test also would aid breeders in making decisions that would decrease the overall prevalence of this disease. Further research is needed to evaluate the equine genome for a causal mutation for NAD/EDM.

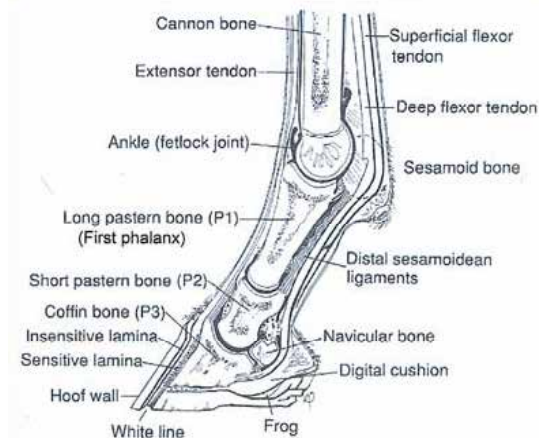
Investigators: Danika Bannasch, Carrie Finno, Monica Aleman, Robert Higgins, and John Madigan

Study ID: 11-06

The discovery of a genetic mutation for NAD/EDM would allow veterinarians to test for the disease in neurologically abnormal horses in order to diagnose the condition. A genetic test also would aid breeders in making decisions that would decrease the overall prevalence of this disease. Further research is needed to evaluate the equine genome for a causal mutation for NAD/EDM.

Development of an X-ray Technique to Detect a Fetlock Injury that Promotes Fetlock Breakdown in Racehorses

Fetlock breakdown injuries are the cause of death in 34% of Thoroughbred and 40% of Quarter Horse racehorses that die from catastrophic injuries. The mild injury that predisposes racehorses to fetlock breakdown was recently discovered in the Veterinary Orthopedic Research Laboratory, but this injury is not detectable using routine x-rays in live horses due to the superimposition of multiple bones in the fetlock. There is a need to develop an x-ray technique that can be used in live horses for detection of this injury. This could greatly enhance our ability to prevent catastrophic injury.



The objective of this study was to evaluate an x-ray taken at a new angle through the fetlock for its ability to reveal mild injury. We envision that this x-ray technique could be used to detect horses with mild injuries that predispose them to catastrophic fetlock breakdown so they could be treated and return to performance.

In this study, we compared x-rays taken at an angle, between bones with the mild injury and bones without the injury, for the ability to detect the mild injury in the fetlock. The results showed that the x-rays, including special radiographic views, were

not sensitive enough to detect lesions that predispose proximal sesamoid bones to fracture. The lesion was too small compared with the overlying bone material.

Although vascular channels were wider and more numerous in

fractured proximal sesamoid bones, we found that these features also were not visible on special clinical radiographic projections. However, this study did reveal a second mild lesion in a new location that also likely predisposes the proximal sesamoid bone to fracture. Because mild injuries are not detectable on clinically feasible x-rays, other methods should be explored for injury detection.

Early detection of overload before it causes catastrophic or permanent injury to the horse is key to successful management of the equine athlete. A new x-ray technique to detect the mild injury that predisposes horses to fetlock breakdown will assist in serious injury prevention and treatment of affected horses.



How does this research benefit horses?

Early detection of overload before it causes catastrophic or permanent injury to the horse is key to successful management of the equine athlete. A new x-ray technique to detect the mild injury that predisposes horses to fetlock breakdown will assist in serious injury prevention and treatment of affected horses. These horses could be treated, rehabilitated and returned to normal performance, thus preventing racehorse deaths due to fetlock breakdowns.

Investigators: Susan Stover, Sarah Puchalski, Tanya Garcia, and Daniela Luethy

Study ID: 11-07



Investigation of the Antibiotic Metronidazole in Newborn Foals

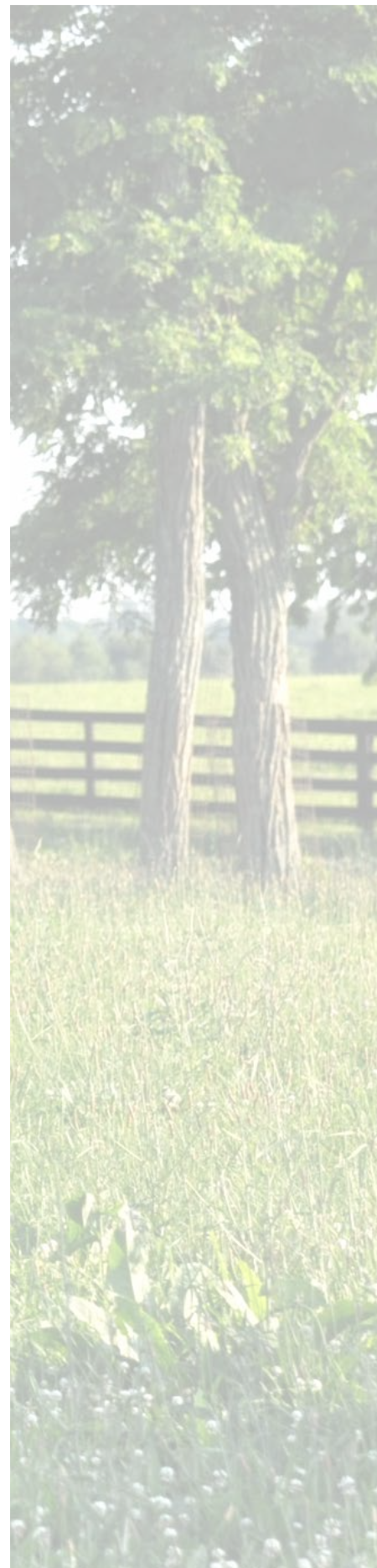
Newborn foals are known to be more sensitive to the actions of drugs compared with adult horses. Due to differences in metabolism and drug pharmacology, newborn foals often require lower drug concentrations than adult horses or even slightly older foals. Metronidazole is a critical antibiotic for highly fatal diseases of newborn foals, including clostridial diarrhea. This compound has a narrow window between an effective dose and a toxic dose. Because a toxic dose causes damage to the nervous system and liver, it is imperative to establish safe, accurate dosages.

The objective of this study was to ascertain that newborn foals require a different dose of metronidazole compared with adult horses in order to achieve effective and safe blood concentrations. We also wanted to determine whether the pharmacology of metronidazole is different between newborn foals and foals that are 10 to 12 days old.

We found that newborn foals and foals that are 10 to 12 days old have quite prolonged metabolism and clearance of metronidazole compared



We have found that newborn foals and foals that are 10 to 12 days old have quite prolonged metabolism and clearance of the antibiotic metronidazole compared with adult horses.



Cushing's disease is the most common equine endocrine disorder. Typically, it occurs in older horses, with the average age of onset being 15 years and the frequency of diagnosis generally increasing with age.



with adult horses. The half-life in foals was 11.2 to 12.3 hours in newborns (1 to 2.5 days old), compared with 2.5 to 3.9 hours in adult horses and 8.2 hours in 10- to 12-day-old foals. This necessitates the need for new dose and frequency recommendations for foals of these age groups, as the adult dose is likely to lead to undesirable toxic side effects. Twice daily dosing is more appropriate for newborn foals (1 to 2.5 days old) than the recommendation of three to four times a day for adult horses.

Foals that are 10 to 12 days of age clear metronidazole slightly faster than the newborn foals, but the maturational effects were not to a degree that would require separate dose recommendations from newborn foals.

How does this research benefit horses?

Metronidazole is a commonly used antibiotic in horses and foals for treating life-threatening infections. However, the metabolism of this drug has never been studied in newborn foals before. The previously recommended adult horse dose and frequency for administration to foals results in drug accumulation that may reach toxic levels. We have established that foals from newborn to 12 days of age require a different dosage recommendation than that for adult horses due to their prolonged metabolism of this drug. Twice daily dosing is more appropriate for newborn (1 to 2.5 days old) foals than the recommendation of three to four times a day for adult horses. Thus, we have established a safer, more accurate dosing regimen for foals.

Investigators: K. Gary Magdesian, Elsbeth Swain, Heather Knych, and Judy Edman
Study ID: 12-02



The Effect of Storage on the Stability of Adrenocorticotrophic Hormone in the Blood of Horses With and Without Cushing's Disease

Cushing's disease is the most common equine endocrine disorder. The veterinary community prefers to call it by the acronym PPID (pituitary pars intermedia dysfunction) because it provides a more accurate name for the disorder, as the cells of pars intermedia of the pituitary gland are dysfunctional. Typically, this disorder occurs in older horses, with the average age of onset being 15 years and the frequency of diagnosis generally increasing with age. Although veterinarians rarely see PPID in horses younger than 10, it has been reported anecdotally.

In PPID-affected horses, the system of pituitary-adrenal gland communication functions abnormally. These horses have an overgrowth of cells in the pars intermedia region of the pituitary gland that is dependent on the neurotransmitter dopamine. A lack of communication in the pituitary-adrenal gland axis contributes to the production of abnormally high levels of many pituitary hormones, including adrenocorticotrophic hormone (ACTH). Excess ACTH likely interferes with a horse's ability to regulate cortisol production by the adrenal glands.

Horses 18 years of age and older are at higher risk of developing PPID. With the recent introduction of a labeled drug for the control of PPID, it is important that any diagnostic modality leading to the diagnosis of PPID be as accurate as possible.



Cushing's disease is characterized by a long and/or wavy hair coat that does not shed appropriately in the spring. Horses can also be affected with this disorder before showing the hair coat change.

ACTH in blood is considered highly unstable because of the breakdown of proteins. Recent studies from companion animals have shown that pre-analytical factors, such as time to centrifugation and temperature storage of blood samples, can influence the pre-analytical stability of ACTH. The equine ambulatory practitioner is faced with several challenges, including the inability to centrifuge samples in the field and to keep samples on ice until processed for plasma collection. However, the combined effect of time to centrifugation and storage of sample prior to centrifugation on the stability of ACTH levels has not been determined for equine blood.

We hypothesized that samples processed for plasma separation within 1 and 4 hours will yield significantly higher ACTH values than samples processed at 8, 24 or 48 hours. We also expected that storage temperature and time to analysis will significantly influence the ACTH results.

In this study, we found that ACTH levels were similar between whole blood and plasma and that time affected ACTH levels with storage beyond 48 hours, dramatically reducing ACTH recovery. Freezing at -20°C and -80°C did not depreciate ACTH levels.

How does this research benefit horses?

Our study has shown that ACTH measurements were subject to degradation, but appreciable changes were seen only at 48 hours and longer in samples stored at 21°C or 4°C . Storage as whole blood or plasma had no appreciable effect on ACTH levels. Freezing samples maintained ACTH levels for at least 30 days. This information will allow equine practitioners to reasonably store blood samples without centrifugation for at least 48 hours and provide a level of confidence in the accuracy of ACTH measurement for diagnosing Cushing's disease.

Investigators: Nicola Pusterla, Sean Owens, Johanna Watson, and Joanne Hodges
Study ID: 12-03

The information gained from this study will allow equine practitioners to reasonably store blood samples without centrifugation for at least 48 hours and provide a level of confidence in the accuracy of ACTH measurement for diagnosing Cushing's disease.

Comparison of Drug Elimination Rates and Recovery Characteristics in Horses Anesthetized with Sevoflurane vs. Desflurane

General anesthesia in horses is associated with moderate risk. The most recent large-scale multi-center study showed a 0.9% risk of death within one week of general anesthesia for all non-colic equine patients. Many of these deaths resulted from injuries sustained during recovery. Having a trained anesthesia team to modify anesthetic protocols to suit the health and temperament of the horse creates the best chance for a positive outcome.



Desflurane may be a particularly advantageous drug for equine anesthetic practice because it has a lower solubility in horse blood than for any other domestic species, and thus it is eliminated from the body more quickly while allowing the horse to regain the coordination it needs to stand safely.

Horses recovering from anesthesia often experience excitement and difficulty standing, which can increase the risk of severe injuries including cuts and bruises, muscle injury, lameness, ligament tears, and even fractures. Ideally, general anesthetics that are eliminated more rapidly from the body and produce little residual ataxia and no excitement may minimize these adverse post-anesthetic events and help reduce deaths.

Desflurane may be a particularly advantageous drug for equine anesthetic practice because it has a lower solubility in horse blood than for any other domestic species, and thus it is eliminated from the body more quickly while allowing the horse to regain the coordination it needs to stand safely. It is significantly less soluble than the next closest agent, sevoflurane.

The objective of this study was to determine whether the elimination rate of desflurane is significantly faster than that of sevoflurane in horses, and whether recovery from anesthesia would be faster and of better quality.

From this study, we were able to quantitatively compare and characterize—for the first time—how horses eliminate sevoflurane and desflurane from their bodies after anesthesia. We found that horses achieved standing recovery in half the time after anesthesia with desflurane compared with sevoflurane. Horses anesthetized with desflurane tended to need fewer attempts to achieve sternal recumbency and to stand than those anesthetized with sevoflurane. Desflurane recoveries were

associated with less head slapping and banging than were sevoflurane recoveries. Subjective assessments of recovery quality were not different between anesthetics.

How does this research benefit horses?

The use of agents that shorten recovery time and improve recovery quality will improve anesthetic safety for all horses. This study showed that desflurane can be used in horses to provide the shortest recovery time and improve some objective indices of recovery quality in horses requiring general anesthesia with an inhaled agent. Improved recovery quality with desflurane may translate into fewer recovery complications, such as muscle and nerve injuries, fractures, bruising, and other trauma.

Investigators: Robert Brosnan, Ana Valente, Alonso Guedes, and Trung Pham

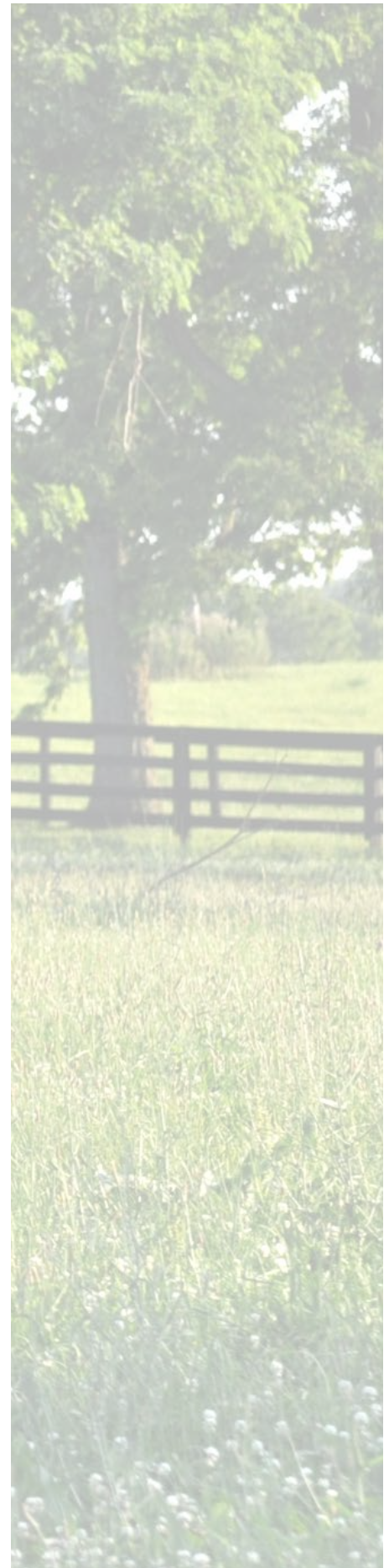
Study ID: 12-05



Control of Suprazero ROS Production During the Early Phases of Sperm Freezing, a Novel Method of Improving Cryosurvival of Stallion Sperm

Given the high level of movement of sport horses, the use of frozen semen is on the rise and research aimed at improving fertility associated with frozen semen is necessary to facilitate this industry trend. However, certain stallions with highly desirable genetics have been unable to benefit from frozen semen due to poor survival of frozen sperm. When sperm are frozen, they are more susceptible to oxidative attack from metabolic byproducts than are unfrozen sperm. Oxidative byproducts are believed to be the major contributing factor to sperm cell death and poor fertility of frozen sperm.

Insight into the ability of sperm to cope with stress is critical for improving cryopreservation techniques for sperm. The objective of this study was to determine whether stallion sperm are actually susceptible to freezing damage in





the initial phases of freezing and whether treatment by restricting oxygen in that period will improve sperm survival.

Our hypothesis was that most of the harmful oxygen-derived byproducts come directly from sperm mitochondria and that they are generated during the initial phases of cooling below ambient temperature and well before the sperm are exposed to ice formation. If oxidative attack can be prevented during the initial phase of cooling, then sperm are likely to better withstand very low temperature storage. We observed cooled cells in two phases, above zero and below zero, at various cooling rates and then measured cell damage outcomes associated with cooling rate variation.

We found that slower cooling from room temperature, compared with medium or fast cooling rates, promotes higher membrane integrity and motility after thawing. We also found that cells exposed to some cooling rates above zero were not different in motility and membrane integrity from sperm cooled below zero at the same rate. However, cells exposed to differing cooling rates above and below zero showed significant differences in motility, membrane integrity and membrane oxidation defects. These results suggest that sperm quality may be more sensitive to the cooling above zero degrees rather than the subzero cooling rate. We highlight the role of the sperm cooling in the above zero range as a cause for irreversible sperm damage in the quality of thawed cryopreserved sperm.

How does this research benefit horses?

The information gained from this research will improve freezing methods for many stallions and lead to the participation of more stallions in cryopreserved sperm breeding programs. This will benefit a variety of breed registries in the United States and abroad, as improvement in preservation of stallion genetics will allow more stallions within breeds to contribute to breeding programs and progeny production.

Investigators: Stuart Meyers, Katie Klooster and Kelly Martorana

Study ID: 12-06



Changes in the Sensitivity of *Corynebacterium pseudotuberculosis* (Pigeon Fever) to Antibiotics Over Time

Corynebacterium pseudotuberculosis, commonly known as pigeon fever, may cause external abscesses, internal abscesses or ulcerative lymphangitis in horses. While treating external abscesses with short courses of antibiotics can be controversial, internal abscesses are fatal without long-term treatment with antibiotics.

There are no reports describing the susceptibility of equine *C. pseudotuberculosis* to commonly used antibiotics over time. To offer the most efficacious treatment for severe infection, we studied the antibiotic susceptibility patterns of *C. pseudotuberculosis* to commonly used antibiotics over the past 15 years to document if *C. pseudotuberculosis* had become more resistant.

The objective of this study was to document changes in antimicrobial susceptibility patterns of *C. pseudotuberculosis*, isolated from horses over a 15-year period, to 20 antimicrobial agents. A secondary objective was to determine whether resistance was related to abscess location (internal versus external).

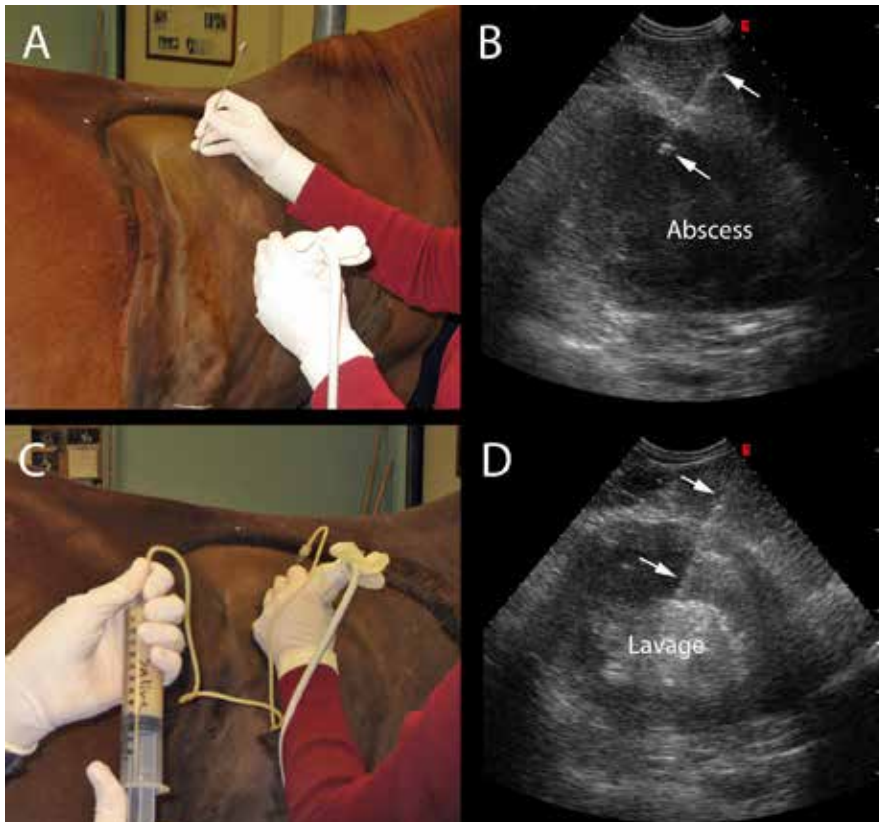
The results of our study showed that the minimum antibiotic concentrations needed to inhibit *C. pseudotuberculosis* did not change significantly over time, suggesting that the organism has not become more resistant. In addition, there was no relationship between the antibiotic concentration needed to inhibit bacteria and abscess location.

How does this research benefit horses?

Corynebacterium pseudotuberculosis infections often require treatment with antibiotics, and the choice of an appropriate antibiotic is paramount to a successful outcome. This study suggests that many commonly used antimicrobials in equine practice are effective against *C. pseudotuberculosis in vitro*. In our study, we did find occasional isolates that were resistant to some antibiotics. Therefore, we recommend that bacterial isolates be tested for antimicrobial susceptibility in order to select the optimal antimicrobial for use in clinical cases of *C. pseudotuberculosis* infection. Future research is needed to determine the role of individual immunity and the propensity to develop recurring or internal abscesses.

Investigators: Sharon Spier, Diane Rhodes, Gary Magdesian, Barbara Byrne, Judy Edman, and Philip Kass

Study ID: 12-07



These photos show ultrasound-guided aspiration and lavage of a large abscess involving the right kidney in a 4-year-old Quarter Horse.

About 8% of horses infected with Corynebacterium pseudotuberculosis develop internal abscesses. This form of pigeon fever has a high mortality rate of 30 to 40%.

Determination of the Genetic Changes Associated with a Bleeding Disorder in Thoroughbred Horses

A major issue facing the Thoroughbred racing industry is a condition known as Exercise-Induced Pulmonary Hemorrhage (EIPH). This condition has resulted in the majority of racehorses in this country receiving an injectable medication on race day, which has opened the sport to public scrutiny and divided the racing community. The underlying cause of EIPH appears to be multifactorial, and identification of predisposing factors, prevention and effective treatment would help to minimize the need for race day medications.

Some Thoroughbred horses have bleeding tendencies ranging from mild to severe that can result in diminished performance or death. We have identified a heritable defect in the blood clotting cells (platelets) of Thoroughbred horses that is characterized by reduced platelet binding of the major blood clotting molecule

This study has provided important information that will ultimately allow us to identify the cause of Atypical Equine Thrombasthenia. The information will also provide a basis for genetic testing and open the door for investigating specific treatment for this disorder.



(fibrinogen) in response to the major platelet activating stimulus (thrombin). We have designated this bleeding disorder as Atypical Equine Thrombasthenia (AET) and believe it may play a role in EIPH.

We believe that a mutation in the equine genome is responsible for causing the abnormal clotting response in horses with AET, and we are seeking to identify mutations that are common among and unique to horses with this condition by direct evaluation of platelets from affected horses.

To date, we have completed genetic screening of four affected horses and four normal control horses. Analysis of the data has identified three possible genes and we are currently working to confirm that these are the genes responsible for the platelet defect. Once confirmed, we hope to develop a genetic screening test to evaluate other horses that have been shown to have exercise-associated bleeding tendencies.

How does this research benefit horses?

Exercise-induced pulmonary hemorrhage is critical to Thoroughbred racing because it can result in diminished performance and sometimes death. Our ability to identify genes that cause platelet dysfunction and bleeding will provide owners and breeders with important information with which to make sound judgments to remove this problem from the Thoroughbred lineage.

This study has provided important data that will ultimately allow us to identify the cause of Atypical Equine Thrombasthenia (AET). The information will also provide a basis for genetic testing and open the door for investigating specific treatment for this disorder. In addition, the genomic data generated in this study will increase the overall equine genome sequence data available to researchers.

Investigators: Fern Tablin, Michael Lenardo, Jeffrey Norris, Ping Jiang

Study ID: 13-01



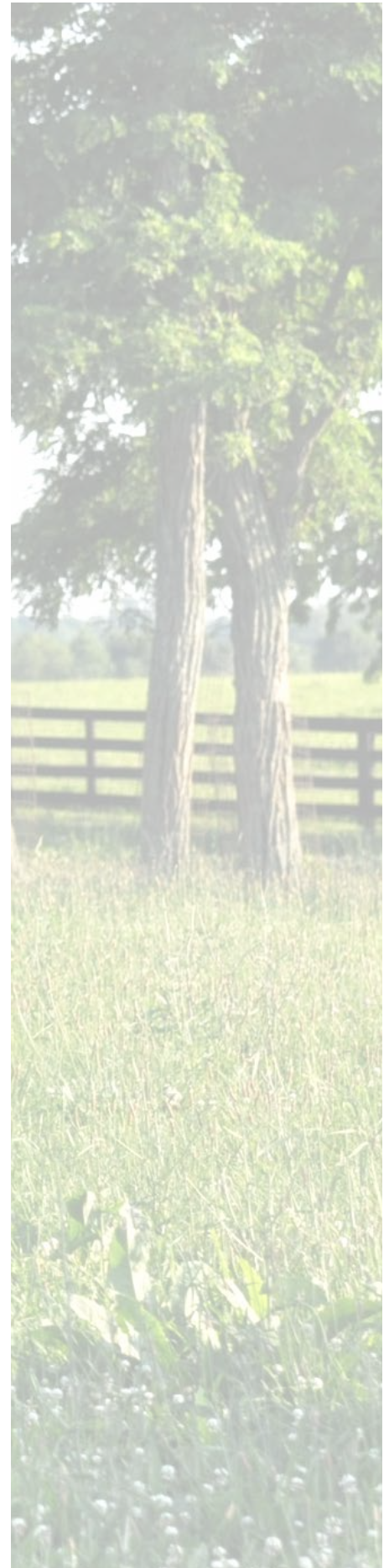
Serum and Spinal Fluid Vitamin E and Selenium Concentrations in Foals with Neuroaxonal Dystrophy During the First Year of Life

During the first year of life, certain foals may develop an inherited neurologic disorder known as neuroaxonal dystrophy (NAD) in which the axons throughout the brainstem and spinal cord undergo degeneration. This condition results in clinical signs defined by a lack of coordination.

Equine NAD is considered the underlying basis of equine degenerative myeloencephalopathy (EDM), a degenerative and irreversible disease of young horses characterized by the voluntary movement of limbs beyond the intended goal (hypermetria). Horses suffering from NAD or EDM typically display a symmetric (left to right) incoordination that may be more severe in the hind limbs than in the forelimbs. Affected horses also have proprioceptive deficits (they do not know where their limbs are as they walk) and may also be abnormally quiet and dull. At the present time, the only way to diagnose NAD/EDM is by examination of the spinal cord and brainstem once the horse has died.

While there is strong evidence that NAD/EDM is an inherited disorder, an important factor in the development of NAD/EDM appears to be the amount of vitamin E that a genetically susceptible foal receives during the first year of life. It may be that vitamin E acts as a modifier to determine the overall severity of the disease in genetically predisposed horses.

There are to date no available studies documenting vitamin E concentrations in blood or spinal fluid during a foal's first year of life. Selenium concentrations in the blood are often measured in conjunction with vitamin E and do not appear to play a role in the development of NAD/EDM. In regions of the country that are deficient in selenium and vitamin E, foals are frequently supplemented with a single intramuscular injection of vitamin E/selenium (E-Se®) during the first week of life.





No studies are available to determine if this injection provides adequate levels of vitamin E and selenium for newborn foals.

In this study, our objective was to compare the values of vitamin E and selenium in the blood and cerebrospinal fluid of healthy Quarter Horse foals with those of foals genetically susceptible to developing NAD/EDM (i.e., out of an NAD/EDM-affected mare that has previously produced confirmed NAD/EDM-affected foals) and to monitor the progression of the disease. The second objective was to determine the effect of a single intramuscular dose of vitamin E/selenium administered at 4 days of age on blood and spinal fluid vitamin E and selenium concentrations.



Neurological abnormalities associated with neuroaxonal dystrophy or equine degenerative myeloencephalopathy can be subtle and may be missed for years unless the horse is specifically examined for neurological disease. Mild cases may present with performance-related problems, where the horse is just not performing up to the standard expected for its breeding and training.

We found a significant decrease in blood and cerebrospinal fluid vitamin E and selenium concentrations during the first year of life in all foals, with the most significant changes in serum vitamin E occurring from age 4 days to 5 months. We also found that vitamin E and selenium concentrations in the dams significantly impacted foal concentrations through 10 days of age. An injection of E-Se[®] did not significantly increase cerebrospinal fluid selenium concentrations or blood/cerebrospinal fluid vitamin E concentrations in healthy foals. Finally, NAD/EDM-affected foals had significantly lower cerebrospinal fluid vitamin E concentrations.

Based on these results, it would appear that a single injection of vitamin E and selenium, administered as E-Se[®] to neonatal foals during the first few days of life, caused a transient and limited increase in both whole blood and cerebrospinal fluid selenium, but not vitamin E, concentrations. Vitamin E supplementation of NAD/EDM-affected mares during late gestation is warranted, as neonatal foals appear to receive most of their vitamin E through the colostrum. In suspect NAD/EDM foals less than 6 months of age, the measurement of both serum and cerebrospinal fluid vitamin E concentrations is advised.

How does this research benefit horses?

While research efforts are currently aimed at identifying the genetic mutation that causes equine NAD, horse owners are requesting information on how to prevent future cases. Vitamin E supplementation to genetically susceptible foals has been demonstrated to reduce the severity of the neurologic disease in herds with previously diagnosed NAD. This study provides owners and veterinarians with information regarding normal blood and CSF concentrations of vitamin E and selenium and helps to guide vitamin E supplementation especially in pregnant mares.

Our ultimate goal is to develop a genetic test to identify NAD/EDM. This would enable veterinarians to diagnose the disease without requiring euthanasia and assist breeders in selecting against the mutation. Research into the genetics of NAD/EDM will enable us to pursue a diagnostic and preventive means to test for the disease as well as provide insight into the mechanism underlying the degenerative process. We can then begin to consider how horses affected with NAD/EDM can be treated effectively.

Investigators: Birgit Puschner, Carrie Finno, Danika Bannasch, Krista Estell, Scott Katzman, and Jamie Textor

Study ID: 13-02



Temporal Detection of Common Equine Intranasal Vaccine Pathogens Using Quantitative PCR

Polymerase Chain Reaction (PCR): PCR is a laboratory technique for "amplifying" a specific DNA sequence. PCR is extremely efficient and sensitive; it can make millions or billions of copies of any specific sequence of DNA, even when the sequence is in a complex mixture. Because of this power, researchers can use it to amplify sequences even if they only have a minute amount of DNA. A single hair root, or a microscopic blood stain left at a crime scene, for example, contains ample DNA for PCR.

Real-time PCR assays have replaced conventional culture methods for quickly and accurately detecting common infectious respiratory pathogens such as equine herpesvirus-1 (EHV-1) and EHV-4, equine influenza virus (EIV), and *Streptococcus equi* subsp. *equi* (*S. equi*). However, its increased sensitivity may occasionally complicate the interpretation of assay results. An example of such an instance would be in testing horses that have been vaccinated recently via intranasal administration and have clinical signs of upper respiratory tract infection. Traces of the vaccine left in the nasal cavity might be mistaken for natural infection.

No data were available regarding the detection of the modified-live vaccine strain of EIV and *S. equi* subsp. *equi* over time, following intranasal administration in healthy young horses. Therefore, the objective of our study was to determine the shedding time of EIV and *S. equi* following intranasal vaccine administration (initial vaccine followed by boosters at 3 weeks and at 6 months) in healthy horses. Real-time PCR was used on nasal swabs to screen for shedding of the organism post-vaccination.

While research efforts are currently aimed at identifying the genetic mutation that causes equine NAD, horse owners are requesting information on how to prevent future cases. This study provides owners and veterinarians with information regarding normal blood and CSF concentrations of vitamin E and selenium and helps to guide vitamin E supplementation, especially in pregnant mares.

The results of this study will allow veterinarians to better differentiate respiratory pathogens in situations where healthy horses are vaccinated intranasally during outbreaks and develop respiratory signs shortly after. The ability to differentiate is important for making adequate biosecurity decisions and for determining the infectious risk such horses may pose to other susceptible herd mates.

Additionally, we sought to determine whether shedding time for these two respiratory vaccine pathogens was different after a booster vaccination compared with the first (initial) vaccine administration.

We found that the duration of *S. equi* shedding was similar after the first and second vaccine administration at 3 weeks but significantly different between the first and third vaccine administration at 6 months. The cumulative amount of *S. equi* shed was similar between the first and third vaccine administration but significantly different between the first and second and second and third vaccine administration. There was no statistical difference in days of shedding or cumulative amount of equine influenza virus shed between the two periods.

The results of this study suggest that previous vaccination can influence the duration of modified-live vaccine pathogen detection, and that ambient temperatures may also affect shedding duration.

How does this research benefit horses?

This information will allow veterinarians to better differentiate respiratory pathogens (wild-type pathogen vs. vaccine strain) in situations where healthy horses are vaccinated intranasally during outbreaks and develop respiratory signs shortly after. The ability to differentiate is important for making adequate biosecurity decisions and for determining the infectious risk such horses may pose to other susceptible herd mates.

In the face of an outbreak, all horses should be swabbed regardless of vaccination status. If the nasal swab is positive in an animal after 5 days post-vaccination, this would suggest natural infection rather than vaccine-derived *S. equi*.

Investigators: Nicola Pusterla

Study ID: 13-04



*In the face of an outbreak, all horses should be swabbed regardless of vaccination status. If the nasal swab is positive in an animal after 5 days post-vaccination, this would suggest natural infection rather than vaccine-derived *S. equi*.*

Investigation of Serum Triglyceride (Fat Metabolite) in Newborn Foals

Triglycerides are a type of fat found in blood. The body uses them for energy and needs some for good health. But high triglycerides might raise the risk of heart disease and may be a sign of metabolic syndrome.

Septic foals have increased serum triglyceride concentrations when they are admitted to the hospital compared with healthy foals. Our research has shown that sick, hospitalized foals on intravenous feeding that have serum triglycerides above 200 mg/dL have an increased risk of death compared with those having levels below 200 mg/dL. However, the physiology of triglycerides is poorly understood in the healthy newborn foal, let alone sick foals.

There are unpublished reports that newborn foals have transient blood samples with a high fat content, but there is little scientific information available to substantiate these observations or their significance to the health of the foal. While increased serum triglycerides in adult horses reflect metabolic derangements due to illness and a negative energy balance, foals have a different body composition and metabolism than adults and thus may release triglycerides into the bloodstream after eating.

The objectives of this study were to determine the triglyceride concentrations and their variation in the bloodstream over time in newborn foals, and to compare this information between healthy foals <48 hours old and foals between 10 to 12 days old.



We found that young neonatal foals did indeed have higher serum triglyceride concentrations than older foals and adult horses, and that these concentrations were affected by foal age and sampling time. Specifically, foals <24 hours old had serum triglyceride concentrations similar to those of the mares (28 mg/dl foals, 20 mg/dl mares). Foals 24 to 48 hours old had significantly higher concentrations as compared with the older foals and adult mares (median 89 mg/dl), and foals

The value of serum triglyceride concentrations as an equine diagnostic is becoming well known. Triglycerides increase with sepsis (the leading cause of death in foals) and liver disease. However, healthy foals are noted to have fatty blood, and this has been poorly understood and undocumented until now.



10 to 12 days old had significantly higher concentrations than the mares but significantly lower concentrations than the 24- to 48-hour foals (median 60 mg/dl).

Different sampling times indicated that triglyceride concentrations in newborns can fluctuate within the same day and up to 2 weeks of age. When triglyceride concentrations from each age group were compared against one another, significant differences were found, especially when the time interactions were taken into account.

Further study to analyze serum insulin and glucose concentrations in the same foal serum samples is currently underway.

How does this research benefit horses?

The results of this study provide normal ranges for serum triglyceride concentrations for newborn foals and foals 10 to 12 days old, as well as for the immediate postpartum mare. This information provides critical baseline values necessary for evaluating triglyceride concentrations in sick foals and postpartum mares in the clinical setting. Examples would include foals with sepsis and mares with Equine Metabolic Syndrome. This information will allow veterinarians to make more accurate decisions regarding abnormally high concentrations of fat or lipids in the blood of sick patients based on their age.

The value of serum triglyceride concentrations as an equine diagnostic is becoming well known. Triglycerides increase with sepsis (the leading cause of death in foals) and liver disease. However, healthy foals are noted to have fatty blood, and this has been poorly understood and undocumented until now. Establishing an understanding of triglyceride concentrations in newborn foals, how concentrations change over the course of the first several hours and days of life, will provide further insight into normal foal physiology as a starting point to understanding lipid metabolism of the sick foal.

Investigators: K. Gary Magdesian, Emily Berryhill and Judy Edman

Study ID: 13-06



Use of Polymerase Chain Reaction for the Detection of *Salmonella* spp. in Pooled Fecal and Environmental Samples

PCR is a simple and powerful method of copying genetic material (DNA). The technique allows scientists to replicate a piece of DNA a million times or more in just two to three hours. These millions of exact copies can then be easily analyzed and certain gene sequences identified. In contrast, other methods that require larger samples for analysis may either be unable to detect or identify the target or require a more lengthy time period to produce sufficient material.

Infection with *Salmonella* spp. is an important cause of intestinal disease in horses. It affects humans, horses, most mammals, and birds and can cause debilitating—and even deadly—diarrhea. *Salmonella* bacteria can affect both foals and adults,

and they spread easily by horse-to-horse contact and through shared tools, water buckets, hands, etc., on which bacteria can "hitch a ride" to the next victim. Seemingly well horses can harbor the bacteria, and when stressed, they can shed it or become ill.

Because it is so contagious, biosurveillance for *Salmonella enterica* in equine hospitals has become a recognized standard of care in order to prevent the spread of infection. In recent years, researchers have evaluated PCR assays used to detect *Salmonella* spp. in fecal samples from horses admitted to veterinary hospitals. Collectively, these studies have shown that PCR has excellent sensitivity and quicker turnaround time compared with microbiological culture. However, the costs associated with PCR testing are higher, especially when environmental and fecal samples are tested for biosurveillance purposes. In this situation, a sensitive and cost-effective test is needed to screen large number of samples.

In this study, our objective was to determine whether pooling of pre-enriched fecal and environmental samples would allow an accurate detection of *Salmonella* spp. by real-time PCR compared with microbiological culture. For study purposes, 677 equine fecal and 686 environmental samples were collected and tested individually (total of 1,363 samples) and in pools of up to ten samples (139 pools) using a *Salmonella* real-time PCR assay.

We found that the pooling strategy was able to detect all fecal and environmental samples that tested positive by both PCR and microbiological culture.

How does this research benefit horses?

Biosurveillance for *Salmonella enterica* in equine hospitals has become a recognized standard of care. The overall agreement between *Salmonella* cultures and pooled PCR analyses in our study was high. This suggests that the pooling strategy is as sensitive as individual cultures and can be used to screen fecal and environmental samples to detect *Salmonella*. Further, the pooling strategy reduced the overall costs and time of *Salmonella* testing while maintaining the same detection accuracy as microbiological culture. This approach is ideal for screening fecal and environmental samples at veterinary hospitals with a low prevalence of *Salmonella*.

Investigators: Nicola Pusterla, K. Gary Magdesian and Barbara A. Byrne

Study ID: 13-07

*Biosurveillance for *Salmonella enterica* in equine hospitals has become a recognized standard of care. The overall agreement between *Salmonella* cultures and pooled PCR analyses in our study was high. This suggests that the pooling strategy is as sensitive as individual cultures and can be used to screen fecal and environmental samples to detect *Salmonella*.*

Evaluation of the Daily Use of an Antiprotozoal Drug for the Prevention of *Sarcocystis neurona* Infection in Foals

Equine protozoal myeloencephalitis (EPM) is a debilitating protozoal disease of the central nervous system that is typically caused by infection with *Sarcocystis neurona*. Several risk factors have been associated with the development of EPM, including young age, seasonality, exposure to wildlife, and high performance. Although treatment of EPM often has a fair to good prognosis, affected horses can retain persistent neurological deficits.

There is a need to establish protocols aimed at preventing *S. neurona* infection in order to improve the well-being of horses and reduce the economic impact of EPM, particularly among performance horses. The prophylactic use of antiprotozoal drugs has become popular in performance horses for reducing the risk of EPM development. However, no randomized study has been undertaken to determine the efficacy of this practice.

To our knowledge, this is the first report showing a safe and effective way to reduce infection to S. neurona via daily administration of low-dose diclazuril pellets. This safe and cost-effective protocol has the potential to improve the well being of horses and decrease the loss of performance in sport horses at risk for developing EPM.



Our research group recently completed a pharmacokinetic study of a preventive dose of diclazuril administered orally as a pelleted top dress in adult horses. At a dose of 0.5 mg/kg body weight, the drug reached 1,000-fold higher plasma and 26-fold higher cerebrospinal fluid levels than the targeted concentration of 1 ng/ml shown to inhibit *S. neurona* merozoite production in laboratory bioassays.

In the current study, our objective was to determine whether foals from farms with a high incidence of infection for *S. neurona* and treated with daily diclazuril pellets would have a reduced infection rate to *S. neurona* compared with untreated herd mates. Infection would be determined by the detection of specific antibodies against *S. neurona* via an indirect fluorescent antibody test (IFAT).

We found that all mares had positive titers to *S. neurona* by IFAT at the time of birthing. At 24 hours post-colostrum ingestion, 96% of untreated foals and 69% of diclazuril-treated foals had detectable antibodies to *S. neurona*. There was no statistical difference in titers between the two foal groups.

Immunology terms:

Seroprevalence = the level of a pathogen in a population as measured in blood serum

Seroconversion = when a specific antibody is detectable in blood and the corresponding antigen/toxin/pathogen becomes undetectable

Titer = the concentration of an antibody in blood

When the titers were measured over a period of months, it became clear that the ideal time to provide prophylactic treatment was after the foal had been weaned due to the interference of maternal antibodies gained through colostrum consumption. There were statistical differences in titers between the untreated and the diclazuril-treated groups in post-weaning foals but not in pre-weaning foals.

The same trends applied to the antibody titers for both foal groups. After the foals were weaned at 4 months, there was a statistically significant difference in the following months not only in seroprevalence but also in median titer. Untreated foals maintained a median titer between 80 and 120 (titers ranging from 40 to 10,240) post-weaning, while treated foals had a median titer ranging from 40 to 60 (titers ranging from 40 to 80) for the same study period.

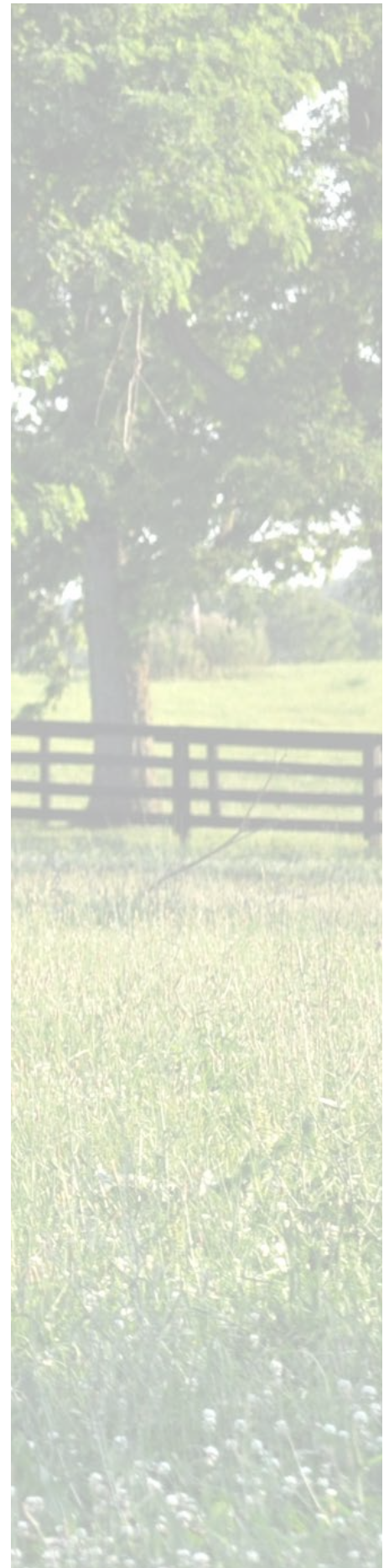
At the end of the study, 88% of untreated and 6.25% of treated foals tested seropositive to *S. neurona*. The lower titer in treated foals suggests that the daily treatment was effective in preventing infection.

How does this research benefit horses?

To our knowledge, this is the first report showing a safe and effective way to reduce infection to *S. neurona* via daily administration of low-dose diclazuril pellets. This safe and cost-effective protocol has the potential to improve the well being of horses and decrease the loss of performance in sport horses at risk for developing EPM.

Investigators: Nicola Pusterla, Patricia A. Conrad and Andrea Packham

Study ID: 13-11



The Marcia MacDonald Rivas Grant program has been in existence for over 25 years in the UC Davis School of Veterinary Medicine. These grants are awarded to new teaching and research personnel.

Development of a New Method to Suppress Heat Behavior in Mares

Study funded by a Marcia MacDonald Rivas Grant

Because of performance problems associated with the estrous period in mares, several treatments have been evaluated for their efficacy in estrous suppression but no single treatment has been shown to be both effective and safe for prolonged use. Many mares fail to perform at their best due to strong sexual behavior that is cyclic. These horses may be more difficult to manage, they may perform irregularly, or they may even appear lame when in heat.

In an unpublished study, a progesterone-like hormone currently used in long-term human contraception, etonogestrel, showed some effect on estrous behavior suppression at human dose levels. This finding suggests that determination of potency and dose levels for horses may provide an alternative therapy to safely suppress heat in mares.

The objective of our present study was to determine whether etonogestrel has progesterone-like activity in the mare such that it might be suitable for long-term applications (similar to the way it is used for long-term contraception in women) in the form of implantable subcutaneous rods.

For this study, we evaluated the bioactivity of etonogestrel in mares in the laboratory (in vitro), using a horse progesterone receptor-expressing cell line. Cells in the bioassay express the equine progesterone receptor, and the activity of the receptor is measured when exposed to different progestins including altrenogest (Regumate®), medroxyprogesterone (Depo Provera®), etonogestrel, and natural progesterone. The activity of the receptor is then measured to compare the potency of the different hormones in the horse progesterone receptor.

Our results showed that etonogestrel had some progesterone-like effect in the equine progesterone receptor, but that natural progesterone was 48 times more potent. We determined that natural progesterone was as biopotent as altrenogest, and that both natural progesterone and altrenogest were more potent than etonogestrel. Medroxyprogesterone was not bioactive enough to measure any activity in the equine progesterone receptor.

This study confirmed the inactivity of medroxyprogesterone (Depo Provera®) currently used by some to control undesirable signs of heat in mares. Etonogestrel had progesterone-like activity in the horse but was much less potent than natural progesterone or altrenogest (Regumate®).

How does this research benefit horses?

The use of long-term progesterone-like drugs would provide a better alternative to the expensive and impractical methods currently available for suppressing estrous behavior in mares, particularly in performance horses during show seasons and training. It would also reduce the risk of handling drugs that can affect human health, such as altrenogest (the most commonly used progestogen in the United



States), and the side effects of drugs that require repeated injections, such as various formulations of progesterone.

Although etonogestrel could provide an alternative for the use of other progesterone-like drugs to maintain pregnancy in high-risk mares—such as in cases of twin reduction, embryo transfer, placentitis, luteal insufficiency, and other conditions—it is not a practical way to suppress estrus in mares due to its high cost and low biopotency compared with progesterone or altrenogest. Further studies are needed to develop cost-effective, safe methods for heat suppression.

Investigators: Ghislaine Dujovne and Alan Conley

Study ID: 13-10M

The use of long-term progesterone-like drugs would provide a better alternative to the expensive and impractical methods currently available for suppressing estrous behavior in mares, particularly in performance horses during show seasons and training.



STUDIES FROM THE J.D. WHEAT VETERINARY ORTHOPEDIC RESEARCH LABORATORY

The J.D. Wheat Veterinary Orthopedic Research Laboratory is an environment in which multidisciplinary studies pertaining to musculoskeletal disorders of animals and humans can be conducted. The goal of researchers participating in the laboratory is to understand the physiologic process of injury and musculoskeletal disease in performance, companion and production animals as well as in humans.

The Center for Equine Health provides funding for studies to identify improved diagnostic, therapeutic and preventive techniques to combat musculoskeletal injury and disease in horses. The following studies were supported by this funding.

Effect of Arena Surfaces on Fetlock Motion in Dressage Horses

The goal of this study was to understand how different arena surfaces affect fetlock motion in elite dressage horses at the extended trot. We expect that different arena surfaces can increase or decrease the propensity for suspensory ligament injury.

Fetlock angle and hoof motions were recorded in six elite dressage horses performing the extended trot using high-speed video. Measurements were made from the video of the horses performing on both dirt and synthetic arena surfaces. The mechanical properties of the arena surfaces were measured independently with an impact device.

We found that fetlock angle was greater (more extended) on the synthetic surface, which had the greater impact force, and concluded that arena surfaces appear to affect fetlock motion and thus propensity for suspensory ligament injury. However, not all dirt and synthetic surfaces have properties identical to those of the surfaces studied.

Effect of Arena Surface on Fetlock Motion in Show Jumping Horses

The goal of this study was to understand how different arena surfaces affect fetlock motion in horses jumping over a jump. We expect that different arena surfaces can increase or decrease the propensity for suspensory ligament injury.

Fetlock angle and hoof motions were captured using high-speed video during take-off and landing in six show jumping horses. Measurements were made from the video of the horses performing on both dirt and synthetic arena surfaces. The mechanical properties of the arena surfaces were measured independently with an impact device. The data are currently being analyzed.

Silicate-Associated Osteoporosis

Silicate-associated osteoporosis (SAO), also known as Bone Fragility Syndrome, is a progressive, debilitating bone disease that affects horses living in certain geographic areas of California. Affected horses develop body stiffness, intermittent shifting lameness, and bone deformities and have an increased risk for bone fracture. The bone disease is apparently associated with inhalation of soils containing toxic particulates, primarily cristobalite which is a form of silicate.

The particulates cause persistent lung and lymph node inflammation. However, the mechanism by which lung disease leads to bone resorption and osteoporosis is not yet understood. Our current study is using genomic tools to detect clues to the mechanism. We hope that by understanding the mechanism, we will be able to design preventive strategies for exposed horses.

This study is also seeking to identify economical ways to detect affected horses. Currently, a device that measures bone strength by indentation with a small needle may hold promise. Nuclear scintigraphy currently remains the diagnostic modality of choice.



Horses with advanced stages of Silicate-Associated Osteoporosis usually have a marked sway-back that is characteristic of the disease.

The goal of researchers in the J.D. Wheat Veterinary Orthopedic Research Laboratory is to understand the physiologic process of injury and musculoskeletal disease in performance, companion and production animals as well as in humans.

INFECTIOUS DISEASE RESEARCH STUDIES SUPPORTED BY THE BERNICE BARBOUR FOUNDATION

The Bernice Barbour Foundation is an independent charitable foundation focused primarily on the preservation and care of domestic and companion animals and the prevention of cruelty to animals. Through their generous support, the Center for Equine Health has allocated funding to the research of Dr. Patricia Pesavento and her laboratory as they study new and changing pathogens affecting domestic animals, sheltered animals and free-ranging wildlife.

Originally established as the Bernice Barbour Communicable Disease Laboratory at the UC Davis School of Veterinary Medicine, researchers like Dr. Pesavento work to identify the mechanisms by which infectious microorganisms cause disease. Because the horse is second only to humans in the speed and frequency with which it travels the world, it served as an ideal initial model for the study of communicable disease transmission. However, other species such as dogs and cats are also studied, and the laboratory's mechanistic approach facilitates the discovery and application of knowledge to all disease regardless of the host species or infectious agents.

The Pesavento Laboratory is a hardworking, productive research group and they have welcomed trainees who are devoted to animal health from all levels of education, from primary school to graduate school. Bernice Barbour funds are vital to the mission of keeping veterinary health tied to excellence in research.

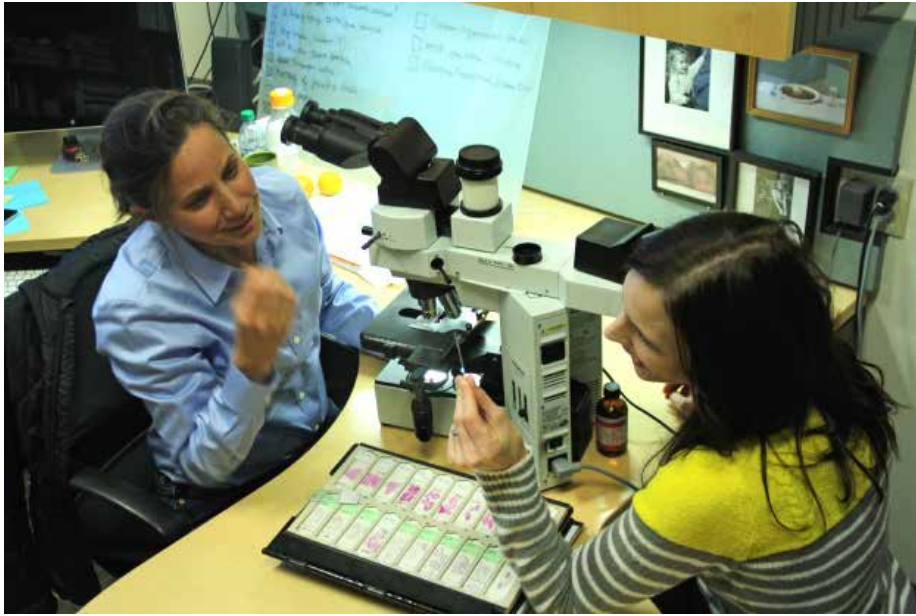
Researchers in the lab study naturally occurring individual and herd animal disease to identify new pathogens in animals. They have conducted several outbreak studies in horses, cats and dogs and their accomplishments are summarized below.

Discovery of New Viruses

Laboratory researchers have discovered four new viruses in dogs with intestinal disease, and they are currently working to estimate the burden that these viruses place on the pet population. For example, they have established the frequency of infection of dogs exposed to canine circovirus, a newly recognized cause of diarrhea in dogs. They are also looking at genetic variation in dog circoviruses so that they can identify those that cause disease.

They have discovered and are characterizing two viruses found in the kidneys in cats. Chronic kidney disease (CKD) is an extremely common disease in older domestic cats, and there has been an increase in prevalence in recent decades. The viruses discovered could be contributing to CKD, and an epidemiologic study is underway to establish association. If viral infection is found to underlie chronic

Through the generous support of the Bernice Barbour Foundation, the CEH has allocated funding to the research of Dr. Patricia Pesavento and her laboratory as they study new and changing pathogens affecting domestic animals, sheltered animals and free-ranging wildlife.



Dr. Pesavento and Dr. Church discuss a case.

renal failure, then preventative measures could be established to significantly reduce the incidence of CKD in cats.

Studies of Viral-Associated Tumors

In addition to viral discovery, the laboratory conducts studies on viral-associated tumors. Their discovery of raccoon polyomavirus has been followed by research that has established this virus as the cause of neuroglial tumors in raccoons.

The laboratory has also analyzed the association of papillomaviruses with skin tumors in horses. Sarcoids are the most common skin tumor of the horse worldwide and can occur anywhere on the horse's skin. These tumors are disfiguring and are difficult to manage. Very little is understood about how sarcoids grow and spread or the way they respond to treatment. The variability in the behavior of sarcoids makes this tumor type particularly challenging for both veterinarians and owners. One thing that we do understand is that it is a virus that initiates and controls growth of the tumor.

The sarcoid-associated virus (BPV) is the same family as the virus associated with cervical cancer in women (HPV). The Pesavento Laboratory is using a novel technique to understand how papillomavirus and other cancer-associated viruses are able to cause and maintain cancer in horses. In the case of human papillomavirus, there is an effective vaccine to prevent some papillomaviruses from causing cervical cancer, and research on a vaccine for horses is underway in several laboratories.

For sarcoids, the work necessary to understand how this papillomavirus is able to cause the tumor is vital to creating a strategy for its eradication. Support from the Bernice Barbour Foundation has allowed these researchers to use a new visualization technique to study the interaction between the virus and the tumor. This resulted in a publication produced through the collaboration of





undergraduate animal science majors, graduate students, and laboratory technicians, as well as two members of the faculty.

The laboratory has successfully demonstrated that BPV nucleic acid is present in equine sarcoids and further identified BPV DNA in the epithelium (surface cells) of a subset of equine sarcoids. The identification of glandular and skin cells positive for BPV DNA in equine cases represents a potential site of viral production and a mechanism for viral dispersal through the equine sebum (oily secretion that lubricates the skin) and hair.

Another common equine skin tumor is squamous cell carcinoma (SCC), which can occur in the genital region of horses as it does in humans. Epidemiologic, clinical and experimental data suggest one possible cause could be *Equus caballus* papilloma-virus type 2 (EcPV2). The potential of papillomavirus to cause tumors is well recognized, but there has been a long-standing debate about whether or not the virus is responsible for these genital SCCs and, if so, whether it is all or just a subset of tumors. The problem is that EcPV2 is present in the genital region of horses without related clinical lesions or disease, and therefore studies designed to evaluate the development of disease must hold a higher burden of proof than simple detection of the virus.

Research shows that a subset of genital SCCs are caused by EcPV2. Additional key observations from this study are that (1) solar damage and viral nucleic acid were detected in genital SCCs in horses, but they were never detected in the same case; (2) all of the metastatic tumors were associated with virus. This is the first time EcPV2 has been detected in metastatic tumor. There could be different behaviors (and different prognoses) for virus-associated SCCs compared with nonviral SCCs, but verification would require additional studies.

Future Studies

While continuing to discover new pathogens, the Pesavento Laboratory is devoted to uncovering deep common links in how viruses persist and/or cause disease in their hosts.



Aural plaques in horses are caused by papillomavirus.

Resident

RESEARCH STUDIES

In an effort to encourage residents to conduct equine studies, the Center for Equine Health provides funding for selected research by residents at the UC Davis School of Veterinary Medicine's teaching hospital. These studies help residents learn to design and conduct research studies of merit. In many cases, these pilot studies are later expanded into larger research projects based on the study results.

Resident research is vital to the success of the equine research program and to creating veterinary leaders of the future. Funding for resident research is currently lacking.





A CRUCIAL ROLE OF THE CEH AND THE SCHOOL OF

Veterinary Medicine is to train young veterinarians through the residency program. Equine medicine residents receive three years of specialized training that culminates in board certification. These residents are vital to training veterinary students, managing caseloads and conducting research at UC Davis.

A top CEH priority is to continue funding for our resident research program with a future goal of expanding graduate student research support as well. Resident research is recognized as one of the key elements of a successful research program for several reasons:

- Resident research projects create pilot data that is crucial for leveraging funding for high-impact research projects designed to prove a concept or develop a diagnostic or therapeutic tool.
- Resident research projects provide information that impacts current treatment options at the William R. Pritchard Veterinary Medical Teaching Hospital. For example, a resident research project studied the effect of antibiotics combined with stem cell injections and demonstrated that the antibiotics actually killed the stem cells. This information changed the way our hospital veterinarians performed this procedure.
- Resident research is a valuable aspect of preparing for successful board certifications.
- Resident research inspires careers in research and academic veterinary medicine.

The following is a list of recently funded projects that have not yet been completed:

Pharmacokinetics of Intramuscularly Administered Ketamine in Adult Standing Horses. This study will provide a dosing regimen necessary for standing sedation in a difficult horse, improving safety for the horse and the veterinarian.

Comparison of 10 and 20 Minutes Tourniquet Application for Distal Limb Perfusion with Amikacin in Horses. This study will clarify the ideal tourniquet time necessary to achieve adequate dosages of local antibiotic and improve outcomes for horses with synovial infections.

Cardiac Troponin Levels in Healthy Neonatal Thoroughbred foals and in Neonatal Thoroughbred Foals

with Rib Fractures. This study will establish normal cardiac Troponin levels (heart enzyme) as a biomarker for severity of rib fractures in newborn foals. Rib fractures can occur secondary to difficult birth and can be life-threatening.

Endometrial Tissue concentrations of Ceftiofur after Intrauterine Infusion in Healthy Mares and Those with Experimentally Induced Endometritis. This study validated the use of Ceftiofur as an intrauterine treatment and will give veterinarians an effective tool to combat infections and improve fertility in mares.

Comparison of Duration of Efficacious Intra-Articular Amikacin Concentrations Following Intravenous Distal Limb Perfusion with Low and High Dose Amikacin in Horses. The results of this study will guide future treatment protocols.

Use of Force Plate Analysis to Quantify Weight-Bearing in Horses with Naturally Occurring Foot Lameness Before and After Perineural Analgesia. The ability to evaluate lameness and healing objectively will enhance research capabilities in this important field.

Validation of a Commercial Stall-Side Test and Analyzer for the Measurement of Equine Serum Amyloid A. Access to quick turnaround, patient-side analytical tests or biomarkers is the wave of the future in human and veterinary medicine.

Investigation of the Safety and Immune Response to the Modified Live Bovine Rota-Coronavirus Vaccine in Healthy Adult Horses. Access to an effective vaccine would help protect horses and provide an option for horse owners traveling to affected areas.

Investigation of the Frequency of Shedding of Respiratory Pathogens in Horses Recently Imported Into the United States. Recently, equine herpesvirus-1 has become a serious health risk to horses comingled for competition. New strains of equine influenza virus pose additional threats to the horse industry and potentially to other species. This work will help to document the incidence and types of respiratory pathogens crossing international borders.

Temporal Variability in Serum Glucose and Insulin Concentrations in Neonatal Foals. This will be the first work of its kind in foals, and it has the potential to expand our understanding of equine metabolic syndrome and insulin resistance in horses.

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Newly Funded
RESEARCH STUDIES



Evaluation of Drug Techniques to Reduce Rapid Involuntary Eye Movements Caused by General Anesthetics

Horses recovering from general anesthesia commonly experience rapid involuntary eye movement (nystagmus) that likely is associated with incoordination and dizziness, increasing the risk of injury. We are hypothesizing that the benzodiazepine midazolam may reduce the frequency and degree of nystagmus induced by the anesthetic isoflurane, and that this effect could be achieved at doses that are sufficiently low to avoid muscle weakness or undesirable side effects. We also predict that the sedative romifine may also reduce nystagmus, and that the two drugs may act together to suppress isoflurane-induced nystagmus in horses recovering from anesthesia.

In this study, we will measure the effects of midazolam and romifidine, administered at different doses separately and in combination, on the severity of nystagmus in lightly anesthetized horses.

How will this research benefit horses?

Decreasing nystagmus and associated incoordination and dizziness in horses recovering from anesthesia will likely translate into improved anesthetic recoveries and reduced post-operative injury. If successful, the drugs we will test in this study may provide a simple and inexpensive method to achieve these benefits.

Investigators: Robert Brosnan, Monica Aleman and D. Colette Williams

Study ID: 14-02



Frequency of Antibody Detection to *Sarcocystis neurona* and *Neospora hughesi* in Healthy Horses from Various Areas of the United States

Equine protozoal myeloencephalitis (EPM) is a debilitating neurologic disease caused by at least two protozoal parasites: *Sarcocystis neurona* and, less frequently, *Neospora hughesi*. Although the biology and epidemiology of *S. neurona* has been well characterized in recent years, less is known about *N. hughesi*.

The geographic range of clinical EPM in horses is defined by the range of the opossum, the definitive host of *S.*

neurona. The range of clinical EPM in horses caused by *N. hughesi* is unknown. In areas where the opossum is common, approximately 50% of horses test seropositive by the Western blot test, indicating exposure to *S. neurona*. In central Wyoming and Montana, outside the natural range of opossums, only 6.5% and 0%, respectively, of wild horses are seropositive.

In many states, the exposure rate of horses to *S. neurona* and *N. hughesi* is unknown. This information is important for determining the geographic incidence of EPM and adequately using serological tests to support or rule out EPM in neurologically affected horses. Some tests currently available do not screen for *N. hughesi* at all, which could result in a false negative test for EPM.

In this study, we will determine the seroprevalence to *S. neurona* and *N. hughesi* in healthy horses residing in various geographic areas of the United States. Exposure to *S. neurona* and *N. hughesi* will be determined by the detection of specific antibodies against these two protozoal pathogens using an indirect fluorescent antibody test (IFAT).

How will this research benefit horses?

This study is intended to establish a baseline for seroprevalence to *S. neurona* and *N. hughesi* in healthy horses from various geographic areas of the country. The resulting information will help better estimate the geographic incidence of EPM and also provide additional data to help direct interpretation of serological tests in neurological horses where EPM is suspected.

Investigators: Nicola Pusterla, Patricia Conrad, Heather Fritz, and Andrea Packham

Study ID: 14-03



Effect of Arena Surface on Fetlock Motion in Jumping Horses

Injuries to structures that support the fetlock and digit (suspensory ligament, superficial and deep digital flexor tendons) are the primary causes of performance limitations in show jumpers. The likelihood of injury to these structures increases with higher limb loads and greater fetlock hyperextension. Characteristics of the arena surface affect maximum limb loads and thus can lower or increase the risk for injury.

We believe that less stiff, more compliant arena surfaces with sufficient strength to support the hoof will result in lower limb loads and less fetlock joint hyperextension and thus pose less risk for common injuries. In this study, we will measure hoof and fetlock motions of horses landing from a jump and compare the motions between jumping on dirt versus synthetic surfaces.

How will this research benefit horses?

Surfaces associated with less fetlock hyperextension could be used to prevent injuries of the flexor tendons and suspensory ligament. This information will advance the design of jumping surfaces to lower the risk of injury to horses. The goal of this kind of research is to engineer safety for equine athletes across the various equestrian disciplines.

Investigators: Susan Stover and Megan O'Brien

Study ID: 14-05



Study of Sudden Death in Racehorses

The number of equine sudden death cases in California horse racing has increased over the past two years. Sudden death is defined as death in a closely observed and previously apparently healthy animal. The increase in incidents has created national and international negative attention on California horse racing, as sudden death adversely affects racehorse welfare, jockey safety, and public perception of horse racing.

Despite thorough postmortem examination and diagnostic workup, a definitive cause of death could not be established in approximately 50% of the cases of sudden death historically. Heart failure is suspected to be responsible for a large number of these deaths, perhaps predisposed by administration of substances such as cobalt, vitamin B12, and/or levothyroxine (T4 thyroid hormone). However, no scientific evidence is available to support this.

We believe that racehorses with sudden death of unexplained cause died due to heart failure and have microscopic lesions in the heart, and that many of these horses have higher values of cobalt and vitamin B12 in liver and levothyroxine (T4) in blood.

This study will determine whether this is true, and if so, we will be able to diagnose the cause of sudden death in a large

number of horses and, more importantly, help prevent a significant percentage of these deaths.

How will this research benefit horses?

Sudden death is a devastating event that severely affects horses, jockeys, owners, the public, and the equine industry in California and elsewhere. Emotional and financial burdens affect the equine industry every time a sudden death occurs. The benefits of this project for the equine industry are twofold:

(1) Finding specific lesions in the heart of at least some of those horses will provide a diagnostic tool to determine the mechanism of death in future cases of sudden death.

(2) Determining that some of the sudden deaths are associated with administration of cobalt, vitamin B12, and or levothyroxine (T4) will provide preventive tools to avoid future sudden deaths.

Investigators: Francisco Uzal, Robert Poppenga, Santiago Diab, Ashley Hill, and Rick Arthur

Study ID: 14-08



Development of a Serological Test to Detect Antibodies to Equine Coronavirus in Adult Horses

Recently, equine coronavirus has been associated with fever and diarrhea in adult horses. Since 2011, our laboratory has been involved with several outbreaks across the United States. The main clinical signs reported were loss of appetite, lethargy and fever. Nine horses from six different outbreaks were euthanized or died due to rapid progression of clinical signs. The cause of death in these horses was associated with septicemia (blood poisoning), endotoxemia (toxins from disintegrated bacterial cells in the blood), and hyperammonemia (excess in ammonia in the blood).

In the majority of the clinical cases, equine coronavirus infection was documented through RT-PCR and viral genome sequencing. Although PCR allows the detection of antigen during the acute disease phase, there is yet no equine specific antibody test that would allow us to determine exposure in infected horses and in horses showing no clinical signs.

We hypothesize that a serological assay specific to equine coronavirus will be able to detect seroconversion (when an

antibody becomes detectable in the blood and the antigen becomes undetectable) in clinically infected horses, and also establish the exposure rate of horses associated with outbreaks of equine coronavirus. The objective of this study will be to develop and validate an assay to detect antibodies against equine coronavirus.

How will this research benefit horses?

The development of a new serological assay would allow us to retrospectively determine exposure using acute and convalescent serum samples. (Acute serum is from an actively ill patient; convalescent serum is from a patient that has recovered from an infection and is considered especially rich in antibodies.) A new assay will also allow us to study exposure rates in various horse populations in order to better understand the epidemiology of this emerging equine virus.

Investigators: Nicola Pusterla and Samantha Mapes
Study ID: 14-09



Assessment of a New Imaging Technique to Detect Active Lesions in the Horse Foot

Imaging of the horse foot has markedly improved over the past 15 years with the development of CT and MRI. We are now able to detect lesions that were unrecognized in the past. The current challenge is to distinguish between active lesions and chronic inactive lesions. This is important both for treatment of specific cases and for assessment of therapies in research projects.

In this study, we will investigate the feasibility of an imaging method known as positron emission tomography (PET) that has never been used in the horse. We will also determine whether PET can identify both soft tissue and bone lesions and provide indications of the level of activity. For this project, we will collaborate with a company that recently designed a small PET scanner for imaging of the human head. The design of the scanner will allow imaging of the equine distal limb.

How will this research benefit horses?

In comparison with currently available imaging systems in the horse, PET has the potential to provide functional information about tissue and lesions. This will help to better target treatment for individual cases and also would have

great applications in better understanding the progression of degenerative and inflammatory changes of bone and soft tissue. In particular, PET could be very helpful for laminitis research.

Investigators: Mathieu Spriet and Larry Galuppo
Study ID: 15-01



Genetic Investigation of Equine Neuroaxonal Dystrophy

During the first year of life, certain foals may develop an inherited neurologic disorder known as neuroaxonal dystrophy (NAD). Horses affected with NAD have very low vitamin E levels, supporting the idea that a genetic mutation involved in vitamin E transport or metabolism is responsible for this disease.

We have been working on developing a genetic test to identify affected horses. However, in order to identify the gene involved in NAD and create a genetic test, an adequate number of horses (NAD-affected and unaffected) and genetic markers (DNA sequence on a chromosome) are required. Previous attempts at identifying the gene involved in NAD were limited due to only 54,000 to 70,000 genetic markers, whereas the newest marker test contains 670,000 markers.

We hypothesize that the recently released equine genetic marker test containing 670,000 genetic markers will provide enough power to identify a NAD-associated genomic region with a gene involved in vitamin E metabolism that is mutated in horses with NAD. In this study, we will test a group of NAD-affected and unaffected horses across the 670,000 genetic markers and identify a region on a specific chromosome that is associated with equine NAD. We will also evaluate the specific chromosome region for any potential genetic mutations using sequences of the entire genome from NAD-affected and unaffected horses.

How will this research benefit horses?

Two previous studies have revealed that equine NAD is the second most common neurologic disease in horses. In the past two years alone, Dr. Finno has consulted on over 50 horses with potential NAD. A genetic test for this disease would assist veterinarians in the diagnosis while providing breeders with invaluable information to make informed breeding decisions and prevent future cases.

Investigators: Carrie Finno and Erin Burns
Study ID: 15-03

Improving Tendon Formation with Biglycan and Decorin Proteins

Normal tendon formation of the horse during development involves the creation of a strong, highly organized collagen-rich tissue capable of withstanding great tension during locomotion. When horses incur an injury to the tendon, recovery is generally incomplete and results in chronic lameness with a corresponding effect on performance. Hence, compared with healthy tendons, the repair response mounted by cells of the tendon and surrounding tissue is inferior.

New strategies to bolster the response of these cell populations and strengthen the organization of the tissue to more closely resemble a healthy tendon are needed. In this study, we will determine the levels of naturally occurring biglycan and decorin in equine tendon cells and then investigate the effect of seeding cells with these proteins. Parameters to be measured include cell status, collagen organization, and tissue functionality.

How will this research benefit horses?

We expect to document improvements in tendon formation that can be carried over to further equine clinical studies by direct treatment of tendon injuries with biglycan and decorin proteins. The proteins could be delivered to affected sites or within scaffolds added surgically. Such treatments would be cost-effective repair strategies that could be dosed and delivered to the repair site over the course of tendon healing.

Investigators: Michael Mienaltowski and Keith Barr
Study ID: 15-06



Investigating the Genetics of Inherited Heart Defects in Arabian Horses

Arabian foals are at increased risk for being born with heart defects. A particular defect called ventricular septal defect (VSD) appears to be inherited in Arabians and has never been investigated from a genetic perspective. Identification of genetic markers associated with VSDs in Arabians may permit the development of a genetic test and ultimately reduce the number of foals that inherit this severe heart defect.

In this study, we will perform a genetic analysis using DNA from Arabian horses affected with VSDs and from healthy

(control) Arabian horses to identify specific regions in the horse genome that are associated with the condition. These regions can then be critically evaluated in future studies to identify genes that are involved in VSD development.

How will this research benefit horses?

This study represents the first step in identifying mutations that cause a serious heart condition in Arabian horses. If successful, this study would provide the foundation for future identification of a mutation that causes VSDs and subsequently allow us to develop a genetic test to screen horses for this condition before breeding.

Investigators: Joshua Stern and Krista Estell
Study ID: 15-07



Efficacy and Safety of Two Glaucoma Drugs at Lowering Intraocular Pressure in Horses

Glaucoma is a disease of the eye that is characterized by increased intraocular pressure and is a common cause of blindness in horses. The disease affects humans as well. Drugs such as brimonidine and brimonidine-timolol have been developed to reduce intraocular pressure in people with glaucoma by decreasing the amount of fluid (aqueous humor) within the eye and thereby preserve functional vision and control pain. The safety and efficacy of brimonidine and brimonidine-timolol have not been tested in horses.

In this study, we will determine whether brimonidine and brimonidine-timolol lower intraocular pressure in normal horses. A second objective is to determine whether brimonidine, either alone or in combination with timolol, causes eye irritation or whether it is as well tolerated as it is in people.

How will this research benefit horses?

Glaucoma is a common cause of blindness and eye removal in horses. There are few medications available to effectively treat this condition in horses. If we can identify new ophthalmic medications that successfully lower intraocular pressure in horses with glaucoma, we may be able to decrease the severity of pain and the incidence of blindness in horses with this devastating disease.

Investigators: Mary Lassaline, Sara Thomasy, Melissa Von Zup, and Paul Miller
Study ID: 15-09

Genetics of Juvenile Idiopathic Epilepsy in Arabian Horses

Juvenile idiopathic epilepsy is a disorder of Egyptian Arabian foals that causes seizures and has potential life-threatening complications, including head injury and aspiration pneumonia. The disorder is inherited and a specific genetic mutation has not yet been identified.

We believe that a mutated gene involved in electrical signaling within the brain is associated with juvenile idiopathic epilepsy in Arabian horses. In this study, we will search the entire horse genome for a region on a chromosome that is associated with the disease. Once that region is identified, sequencing will be performed to identify possible genetic mutations in that region that could be responsible for juvenile idiopathic epilepsy.

How will this research benefit horses?

Epilepsy can result in loss of animals and a major financial burden from the costs of antiepileptic treatment and hospitalization. Thus, identification of genetic variants will aid in strategic breeding and avoid the perpetuation of genetic mutations that affect the overall health and well being of the Arabian breed.

Investigators: Monica Aleman and Carrie Finno

Study ID: 15-10



Identifying Genetic Risk Factors for Eye Cancer in Haflinger Horses

Squamous cell carcinoma is the most common cancer of the equine eye and the second most common tumor of the horse overall. It frequently originates in a region of the eye known as the limbus and can quickly spread to other parts of the eye, leading to vision loss and destruction of the eye. Haflinger horses are over-represented for this disease and are affected, on average, at a younger age. Affected horses can be traced back to a common ancestor, which makes this an important breed in which to study the genetics of the disease.

We hypothesize that a single recessive mutation contributes to the risk of squamous cell carcinoma in Haflinger horses. The objective of this study is to investigate and identify any DNA mutations that might be associated with increased prevalence of limbal squamous cell carcinoma in this breed.

How will this research benefit horses?

Identification of the “at risk” mutation that causes ocular squamous cell carcinoma in Haflinger horses will allow for DNA testing to inform breeding decisions, thus lowering the incidence of disease in the breed and allowing for earlier detection and better prognosis for the animals. In addition, clinicians could use the DNA test as a basis of identifying “at risk” horses for earlier and more frequent examinations, thus aiding in earlier diagnosis and treatment and a better prognosis. In the long term, however, understanding the genes and biological pathways that are disrupted in ocular squamous cell carcinoma may lead to the development of new and more effective treatments and thereby prevent visual impairment associated with loss of the eye.

Investigators: Rebecca Bellone, Mary Lassaline, Jiayin Liu, and Michael Mienaltowski

Study ID: 15-12



Arterial Injection of Stem Cells in the Hind Limb of Horses

Recent research in regenerative medicine has revealed that intra-arterial injection of mesenchymal stem cells into the medial artery of the front limb provides the best distribution of stem cells to the limb. There is a need to develop a similar route in the hind leg.

Injection of the cranial tibial artery at the dorsal proximal aspect of the hock might be a feasible route for administering stem cells in the treatment of distal hind limb injury. A technique for intra-arterial injection of stem cells to the horse front limb was developed recently by our group, but because the anatomy of the hindlimb is different, this technique should be evaluated for feasibility.

The objective of this study is to demonstrate that injection of the cranial tibial artery is feasible and assess the distribution and persistence of stem cells after administration.

How will this research benefit horses?

This technique could provide a valuable approach for treating hind limb proximal suspensory desmopathy or other tendon and ligament injuries in the distal limb.

Investigators: Albert Crosa, Mathieu Spriet, and Larry Galuppo

Study ID: 15-13

Heel Movement and Hoof Wall Deformation with Different Nail Positions Applied to the Horseshoe

Racehorses commonly exhibit long toe/low heel hoof conformation, which has been associated with increased risk for fetlock injuries and breakdown. Shoeing techniques have an impact on hoof growth that can lead to this long toe/low heel hoof conformation. Application of horseshoes is a factor that can be manipulated easily and could be a viable method to prevent injury in racehorses and performance horses.

In this study, we expect to show how horseshoe nail distribution may be a factor in changing hoof conformation to a long toe/low heel type.

How will this research benefit horses?

If we can understand the mechanisms that cause long toe/low heels in horses, it is possible that we can make changes in shoeing techniques that will reduce the incidence of injuries in racehorses. Since this hoof conformation is also observed in performance horses, the information obtained could help to reduce injuries in additional disciplines as well.

Investigators: Vanessa Dahl, Susan Stover and Tanya Garcia-Nolen

Study ID: 15-14



Normal Appearance of the Caudal Spinal Canal in Healthy Horses

Cervical stenotic myelopathy, or compression of the spinal cord, is a common cause of neurologic disease in horses but it is difficult to diagnose. Myelography is the current gold-standard diagnostic technique used to establish spinal cord compression. This procedure involves injecting contrast dye into the area around the spinal cord to identify areas of compression based on narrowing of the contrast column. Evidence of spinal cord compression is commonly seen at the seventh cervical vertebra and first thoracic vertebra (C7-T1). However, because normal values have not been established for this location, we do not know if this narrowing is an abnormality.

We hypothesize that myelography in healthy horses will identify a contrast column that narrows at the C7-T1 space, indicating that this is a normal finding in some horses and is not necessarily indicative of spinal cord compression that

will result in neurologic disease. The primary objective of this study is to perform myelograms on normal, non-neurologic horses to establish normal findings for the C7-T1 space. An additional objective of this study is to establish normal myelographic findings for Thoroughbreds and Warmblood horses. Warmbloods are commonly affected by cervical stenotic myelopathy but have not been well represented in previous studies.

How will this research benefit horses?

By determining the appearance of the C7-T1 space, veterinarians will be able to fully evaluate the spinal cord and more definitively diagnose the presence or absence of compression. This data is currently lacking and the significance of narrowing of the contrast column at C7-T1 is currently unknown. Additionally, most previous studies have been performed on Thoroughbred horses alone, although Warmblood horses are commonly affected by this disease. By establishing normal values for C7-T1 and the entire cervical spine in Warmblood horses, we hope to decrease the chance of misdiagnosis of spinal cord compression.

Investigators: Krista Estell, Mathieu Spriet, Carrie Finno, and Monica Aleman

Study ID: 15-16



Using Stem Cells to Engineer Cartilage for Joint Repair in Horses

Equine athletes frequently incur injuries to the joint, but effective treatments for repairing these injuries are lacking due to the inherent difficulty of healing cartilage. Tissue engineering is a relatively new field that uses a combination of cells, engineering and materials methods, along with suitable biochemical and physicochemical factors, to improve or replace biological functions. It frequently involves implantation of stem cells to generate bone, tendon or cartilage.

In this study, we will use tissue engineering to grow healthy new cartilage constructs in a controlled laboratory environment, using mesenchymal stem cells (MSCs) from various tissue sources. However, methods will need to be developed not only to select an appropriate tissue source, but also to ensure that the engineered cartilage possesses adequate properties for implantation.

We hypothesize that MSCs harvested from equine cord blood will produce cartilage that more closely resembles healthy native articular cartilage than that of MSCs harvested from bone marrow. Furthermore, we hypothesize that the quality of engineered cartilage from MSCs can be further enhanced by applying biochemical and mechanical stimuli.

The goals of this study are to determine which source of MSCs (bone marrow or umbilical cord blood) produce superior cartilage and the optimal conditions for growth of functional new cartilage from MSCs.

How will this research benefit horses?

Tissue engineering for cartilage repair aims to increase the success rate of surgical intervention for repairing damaged cartilage as well as to hasten recovery times by replacing defective tissue with a healthy neocartilage implant. Such technologies for cartilage repair are currently in Phase II human clinical trials. With this project, we hope to develop a similar approach for equine orthopedic applications by applying our group's scaffold-free approach that mimics the cartilage developmental process.

Investigators: Kyriacos Athanasiou, Dori Borjesson, Jerry Hu, Larry Galuppo, Heenam Kwon, Naomi Walker, and Jamie White

Study ID: 15-17



Investigation of Iodide Supplementation as a Preventative and Treatment for *R. equi* Pneumonia in Foals

Foal pneumonia caused by *Rhodococcus equi* infection can be an expensive, devastating and sometimes fatal disease. Two methods of prevention are currently available, each with its own drawbacks. Hyperimmune plasma can be administered to foals prior to the onset of disease but is labor-intensive and expensive. Alternatively, prophylactic antibiotics can be administered to foals, but this increases the risk of antibiotic resistance of *R. equi* and other microbes on a farm. We are interested in exploring other viable options for prevention and treatment of *R. equi* infection.

Cells lining the respiratory tract and immune cells within the lung both use enzyme systems to kill invading bacteria.

In humans, this system is more effective when supplied with iodide. It is possible that augmentation of the innate respiratory defense system of foals with sodium iodide will aid in the prevention and treatment of *R. equi* and we will explore this as a viable option in this study.

How will this research benefit horses?

Horse farms rely heavily on antimicrobial drugs to control and prevent pneumonia. In an era where the threat of antimicrobial resistant bacteria is increasing, it is important to investigate alternative strategies for easily fortifying the innate immune response and preventing disease.

Investigators: Meera Heller, Johanna Watson, Kenneth Jackson, and Fauna Smith

Study ID: 15-21



Clinical Trial to Determine if the Madigan Foal Squeeze is an Effective Treatment for Neonatal Maladjustment Syndrome and Comparison of Two Methods of Measurement of Foal Hormones Before and After the Squeeze Procedure

Neonatal maladjustment syndrome (NMS) occurs in approximately 1 to 3% of equine births, resulting in nonspecific symptoms and requiring intensive care to ensure foal survival. There is no diagnostic test for NMS or specific treatment, but recent work has demonstrated that NMS foals have high levels of *in utero* sedative neurosteroids persisting after birth. A rapid method squeeze procedure to lower the neurosteroids by mimicking the birth canal pressures could be a field treatment used by veterinarians.

In this study, we will determine if the squeeze procedure causes a change in neurosteroid levels and behavior in foals with maladjustment syndrome and compare two methods of measuring the neurosteroid levels as an indicator of maladjustment syndrome in foals.

How will this research benefit horses?

Identification of an effective simple treatment for neonatal maladjustment syndrome would greatly improve survival of many foals that cannot be managed with intensive care and would reduce costs of those foals that currently receive

intensive care. A rapid field test to diagnose NMS would be of benefit to veterinarians and horse owners to guide prognosis and therapy for a foal with clinical signs of NMS.

Investigators: John Madigan, Monica Aleman, Kirstie Pickles, and Nilesh Gaikwad

Study ID: 15-22





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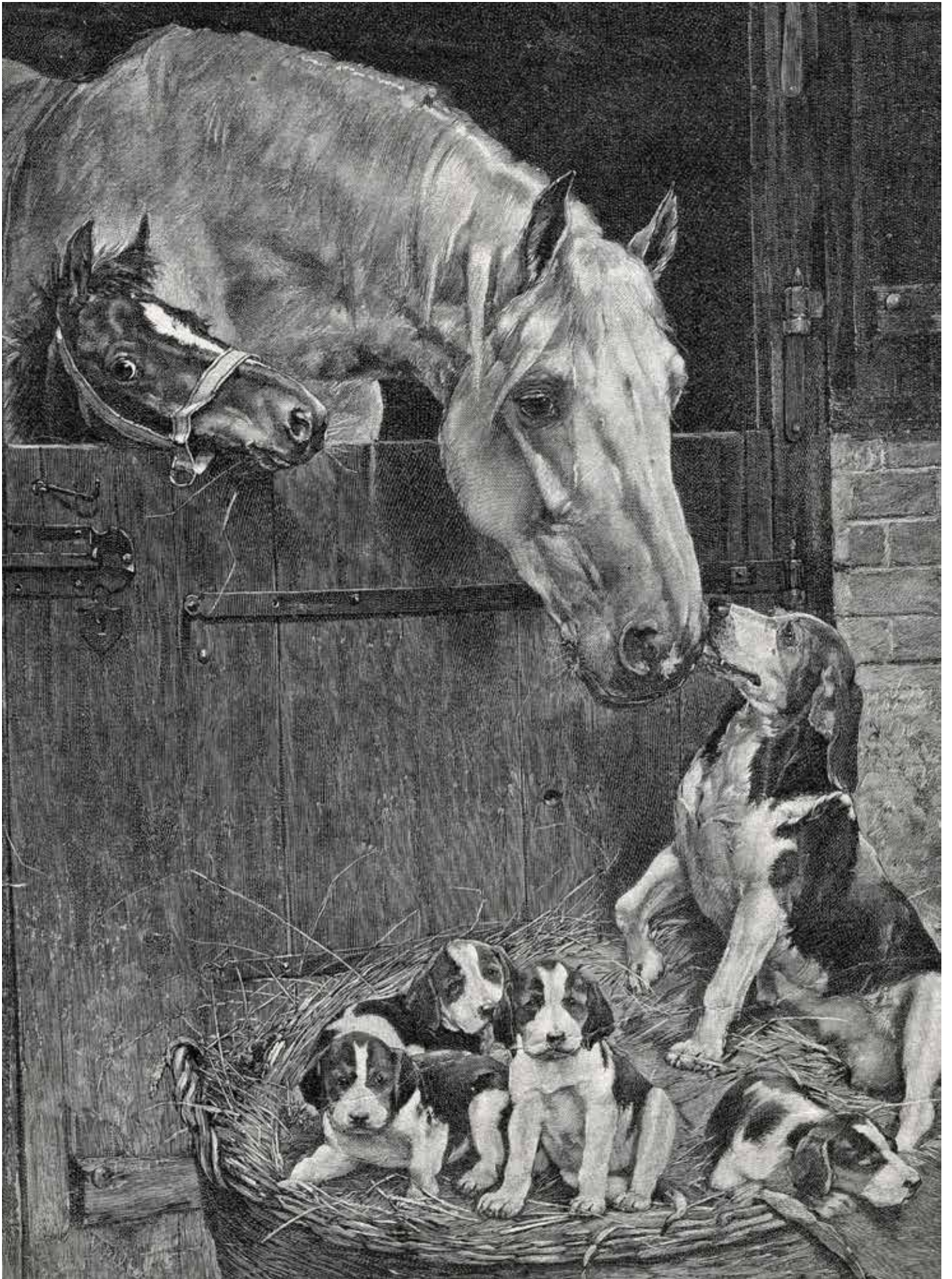
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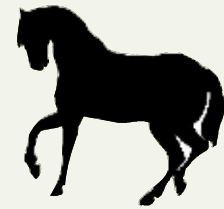


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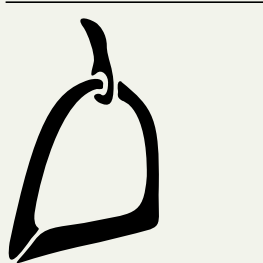
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