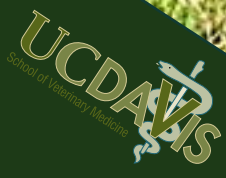




Center for Equine Health

2005–2006 RESEARCH PREVIEW

School of Veterinary Medicine



2005–2006 RESEARCH PREVIEW

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Center for Equine Health

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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, 13 such endowments have been established, ranging from \$10,000 to more than \$1 million. The endowments are described below.

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. The minimum amount required to establish an endowment under this system is \$10,000. For more information regarding the endowment programs, contact Dr. Gregory Ferraro at (530)752-6433 or send an e-mail to gferraro@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.)

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CEH Focused Research Endowments



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

Research Endowments—Continued

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



On May 2, 2005, the Claire Giannini Hoffman Equine Athletic Performance Laboratory was dedicated at the UC Davis School of Veterinary Medicine. This facility will provide a focal point for establishment of a new Equine Performance and Sports Medicine clinical program. One of the unique features of this laboratory is that it offers two Swiss Mustang 2200 motorized equine treadmills for evaluating performance problems and implementing new therapeutic approaches for treating them. These treadmills are among the most sophisticated in the world today, capable of reaching racing speeds of over 37 mph.

As part of the laboratory's mission to develop new diagnostic and therapeutic approaches to performance-related problems, the laboratory will interface with researchers in a number of scientifically related disciplines, including cardiovascular and respiratory physiology and medicine, biomechanics (J.D. Wheat Veterinary Orthopedics Laboratory), pharmacology and drug effects (Equine Analytical Chemistry Laboratory), and evaluation of muscle structure and function (Neuromuscular Disease Laboratory).

and surgery that is relevant to the development of their specialty board certification.

Equine Enduring Legacy Endowment

This fund provides for the development of new treatment methods and techniques in equine medicine or surgery. It supports the application of experimental treatments to horses that may have untreatable or life-threatening conditions, as well as the conduct of clinical research trials to aid in the development of new therapies.

Marcia MacDonald Rivas Research Endowments

These funds are available to teaching and research personnel, including all faculty at the Assistant 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. ❁

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/or 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 is 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 horse-racing:

► Discovered that stress fractures precipitate catastrophic fractures.

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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.

Great strides have been made in discovering the causes of catastrophic injury in racehorses. We now have a better understanding of the events leading to bone fracture and better techniques to detect stress fractures in live horses.



Research Initiatives—Continued

- ▶ Discovered new sites, now routinely examined, for stress fractures.
- ▶ Advocated bone scan (scintigraphy) installation at Santa Anita Racetrack.
- ▶ Developed new bone scan views to enhance detection of stress fractures.
- ▶ Associated high-intensity exercise with increased risk for skeletal injury.
- ▶ Determined that high-intensity exercise increases risk for layup.
- ▶ Determined that layup increases risk for catastrophic humeral fracture.
- ▶ Associated horseshoe toe grabs with increased risk for injury, especially suspensory apparatus failure (fetlock breakdown).
- ▶ Discovered osteoarthritis in the back and pelvis of over 25% of racehorses.
- ▶ Developed new, improved techniques for sampling and treating the fetlock and pastern joints.
- ▶ Improved methods for fracture repair.
- ▶ Improved understanding of joint cartilage inflammation and function.

Great strides have been made in discovering the causes of catastrophic injury in racehorses. We now have a better understanding of the events leading to bone fracture and better techniques to detect stress fractures in live horses. We can identify 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 horse-racing a safe sport for horses and people.

To contact the J. D. Wheat Veterinary Orthopedic Research Laboratory, visit their Web site at www.vetmed.ucdavis.edu/vorl, or telephone Dr. Susan Stover at (530)752-8388 (e-mail: smstover@ucdavis.edu).

In January 2001, the **Bernice Barbour Communicable Disease Laboratory (BBCDL)** was established to conduct research devoted to investigating the mechanisms by which infectious diseases are produced. The Bernice Barbour Foundation, Inc., was established by the late Bernice Wall Barbour of New Jersey, who devoted her life to making the lives of animals happier and healthier. The Foundation's trustees are concerned that the increasing voracity of infectious agents poses a serious threat to the well-being of all creatures. The BBCDL was established to address this problem.

Infectious communicable diseases pose one of the major threats to worldwide health in the twenty-first century. Currently, the capacity of many infectious organisms to adapt and mutate far

exceeds the medical community's ability to respond with new strategies for control. The resilience of these pathogenic microbes, combined with the rapidity with which humans and animals currently circumvent the globe, present today's biomedical scientists with a most difficult challenge.

The BBCDL employs an innovative approach to accomplish its goals. Instead of studying specific diseases in isolation, the laboratory provides a research umbrella under which scientists from varying disciplines work together as a team to study disease-causing microbes throughout the world. The BBCDL's work focuses on three critical areas of infectious disease research: (1) the microorganism's life outside the host, (2) the pathogenic mechanisms used by microorganisms to invade the host and cause disease, and (3) the defense mechanisms used by hosts against microorganisms.

Some major accomplishments to date include:

- ▶ Acquisition of new information on *Cryptosporidium parvus*, the waterborne protozoal disease, which remains a significant public health threat in the United States. Specifically, researchers conducted a comprehensive evaluation of the hydrological transport of *C. parvus* oocysts in groundwater and riverine systems.

- ▶ Development of predictive models on the rate of environmental inactivation of infective *C. parvum* oocysts for use in improving environmental regulations for livestock grazing on watersheds and in ensuring water quality.

- ▶ Development of methods to study disease outside of the horse—the "horse in the Petri dish." We are now able to study the cells involved in infections in the horse in laboratory cultures. We use monocytes from blood, alveolar macrophages from the lung, endothelial cells that line the blood vessels, and dendritic cells (the first and most important cells that need to interact with an invader) to study disease virulence and host immune response. These cell culture systems, which allow us to study the interactions of pathogens in the horse without the need to use whole animals, are a significant boost to advancing the study of equine infectious diseases.

- ▶ This lab supports one full-time cell biologist specializing in disease infection and host response.

- ▶ Training the next generation of scientists in the area of waterborne zoonotic diseases, environmental health assessment, and host/pathogen interactions.

To contact the Bernice Barbour Communicable Disease Laboratory, visit their Web site at www.vetmed.ucdavis.edu/ceh/BBCDL, or telephone Dr. Gregory Ferraro at (530)752-6433 (e-mail: glferraro@ucdavis.edu).



The case of *Sarcocystis neurona* appearing in two quite different species (sea otters and horses) underscores the importance of understanding the basic mechanisms by which all disease spreads.

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Research Initiatives—Continued

Viral diseases of humans and animals are becoming increasingly important to the maintenance of worldwide health. The changing demographics of the horse industry, particularly the international movement of sport performance horses, clearly places horses in an especially high-risk category for both infection and transmission of any new and/or emerging viral disease. With initial funding support provided by Dr. Bernard and Mrs. Gloria Salick, the **Equine Viral Disease Laboratory** was dedicated in April 1999 to facilitate the diagnosis, control, and study of the global spread of viruses that have the potential to cause disease in horses and humans. The mission of the Equine Viral Diseases Laboratory is to promote equine health by undertaking research on diseases of the horse caused by viruses. Specific objectives are to provide state-of-the-art diagnostic expertise, reagents, and technology dedicated to the horse and to facilitate transfer of this technology and expertise to appropriate partners within this and other universities, in the state government, federal government, and international health organizations.

Under the direction of Dr. N. James MacLachlan, the Equine Viral Disease Laboratory is leading the international effort to develop better diagnostic technology to identify diseases. The laboratory is working to improve vaccines to prevent these diseases and is coordinating efforts to better monitor and control them. It disseminates information on a regular basis and provides a facility that is a global hub for the interaction of scientists involved in the study of disease.

Some major accomplishments since the laboratory's inception include:

- ▶ Pioneering work in the characterization of Equine Viral Arteritis (EVA).
- ▶ Development of improved assays to expedite accurate diagnosis of EVA infection in horses.

- ▶ Development of a new-generation vaccine for immunizing horses against EVA.
- ▶ Initiation of comprehensive epidemiologic studies to examine the impact of West Nile Virus (WNV) on horses in California.
- ▶ Continued work with molecular studies of WNV to identify key genetic determinants of phenotypic properties, including virulence of field strains and the role of reservoir hosts (mosquitoes, birds) in fostering evolution of WNV in the field.
- ▶ Initiation of an extensive study of virus-induced respiratory disease of young thoroughbred horses in Southern California to determine the precise role of viruses in the occurrence of respiratory disease in yearlings.
- ▶ Progress in the development of a vaccine for African Horse Sickness Virus.

Future goals for the Equine Viral Disease Laboratory are to broaden diagnostic capabilities through strategic partnering with preeminent groups nationally and internationally in order to address every major viral disease of the horse. The laboratory will continue cutting-edge research on viral diseases of the horse that are important to the regional industry. There will be a focus on new diagnostic and immunization technologies, characterization of the epidemiology and pathogenesis of important viral diseases of the horse, and identification of new and emerging viral diseases of the horse.

To contact the Equine Viral Disease Laboratory, visit their Web site at www.vetmed.ucdavis.edu/evdl, or contact Dr. James MacLachlan at (530) 752-1385 (e-mail: njmaclachlan@ucdavis.edu). ✨



Viral diseases of humans and animals are becoming increasingly important to the maintenance of worldwide health. The changing demographics of the horse industry, particularly the international movement of sport performance horses, clearly places horses in an especially high-risk category for both infection and transmission of any new and/or emerging viral disease.

Genetics

Is Scn8a the Gene That Causes Equine Cerebellar Abiotrophy? (05-10)

M. Cecilia T. Penedo, Leah S. Brault, and Thomas R. Famula

Cerebellar abiotrophy (CA) is a neurological condition found almost exclusively in Arabian horses. Around six weeks of age, the disease causes the death of neurons in the cerebellum of affected foals, leading to head tremors and a loss of balance. Preliminary data indicate an approximate location for CA on the horse genome, indicating that a gene exists in this region that may be responsible for CA. In mice, mutations in this gene—known as Scn8a—cause tremors, muscle weakness, and a lack of balance.

The goal of this project is to determine whether the genetic marker for Scn8a is associated with cerebellar abiotrophy. To accomplish this, we will develop markers within the Scn8a gene using restriction enzyme digests and sequencing methods. These markers will be tested against three families of 46 horses segregating for CA to determine whether the marker for Scn8a is associated with CA. More markers will also be developed in the region around Scn8a so that the location of CA can be more accurately identified, or so that the region may be excluded as the location for CA. Ultimately, we hope to identify the specific marker for cerebellar abiotrophy so that affected animals can be identified early.

Breeders are understandably reluctant to disclose that their breeding stock has produced a foal with a neurological disease, so the incidence of cerebellar abiotrophy is not well known by breeders or the Arabian Registry. Affected foals are often euthanized or restricted to life as pasture pets because they are never coordinated enough to be ridden safely. Horses with CA also are a danger to themselves as they are prone to accidents and injury. Genetic screening will allow a positive diagnosis of CA and provide data for estimation of the carrier rate in Arabians. Breeders can then use this information to avoid mating of carriers.

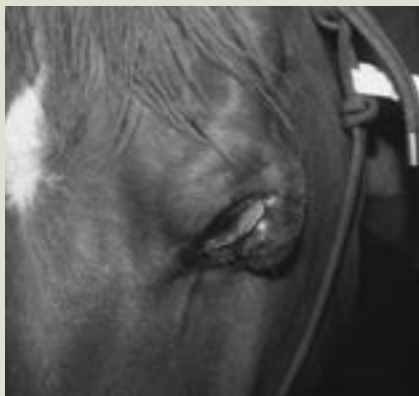
Experimental Approach

Primers have been developed to amplify a region of the Scn8a gene using long-range polymerase chain reaction (PCR). This PCR product will be treated with a variety of restriction enzymes that

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Cerebellar Abiotrophy—Continued



Sarcoid tumors appear in various forms and range from small, contained growths to large, uneven growths. These photographs show three types of sarcoids. The top photo shows a fibro type, the middle photo shows a small verrucous type, and the bottom shows an occult type.

recognize very specific DNA sequence patterns and cut DNA at these sites. Sequence recognition is so specific that single-base mutations can eliminate a restriction site. These identified sequence changes, or polymorphisms, can then be used as markers for the gene. Three families of horses segregating for CA will be tested using these new markers to determine if Scn8a is associated with CA. At the same time, more markers will be identified in the region around Scn8a in order to narrow the search for the gene that causes CA. *

Genetic Analysis of the Role of Bovine Papilloma Virus in the Development of Equine Sarcoids (05-02)

Alain P. Théon, Christian M. Leutenegger, and Lee V. Millon

Sarcoids are the most common skin tumors in horses. Based on their appearance and growth rate, sarcoids range from benign, indolent tumors to aggressive, infiltrative tumors. Bovine papilloma virus (BPV), which causes warts in cattle, is thought to be an important factor in the formation of these sarcoid tumors in horses.

In this study, investigators will conduct an in-depth analysis of the association between bovine papilloma virus infection and the development of equine sarcoids. The effects of BPV viral type will be determined in affected horses, as will the viral intracellular location on genetic anomalies in tumor cells and tumor biology.

Sarcoids are not life-threatening, but they adversely affect the commercial value of horses and compromise animal use because of their location (girth and around the base of the ears, eyelids and distal extremities). Surgery is the primary treatment. However, tumor regrowth commonly occurs. Although treatments are available, they are often used once the tumor has recurred and are then less effective. Researchers hope that a better understanding of the role of bovine papilloma virus in the development of these tumors will contribute to improving the treatment options for sarcoids.

Experimental Approach

Tissues and blood will be collected from 30 horses referred to the VMTH for treatment. Diagnosis of sarcoid will be confirmed by histopathology. Freshly excised tumor tissue will be processed for in vitro tissue culture. Representative samples of tumors grown in tissue culture from benign and aggressive lesions in horses will be analyzed for chromosomal changes and determination of bovine papilloma virus type and intracellular location. Statistical analysis will be performed to evaluate the association between the characteristics of viral infection (type and location), genetic changes, and tumor behavior. *

The Role of the Initial Immune Response to *Rhodococcus equi* Infection (05-04)

Johanna L. Watson, Kenneth A. Jackson, Sharon K. Hietala, and W. David Wilson

Foal pneumonia caused by *Rhodococcus equi* infection can be an expensive, devastating and sometimes fatal disease. Currently, the only way to prevent *R. equi* infection in foals is to administer plasma prophylactically, before onset of the disease, but this treatment is labor-intensive and expensive.

In this study, we will work to gain a better understanding of the early immune response to *R. equi* by (1) determining the defect in the early immune response of foals to the organism, and (2) by determining which genes are associated with susceptibility to *R. equi*. This research is significant because it will provide new information about the initial events in the course of *R. equi* infection. Once we understand the defects in the initial immune response of the foal we can investigate how to overcome them with vaccination. Our ultimate goal from these studies is to provide the information needed to develop a vaccine.

Experimental Approach

We will study cells from the lung (alveolar macrophages) and blood (dendritic cells) of adult horses and foals, which are crucial for protection of foals from *R. equi* infection. The cells will be grown in the laboratory and incubated with live *R. equi* organisms capable of causing pneumonia. In a previous study, we identified 11 genes that have not been associated previously with protection from *R. equi*. In this study, we will use our cell culture systems to study potential differences in the activity of these genes between adult horses and foals. Genes with the highest level of differential activity will be cloned and screened for inherited differences in their sequences. These differences in a given gene (polymorphisms) will be investigated for their association with *R. equi* pneumonia in foals using DNA samples from a family of horses. *

Immunology



Preliminary Testing of a *Rhodococcus equi* Vaccine (05-09)

Barbara A. Byrne and Johanna L. Watson

Researchers are optimistic that vaccination of foals early in life using a single *R. equi* protein can result in an immune response capable of protecting foals from *R. equi* pneumonia.



Pneumonia due to *Rhodococcus equi* is believed to affect as many as 5 to 17% of the foal crop each year with significant death loss. Currently, there is no effective vaccine available to prevent this devastating disease. Although there are methods for prevention and treatment, they are expensive, labor-intensive and not always successful. Because infection may occur early in life, a vaccine is needed that can be used in foals within the first month of life. Researchers are optimistic that vaccination of foals early in life using a single *R. equi* protein can result in an immune response capable of protecting foals from *R. equi* pneumonia.

The long-term goals of this research are to develop an effective vaccine for rhodococcal pneumonia and to understand the equine immune responses to these bacterial proteins. To this end, researchers will examine the ability of a vaccine previously tested in mice to develop immune responses in foals in the presence of colostral antibody. These experiments will further test and define the immune responses in mice and allow us to move to testing in horses. Initially, mice will be immunized with the protein rsVap and their immune responses will be evaluated. The data gathered from these studies with mice will guide the choice of vaccine make-up used to vaccinate foals. The type of immune response in foals following vaccination will then be determined.

Experimental Approach

In this study, investigators will demonstrate that vaccination of mice with a single protein (rsVap) sharing sequences with multiple virulence proteins is capable of stimulating an effective immune response. Subsequently, the ability of horse and mouse primary stimulating cells of the immune system to respond to rsVap in the laboratory will be evaluated. Finally, foals will be vaccinated with rsVap to test its ability to stimulate an immune response. The antibody and cellular immune responses of foals to rsVap will be examined. If successful, rsVap can be tested in future studies for its ability to protect foals against *R. equi* pneumonia. *

Do Serum and CSF Testing of Neurologic Horses by Indirect Fluorescent Antibody Increase the Probability of Detection of Equine Protozoal Myeloencephalitis? (05-03)

Ian A. Gardner, Patricia A. Conrad, W. David Wilson, and Paulo Duarte

Equine protozoal myeloencephalitis (EPM) is a neurologic disease in horses caused by protozoal parasites that infect the central nervous system. Clinical signs can include head-tilt, facial paralysis, disorientation, visual deficits, incoordination, and muscle atrophy. Diagnosis is difficult to make because there is no specific assay for this disease and clinical signs of EPM mimic other neurologic diseases of horses. Therefore, this disease is an important differential diagnosis (the determination of which one of two or more diseases or conditions a patient is suffering from by systematically comparing and contrasting their clinical findings) among horses presenting with neurologic signs.

We hypothesize that decision analysis, a formal method for critical appraisal of clinical decisions, can be used to assist in the diagnosis of EPM. By incorporating the magnitude of diagnostic test results to evaluate indirect fluorescent antibody (IFA) testing strategies, we hope to obtain stronger evidence of the likelihood of EPM than we have been able to achieve through simple positive/negative test results and associated predictive values. Previous studies have shown that the IFA test for *Sarcocystis neurona* (one of the parasites that causes the disease) is more sensitive and specific for EPM than the Western blot test. In addition, we found that the results of IFA testing of serum and cerebral spinal fluid (CSF) were correlated. This finding raised questions about whether CSF testing would substantially increase the certainty of EPM diagnosis given a serum IFA result of a certain magnitude.

To develop a decision analysis model that can be used to determine the optimal testing strategy for EPM caused by *S. neurona*, we will evaluate three testing options in the decision tree model (serum only, CSF only, serum and CSF). The model will capture the uncertainty in the pretest probability of EPM and the magnitude of IFA test results (titer values). If successful, the methods developed in this project will not only represent

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Medicine/ Epidemiology



substantial progress toward the management of EPM but will be applicable to other diagnostic problems where quantitative test results are available, such as in joint and peritoneal disease.

Experimental Approach

Laboratory testing is an integral part of the diagnostic strategy for equine neurologic disease because many diseases present with similar clinical signs. In this study, we will use IFA test results from a previously published EPM test validation study that used samples from naturally infected, experimentally infected, and vaccinated horses. The statistical relationship between serum and CSF results will be modeled and formally incorporated into a decision tree model that will allow us to determine whether addition of results of CSF testing substantially increases the probability of EPM being the correct diagnosis. The model will allow for uncertainty in test results, and diagnostic certainty (from using tests on both serum and CSF compared with tests on one or the other) will be assessed in relationship to the increased testing costs and possible negative consequences associated with CSF taps. *

Screening of Thoroughbred Racehorses for Abnormal Platelet Function and Related Clinical Disease by a Unique Platelet Function Assay (05-14)

Fern Tablin, Ian A. Gardner, Jeffrey W. Norris, and Jeanne Bowers-Lepore

Thoroughbred racehorses suffer from bleeding tendencies ranging from mild to catastrophic and resulting in diminished performance and even death. We have characterized a congenital platelet defect in a Thoroughbred mare similar to a bleeding disorder in humans, which is genetically transmitted and occurs with higher frequency in closely related populations. In this disorder, platelet function is greatly impaired. We have developed an assay that identifies this disorder in horses. We propose to use this assay to screen a discrete portion of the California Thoroughbred population in order to assess the incidence of this defect as well as its potential impact on the Thoroughbred population.

In this study, we will use the assay developed in our laboratory (fibrinogen binding assay, FBA) to test platelet function of Thoroughbred horses from a breeding and training farm in California. This assay is sensitive, reliable, reproducible and

able to detect normal and varying degrees of platelet dysfunction in horses. The information we obtain will allow us to estimate the prevalence of platelet disorders in Thoroughbred horses. When information on clinical history, athletic performance and family history is available, we will compare it with the results from the BFA to determine whether the screening assay is predictive of potential or existing problems.

Identification of horses with a predisposition for clinical bleeding disorders will benefit the entire racing industry. This information can be used by breeders, trainers and owners to balance the complicated equations of speed, talent and heritage with normal platelet function and overall health and well-being of equine populations. Although this study would target Thoroughbred horses, this test may have potential predictive value in other breeds.

Experimental Approach

Our laboratory has developed and refined an equine platelet-fibrinogen binding assay that has allowed us to detect the differences between normal, subnormal and abnormal equine platelet function. The FBA will be used to test the blood from Thoroughbred horses at a breeding and training facility in central California. This facility has records of clinical disease and performance in training and racing for the horses tested. When the data sets are complete and have undergone statistical analysis, they will be compared with the clinical history to determine whether the test can predict an increased risk of bleeding disorders or suboptimal racing performance. *

Establishing Normal Ranges of Commonly Analyzed Blood Values of Draft Horses (05-12)


Musa Tivapasi, Joseph G. Zinkl, and Gregory L. Ferraro

Veterinarians frequently use data obtained from analysis of blood to help evaluate the health of patients. In order to be most effective with such evaluations, it is necessary to compare the values with those of healthy animals of similar characteristics. Since there is no recent information on blood parameters in Draft horses in the literature, many veterinarians use data derived from light breed horses. However, reference ranges of blood analytes for Draft horses differ from those of light breed horses. As Draft horses are becoming more popular and veterinarians are seeing them more often for health problems, there is a need for reference ranges from healthy Draft horses.

The goal of this project is to obtain reference ranges for hematology and serum chemical analytes from Clydesdale, Percheron and Shire Draft horses. Blood samples will be obtained from such horses at the cooperating ranches in order to prevent stress of transportation and unfamiliar environment, which can alter some values. Parameters will be determined on 25 horses of each breed using methods established at the VMTH. For comparison purposes, approximately 20 analyses will be performed on light breed horses. Additionally, a subset of values will be determined using the i-Stat® portable clinical analyzer. Establishing data for Draft horses will provide veterinarians with information useful for clinical evaluation.

Experimental Approach

Whole blood and serum will be sent by courier or transported by one of the investigators to the VMTH. Hematologic analysis will be performed using the Advia 120® hematology instrument. Additionally, WBC differential counts, erythrocyte and platelet morphology, and reticulocyte enumeration will be determined by microscopic evaluation of stained blood smears. Serum chemical analysis will be performed using the Hitachi 917® analyzer. A subset of samples will be analyzed at the ranches using an i-Stat® analyzer. Data analysis will include determination of mean and standard deviation of all analytes and data will be rank-ordered. Reference ranges will be calculated using the mean \pm two standard deviations or middle 95th percentile. Comparison between Draft horses and light breed horses will be performed using appropriate statistical methods. Correlations of similar analytes from the VMTH laboratories and the i-Stat® will be performed by regression analysis. *



As Draft horses are becoming more popular and veterinarians are seeing them more often for health problems, there is a need for reference ranges from healthy Draft horses.

Orthopedics



Laboratory Comparison of the Repair Strength of Two Different Screw Types for Fixing Fractures of the Long Pastern Bone in Horses (05-01)

Larry D. Galuppo, Susanne Dykgraaf, and Susan M. Stover

Fractures of the long pastern bone (P1) in racehorses are commonly repaired by internal fixation using standard bone screws inserted in lag fashion. This method usually allows for adequate healing of the fracture. However, complications can occur if the screws lose strength in the soft bone of P1. In this study, we will investigate using headless compression screws made of titanium to repair long pastern bone fractures with the intended goal of reducing or eliminating complications that sometimes arise from using standard bone screws. We anticipate that the headless titanium screw will produce a stronger fixation and avoid soft tissue impingement from a screw head.

With the standard bone screws currently used to repair P1 fractures, complications arising as a result of the screws can result in increased convalescent time, persistent lameness, and the need for a second surgery to remove the screws. Use of headless titanium screws as the sole method of repair or in combination with the AO screw system may help reduce the costs associated with repair as well as increase the chances for the horse to achieve previous performance levels.

Experimental Approach

A simulated long pastern bone fracture will be created in ten pairs of equine cadaver forelimbs (prepared from mid-radius). One bone from each pair will be repaired using two 45-mm tapered compression (Acutrak Equine, AE) screws. The other will be repaired using two standard 4.5-mm AO cortical bone screws of appropriate length and standard technique. The pastern bones will be x-rayed as well as scanned by computed tomography (CT) before repair, and x-rayed before and after mechanical testing. Specimens will be instrumented and tested in a materials testing system to compare the fixation strength of each method. The bone mineral density of P1, determined by CT, will be analyzed to determine whether a relationship exists between screw fixation strength and bone density. Mechanical testing data will be analyzed for statistical differences. A pilot study using five limbs has been performed. The fracture model, screw insertion, and mechanical testing techniques have been standardized. ✱

Computer Simulation of Exercise Management for Prevention of Humeral Stress Fracture (05-05)

Susan M. Stover and Scott J. Hazelwood

Musculoskeletal injuries and lameness are the greatest causes of wastage, training failure, racehorse turnover, and racehorse death in the racing industry. Injuries result in loss of horses from training and racing, jockey injuries, and poor public perception of racing. We believe that the risk for bone fractures in Thoroughbred racehorses can be reduced by distributing the high-speed exercise that is traditionally incurred once a week over two shorter high-speed exercise events within each week. The basis for this hypothesis is that the rate that bone damage accumulates would be lower than the rate of repair.

In this study, we will use computer modeling to evaluate humeral stress fractures because the exercise history, abnormal findings, and mechanical loading environment associated with this specific injury are known. We have chosen to use computer modeling and exercise simulation since it is impractical to test all potentially feasible exercise regimens on live horses. The specific aims of this project are to develop the actual steps required to build and run the model and determine which exercise regimens are best for reducing the incidence of bone fractures. Because exercise can be managed, knowledge of exercise regimens that prevent injury will improve the welfare and economics of racehorses.

Experimental Approach

The size and shape of the humerus will be replicated in a three-dimensional computer model using computed tomography (CT) scans of cadaveric bones. Physiologic loads will be simulated in the model to reproduce different exercise regimens, so that regions of bone that undergo high stress will be “damaged” by an amount related to the exercise incurred. Damaged regions of bone will be removed and replaced over time, as occurs naturally during bone repair. If bone removal exceeds bone replacement, “severe injury” will occur. This approach will allow us to determine which exercise regimens result in high risk for injury. *



We believe that the risk for bone fractures in Thoroughbred racehorses can be reduced by distributing the high-speed exercise that is traditionally incurred once a week over two shorter high-speed exercise events within each week.

Performance

Linda Starkman Research Grant Award



This research study on downhill trotting and its effects on performance is being supported by an honorary grant to acknowledge Linda

Starkman. Established in 2003, the grant recognizes Ms. Starkman's contributions toward the success of the Center for Equine Health. Each year, research projects are reviewed by a Scientific Advisory board, and one that is most appropriate to the interests of Ms. Starkman is selected in her honor.

Since 1995, Ms. Starkman has provided significant leadership, guidance and service in support of the Center for Equine Health, its research and educational mission. She has also been a tireless advocate on behalf of the School of Veterinary Medicine. We gratefully acknowledge Linda Starkman for her dedication to the welfare of horses.

Consequences of Trotting Downhill: How Trotting Downhill Affects Muscle Function and Force Production (05-06)

Steven J. Wickler, Donald F. Hoyt, and Darren Dutto

There is a strong association between running downhill and incurring muscle injury. In fact, downhill running has often been used as a model of muscle injury in humans—to the extent that a basic analysis of muscle function during decline locomotion has received little attention. We know that metabolism is lower while moving down a moderately declined surface (10% decline) than on a level surface due to changes in the actions of the various limb muscles, but the specifics of these changes have not yet been determined. In this study, we will examine the hypotheses that, with proper conditioning, horses can trot downhill without injury, and that the decreased energetic costs of decline locomotion can be related to a decrease in shortening contractions of hind limb muscles.

We will evaluate muscle lengths, forces on the limbs, and joint angles in three hind limb muscles in horses trotting on a level surface and downhill to determine the effect of each activity on the muscles. From this information, we hope to improve our understanding of the basic physiology of the horse during locomotion, specifically how musculoskeletal elements respond to stress and how a horse's locomotor system adapts to different environments. This research can ultimately provide practical approaches to increase athletic performance in the horse and at the same time reduce the incidence of injury that occurs during sporting events.

Experimental Approach

Horses will be slowly conditioned to trot at a range of speeds on a declined treadmill with the intent of minimizing muscle injury associated with downhill exercise. Once conditioned, muscle lengths during locomotion will be measured using small recording devices (sonomicrometry) over a range of trotting speeds. Horses will be led down a 10% decline over a force plate (which records forces on the limbs) at a similar range of speeds while being filmed for motion analysis of the joints. The combination of forces, joint motion, and muscle lengths will permit a comprehensive analysis of how the muscles are functioning. *

Reproduction

Utilization of Müllerian-Inhibiting Hormone in the Diagnosis of Granulosa-Theca Cell Tumors in the Mare and Undescended Testes in the Stallion (05-08)

Irwin K.M. Liu, Alan J. Conley, and Sophie A. Grundy

Granulosa-theca cell tumors (GTCTs) are the most common ovarian tumors observed in mares. This condition may have a variety of effects, from prolonged or absent estrus cycles to stallion-like behavior. GTCTs are typically composed of multiple fluid-filled ovarian cysts lined by granulosa cells. Currently, diagnosis is supported by rectal palpation and serum inhibin and testosterone testing, but these methods do not always yield a conclusive diagnosis because the hormonal activity of GTCTs is variable.

Our previous research leads us to believe that mares with GTCTs, as well as cryptorchid stallions, have increased serum concentrations of Müllerian-inhibiting hormone compared with their normal counterparts. In cryptorchid stallions, it is often difficult to diagnose an undescended testicle in a castrated animal using hormone stimulation testing, because hormone levels may not be as sensitive as desired. In this study, we will use Müllerian-inhibiting hormone to develop a specific clinical diagnostic application for both mares with GTCTs and for stallions with undescended testicles. Such an application could provide a sensitive and specific noninvasive diagnostic tool for clinical use and could be available to clinicians in outpatient settings and field services. Additionally, future applications could include evaluations of XO, XY abnormalities and infertility in the mare.

Experimental Approach

During the first year of this two-year study, we will use immunohistochemistry to demonstrate the expression of Müllerian-inhibiting hormone (MIH). Granulosa-theca cell tumors and ovarian and testicular tissues will be evaluated for the expression of MIH. Fixed tissue sections will be stained using antibodies against human MIH and examined under light microscopy. Our plans for the second year of the study are to clone the MIH gene using polymerase chain reaction and to express the protein in an insect-based expression system. Subsequently, the MIH protein produced will be used to develop antibodies in rabbits, which will then be tested in equine sera in an ELISA reaction. *



Using Sperm-Related Proteins to Predict Reproductive Success (05-07)

William E. Plummer, Peter Sutovsky, and Samantha Hollingshead

Preliminary data indicate that the expression of specific proteins on the surface of sperm cells is correlated with a stallion's reproductive success. We will confirm that finding in this study.

The fertility of stallions is currently assessed subjectively by analyzing semen parameters such as sperm concentration, motility, and morphology and by considering previous breeding records. However, these evaluations are often inaccurate because they are subject to varying interpretations among the evaluators. Preliminary data indicate that the expression of specific proteins on the surface of sperm cells is correlated with a stallion's reproductive success. In this study, we propose to confirm the hypothesis that protein levels may be indicative of fertility in stallions.

Two sperm-related proteins—platelet activating factor receptor (PAFr) and ubiquitin—indicate whether a sperm cell is functionally normal. Ubiquitin is a “housekeeping” protein detected mainly in defective sperm, whereas PAFr is found in normal sperm and is greatly diminished in defective sperm with high ubiquitin content. We will evaluate levels of these proteins in stallions with known fertility and subsequently validate this measure as a predictor of fertility by examining the horses' breeding records. Information gained from this study could provide an efficient and objective means to predict the fertility of stallions lacking prior breeding records. This information could also be useful for determining breeding bookings for stud farms and for evaluating stallions that are considered subfertile as well as those coming off the show circuit or racetrack or recovering from injury or disease. In addition to recognizing all types of sperm defects, this assay has the potential to reduce problems with false detection of normal sperm damaged during processing and detect sperm that are abnormal but appear normal during traditional analysis.

Experimental Approach

This study will be conducted using semen samples from approximately 60 stallions of various breeds. Sperm will be stained with a fluorescent dye and sorted to assess the quantity of the proteins PAFr and ubiquitin contained in the sperm's outer membrane. These initial tests will also help determine the amount of staining required to correlate with the breeding record of the stallion. *

The Effects of Soybean Lipid and Cholesterol on Low-Temperature Damage to Stallion Sperm (05-11)

Stuart A. Meyers and Josette V. Ricker

Cryopreservation of sperm reduces both the lifespan and viability of these cells. Semen processing for cryopreservation involves the addition of some form of lipid, the most common being egg yolk lipids. While such formulations are successful for some stallions, they may be less effective for others. Moreover, these lipids can be undefined, vary from batch to batch, and be subject to microbial contamination. New and effective agents that protect stallion sperm against injury due to freezing are needed. We believe that a chemically defined semen extender containing soybean phosphatidylcholine may significantly improve the quality of cooled and cryopreserved stallion sperm.

In this study, we will determine the effects of extenders containing soybean lipid (soy PC) on sperm survival and function after low-temperature storage. We will also examine the effects of cholesterol supplementation of a soy PC extender on sperm survival and function relative to low-temperature storage. The development of an optimal nonanimal-based extender for low-temperature storage of stallion sperm could be universally applicable to achieve superior sperm quality for artificial insemination. Such a defined product would also decrease or eliminate microbial contamination and minimize any transport restrictions associated with the use of animal-based products.

Experimental Approach

We will evaluate the morphological and functional qualities of stallion sperm cooled and cryopreserved in soy PC- and cholesterol-based semen extenders. Sperm viability, motility and acrosome status will be determined for the various treatments, along with visualization of sperm cell integrity and morphology. The combined results from these assays will be used to specify a defined product(s) of nonanimal origin that will yield optimal sperm quality for artificial insemination. ✱



The development of an optimal nonanimal-based extender for low-temperature storage of stallion sperm could be universally applicable to achieve superior sperm quality for artificial insemination.

Surgery/ Anesthesia



Development of Novel Wound-Healing Products for Horses (05-13)

Fern Tablin, Linda M. Van Hoogmoed, Verena Affolter, and Jeffrey W. Norris

Wounds on the lower limbs of horses present a therapeutic challenge because of prolonged healing times and complications that sometimes occur. Gels composed of fresh platelets and plasma have been used to promote wound healing in a wide variety of species, including horses. We propose that freeze-dried platelets could be used to improve healing in equine limb wounds. This potential product containing freeze-dried platelets would be stable at room temperature and could be used by field veterinarians for treatment of injured horses.

In this study, we will use previously established procedures to develop a method of loading equine platelets with trehalose, a sugar that stabilizes the cells during freeze-drying. We will analyze the freeze-dried platelets compared with fresh platelets for quantities of growth factors present, ability to stimulate cell growth, and wound contraction. We will compare wound healing on horses' lower limbs when treated with platelet preparations versus conventional treatment. Characteristics of wound healing such as time to first epithelialization, rate of wound contraction, and days to complete re-epithelialization will be documented. Finally, we will determine the microscopic characteristics of the wounds at the completion of healing.

Improved wound healing will benefit the entire equine industry by reducing the costs associated with treatment and loss of use. Platelet gels have been used previously in horses in a research setting, but the gels were freshly produced and were not stable for long periods of time. The product for development in this study would have a room-temperature shelf life of many months and would be available for routine use in treating wounds in horses.

Experimental Approach

Our laboratory has developed protocols for creating trehalose-loaded freeze-dried platelets for humans, pigs and mice. Using this expertise, we will develop a protocol for equine platelets.

Freeze-dried platelets will be analyzed for the presence of growth factors by commercially available assays. Prior to evaluation on horses, the activity of the platelet growth factors in the preparation will be assayed using cultured fibroblasts for the growth factor's ability to stimulate cellular proliferation and collagen gel contraction. Subsequently, four standard-sized wounds will be created on the lower limbs of horses under general anesthesia using sterile surgical procedures. The wound treatments will include (1) no treatment other than standardized bandaging applied to all wounds, (2) fresh platelet gel with stimulating agent to cause secretion of growth factors from the horse's own platelets, (3) stimulating agent alone as a control, and (4) trehalose-loaded, freeze-dried platelet preparation also made from the horse's own blood. *



***Improved wound healing
will benefit the entire equine
industry by reducing the costs
associated with treatment and
loss of use.***

Resident Research Grants

Resident Research Grants are awarded to residents within the equine program and are designed to assist them in learning their field of specialty. Often these small projects provide preliminary information that leads to larger, more in-depth research studies.

Fertility Trial of Stallion Semen Frozen in a Soy-Based Extender (04-15R)

Elizabeth L. Scholtz, Barry A. Ball, and Stuart Meyers

The plasma membrane in equine semen is a primary site for damage as a result of freezing. In preliminary studies conducted in our laboratory, we found that a soy phosphatidylcholine freezing diluent may have a stabilizing effect on the sperm membrane. In addition, a soy-based freezing extender could replace animal-derived substances for use in cryopreservation of equine semen, thus increasing biosecurity for the breeding industry and facilities processing equine semen for export purposes. A fertility field trial is needed to validate this extender for widespread industry use.

In this study, we will determine whether fertility rates of stallion semen cryopreserved with a soy-based extender are within the accepted industry standard for frozen-thawed stallion semen and how the fertility rates compare with stallion semen cryopreserved with a conventional freezing extender. If we find that fertility is improved, the soy-based freezing extender might allow a greater number of stallions to undergo semen cryopreservation with a commercially acceptable outcome. Additionally, the livestock industry would benefit greatly from the availability of freezing extenders containing no animal-derived substances for reasons of biosecurity and microbial contamination.

Experimental Approach

This is an initial field fertility trial of stallion semen cryopreserved using a soy-based semen extender. The semen from one stallion of known fertility will be divided into two aliquots and frozen using either a conventional freezing extender or a soy-based freezing extender. Approximately 20 mares of normal fertility will be randomly assigned to one of two groups in a single blind, switchback protocol. Mares will be inseminated using a deep intrauterine insemination technique. Group 1 will be inseminated with frozen-thawed semen cryopreserved in the conventional extender (Control). Group 2 will be inseminated with frozen-thawed semen cryopreserved in the soy-based extender. Pregnancy will be determined via rectal palpation and ultrasound 14 to 16 days after ovulation. *

Predicting Regrowth of Sarcoid Tumors that Have Been Completely Removed by Testing for the Presence of Bovine Papilloma Virus in the Edges of Tumor Specimens Using DNA Analysis (04-16R)

Sandra D. Taylor, Alain P. Théon, Stephen Griffey, and Christian Leutenegger

Equine sarcoids are the most common skin tumors in the horse. The bovine papilloma virus is unequivocally linked with the development of sarcoids and is always detected in tumors and the surrounding skin. However, there is no documented correlation between histologic findings in tissues surrounding tumors and viral infection. This may explain why 20% of sarcoids deemed completely resected histologically grow back. We believe that the presence of bovine papilloma virus in normal-appearing tissue surrounding excised tumors may be a predictor of tumor recurrence. The development of a test to detect transformed cells despite microscopically undetectable tumor would allow us to identify horses at risk for tumor recurrence.

In this study, we will evaluate a new molecular test to predict the risk of tumor regrowth after surgery in horses with sarcoids. The test is based on the detection of bovine papilloma virus DNA in normal-appearing tissue surrounding the tumor. This new diagnostic test will provide information on the association between viral type, infectivity and clinical outcome in horses that remain at risk of failure after a complete surgical resection. The predictive value of the test will be analyzed statistically.

Sarcoids adversely affect the value of horses and often limit the animal's use because of their location—commonly the girth and around the base of the ears, eyelids and distal extremities. Complete surgical resection represents the best chance for a cure. The ability to predict the risk of tumor recurrence would decrease management cost, increase cure rates, and decrease treatment complications.

Experimental Approach

Records of 22 horses from the Veterinary Medical Teaching Hospital database will be used in this study. These horses have had surgery and tissue histopathologic evaluation at the VMTH and have a known outcome after surgical treatment. Archival tissue will be used to determine the presence, amount and type of virus in the tumor as well as surrounding normal tissue. We will statistically analyze the data to determine whether an association exists between the presence and amount of the virus in surrounding tissue and the risk of tumor recurrence. *

We believe that the presence of bovine papilloma virus in normal-appearing tissue surrounding excised tumors may be a predictor of tumor recurrence. The development of a test to detect transformed cells despite microscopically undetectable tumor would allow us to identify horses at risk for tumor recurrence.

Laboratory Comparison of the Repair Strength of Two Different Screw Types for Fixing Fractures of the Long Pastern Bone in Horses (04-17R)

Susanne Dykgraaf, Larry D. Galuppo, and Susan M. Stover

If successful, this technique should reduce complications related to the use of standard bone screws for repairing fractures of the long pastern bone.



Photo showing a typical fracture of the long pastern bone (proximal phalanx) in a horse. This type of fracture is repaired by internal fixation with bone screws.

Internal fixation using standard bone screws inserted in lag fashion is the recommended method of repair for fractures of the long pastern bone (proximal phalanx or P1) in Thoroughbred and Standardbred racehorses. Although this method usually produces adequate fracture healing, complications can occur because the head of the screw can interfere with soft tissues as well as pull out of the soft bone. These complications can result in increased convalescence time, persistent lameness, and the need for a second surgery to remove the screws. Because the tapered compression screw has increased bone-to-screw contact, we believe it may provide greater holding power in soft bone.

In this study, we will determine whether the headless tapered compression screw can be inserted successfully into the long pastern bone of horses and whether it will provide similar or greater fixation strength than the standard lag screw technique. If successful, this technique should reduce complications related to the use of standard bone screws.

Experimental Approach

A simulated long pastern bone fracture will be created in two pairs of equine cadaver forelimbs from horses euthanized for reasons unrelated to fetlock joint problems. One bone from each pair will be repaired using two 6.5-mm to 5.0-mm tapered compression screws (Acutrak Equine), while the other will be repaired using two standard 4.5-mm AO cortical bone screws using standard technique. The pastern bones will be x-rayed before repair and then before and after mechanical testing. All repaired specimens will be tested in a materials testing system to compare the fixation strength between each method. We will assess bone mineral density of the long pastern bone using computed tomography to determine whether any relationship exists between fixation strength and bone density. Joint motion and mechanical testing data will be analyzed for statistical differences in this pilot study. *

Effects of a New Agent (Zelnorm) That Increases Motility of the Bowel in Horses (04-18R)

Michelle Delco, Jorge E. Nieto, Scott Stanley, Brenna Timmerman, and Jack R. Snyder

Colic is the leading cause of death among horses. It is expensive to treat surgically (around \$6,000) and often there are complications after surgery. Treatment consists of prokinetic drugs, which increase motility. However, two of the four most commonly used prokinetic agents used to treat constipation (Cisapride and erythromycin) are no longer available to the veterinary market. Tegaserod, a recently approved medication used to treat constipation in women (Zelnorm), has been shown to be safe and effective in stimulating bowel motility in other species.

In this study, we will evaluate Tegaserod in normal horses to determine whether it increases intestinal movement in different regions of the bowel. We will determine whether it is well absorbed after oral administration in fasted horses and also how often it needs to be administered to maintain blood levels similar to those reported to increase bowel motility in other species. If this drug can be shown to be safe and effective in horses, it may have an advantage over other prokinetics. Tegaserod has demonstrated analgesic effects in humans, acting to decrease the perception of pain. This property may prove particularly useful in the management of impaction colic or postoperative ileus in horses because most of the analgesics currently used in such cases have negative effects on gastrointestinal motility.

Experimental Approach

This study will consist of *in vitro* and *in vivo* parts. For the *in vitro* study, segments of bowel from horses that died at the VMTH will be collected and suspended in special tissue baths under physiological conditions. Increasing concentrations of Tegaserod will be added to the baths, and the movement of the intestine before and after drug administration will be recorded. Results will be evaluated to determine the effect of Tegaserod on different regions of the intestinal tract. For the *in vivo* study, Tegaserod will be administered orally and intravenously to clinically normal horses fasted overnight. An intravenous catheter will be placed in the horse's jugular vein and blood and urine samples will be obtained at different time points. The blood will be processed to obtain the plasma, and the concentration of Tegaserod will be measured in serum. We will then analyze the data to determine appropriate doses and frequency of administration in horses. *

Tegaserod has demonstrated analgesic effects in humans, acting to decrease the perception of pain. This property may prove particularly useful in the management of impaction colic or postoperative ileus in horses because most of the analgesics currently used in such cases have negative effects on gastrointestinal motility.

Evaluation of the Effectiveness of Resins to Recover Microorganisms from Blood of Neonatal Horses (04-23R)

Mireia Lorenzo and Nicola Pusterla

Blood culture is the current “gold standard” test to diagnose sepsis in foals. However, these patients often receive antibiotics before blood is collected for culture, thus masking the bacterial etiology and complicating effective therapy. It is possible that the culture media system used can be improved so that the time required to detect blood-borne bacteria in septicemic foals being treated with antibiotics can be shortened. In particular, we have not evaluated the use of antibiotic-absorbing resin-containing media in the setting of ill neonates given antimicrobials and presented at a referral hospital. In addition, the use of real-time polymerase chain reaction (PCR) analysis may represent a novel and reliable approach to obtain prompt isolation and identification of bacterial pathogens. We will investigate these approaches in this study. These determinations may greatly facilitate selection of the best antimicrobial therapy for foals with septicemia.

Experimental Approach

Blood from horses will be spiked with *Escherichia coli* and three different doses of gentamicin. Samples from each of the three groups will be inoculated into a conventional blood culture medium, a resin-containing medium, and a lysis-centrifugation-based medium and then cultured on agar plates. Bacterial growth will be monitored over a period of 72 hours. A sample for PCR quantitation will be taken from any blood specimen before inoculation and before plating to determine the load of bacterial DNA. Detection of bacteria and time to detection will be compared among the culture media systems. Different controls will also be included to determine the amount of bacterial contamination. *

Investigation of the Distribution, Elimination and Clinical Effects of Midazolam in Newborn Foals (04-24R)

K. Gary Magdesian, Darien J. Feary, and Scott Stanley

Benzodiazepines are frequently used in newborn foals for their centrally mediated sedative, muscle relaxant, and anticonvulsant effects. The benzodiazepine diazepam is a commonly used sedative agent and has been the first-line drug recommended for treatment of seizures in critically ill newborn foals. However, we believe that another drug, midazolam, which is the first-line drug of choice in critically ill human neonates, may

offer a safer and more reliable alternative to diazepam in foals.

In this study, we will investigate the safety and effectiveness of a single intravenous dose of midazolam in normal neonatal foals. We will determine the distribution and elimination of the drug as well as its effects on the cardiorespiratory system. Because the metabolism of drugs in newborns is different than it is in adults, these findings will provide useful baseline information for selecting appropriate and safe doses of midazolam for critically ill foals admitted to the neonatal intensive care unit at the VMTH. The information will also provide baseline data for further investigation into the use of this sedative in neonatal foals.

Experimental Approach

Six healthy foals between 12 and 24 hours old will be administered a single intravenous dose of midazolam (0.2 mg/kg). Serial blood samples will be taken via an intravenous catheter to determine blood concentrations and elimination of midazolam from the body. The cardiovascular and respiratory systems will be monitored frequently during the study to determine the effects of the drug. Foals will be allowed to nurse from the dam as normal during the study. *

Detailed, Three-Dimensional Imaging of Extracted Equine Upper Cheek Teeth by High-Resolution Computed Tomography: Pulp Cavity Anatomy and Its Age-Related Changes (05-01M)

Helena Kuntsi-Vaattovaara, Frank J.M. Verstraete, and Andres Laib

Equine dental disease is a common, severe medical problem leading to suboptimal performance or even life-threatening medical conditions. Extractions of cheek teeth are difficult and often complicated, whereas root canal treatment can preserve a functional tooth and is a less traumatic procedure. However, the anatomy of the upper cheek teeth in horses is complex and we do not have sufficient knowledge of it for routinely applying root canal therapy.

In this study, we will acquire information on the pulp cavity anatomy of equine upper cheek teeth to further our understanding and improve the accuracy of diagnosis for dental disease. We will use a modern, high-resolution, three-dimensional computer tomography technique to image the cheek teeth of one quadrant of a small group of horses of different ages. The images will reveal the number and shape of root canals and pulp horns, their architectural complexity, and connection with each other at different ages. This information will be used to plan further studies as well as determine the required capacity of imaging techniques to achieve clinically adequate information.

Experimental Approach

Extracted right- or left-side upper cheek teeth of five horses (5 to 13 years old) will be used for this study. The horses will be selected from animals that were euthanized for reasons unrelated to problems with the teeth or upper cheek. The age of the horses will be verified from medical records and registration files, and one horse of 5, 7, 9, 11 and 13 years of age will be selected. The teeth will be examined with a high-resolution microcomputed tomography device and software that allows creation of high-quality, three-dimensional images and animation of objects. The images will allow definition of the number and shape of pulp compartments, their possible connection with each other, and age-related changes. *

Marcia MacDonald Rivas Research Grants

The Marcia MacDonald Rivas Grant program has been in existence for over 20 years in the UC Davis School of Veterinary Medicine. Recently, this program was transferred to the Center for Equine Health for oversight and management of the selection of projects. These grants are awarded to new teaching and research personnel (less than 5 years in the School of Veterinary Medicine).

Investigation of the Severity of Sepsis in Equine Neonates by Determining the Bacterial Load and the Inflammatory Mediator Tumor Necrosis Factor- α Using TaqMan PCR (05-2M)

Nicola Pusterla, Christian M. Leutenegger, and K. Gary Magdesian

Despite advances made in the care of neonatal foals, sepsis—the systemic inflammatory response due to bacterial infection—remains the principal cause of death. Sepsis is often diagnosed based on clinical signs, blood abnormalities, and positive bacterial culture of blood. However, infected foals may have minimal or nonspecific signs that made definitive diagnosis of sepsis difficult. Furthermore, bacterial culture is time-consuming and sometimes unrewarding, especially if the foal has been pretreated with antimicrobials. Molecular techniques such as quantitative real-time TaqMan PCR make it possible to detect and quantitate the bacterial load as well as markers of inflammation such as tumor necrosis factor- α (TNF- α).

In this study, we will investigate fast and reliable molecular markers of sepsis in foals presented to the VMTH. We will compare the bacterial load and gene transcription of TNF- α in the peripheral blood between neonatal foals with sepsis and healthy foals. In the search for more accurate measures of sepsis, we expect that these markers of inflammation determined by TaqMan PCR will aid in the early diagnosis and improved prognosis of this condition.

Experimental Approach

We propose to perform the study on 50 to 60 neonatal foals expected to be presented during the 2005-2006 foaling season to the VMTH with life-threatening conditions such as sepsis (test group). An additional 20 healthy foals seen at 12 to 24 hours postfoaling as part of their health check will constitute the healthy control group. Foals of both groups will undergo a thorough physical examination. Further blood collection for blood cell count, immunoglobulin G levels and bacterial culture(s) will be collected as part of the routine check-up. Blood collected on initial presentation will be stabilized in vacutainers and processed for nucleic acid extraction with subsequent analysis for bacterial load and gene transcription of TNF- α using existing TaqMan PCR assays. The molecular results (bacterial load and TNF- α transcription) will thereafter be compared with clinical and routine blood work (CBC, blood culture) and clinical criteria of sepsis to determine their diagnostic and prognostic values. *

In the search for more accurate measures of sepsis, we expect that these markers of inflammation determined by TaqMan PCR will aid in the early diagnosis and improved prognosis of this condition.



Can Abdominal Pressure Measurements in Horses with Colic Be Used to Predict Treatment and Outcome? (05-03M)

Julie E. Dechant, Jorge E. Nieto, and Sarah S. Lejeune

A distended abdomen often indicates increased abdominal pressure, but critical increases in abdominal pressure can occur without such signs. Increased intra-abdominal pressure is a significant finding, because the increased pressure can compromise blood flow to critical organs, resulting in organ malfunction and failure and increasing the absorption of bacteria and bacterial toxins from the intestinal tract. The measurement of intra-abdominal pressure has not been routinely performed in horses, although this information could significantly improve the monitoring and treatment of sick horses. Increased intra-abdominal pressure is expected to be a common occurrence in colicky horses, and the severity of the condition is likely to indicate postsurgical complications or predict the development of complications.

In this study, we will validate the measurement of intra-abdominal pressure by measuring pressure on the urinary bladder in mares and comparing it with direct pressure measurements during standing laparoscopic surgery. We will determine intra-abdominal pressure measurements in normal adult mares as well as in horses with medical and surgical colics. In the latter case, we will determine whether a relationship exists between intra-abdominal pressure measurements and treatment decisions and the development of complications. A simple, inexpensive and easily performed diagnostic test such as intra-abdominal pressure measurement could greatly aid the decision-making process for horse owners, referring veterinarians and specialists by predicting the need for referral and critical care management and allowing rapid recognition and correction of abnormalities.

Experimental Approach

The bladder of mares will be catheterized and drained of urine using a rubber catheter. Sterile water (200 ml) will be infused into the bladder and the catheter will be connected to a pressure gauge and measured relative to the level of the pelvis. This test will be validated in 10 mares undergoing standing laparoscopic surgery at the VMTH and compared with direct-pressure measurements in 20 normal mares from the CEH using an abdominocentesis catheter. If the pressure measured using the abdominocentesis catheter can be correlated with the bladder pressure measurement, the former method would be preferable because it could be measured during a colic workup and the technique could be performed in mares, stallions or geldings. If the abdominocentesis technique is successful, it will then be performed in 20 horses presenting for colic at the VMTH. This

procedure will be repeated at different stages during hospitalization to determine whether a relationship exists between abdominal pressure and changes in blood tests and clinical condition. On the other hand, if the abdominocentesis technique does not correlate well with bladder pressure measurements, then this part of the study will be performed only in mares with colic. *

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