The Equine Health Program at North Carolina State University’s College of Veterinary Medicine brings together horse owners, veterinarians, and CVM faculty on the issues, research, and advancements concerning horse health in North Carolina and beyond.

www.cvm.ncsu.edu/docs/ehp.html
To horse owners, practitioners and faculty,

As the Assistant Department Head of Equine Programs, I would like to take this opportunity to welcome you all to the annual Equine Health Program Research Update. I firstly want to acknowledge the tremendous suffering of those individuals and their animals caught in wrath of Hurricane Katrina. NC State University is doing its utmost to offer assistance to horse owners, including those that may want to ship their horses to our state for care while their homes and farms are restored.

Our research programs are critical to the mission of the College of Veterinary Medicine because they form the basis of the way in which we study and understand those problems that take the lives of horses, and those that keep our horses out of work. In addition, we actively research new methods of increasing and improving breeding programs so that we can become less dependent on breeding programs that are overseas or out of state. We are constantly assessing new ways of funding this research effort as federal funds become scarce, and private funding becomes more and more competitive. Presently, most of our funding ultimately comes from the horse owner, either by direct donation, via contributions from feed sales, or from national animal health foundations. Anyone interested in assisting in any way should feel free to contact the College of Veterinary Medicine Foundation Office.

As far as our specific research programs, the Colic and Digestive Disease Program has a mission of understanding colic so that we can reduce the unacceptably high number of deaths from colic. This is the leading cause of loss of life, and has been for centuries. We aim to change that by focusing on prevention, with projects assessing de-worming and feeding programs, and reduction of mortality, by developing new treatments for those horses with severe cases of colic caused by intestinal strangulation. Other programs have more of an emphasis on the problem on loss of use. The Sports Medicine Program has a newly acquired Iams Pet Imaging Center, which contains one of only two high-power Magnetic Resonance Imaging (MRI) units in the country that can be used for horses (the other is in Washington State). MRI provides the best possible views of regions of horses’ legs diagnosed with lameness problems. A lot of research needs to be done to understand the new findings from this technology, although we have already made great strides in our understanding of MRI. Within the Sports Medicine Program, we also study diseases of the respiratory tract. There are two major thrusts to this – horses with poor lung function associated with reactive airway disease (formerly ‘heaves’) and improvement of
upper airway function, such as in those horses that have pharyngeal or laryngeal problems (‘roarers’). The major innovations in these areas include lung function testing equipment, and laser surgery respectively. NC State is one of the few institutions in the United States that has the faculty and equipment to offer these options for respiratory patients. The Ophthalmology Program continues to work on preserving the eye site of horses with problems such as corneal ulcers and recurrent uveitis (‘moon blindness’). Horses have a propensity for developing eye injury because their eyes are large and prominent, although the high prevalence of uveitis is harder to explain. We have the only equine-dedicated ophthalmology service in the United States, and our faculty continue to assess new ways of improving treatments for important eye diseases. Finally, we have developed an embryo transfer program in Southern Pines so that successful show horses can continue to be bred even if they cannot carry the foals themselves. This area is critical as North Carolina becomes one of the leading centers for breeding of performance horses, considering prior efforts have largely been directed at racing Thoroughbreds.

All of our research programs branch directly off our clinical programs. The hospital is therefore the driving force for our research, and we are indebted to the dedicated horse owners and their veterinarians for bringing us their horses so we can continue to break new frontiers in equine medical care. I am particularly grateful to our new dean, Dr. Warwick Arden, for strongly supporting our equine programs, Dr. Dick Mansmann for linking us with the outside world, and our hard working clinicians, students and technicians, who work well beyond the normal work week to promote our programs. I hope to meet with many of you during the research overview, and invite you to contact me with any questions about our equine programs.

Best regards to you and your horses,

Anthony Blikslager, DVM, PhD
Diplomate, American College of Veterinary Surgeons
Assistant Department Head, Equine Programs
Director’s Report

September 14, 2005

To equine practitioners, horse owners, veterinary students and CVM faculty,

We are completing our fourth year in the new Equine Health Program and things continue to grow and expand. At this very minute I am in the middle of organizing potential horse evacuees from Hurricane Katrina with the College of Veterinary Medicine, SART, an AAEP member practitioner in Memphis, Dr. Kelli Ferris from NC State who is Baton Rouge, NC State Extension Horse Husbandry, and the NC Horse Council all working together to help our fellow horse owners and their troubled horses.

This year was a year of organization under the guidance of our new Dean, Warwick Arden. He has a strong understanding of horses, equine practitioners and horse owners and feels, like I, the best way forward for horse health is all of us working together. He spent a full day in retreat this past May with the equine faculty hearing their ideas both from the past; but more importantly, what they wanted to do in the future. He has named Dr. Anthony Blikslager as Assistant Head of the Department of Clinical Sciences in charge of all equine related programs in the College and has made the Equine Health Program and myself primarily related to Outreach. In that role we continue to expand our annual programs such as the Research Overview, where we now have more abstracts, which means more research, helping advance North Carolina horse health. The AAEP reception last year has grown with Michael Schramme giving an overview of our new Iams Equine MRI to alumni and other veterinarians at the reception in Denver. There have been lots of educational opportunities for veterinarians and horse owners during the year.

One area that I have focused a great deal of concern is in the shortage of equine practitioners. There are less students graduating wanting to have equine medicine and surgery as a career. They graduate with $60,000 on average debt from NC State which is $12,000 less than the national average. Some young veterinarians practice for a few years and then go into other areas of veterinary medicine. What can you do to help:

1) Consider scholarship donation to help reduce their debt. The equine scholarships are improving in numbers from the Randall Terry Foundation, the NC Hunter/Jumper Association, the Raleigh Spring Premiere Horse Show and the NC Thoroughbred Association and have now become ongoing scholarships.

2) Consider telling us about a summer job with some compensation at a training, boarding or breeding facility that has at least one veterinarian coming there.
weekly to which students could apply. The student would be learning husbandry
and handling skills but also be allowed to observe with the professionals.

3) Connect us with a young agriculturally minded horse person who has expressed
some interest in biology or veterinary medicine. It is important that this person
love to care for all types of horses – stallions and foals, too – not just love horses.
Let me know. Any time contact me with good news, ideas or suggestions on
improvement.

Dick

Richard A. Mansmann, VMD, PhD
Clinical Professor and Director of Outreach
Equine Health Program
NC State College of Veterinary Medicine

www.cvm.ncsu.edu/docs/ehp.html
From the CVM Office of Development
2004 – 2005

The Bernice Barbour Foundation

The Bernice Barbour Foundation was established in 1986 by the late Bernice Wall Barbour. The Bernice Barbour Foundation is a family foundation that supports animal welfare and in many cases supports equine related projects across the country. In February 2004, the North Carolina State University’s College of Veterinary Medicine was one of four veterinary colleges asked to present to invitees of the Bernice Barbour Foundation in Florida the latest equine health studies. Dr. Rich Redding presented on the clinical significance of suspensory ligament problems. Once back in Raleigh, Dr. Redding submitted a proposal asking to fund the study of Equine Suspensory Ligament Injuries – Access to MRI Diagnostics for Horses in Need. In December of 2004, the College was granted $25,000 from the Bernice Barbour Foundation. This funding has helped provide access to the latest MRI diagnostic equipment at NC State for horses suffering from suspensory ligament injuries as well as to initiate the first of four years of SLI clinical trials and to support Dr. Redding’s ongoing research in equine sports medicine that will benefit the global equine community.

Equine Oriented Veterinary Scholars

Scholarships motivate, reward student recipients and reduce their educational debt making it more attractive to enter equine practice. Scholarship donations keeps the cost of a world class veterinary medical education within reach. This year four equine scholarships were awarded. The North Carolina Hunter Jumper Association Equine Scholarship was awarded to 3rd year student Lisa Kivett. The North Carolina Thoroughbred Association Scholarship was awarded to 4th year student Brandi Phelps. The Randall B. Terry, Jr. Horse Racing Scholarship was awarded to 4th year student Tami Turley. The Raleigh Spring Premier Scholarship was awarded to 4th year student Gretchen Laws. Congratulations students and thank you scholarship donors!
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NORTH CAROLINA STATE UNIVERSITY’S 2-YEAR-OLD EQUINE REPRODUCTIVE PROGRAM FINDS ITS STRIDE

Champion eventer Denny Emerson and champion show hunter Rox Dene share in successes

Southern Pines, N.C., August 29, 2005—Equine veterinarians and horse owners across the southeast are welcoming a new array of advanced equine reproductive services now available from North Carolina State University’s (NCSU) equine research center located in Southern Pines, N.C. In addition to the services offered at the college’s main campus in Raleigh, the expanded reproductive program was initiated two years ago and supports the growing sport horse breeding programs in the region. The NCSU Southern Pines facility offers comprehensive services that include semen freezing, embryo transfer, oocyte and gamete intrafallopian transfer (GIFT), as well as advanced methods of sperm analysis. According to Dr. Carlos Pinto, who designed and now oversees the program, “We also plan to offer in-vitro fertilization in one to two years.”

“Hunter of the Century” produces foal after several failed attempts

Last April, the veterinary team in Southern Pines welcomed a newborn foal, “Eyelet,” nicknamed “Poppy,” a filly helped into the world through the miracle of modern reproductive techniques. The filly’s dam is the legendary Dutch Warmblood mare, Rox Dene. Called the “Hunter of the Century,” Rox Dene dominated the U.S. hunter show ring in the 1990s, winning multiple championships in both conformation and working divisions at all the largest shows. She was awarded several Horse of the Year titles, and in 2003 was inducted into the National Show Hunter Hall of Fame. In 1996, Rox Dene’s owners, Elaine and Chanda Boylen, retired her from showing and moved her to Ed and Parker Minchin’s Pine Meadows Farm in Vass, N.C., just a few miles from NCSU’s research center in Southern Pines. In their care, Rox Dene delivered a colt in 1999 and a filly in 2001.

Despite several additional attempts to breed Rox Dene from 2002 to 2004, the mare was unable to carry any subsequent pregnancies to term. The Minchins’ equine practitioner, Dr. Tom Daniel of Southern Pines Equine Associates, referred them to NCSU’s research center where they met board-certified theriogenologists Dr. Pinto and Dr. Michael Whitacre and decided to attempt to breed Rox Dene one last time. The mare was artificially inseminated using frozen semen from Popeye K, a Dutch warmblood that was the 2004 USEF Horse of the Year for the Green Conformation Division. Seven and one-half days after ovulation, Dr. Pinto transferred the embryo to Spyglass, one of the surrogate mares in the center’s herd. Fifty days after gestation, Spyglass was moved to the Pine Meadow Farm where she stayed under the watchful eye of the Minchins until the gleeful day when Poppy arrived.

“We are thrilled with the reproductive assistance that NCSU provided,” says Parker Minchin. “Our experience with Dr. Pinto and Dr. Whitacre was exceptional. Everyone we
worked with on the staff was not only very knowledgeable, but took a personal interest as well.”

“Rox Dene is a champion and Popeye K is one of the top stallions in the nation,” says Dr. Pinto. “This filly has championship bloodlines and the genetic potential to become a superstar.”

**Former gold medalist now involved in breeding**

Three-day event rider and trainer Denny Emerson, a former U.S. Olympian, USET world champion gold medalist, and now chairman of the Breeders Committee of the USA Equestrian, also has firsthand knowledge of NCSU’s equine reproductive support. Working with stallions from Emerson’s Tamarack Hill Farm, the center has collected semen from Reputed Testamony, a thoroughbred; Aberjack, a New Zealand sport horse; and Formula One, an Irish sport horse.

Emerson explains, “Our relationship with NC State’s equine reproductive team has enabled us to expand our stallion services to a wider network of clients, and has led to a much greater professionalism in our business. Their cutting-edge technology, reliability and friendly staff have all helped make equine reproduction a much more successful and significant piece of our business,” he adds.

**Continuing education for practitioners; student training; research**

In addition to clinical services offered at both the main campus in Raleigh and the research center in Southern Pines, NCSU’s Equine Health Program provides continuing education to practicing veterinarians, trains veterinary students and conducts medical research. Dr. Pinto was hired in 2002 to develop the college’s equine reproductive program. A native of Brazil, Dr. Pinto received his veterinary degree from Sao Paulo State University, and worked there in mixed animal practice before coming to the U.S. in 1991 to further his education. He studied at Louisiana State University, where he completed a residency in _heriogenology, and later, a PhD in reproductive physiology.

As pleased as he is with the success of the clinical services, Dr. Pinto seems most proud of the teaching services offered at the college. “We now have three newly graduated veterinarians working in reproductive internships,” he beams. “The students have been stimulated by the learning opportunity here and want to pursue further training in _heriogenology.”

**About North Carolina State University’s Equine Health Program**

Established in 1979, North Carolina State University’s College of Veterinary Medicine (CVM) is one of the youngest veterinary schools in the United States, yet is currently ranked fourth among the nation’s 28 veterinary schools by *U.S. News and World Report*. The College’s Equine Health Program offers nationally-recognized tertiary equine care in ophthalmology, reproduction, sports medicine, and colic and digestive diseases. The program attracts distinguished equine specialists from around the world to its faculty. In
addition to training students and offering continuing education for equine practitioners, the program supports equine veterinarians and their clients by offering advanced equine diagnostics and therapeutic options that may not be available in private practice. For more information, or to make a tax-deductible contribution, call 919-513-0035 or visit www.cvm.ncsu.edu/docs/ehp.html.

Dr. Carlos Pinto with Poppy, a filly he helped into the world through the miracle of modern reproductive techniques. Carried to term by a surrogate mare, Poppy has championship bloodlines from both her dam (the legendary show hunter, Rox Dene) and sire (2004 USEF Horse of the Year, Popeye K).
Colic and Digestive Diseases

Abstracts:


Gerard M, Blikslager A, Roberts M. Prospective study of risk factors for the development of ventral midline incisional infection following colic surgery in horses.

Little D, Tomlinson J, Blikslager A. Postoperative inflammation in equine small intestine after manipulation and ischaemia.

Little D, Gardner SY, LaFevers DH. Emergence of cyathostomins resistant to ivermectin on a breeding farm in North Carolina.

Little D, Mansmann RA. Investigation of suspected moxidectin resistance on a boarding farm in North Carolina.

Little D, Brazik EL, Luquire JT. Pyrantel pamoate resistance in horses receiving daily administration of 2.64 mg/kg (1.2mg/lb) PO pyrantel tartrate on a boarding farm in Eastern North Carolina.
Callie Fogle, DVM  
Resident  
Equine Surgery  

Mathew Gerard, BVSc  
Yvonne Elce, DVM  
Alison Morton, DVM  
Anthony Blikslager,  
DVM  

Description of the Problem:  
Small intestinal surgery in the horse traditionally has a high rate of postoperative complications and consequently a lower survival rate as compared to large intestinal surgery. Ileus, colic, adhesions, and anastomotic obstructions are the most common complications for horses recovering from small intestinal surgery. Carboxymethylcellulose products have recently become available to reduce onset of adhesions, although the clinical efficacy of these products is unknown. This retrospective study was performed to analyze the association of sodium carboxymethylcellulose (CBMC), and a bioresorbable hyaluronate-carboxymethylcellulose membrane (Seprafilm®) on postoperative complications; particularly colic, as an early indicator of adhesion formation.

Study Objective:  
➢ To determine if new treatments available for reducing postoperative complications make a difference in a clinical setting

Experimental Approach:  
A database of horses having colic surgery from 2003-2004 at North Carolina State University-College of Veterinary Medicine was examined. A total of 33 horses that recovered from anesthesia for small intestinal surgery, and had use of either carboxymethylcellulose or Seprafilm intraoperatively, were selected for inclusion in the study. A control population of 164 horses was selected from databases on horses undergoing small intestinal surgery prior to
the use of carboxymethylcellulose at NC State University from 1994-2002. Data extracted included age, sex, breed, lesion diagnosis, resection and location, use of Seprafilm and/or CBMC, postoperative clinical pathologic data, short-term postoperative complications, and short-term survival. Data was analyzed using univariate and multivariate logistic regression using a statistical software package (SigmaStat, Jandel Scientific, San Rafael CA).

**Accomplishments/Results:**

The average age of the horses that received an intraoperative preventive treatment for adhesions was 12.5 years (range 0.5-30 years), with 48% (16/33) cases in the 0-9 year range, and 51% (17/33) in the 10-30 year range. The breed distribution was representative of the hospital population: 10 Quarter Horses, 4 Tennessee Walking horses, 3 Warmbloods, 3 Arabians, 2 Paint horses, 2 Appaloosas, 2 Thoroughbreds, 2 Morgan horses, 2 ponies, 1 Standardbred, 1 Belgian, and 1 mule. Geldings represented 58% (19/33) of the population, whereas 33% (11/33) of the horses were mares, and 9% (3/33) were stallions. There were 16 cases of ileal impaction, 9 strangulating lipomas, 5 miscellaneous strangulations, 2 gastrosplenic entrapments, and 1 small intestinal volvulus. Of the 33 cases treated with CBMC or Seprafilm, 16 (48%) required resections. Of 164 horses in the control small intestinal surgery population, 100 (61%) required resections. Univariate analyses with postoperative colic, incisional drainage, and survival as individual dependent variables were used to select independent variables for use in multivariate logistic regression analysis. The results were reported as odds ratios (OR), including the 95% confidence interval (CI), indicating the risk for or protection from postoperative colic and incisional drainage. Horses that had small intestinal resections (OR 4.4, CI 2.4-8.1) and postoperative ileus (OR 4.5, CI 2.5-8.2) were more likely to suffer from postoperative colic. Alternatively, horses in which CBMC (OR 0.6, CI 0.2-1.9) or Seprafilm (OR 0.4, CI 0.1-1.2) were used intraoperatively were less likely to have short term postoperative colic. Multiple logistic regression revealed that the combination of resection (OR=4.9, CI=2.5-9.4), CBMC (OR=0.6, CI=0.2-1.9), and Seprafilm (OR=0.4, CI=0.1-1.2) provided the provided the strongest association with colic. Despite the lack of significance of CBMC and Seprafilm in this model, inclusion of these factors substantially improved the model, and increased their protective effect. Horses that had a resection (OR=2.7, CI=1.5-5.1) or postoperative ileus (OR=1.8, CI=1-3.3) were at an increased risk of developing incisional drainage, whereas use of CBMC (OR=0.6, CI=0.2-1.7) tended to be protected from incisional drainage. However, no multiple logistic regression model enhanced the significance of these factors in assessing risk of incisional drainage. The high short term survival in the 33 cases that received either CBMC or Seprafilm (81%) precluded accurate statistical modeling.
Benefits to the Equine Industry:

Horses with postoperative ileus or a surgical resection have a significantly increased risk of postoperative colic and incisional drainage. While this was not surprising, it was interesting to note that CBMC and Seprafilm appeared to have a protective effect in the horses with surgical resections against postoperative colic, suggesting the possibility of reduced formation of adhesions. It was also interesting to note that CBMC did not place horses at increased risk of incisional drainage, a concern of some surgeons because of the spillover of this product into the surgical abdominal incision during application. Additional studies will be required to assess the long-term implications of use of CBMC and Seprafilm on postoperative colic and adhesion formation. However, based on the present data, it appears that use of both CBMC and Seprafilm tend to be protective against postoperative colic when used in horses undergoing intestinal resection.

Lisa Frederico, DVM
Resident
Equine Surgery

Anthony Blikslager, DVM

Description of the Problem:
Small colon impactions account for 1.9-18% of colic cases admitted to referral centers. Diffuse small colon impactions account for 34.3% of all equine small colon disease. Concomitant diagnosis of colitis raises suspicion that small colon impaction may result from motility disorder. Outcome varies between surgical and medical treatment. Difficult to give accurate prognosis for post-operative recovery or short and long-term survival due to low incidence, as well as to sparse and conflicting data.

Study Objective:
- To determine risk factors for development of small colon impaction.
- To determine useful parameters in deciding whether to manage horses with small colon impaction medically or surgically.

Experimental Approach:
Samples of normal jejunum, jejunum from the proximal resection margins of clinical cases and jejunum obtained 18 h after 1 or 2 h ischaemia or manipulation alone were evaluated for neutrophil infiltration. Samples obtained 18 h after surgery were additionally evaluated for leucocyte activation using calprotectin immunohistochemistry. Results were evaluated by ANOVA and P < 0.05 was considered significant.

Accomplishments/Results:
Out of 44 cases of small colon impaction, 21 were treated surgically and 23 medically; whereas of the 83 large colon impaction cases, 21 were treated surgically and 62 medically. There was a five-fold increased risk for horses with diarrhea prior to presentation at the
referral center to develop impaction of the descending colon 74.4% of small colon impaction cases had >24 hour duration of colic prior to presentation versus 45.8% of large colon impaction cases. There was a ten-fold increased likelihood that patients with abdominal distention would require surgical correction. Surgical cases had longer hospitalization times compared to cases managed medically. The average time until hay was fed to medically and surgically managed patients was 3.26 days and 9.95 days, respectively. There was a trend for horses with greater duration of colic, increased temperature, and increased pulse to require surgery. Short term survival rates for medically and surgically managed cases of small colon impaction were 95.2% and 91.3%, respectively. The average cost of medical management of small and large colon impaction was $1688 and $1152, respectively. The average cost of surgical management of small and large colon impaction was $5017 and $3725, respectively.

**Benefits to the Equine Industry:**

Of the environmental risk factors for developing small colon impaction, diarrhea was the only one of significance. The development of diarrhea associated with small colon impaction may be related to the pathogenesis of this type of colic, which is unknown. Possible theories of pathogenesis include colonic edema, motility dysfunction, increased delivery rate of an increased amount of ingesta, or a combination of these abnormalities. The short term survival rates for surgically managed cases in this study (91.3%) were slightly higher compared to other studies (86% and 77.8%). Short term survival rates of the medically managed cases (95.2%) were similar to those of other studies (87% and 100%). Clinicians should pay close attention to the presence of abdominal distention, as this is a significant factor in deciding whether to manage small colon impaction cases medically or surgically.
Prospective study of risk factors for the development of ventral midline incisional infection following colic surgery in horses

Mathew Gerard, BVSc, PhD, DACVS
Assistant Professor, Equine Surgery

Anthony Blikslager, DVM, PhD, DACVS
Associate Professor, Equine Surgery

Malcolm Roberts, BVSc, PhD, FACVSc
Professor, Equine Medicine

Assistance in data collection is provided by the entire Equine group and veterinary students (Matt Blevins and Kaelynn Moury)

This study is funded by the North Carolina Horse Council

Description of the Problem:

The ventral midline incision approach is used most commonly in North America to explore the horse’s abdomen for diagnosis and treatment of colic. Emergency colic surgery accounts for approximately 50% of all equine surgeries performed at NCSU-CVM. According to published studies about 25% of horses develop incisional infection following colic surgery. The presence of incisional infection increases patient morbidity and costs of treatment. Risk factors for the development of incisional infection at one surgical facility are not necessarily the same for another due to slight variations in surgical techniques and protocols.

Study Objectives:

- To determine risk factors for the occurrence of incisional infection following an exploratory celiotomy for the diagnosis and treatment of colic in horses at NCSU-CVM

Experimental Approach:

This investigation is designed as a prospective study. Data collection forms were devised and are used to record information on all horses admitted for colic management at NCSU. Horses that undergo surgery for treatment of the colic and that are discharged from the hospital will be included in the study population analysis. The data collection is comprehensive in an effort to record all variables that may influence incisional healing, both intraoperatively and perioperatively. Follow-up phone calls are made to owners after the horse is discharged from the CVM to collect information on any incisional healing complications and postoperative outcome. Risk analyses will be performed when the study population is large enough.
Accomplishments/Results:

- The study was initiated in March 2003 and is currently ongoing
- As of August 2005, operated on 264 colic patients and incisional complications (includes serous drainage, infection) have been recorded in 22% of surviving horses
- Risk analyses require more study population numbers before they can be accurately performed

Benefits to the Equine Industry:

Identification of risk factors will facilitate alterations in colic treatment both intraoperatively and perioperatively to help reduce the incidence of postoperative incisional infection. Decreased occurrence of incisional complications will reduce patient morbidity, hospitalization, and treatment costs and therefore improve the overall outcome for horses undergoing colic surgery at NCSU-CVM.
Postoperative inflammation in equine small intestine after manipulation and ischaemia

Dianne Little, BVSc
Research Associate
Equine Gastrointestinal Physiology

Julia Tomlinson, BVSc
Anthony Blikslager, DVM

Description of the Problem:

Post operative ileus (POI) remains an important cause of post operative morbidity and mortality in the horse. However, clinical progression of naturally occurring cases of POI in both horse and man does not entirely support the 'neurogenic' hypothesis as the sole mechanism of POI; and the hypothesis that inflammation plays a major role at 12-24 h after surgery requires validation.

Study Objective:

- We hypothesized that an inflammatory infiltrate in the muscularis externa and myenteric plexus of equine jejunum is present 18 h following a period of ischaemia.

Experimental Approach:

Samples of normal jejunum, jejunum from the proximal resection margins of clinical cases and jejunum obtained 18 h after 1 or 2 h ischaemia or manipulation alone were evaluated for neutrophil infiltration. Samples obtained 18 h after surgery were additionally evaluated for leucocyte activation using calprotectin immunohistochemistry. Results were evaluated by ANOVA and P < 0.05 was considered significant.

Accomplishments/Results:

Significant neutrophilic inflammation was identified in the samples from the proximal resection margins of clinical cases compared to uninjured jejunum. In experimental cases, neutrophilic inflammation appeared to be increased further by 18 h and was identified through all intestinal layers, particularly in
the serosa, fascial planes around circular and longitudinal muscle fibres, and myenteric plexus. This elevated level of neutrophilic inflammation was mirrored by an increased number of calprotectin-positive cells in these intestinal layers, indicating leucocyte activation. CONCLUSIONS: Significant neutrophilic inflammation occurs in equine jejunal myenteric layers 18 h after surgery.

Benefits to the Equine Industry:

This neutrophilic inflammation coincides with the clinical time point at which POI is identified and may indicate that inflammatory pathways, rather than solely neurogenic pathways, are responsible for POI in the horse.
Emergence of cyathostomins resistant to ivermectin on a breeding farm in North Carolina

Dianne Little BVSc MS
MRCVS DACVS
Research Associate
Gastrointestinal
Physiology

Sarah Y. Gardner DVM
PhD DACVIM Associate
Professor of Equine
Medicine

D. Heath LaFevers, BS
Equine Medicine
Research Technician

Description of the Problem:

The small strongyles or cyathostomes are the major pathogenic gastrointestinal parasite of the horse today. Cyathostome infection can cause a wide variety of clinical and sub-clinical disease from severe diarrhea and potentially fatal larval cyathostomiasis to colic, ill-thrift, weight-loss and poor performance. There are only three classes of drugs available that effectively treat cyathostomes, the benzimidazoles (fenbendazole, oxfendazole, oxibendazole, mebendazole), the pyrantel salts (pyrantel pamoate) and the avermectins/milbemycins (ivermectin and moxidectin). Cyathostome populations resistant to the macrocyclic lactones have not yet been reported in the horse, despite many years of ivermectin use and reports of widespread resistance to benzimidazoles and pyrantel salts. The current investigation was initiated 7 years ago when cyathostome populations resistant to fenbendazole and pyrantel pamoate were detected. At that time, a low level of resistance to ivermectin was suspected, but could not be proven by fecal worm egg count reduction test.

Study Objectives:

- Determine if cyathostomes resistant to treatment with ivermectin were present on the farm.
- Develop a management program that would prevent clinical disease in horses and achieve some control of populations of cyathostomes resistant to all available drug classes.

Experimental Approach:

Sequential fecal worm egg counts were performed on all horses on the farm before and 14 days after treatment with ivermectin and percent reduction was calculated. In addition egg reappearance times were monitored after ivermectin treatment.
Accomplishments/Results:

In several horses fecal worm egg count reduction tests indicated resistance to ivermectin. In addition, over the 7 years of the study, during which pasture hygiene and farm management recommendations were not implemented, the time taken for eggs to reappear in feces after treatment with ivermectin became progressively shorter. Despite ceasing routine use of pyrantel pamoate and fenbendazole, susceptibility of cyathostome populations to these drugs did not return.

We are working with current farm management to reduce stocking density of grazing horses, rest and rotate pastures, cross-graze pastures with cattle, improve grassland management in an effort to reduce the levels of pasture contamination. Selected treatment of horses with high fecal worm egg counts with anthelmintics will be used to treat horses that need anthelmintics to prevent clinical disease.

Benefits to the Equine Industry:

We have identified a farm on which no class of anthelmintic currently available can be relied upon to treat cyathostome infections.

The traditional method of detecting drug resistance in gastrointestinal parasites, the fecal worm egg count reduction test is very insensitive at detecting low levels of resistance, and does not detect resistance until about 25% of the parasites are resistant to a given drug. Monitoring the time taken for parasite eggs to reappear in feces after treatment is a much more sensitive method of detecting low levels of resistance, since as a drug becomes less effective for treatment of cyathostomes, the time for which egg output is suppressed in an individual horse becomes shorter.

Improving pasture hygiene and farm management reduces the total number of cyathostomes present on a farm, thereby also reducing the chances that an individual horse will need treatment at any given time point.

Our experience with this farm highlights the urgent need to monitor the effectiveness of parasite control programs on horse farms, the urgent need to reduce the selection pressure for development of resistance, and the need for good farm management practices to reduce the cyathostome populations that a grazing horse will ingest.
Investigation of suspected moxidectin resistance on a boarding farm in North Carolina

Dianne Little BVSc MS
MRCVS DACVS
Research Associate
Gastrointestinal Physiology

R.A. Mansmann
Director Equine Health Program

Description of the Problem:

The small strongyles or cyathostomes are the major pathogenic gastrointestinal parasite of the horse today. Cyathostome infection can cause a wide variety of clinical and sub-clinical disease from severe diarrhea and potentially fatal larval cyathostomiasis to colic, ill-thrift, weight-loss and poor performance. There are only three classes of drugs available that effectively treat cyathostomes, the benzimidazoles (fenbendazole, oxfendazole, oxibendazole, mebendazole), the pyrantel salts (pyrantel pamoate) and the avermectins/milbemycins (ivermectin and moxidectin).

Cyathostome populations resistant to the macrocyclic lactones have not yet been reported in the horse, despite many years of ivermectin use and reports of widespread resistance to benzimidazoles and pyrantel salts.

The current investigation was initiated after apparent failure of moxidectin to effectively suppress fecal worm egg counts in horses on a boarding farm with 46 resident horses, only 4 years after moxidectin was first used on the farm.

Study Objectives:

- Determine if cyathostomes resistant to treatment with moxidectin were present on the farm.

Experimental Approach:

Sequential fecal worm egg counts were performed on all horses on the farm before and 14 days after treatment with moxidectin and the percent reduction in fecal worm egg count after moxidectin treatment was calculated. In addition, horses were tested for the presence of resistance to fenbendazole and pyrantel pamoate. The time taken for
Fecal worm egg counts to become positive after treatment with ivermectin or moxidectin was also monitored.

**Accomplishments/Results:**

Cyathostomes resistant to both fenbendazole and pyrantel pamoate treatment were identified on the farm. Resistance to moxidectin was not identified by fecal worm egg count reduction testing, but we suspect that a low level of resistance to moxidectin was present on the farm, because the length of time that cyathostome egg output was suppressed for after treatment was considerably less than the optimal 12 weeks in many cases.

Pasture hygiene and management on the farm was improved to reduce the levels of cyathostome infections to which horses on the farm were exposed on pasture. Fecal worm egg counts were monitored by the farm managers to determine which horses would benefit from moxidectin treatment to minimize the selection pressure for development of further resistance problems on the farm.

**Benefits to the Equine Industry:**

The traditional method of detecting drug resistance in gastrointestinal parasites, the fecal worm egg count reduction test is very insensitive at detecting low levels of resistance, and does not detect resistance until about 25% of the parasites are resistant to a given drug. Monitoring the time taken for parasite eggs to reappear in feces after treatment is a much more sensitive method of detecting low levels of resistance, since as a drug becomes less effective for treatment of cyathostomes, the time for which egg output is suppressed in an individual horse becomes shorter.

Approximately 20% of horses carry 80% of the parasite load on any given horse farm. It is critical to identify these horses by performing fecal worm egg counts so that these individuals can be targeted for drug treatment. Selected treatment of only those horses with a high fecal worm egg count reduces the number of treatments administered to the herd of horses resident on a farm as a whole, and is expected to reduce the selection pressure for development of drug resistance amongst cyathostomes. Reduction of selection pressure is critical to prevent development of further drug resistance because on this farm there are no other drug classes available for control of cyathostomes.

Improving pasture hygiene and farm management reduces the total number of cyathostomes present on a farm, thereby also reducing the chances that an individual horse will need treatment at any given time point.
Pyrantel pamoate resistance in horses receiving daily administration of 2.64 mg/kg (1.2mg/lb) PO pyrantel tartrate on a boarding farm in Eastern North Carolina

Dianne Little BVSc MS
MRCVS DACVS
Research Associate
Gastrointestinal
Physiology

Description of the Problem:

The small strongyles or cyathostomes are the major pathogenic gastrointestinal parasite of the horse today. Cyathostome infection can cause a wide variety of clinical and sub-clinical disease from severe diarrhea and potentially fatal larval cyathostomiasis to colic, ill-thrift, weight-loss and poor performance. There are only three classes of drugs available that effectively treat cyathostomes, the benzimidazoles (fenbendazole, oxfendazole, oxibendazole, mebendazole), the pyrantel salts (pyrantel pamoate) and the avermectins/milbemycins (ivermectin and moxidectin). Pyrantel tartrate is labeled for control of recently ingested cyathostome larvae from pasture. Unfortunately the cyathostomes are becoming increasingly resistant to anthelmintics used for their control. Resistance to treatment by the benzimidazoles is widespread around the world. In the southeastern USA resistance to treatment by the pyrantel salts has been identified on 20-40% of all horse farms. We have evidence that resistance to the third group of drugs, the avermectins, may also be developing.

Study Objectives:

- Determine if cyathostomes resistant to treatment with 6.6mg/kg pyrantel pamoate were present on a farm where horses were treated with daily pyrantel tartrate.

Experimental Approach:

Sequential fecal worm egg counts were performed on all horses on the farm to determine if daily administration of pyrantel tartrate was achieving adequate control of fecal worm egg counts on the farm. Horses with high fecal worm egg counts were treated with 6.6mg/kg pyrantel pamoate, then a further fecal worm egg count was
performed 14 days later. The percent reduction in fecal worm egg count after treatment was calculated.

**Accomplishments/Results:**

Unacceptably high fecal worm egg counts were identified in some horses treated with daily pyrantel tartrate. Furthermore, cyathostomes resistant to pyrantel pamoate were identified in these horses. The use of pyrantel tartrate was abandoned as a method of parasite control on this farm and routine fecal worm egg counts were initiated on all horses resident on the farm. Only horses with a fecal worm egg count of greater than 200 eggs per gram were treated with anthelmintic.

**Benefits to the Equine Industry:**

Approximately 20% of horses carry 80% of the parasite load on any given horse farm. It is critical to identify these horses by performing fecal worm egg counts so that these individuals can be targeted for drug treatment. Selected treatment of only those horses with a high fecal worm egg count reduces the number of treatments administered to the herd of horses resident on a farm as a whole, and is expected to reduce the selection pressure for development of drug resistance amongst cyathostomes. Reduction of selection pressure is critical to prevent development of further drug resistance because no other drug classes are likely to become available for treatment of cyathostome infection in horses.
Ophthalmology

Abstracts:


Miller TM, Gilger BC. Glaucoma in Horses.

Miller TM, Elce Y, Gilger BC, Salmon J. Ocular Squamous Cell Carcinoma in Horses.
Equine Recurrent Uveitis – current studies

Brian C. Gilger  
DVM, MS, DACVO  
Professor, Ophthalmology

Tammy Miller  
DVM, MS, DACVO  
Assistant Professor, Ophthalmology

Jacklyn Salmon, BS

Description of the Problem:

Equine recurrent uveitis (ERU) is the most common cause of blindness in horses with an 8-10% prevalence in horses in the United States. ERU is characterized by inflammation in one or both eyes that recurs at unpredictable intervals until the eye becomes blind. Traditional treatment consists of topical and systemic anti-inflammatory medications, which helps decrease active episodes of inflammation, but does not prevent recurrence. The cause of ERU is not known. Studies in our laboratory have revealed that eyes with ERU develop an immune mediated inflammation typical of a Th1 inflammatory response. This suggests that the disease is not a result of specific causative agents, but is set off by a “trigger”. Recent studies have suggested that Leptospira organisms are the culprits of the recurrent episodes. However, Leptospira organisms were not identified in up to 75% of eyes evaluated. This suggests that other bacterial organisms may be responsible for the recurrent episodes in the Leptospira negative eyes. But, if other bacteria are responsible for initiating ERU, do they also play a role in chronic recurrent disease?

Study Objectives:

- Determine whether or not bacterial organisms are associated with ERU by use of PCR primers to bacterial 16S ribosomal DNA and GenBank alignment identification
- Determine if specific types of bacteria correlate to clinical features of uveitis, such as anterior or posterior involvement, and if specific types of bacteria are present in certain breed related ERU, such as that seen in the Appaloosa horse.
If bacteria are identified in Specific Aims 1 and 2, to quantify the amount of bacterial DNA present in the samples.

**Experimental Approach:**

**DNA isolation:** Total DNA will be isolated from ocular tissues and fluids with proteinase K in DNA digestion buffer for 4 hours at 42°C, then DNA will be extracted using routine phenol-choroform methods.\(^{23}\) DNA will be quantitated by spectrophotometry.

**PCR Amplification and Cloning of 16S ribosomal RNA Genes (rDNA):** To target the 16S rDNA in the bacterial chromosomes, a combination of the universal primers 8F (5’AGAGTTTGATCCTGGCTCAG)/1492R (5’GGTTACCTTGGTTACGACTT) will be used in the PCR, as described. In case of negative PCR results, one of the following primer combinations: 8F/1391R (5’GACGCGCGTTGACGTRCA; W=A or T, R=A or G), 515F (5’GTGCGAGCMGCCGCGGTAA; M=A or C)/1391R, or 515F/1492R, respectively, will be used. Amplified 16S rDNA fragments will be cloned into the newly constructed T-vectors pCPT9 for plasmid-based 16S rDNA analysis or into the T-vector pGEM-T (Promega). Recombinant plasmid DNA will be used to transform E.coli XL-1 Blue strain (Stratagene) or JM109 (Promega).

**Screening and Sequencing of 16S rDNA clones:** To determine if clones were derived from pure cultures, rDNA-inserts of positive clones will be reamplified by PCR using the plasmid specific primer T7 (5’GTAATACGACTCACTATAGGG) and primer SP6 (5’ATTAGGTGACACTATAG). PCR products will be digested with specific restriction endonucleases and analyzed by gel electrophoresis.

**Identification and Classification of Organisms:** Sequence identity to other 16S rRNAs will be determined by the BLAST search program. Sequence data will then be edited to the 16S rRNA sequence database of the program package ARB. Data sets that are missing in ARB but were determined by the BLAST search will be retrieved from the GenBank database (http://www.ncbi.nlm.nih.gov) and will also be edited.

**Accomplishments/Results:**

- Several organisms have been identified and current studies are being done to determine the DNA sequence and further identify these organisms and their significance.

**Benefits to the Equine Industry:**

ERU is responsible for large economic losses (estimated annual US loss of $ 0.5 to 1 billion) in the equine industry because it disrupts training, decreases performance, disqualifies horses from competition (because of medication use), and decreases the
horses’ value. Existence of bacterial DNA would strongly implicate bacteria’s role in development of recurrent episodes in ERU, therefore, specific antimicrobials or anti-DNA treatment (e.g. specific DNases, complimentary “antisense” oligonucleotides, etc - delivered via novel ocular drug delivery systems developed in our laboratory) may offer an effective treatment and possibly even a cure. Furthermore, better understanding of the pathogenesis of ERU may lead to preventative measures such as farm management, vaccination, or training changes.
Fungal keratitis in horses

Description of the Problem:
Equine fungal keratitis accounts for approximately 13% of the corneal problems reported in horses over the past 40 years. It often occurs in association with corneal trauma, which allows the normal equine ocular microflora, including fungal organisms, to invade the cornea and become pathogenic. Fungal organisms initially colonize the superficial cornea, producing ulcerative keratitis and secondary anterior uveitis. Subsequently, tropism for the posterior stromal glycosaminoglycans results in burrowing of the fungal elements toward the deeper cornea, causing rapid progression with risk of corneal perforation and iris prolapse. Aggressive topical and systemic medical therapies, as well as surgical therapy, are often required to preserve vision and can fail.

The pathogenesis of infectious keratitis involves both agent and host factors. Initiation and progression of infectious keratitis are mediated by inflammatory cytokines released by the infectious agent, injured corneal tissue, and/or infiltrating inflammatory cells. Infectious keratitis is reported in many species, however, the equine cornea is exceptionally susceptible to fungal keratitis. In addition to environmental factors that predispose to infection, it is possible that the equine cornea suffers from an inherent defect in resistance.

Study Objectives:
- To understand the pathophysiology of fungal keratitis in the horse
  - determine transforming growth factor beta levels, an immune modulatory cytokine, in the tear film and cornea of normal horses and horses with fungal keratitis
• determine COX-1 and COX-2, levels in the cornea of normal horses and horses with fungal keratitis
• determine via PCR the involvement of fungal organisms in deep corneal stromal abscesses in the horse
➢ To develop and test new drugs for the treatment and prevention of fungal keratitis

Experimental Approach:

➢ To determine the ocular penetration of topically and orally administered voriconazole. Peripheral blood levels and toxicity resulting from topical application of voriconazole were also evaluated. Six horses received topical voriconazole (0.5, 1.0, or 3.0%) solution, administered every 4 hours for 7 doses. Aqueocentesis was performed and plasma samples were collected following the final dose. Voriconazole levels in the aqueous humor (AH) and plasma were measured by high pressure liquid chromatography (HPLC). Five horses received a single oral voriconazole dose of 4 mg/kg, aqueocentesis was performed, and AH voriconazole levels were measured by HPLC.
➢ To determine the tear film concentration of TGF-β1 and TGF-β2, tears from normal horses and horses with fungal keratitis and stromal abscesses were collected atraumatically with cellulose sponges. Tear samples were collected with cellulose sponges, and analyzed with and without acid-activation to measure the total (latent plus active) and active concentration of TGF-β2 in the sample. Concentration of TGF-β2 was then determined using a commercially available enzyme linked immunoassay kit (ELISA) developed for humans.
➢ To determine the presence of COX-1 and COX-2, TGF-β1 and TGF-β2 and its receptors in the cornea of normal horses and horses with fungal keratitis and stromal abscesses. Corneal samples are currently being collected from horse’s undergoing surgery as a part of the therapy for their disease. Normal corneal samples are also being collected from horses euthanized for reasons unrelated to this study. Once collected, Immunohistochemistry and Western blot will be performed to identify differences between normal corneas and diseased corneas.

Accomplishments/Results:

➢ A new clinical drug, voriconazole, has been tested on normal horses and a clinical drug trial on horses with fungal keratitis is underway. Voriconazole is able to penetrate intact corneas and non-inflamed equine eyes to result in AH levels that are likely to be therapeutic, based on comparisons with previously determined MICs.
➢ PCR on samples of deep stromal abscess in the horse has identified the DNA of fungal organisms in all samples. This is significant, as identification of the underlying etiology in these cases is extremely difficult due to the location within the cornea. Knowledge that they are probably all fungal in origin indicates that aggressive antifungal therapy should be initiated in all cases.
TGF-β1 and TGFβ-2 have been identified in the tear film of normal horses and horses with fungal keratitis. The percentage of TGF-β2 found in the active form in horses with fungal ulcers was significantly decreased. There was a significant decrease in eyes with both ulcerated and non-ulcerated fungal infections from normal horses, but not from each other.

**Benefits to the Equine Industry:**

Loss of a horse’s vision can be devastating to it’s quality of life as well as it’s ability to remain a performance animal. Loss of the eye, in addition to loss of vision, can be a significant blow to the owner due to the change in the horse’s appearance. New, efficacious drugs will help to improve our chances of fighting fungal keratitis and saving the vision and eyes of horses afflicted with this disease. Beginning to understand the pathophysiology of this catastrophic corneal infection will hopefully lead to better ways to diagnose, prevent, and treat this disease.
Glaucoma in horses

Tammy Miller Michau, DVM, MS, DACVO
Assistant Professor, Ophthalmology

Brian C. Gilger, DVM, MS, DACVO
Professor, Ophthalmology

Description of the Problem:

Glaucoma is one of the leading causes of blindness in humans and dogs. It is a disease in which the intraocular fluid cannot exit the eye, resulting in a buildup of fluid within the eye. It is becoming increasingly more apparent that horses suffer frequently from this disease as well. Medical and laser therapy often fail to control intraocular pressure and the elevated intraocular pressure results in pain and blindness. Once glaucoma is diagnosed, there is a roughly 70-100% rate of blindness that develops secondary to the disease. Horse’s present a particular therapeutic challenge, in that they do not respond well to most of the drugs developed for glaucoma in humans.

Transforming growth factor-beta is a cytokine that acts upon cellular proliferation and inhibition, migration, differentiation, apoptosis, adhesion, and accumulation of extracellular matrix components. The intraocular fluids contain high concentrations of TGF-β2 relative to plasma and this concentration is altered in various disease states. Elevated levels of TGF-β2 are found in the aqueous humor from human and dog eyes with primary glaucoma. In glaucomatous eyes, the production of latent TGF-β2 in trabecular cells may be enhanced for some reason. In addition, latent TGF-β2 may be converted to mature by some mechanism different from normal eyes. A high concentration of aqueous humor TGF-β2 may also enhance production of TGF-β2 in trabecular meshwork cells, stimulate increased ECM deposition, and increase resistance to outflow even more.

It is possible, as it is currently under investigation in humans, that TGF-β2 may play a significant role in glaucoma in the horse as well. The use of antibodies or antisense oligonucleotides to therapeutically block TGF-β activity is currently under investigation.
In addition, high frequency ultrasound biomicroscopy (UBM) is a relatively new tool that has been used to define and treat human glaucoma based on numerous iridocorneal and anterior chamber structural changes that can now be identified using this technique.

**Study Objectives:**

- To elucidate the pathogenesis of glaucoma in the horse.
  - determine transforming growth factor beta-2 levels in the aqueous humor and trabecular meshwork of normal horses and horses with glaucoma
  - determine the anatomy of the anterior segment of horses with glaucoma using high frequency ultrasound.

**Experimental Approach:**

- Aqueous humor samples will be obtained from horses undergoing laser therapy for glaucoma. Control aqueous humor samples will be obtained from normal horses that are euthanized for reasons unrelated to this study. Samples of aqueous humor will be analyzed with and without acid-activation to measure the total (latent plus active) and active concentration of TGF-ß2 in the sample. Concentration of TGF-ß2 will then be determined using a commercially available enzyme linked immunoassay kit (ELISA) developed for humans.
- To determine the presence of TGF-ß2 and it’s receptors in the trabecular meshwork of normal horses and horses with glaucoma, IHC will be performed on banked histopathology specimens.
- All horses with glaucoma will have high frequency (30 mHz) ultrasonography performed and the results compared to high-frequency ultrasound performed on normal horses.

**Benefits to the Equine Industry:**

Glaucoma is a blinding and painful disease in the horse with few therapeutic options. Horses do not respond well to the topical anti-glaucoma drugs developed for human glaucoma. By elucidating the pathogenesis of glaucoma in the horse, a better understanding of the disease and possibly a new therapeutic modality can be obtained.
Ocular squamous cell carcinoma in horses

Tammy Miller Michau, DVM, MS, DACVO
Assistant Professor, Ophthalmology

Yvonne Elce, VMD

Brian C. Gilger, DVM, MS, DACVO
Professor, Ophthalmology

Jacklyn Salmon, BS

Description of the Problem:

Squamous cell carcinoma (SCC) is the most common tumor of the eye and its adnexa in horses. The most common locations for periocular SCC include the medial canthus, nictitans, and corneal limbus. Light horse breeds (e.g. Appaloosas, Thoroughbreds, Arabians, and Quarter Horses), as well as Draft breeds such as the Belgian, are predominantly affected. Treatment modalities include surgery (curative resection, cytoreductive surgery before adjunctive therapy), strontium 90 beta irradiation, interstitial radiotherapy (cesium137, cobalt 60, gold 198, iridium 192, and radon 222), cryotherapy, radiofrequency hyperthermia, immunotherapy, carbon dioxide laser ablation, and intra-lesional matrix chemotherapy. Each of these therapies have their drawbacks, including high cost (e.g., radiation therapies), potential for ocular damage (e.g., cyrotherapy, hyperthermia, laser treatment), and need for repeated treatments (e.g., chemotherapy). Location of the neoplasm adjacent to or on the eye presents special management problems during treatment. Specifically, the goal is to prevent ocular damage and preserve vision while at the same time eliminating the neoplasia. Surgery alone, has been reported to result in a cure rate of only 55%.

Transforming growth factor –β1, an extracellular matrix modulating cytokine, has been shown to be intimately involved in the progression of pre-cancerous to cancerous epithelial tumors in humans. It has also been shown to be a primary mechanism through which the tumor invades healthy tissue.
Study Objectives:

- To understand the pathophysiology of the development of SCC in the cornea of the horse
  - determine transforming growth factor beta-1 in the tear film and cornea of normal horses and horses with corneal and conjunctival SCC.
- To evaluate more effective therapies at prevention of progression or recurrence of ocular SCC in the horse.

Experimental Approach:

- To determine the tear film concentration of TGF-ß1 in normal horses and horses with ocular SCC. Tear samples are collected with cellulose sponges, and will be analyzed with and without acid-activation to measure the total (latent plus active) and active concentration of TGF-ß1 in the sample. Concentration of TGF-ß1 will be determined using a commercially available enzyme linked immunoassay kit (ELISA) developed for humans.
- To determine the presence of TGF-ß1 and its receptors in the cornea and conjunctiva of normal horses and horses with SCC. Corneal and conjunctival samples are currently being collected from horse’s undergoing surgery as a part of the therapy for their disease. Normal corneal samples are also being collected from horses euthanized for reasons unrelated to this study. Once collected, Immunohistochemistry and Western blot will be performed to identify differences between normal corneas and diseased corneas.

Benefits to the Equine Industry:

Corneal and conjunctival SCC can result in loss of the horse’s eye. Loss of a horse’s vision can be devastating to it’s quality of life as well as it’s ability to remain a performance animal. Loss of the eye, in addition to loss of vision, can be a significant blow to the owner due to the change in the horse’s appearance. Beginning to understand the pathophysiology of SCC in the horse’s eye will hopefully lead to better ways to prevent and treat this disease.
Abstracts:

Pinto CR, Davis M, Kivett L. Pregnancy Diagnosis by Ultrasonography Before Collection and Transfer of < 10mm Horse Embryos.
Pregnancy Diagnosis by Ultrasonography Before Collection and Transfer of < 10mm Horse Embryos

Carlos R. Pinto, DVM, PhD, DACT
Assistant Professor of Equine Theriogenology

Melody Davis, BS, 2nd year vet student

Lisa Kivett, BS 3rd year vet student

Description of the Problem:

In commercial embryo transfer programs, horse embryos are typically collected and transferred at 7 or 8 days of age. Reported success rates of transfer of day 9 horse embryos are conflicting (Imel et al, 1981; Fleury and Alvarenga, 1999). Although it has been reported that ultrasonographic detection of horse embryos is possible as early as at day 9 post ovulation (Ginther, 1984), there have been no reports about the ultrasonographic detection of embryos prior to embryo collection attempts.

Study Objectives:

The aims of the present study were to determine: 1) detection rate of < 10 mm embryos diagnosed by ultrasonography; 2) embryo recovery rate in mares positively diagnosed pregnant with < 10 mm embryos; 3) embryo transfer success rate for < 10 mm embryos; and 4) pregnancy rate after transfer of < 10 mm embryos.

Experimental Approach:

Twelve mares were artificially inseminated during 16 estrous cycles with fresh or shipped cooled semen using current breeding techniques. Beginning on day 7 post ovulation or day 9 after hCG administration (to induce ovulation) mares were examined daily by transrectal ultrasonography (5 MHz transducer) and twice daily on day 9 post ovulation until day 11. Mares diagnosed pregnant had their cervices catheterized with a 37 Fr. silicone catheter and their uteri flushed with Ringer’s solution with 1% (v/v) bovine calf serum at 500 or 1000 ml increments. All but one embryo measured 3 to 5 mm at the time of collection and were transferred transcervically using a disposable artificial insemination rod; one embryo measured 6 to 7 mm in diameter and was transferred.
loaded into a 34 Fr. flushing catheter. The success rates of transfer were documented by transrectal ultrasonography immediately after transfer and daily thereafter to follow embryo development.

**Accomplishments/Results:**

- Pregnancy rate = 23/45 (51.1%);
- Embryo recovery rate = 19/21 (90.5%);
- Embryo transfer success rate = 13/19 (68.4%);
- Embryo transfer pregnancy rate = 5/13 (38.5%)

**Benefits to the Equine Industry:**

< 10 mm embryos were successfully detected by ultrasonography before embryo collection and transfer. Pregnancies resulting only from transfer of embryos to recipients with 4 to 5 days of asynchrony warrant further investigation. The development of equipment designed to hold and transfer larger horse embryos may improve success rates.

**Acknowledgements:**

We thank Drs. Whitacre and Schramme, and the VERC staff for technical assistance.
Sports Medicine

Abstracts:


Schramme M, Little D, Redding WR, Linder K, Smith R. Regenerating equine tendon using autologous, bone marrow-derived mesenchymal stem cells.

Schramme M, Redding WR, Robertson I, Thrall DE. The use of MRI in the diagnosis of musculoskeletal abnormalities in the horse.
Characterization of a new surgical model of tendinitis of the superficial digital flexor tendon in horses

Michael Schramme
DrMedVet CertEO
PhD DipECVS
Associate Professor of Equine Surgery

Dianne Little DVM
DipACVS
Research Associate

W. Rich Redding DVM
Dip ACVS
Associate Professor Equine Surgery

Keith Linder DVM PhD
Dip ACVP
Assistant Professor Pathology

Callie Fogle BSc DVM
Resident Equine Surgery

Description of the Problem:

Equine tendinitis is a common devastating injury in horses. Although the collagenase model of tendinitis (Spurlock et al. 1989) has been used for many years to study the effect of different treatment modalities on the rate and quality of tendon healing in horses (Dahlgren et al. 2002), this model is dissimilar to naturally-occurring tendinitis. As a result, extrinsic healing factors from the peritendinous connective tissues contribute to cellular infiltration and neovascularisation of the lesion, rather than just intrinsic healing factors arising from the tendon’s own cell population and blood supply, as is more likely to happen in naturally occurring disease. Furthermore, treatment of SDF tendinitis may rely on accurate delivery and containment of the therapeutic agent into the tendon’s core (Smith 1992, Dahlgren et al. 2002, Hertsch et al. 1989, Dyson 2004). As an example, the differentiation of therapeutically delivered mesenchymal stem cells (Smith et al. 2003) into tenocytes is likely to be mediated by their location within the tendon’s core, where the appropriate mechanical (tensional forces) and biochemical (growth factors) environment exists for optimal differentiation. It is therefore important that the MSCs can be placed in a contained ‘lesion receptacle’ where they can be expected to stay and be subjected to this local differentiating environment. Naturally occurring tendinitis typically presents with such a centrally located core lesion that forms a perfect receptacle for the therapeutic MSCs. The proposed model of tendinitis is a modification of surgical window models in laboratory animals that have used variable amounts of tendon transection (Young et al. 1998, Awad et al. 1999).

Study Objectives:

1. to create a core lesion within the central third of the metacarpal region of the SDFT
2. to monitor the clinical progression of pain, lameness, swelling and tenderness
3. to monitor ultrasonographically the size of the SDFT, the size and the echogenicity of the core lesion and the fiber alignment in the core lesion
4. to monitor the MR signal characteristics of the core lesion and the parent SDFT with MRI
5. to evaluate and grade the structural characteristics of the tissue contained within the core lesion with histology
6. to compare the ultrasonographic characteristics and the MRI findings with each other and with histological observations

**Experimental Approach:**

Four horses of 3-6 years of age were recruited for the study and a core lesion was created in the SDF tendon of each front limb. The legs were assessed qualitatively for heat, lameness and pain on palpation of the tendon each day for the first week and thereafter once a week for the remainder of the study. The core lesion in each SDF tendon were monitored ultrasonographically at 1, 2, 4, 6, 8 and 12 weeks after injury. The core lesion in each SDF tendon was also monitored with high-field MRI (Siemens Symphony 1.5 Tesla) at 2, 4, 8 and 12 weeks after injury in transverse, dorsal and sagittal planes. 2D and 3D T1-weighted and T2 weighted sequences with and without fat saturation and a 2D short tau inversion recovery (STIR) sequence were used. Tendon segments were embedded in paraffin, sectioned to 6 micron sections on a rotary microtome and stained with haematoxylin and eosin and Masson trichrome. Sections are being evaluated under plain and polarized light. Levels of the tendon with homogenous histological abnormality were chosen for correlation with imaging studies.

**Accomplishments/Results:**

Preliminary data show that a consistent, mild to moderate core lesion can be created using this technique followed by one week of lunging exercise. Horses were not lame at walk at any stage of the study and tendon swelling or pain on palpation were minimal. The maximum size of core lesions ranged from 19 to 24% of the cross sectional area of the tendon and was reached between 4 and 6 weeks of initiation of the lesion. The maximum length of the lesion ranged from 8 to 12 cm on ultrasonographic examination and from 12 to 13 cm on MRI. Histological data are still being analysed.

**Benefits to the Equine Industry:**

Use of this model will make application and investigation of novel intra-tendinous therapies such as stem cell injection easier, by reducing the amount of concurrent peritendinous reaction and allowing for better comparison, so that ultimately stem cell therapies can be tailored properly to the specific demands of equine tendinitis.
Improved monitoring of tendon integrity with MRI will enable veterinarians to identify horses at risk of injury better at an earlier stage of the disease and patients with a high risk of re-injury better during the late stages of convalescence.

Figure 1. Ultrasonographic image of the core lesion at 19 cm distal to the ACB, 4 weeks after lesion induction

Figure 2. MR images (Sagittal 2D T1 SE and transverse 3D T1 FLASH) of the core lesion at 19 cm distal to the ACB, 4 weeks after lesion induction
Regenerating equine tendon using autologous, bone marrow-derived mesenchymal stem cells

Michael Schramme
DrMedVet CertEO
PhD DipECVS
Associate Professor of Equine Surgery

Dianne Little DVM
DipACVS
Research Associate

Rich W. Redding DVM
Dip ACVS
Associate Professor Equine Surgery

Keith Linder DVM PhD
Dip ACVP
Assistant Professor Pathology

Roger Smith BVetMed
PhD DEO DipECVS
Professor, Equine Surgery

Description of the Problem:

Superficial digital flexor tendon injuries contribute a major proportion of lameness in racehorses and other performance horses, having an incidence of 7-43% in Thoroughbred racehorses. Regardless of treatment, the severity of the injury is the most important prognostic indicator, resulting in return to performance of only 20-71% affected horses, and re-injury is common (Dowling et al. 2000). During repair of damaged SDF tendon but also of damaged ligaments, normal parallel elastic tendon or ligament fibers are replaced by dysfunctional, stiffer, disorganized fibrous tissue with inferior biomechanical properties, which is considered the reason for the high incidence of loss of performance and re-injury. Recently however, promising results have been obtained with direct injection of cultured bone marrow-derived mesenchymal stem cells (MSCs) (Smith et al. 2003; Smith 2004) into damaged tendons and ligaments. This study evaluates the clinical, ultrasonographic, MRI, histological and mechanical response of the SDFT to direct mesenchymal stem cells implantation into surgically induced core lesions. Our results further validate the so-far positive but anecdotal clinical experience with stem cell therapies (Smith et al. 2003; Smith 2004). We are testing the hypothesis that autologous mesenchymal stem cells, implanted into surgically-induced tendinitis, synthesise a matrix with greater similarity to normal tendon than can be found in untreated control limbs.

Study Objectives:

1. To induce tendinitis of the superficial digital flexor tendon using a defined surgically created central window defect with similarities to the natural disease.
2. To implant autologous MSCs into the central tendon defect.
3. To determine if MSC-treated core lesions have improved clinical and sonographic parameters compared to control lesions in vivo.
4. To determine if intra-lesional implantation of autologous MSCs results in enhanced repair of surgically induced SDF tendon core lesions compared to control limbs using established histological, biological and biomechanical analysis.

**Experimental Approach:**

Tendinits will be surgically induced in both forelimbs of 6 horses. Bone marrow will be collected from all horses. Mesenchymal stem cells will be isolated, expanded in the laboratory using established techniques, resuspended in bone marrow supernatant, and implanted into the damaged tendon of both legs under ultrasound guidance. Control limbs will receive sham injection of phosphate buffered saline as treatment. All horses will be euthanased at 12 weeks after treatment and tendons recovered for histological, biological and biomechanical analysis.

**Accomplishments/Results:**

- The study is currently underway and techniques are being developed for collection and multiplication of mesenchymal stem cells in our new comparative orthopaedic research laboratory. Once these have been optimized, tendinits lesions will be treated with both mesenchymal stem cells and control injections.

**Benefits to the Equine Industry:**

Anecdotal information suggests that injection of MSCs may offer new perspectives in the restoration of health to injured ligaments and tendons in horses. This exciting new technique deserves further scientific assessment. Once we have determined whether MSC implantation does indeed induce regeneration of normal tendon matrix in our controlled injury model, clinical cases can be recruited on a larger scale for a scientific evidence-based clinical trial to determine efficacy in the clinical arena. Subsequently MSC implantation may become more universally used for optimal repair of injured ligaments and tendons in horses.

![Figure 1. Bone-marrow derived equine mesenchymal stem cells in early culture during expansion.](image-url)
The use of MRI in the diagnosis of musculoskeletal abnormalities in the horse

Michael Schramme
DrMedVet CertEO
PhD DipECVS
Associate Professor of Equine Surgery

W. Rich Redding DVM
MS Dip ACVS Associate Professor Equine Surgery

Ian Robertson BVSc
DipACVR
Assistant Professor Diagnostic Imaging

Donald E. Thrall
DVM MS PhD
DipACVR Professor Diagnostic Imaging

Description of the Problem:

MRI produces a grey-scale image of hydrogen protons in tissues, based on the measurable energy release when protons alter their orientation in a large magnetic field. Depending on the number and density of these protons, and on the weighting of the particular MR sequence, different tissue types will produce MR signal of different intensity. In addition tissue alterations caused by inflammation and tissue remodeling will change the proton content and density, and therefore the MRI characteristics of a particular tissue.

Although the use of regional analgesia is relatively successful at localizing the region where pain arises in the limb, the exact cause of lameness in horses often remains elusive with current imaging methods. This is especially true in areas of the limb where radiography is insufficiently accurate or where soft tissue structures are deeply buried and therefore inaccessible to satisfactory ultrasonographic examination (i.e. the foot, the origin of the suspensory ligament, the palmar cortex of the metacarpal condyles). MRI is a cross-sectional imaging modality that can produce digital imaging slices in any plane, as thin as 1.5 mm, of body regions that can be positioned in or close to the isocentre of the magnet. MRI results in superior anatomical detail and soft tissue contrast and provides information on the fluid and mineral content of bone.

Study Objectives:

1. To validate a Siemens Symphony 1.5 T Magnet for use with horses and explore the accessibility of different anatomical areas of interest.
2. To develop a set of MR sequences for different anatomical areas of interest, that maximize the diagnostic information without prolonging the anesthesia time unnecessarily.
3. To document the frequency of examinations of different anatomical areas of interest.
4. To document the incidence of specific diagnoses for each area of interest.
5. To determine the proportion of patients in which MRI was able to produce a conclusive diagnosis that could not be obtained with other diagnostic modalities

Experimental Approach:

Horses presented for MRI examination between October 1st 2004 and August 25th 2005 were included in the study. MRI was considered indicated if other imaging modalities (radiography, ultrasonography and/or scintigraphy) had failed to reveal any significant abnormalities or produced equivocal results. A final diagnosis was based on the integration of the clinical history and the results of physical examination, regional analgesia and imaging methods including MRI.

Accomplishments/Results:

- Sequences were developed for examinations of the foot, the fetlock, the suspensory ligament, the hock and the skull (including the brain). It was determined that all areas distal to and including the carpus and tarsus in front and hindlimbs could be examined satisfactorily using MRI. MRI was performed on 48 horses during the period of investigation. Twenty nine MRI examinations involved both front feet, 9 examinations the origin of the suspensory ligament, 5 examinations the fetlock, 3 examinations the head and 2 examinations the hock. Three horses were examined twice with an average interval of 6 months between both examinations.

- The following diagnoses were made in 29 horses with foot lameness.

<table>
<thead>
<tr>
<th>FOOT MRI DIAGNOSIS (n=29)</th>
<th>PRIMARY</th>
<th>TOTAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary DDF tendinitis (occasionally with DSIL or CSL abnormalities)</td>
<td>1</td>
<td>13</td>
</tr>
<tr>
<td>DDF tendinitis and navicular disease</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>Primary navicular disease</td>
<td>4</td>
<td>17</td>
</tr>
<tr>
<td>Impar ligament Desmitis</td>
<td>2</td>
<td>7</td>
</tr>
<tr>
<td>Navicular suspensory Desmitis</td>
<td>3</td>
<td>7</td>
</tr>
<tr>
<td>Pedal osteitis</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Collateral ligament desmitis</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Septic arthritis</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>No abnormalities detected</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>
The following diagnoses were made in 9 horses with proximal metatarsal/suspensory pain:

<table>
<thead>
<tr>
<th>MRI DIAGNOSIS (n=9)</th>
<th>PRIMARY</th>
</tr>
</thead>
<tbody>
<tr>
<td>Suspensory ligament desmitis</td>
<td>4</td>
</tr>
<tr>
<td>Osteitis proximal plantar metatarsal cortex</td>
<td>1</td>
</tr>
<tr>
<td>Osteitis proximal plantar metatarsal cortex and suspensory ligament desmitis</td>
<td>1</td>
</tr>
<tr>
<td>Osteitis 2\textsuperscript{nd} or 4\textsuperscript{th} metatarsal bone with focal suspensory desmitis</td>
<td>1</td>
</tr>
<tr>
<td>Central tarsal bone cyst</td>
<td>1</td>
</tr>
<tr>
<td>No obvious abnormalities</td>
<td>1</td>
</tr>
</tbody>
</table>

Benefits to the Equine Industry:

MRI is a realistic and rewarding imaging technique in horses available at NCSU-VTH, especially for musculoskeletal imaging of areas that are poorly accessible to other imaging modalities. The Siemens Symphony 1.5 T magnet offers the advantage of fine anatomical detail and improved accessibility over other magnets, as objects can be imaged to within 20 cm of the isocenter of the magnet. In addition the diagnostic rate is high and the incidence of examinations without abnormal findings low. However, the significance of subtle abnormalities is still uncertain and further studies are on-going to determine the sensitivity and specificity of MRI for the identification of known pathological abnormalities.
Figure 1. Sagittal 2D PD SE image of a right foot with a core lesion (high signal) in the lateral lobe of the DDFT, extending from the proximal border of the navicular bone to the distal aspect of the digital synovial sheath. Reference level of transverse image shown.

Figure 2. Transverse 3D T1 Flash image of a right foot with a core lesion in the lateral lobe of the DDFT. The core lesion is characterized by high signal intensity, suggesting a high focal concentration of hydrogen protons.
Abstracts:

Breuhaus BA, LaFevers DH. Thyroid function in healthy full term foals, sick term foals, and premature foals.

Davis JL, Weingarten A, Papich MG. The Pharmacokinetics of Orbifloxacin in the Horse.

Davis JL, Salmon J, Papich MG. The Pharmacokinetics of Voriconazole in the Horse.

Elce YA. Expression of Cyclooxygenase-1 and -2 in Equine Squamous Cell Carcinoma and Corresponding Normal Skin.

Gardner SY, Johansson AM, Atkins CE, LaFevers DH, Breuhaus BA. Cardiovascular effects of acute pulmonary obstruction (heaves) in the horse.

Gardner SY, Johansson AM, Roberts MC. Acute renal failure as a consequence of other systemic problems in a referral equine hospital population (1990-2002): 13 cases.

Jones S, Breitschwerdt E, Valenzisi A. Molecular Detection of Bacteria in Pericardial Fluid from Horses with Pericarditis.


Thyroid function in healthy full term foals, sick term foals, and premature foals

Babetta A. Breuhaus, DVM, PhD
Associate Professor of Equine Medicine
D. Heath LaFevers, BS
Equine Medicine Research Technician

Description of the Problem:

Normal equine neonates are precocious. Their eyes are open, they have a complete haircoat, and their neural, muscular, and skeletal systems are well developed. By contrast, premature foals are characterized by small body size, a domed forehead, a short silky haircoat, floppy ears, weak flexor tendons, and decreased or absent ossification of the cuboidal bones of the carpi and tarsi. Premature foals have trouble maintaining body temperature and blood glucose concentrations, and require a much greater investment of time and money to save their lives, with death most commonly occurring secondary to immature lung development and sepsis. Foals that survive these first challenges face additional complications from decreased gastrointestinal, neural, and musculoskeletal development. While causes of problems experienced by premature foals are multifactorial, an immature hypothalamic / pituitary / thyroid axis probably contributes. Thyroid hormones increase metabolism by stimulation of a variety of cell types, and are essential for normal growth and maturation. In many species, fetal serum thyroid hormones increase just before birth and probably play a role in the rapid growth and organ system development that occur in late gestation. Organ systems that are developmentally immature contribute to early morbidity and mortality of premature foals, and decrease the prognosis for long term soundness and athletic potential. This study was designed to test the hypothesis that premature foals experience transient postnatal hypothyroidism.

Study Objectives:

- To determine whether or not premature newborn foals are hypothyroid (compared to full term foals) secondary to an immature hypothalamic/pituitary axis.
To determine the contribution of nonthyroidal illness to thyroid dysfunction in premature foals by comparing serial serum concentrations of total and free thyroxine (T4), total and free triiodothyronine (T3), and thyroid stimulating hormone (TSH) and their responses to thyrotropin releasing hormone (TRH) in premature foals suffering from systemic illnesses to term foals suffering from similar illnesses.

Experimental Approach:

Serum concentrations of total and free thyroid hormones and TSH, both at rest and in response to TRH, were measured in normal, healthy neonatal foals that were full term (normal foals), full term neonatal foals that were ill and hospitalized for conditions similar to premature foals (sick foals) and in premature neonatal foals (premature foals) to determine the possible contributions of an immature hypothalamic-pituitary axis and nonthyroidal illness to thyroid dysfunction in premature foals. Normal foals did not receive any medications. Both sick and premature foals received medications routinely used to treat conditions including (but not limited to) failure of passive transfer, sepsis, and perinatal asphyxia syndrome. Blood samples were collected for measurement of baseline concentrations of thyroid hormones and TSH at predetermined ages, and TRH stimulation tests were performed in foals at less than 3 days of age. Thyroid hormone and TSH concentrations were compared among the 3 groups of foals by ANOVA. Post hoc comparisons were performed using the Bonferroni correction.

Accomplishments/Results:

Premature foals had significantly lower serum concentrations of total and free fractions of thyroid hormones than normal foals. Baseline serum concentrations of TSH were not different, but TSH responses to TRH were exaggerated in premature foals compared to normal foals. Serum concentrations of T3 and TSH were similar in sick term foals and premature foals, but serum concentrations of T4 in sick term foals were intermediate between premature and normal foals.

Results suggest that sick term foals experience non-thyroidal illness syndrome, primarily a low T3 state. Alterations in thyroid function in premature foals may be caused by primary hypothyroidism, decreased peripheral conversion of T4 to T3, non-thyroidal illness syndrome, or by a combination of the three.

Benefits to the Equine Industry:

Early thyroid hormone supplementation in premature foals might accelerate organ system maturation, thereby improving short-term survivability and preserving long-term athletic function.
The Pharmacokinetics of Orbifloxacin in the Horse

Description of the Problem:

Many oral antimicrobial drugs cause severe, sometimes life-threatening gastrointestinal disturbances in the horse and therefore our choices of treatment are often limited. Fluoroquinolone antibiotics are one class of antimicrobials that are often safe and effective for use in the horse. Orbifloxacin is a fluoroquinolone antibiotic that has excellent activity of many of the gram-negative bacteria as well as some Staphylococcus sp. important in equine medicine. It is reported to be more active than enrofloxacin against many species of E coli. In this study, we describe the pharmacokinetics of oral and intravenous orbifloxacin in the adult horse.

Study Objectives:

- To determine the plasma pharmacokinetics of orbifloxacin following a single oral and intravenous dose to horses.
- To determine the physicochemical properties of orbifloxacin that may influence the oral absorption and tissue distribution of the drug.
- To develop dosing guidelines and strategies based on the pharmacokinetic/pharmacodynamic markers predictive of clinical outcome.

Experimental Approach:

Six healthy adult horses were used in this study. One horse was excluded from the oral study due to problems unrelated to drug administration. Using a two-way crossover design, horses received 2.5 mg/kg of orbifloxacin intravenously or orally. There was a minimum 2-week washout period between drug administration.
Plasma samples were collected at predetermined times following administration. *In vitro* plasma protein binding and lipophilicity assays were also performed. All samples were analyzed by reverse-phase high performance liquid chromatography (HPLC). Data obtained from the study was analyzed and suitable pharmacokinetic parameters were calculated using computerized software (WinNonlin, Version 4.0, Pharsight Corporation, Mountain View, CA). The stability of orbifloxacin in different vehicles, including molasses and corn syrup, was also tested.

**Accomplishments/Results:**

- A summary of the pharmacokinetic parameters following oral and intravenous administration of orbifloxacin is shown below.

<table>
<thead>
<tr>
<th>Pharmacokinetic Variable</th>
<th>IV (mean ± SD)</th>
<th>Oral (mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$T_{MAX}$ (hr)</td>
<td>---</td>
<td>1.21 ± 0.6</td>
</tr>
<tr>
<td>$C_{MAX}$ ($\mu$g/mL)</td>
<td>---</td>
<td>1.25 ± 0.5</td>
</tr>
<tr>
<td>$V_{d_{MAX}}$ (L/kg)</td>
<td>2.35 ± 0.55</td>
<td>---</td>
</tr>
<tr>
<td>MRT (hr)</td>
<td>5.81 ± 2.11</td>
<td>5.2 ± 0.85</td>
</tr>
<tr>
<td>AUC (hr*µg/mL)</td>
<td>9.04 ± 0.9</td>
<td>6.16 ± 2.35</td>
</tr>
<tr>
<td>AUMC(hr<em>hr</em>µg/mL)</td>
<td>53.86 ± 25.82</td>
<td>32.48 ± 13.33</td>
</tr>
</tbody>
</table>

- Bioavailability of orally administered orbifloxacin was 68.35 ± 27.32.
- Plasma protein binding was 20.64 ± 3.69%.
- The lipophilicity, as determined by the octanol:water partition coefficient of 0.2 ± 0.11, was low.
- No adverse side effects were noted during this study.
- Orbifloxacin is stable in vehicles commonly used to administer oral drugs to horses.
- Based on the results of this study, a dose of 5 mg/kg of orbifloxacin given orally once every 24 hours would be effective for the treatment of gram-negative bacteria with an MIC of 0.12 µg/mL, or gram-positive bacteria with an MIC of 0.25 µg/mL.

**Benefits to the Equine Industry:**

Orbifloxacin is well absorbed following oral administration in the horse and may be a useful drug for the treatment of susceptible bacterial infections. It presents an excellent alternative for oral dosing when a fluoroquinolone is needed for therapy. Because of the high water solubility and the nature of the tablet, it may be easier to compound into formulations for horses than enrofloxacin.
The Pharmacokinetics of Voriconazole in the Horse

Description of the Problem:

Choices for oral antifungal drugs in the horse are limited due to expense, unacceptable side effects, and a lack of available pharmacokinetic information. Voriconazole is a new generation triazole antifungal drug that has excellent activity of many of the fungi and molds important in equine medicine. It has excellent pharmacokinetic and safety profiles in other species. Therefore, we propose to characterize the pharmacokinetics and physicochemical properties of oral and intravenous voriconazole in the adult horse.

Study Objectives:

- To determine the plasma pharmacokinetics of voriconazole following a single oral and intravenous dose to horses.
- To determine the physicochemical properties of voriconazole that may influence the oral absorption and tissue distribution of the drug.
- To develop dosing guidelines and strategies based on the pharmacokinetic/pharmacodynamic markers predictive of clinical outcome.

Experimental Approach:

Six healthy adult horses were used in this study. A randomized two-way crossover design with a minimum 21 day washout period was used. Horses received a single dose of either 1 mg/kg intravenous voriconazole (Vfend® I.V., Pfizer Pharmaceuticals) as a slow bolus over 10 minutes, or 4 mg/kg of pure voriconazole powder mixed in 60 mL of corn syrup orally.
Plasma samples were collected at predetermined times following administration. *In vitro* plasma protein binding and lipophilicity assays were also performed. All samples were analyzed by reverse-phase high performance liquid chromatography (HPLC). Data obtained from the study was analyzed and suitable pharmacokinetic parameters were calculated using computerized software (WinNonlin, Version 4.0, Pharsight Corporation, Mountain View, CA).

**Accomplishments/Results:**

- A summary of the pharmacokinetic parameters following oral and intravenous administration of voriconazole is shown below.

<table>
<thead>
<tr>
<th>Pharmacokinetic Variable</th>
<th>Oral (mean ± SD)</th>
<th>Intravenous (mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$T_{\text{max}}$ (hr)</td>
<td>2.92 ± 1.2</td>
<td>---</td>
</tr>
<tr>
<td>$C_{\text{max}}$ (µg/mL)</td>
<td>2.43 ± 0.4</td>
<td>---</td>
</tr>
<tr>
<td>$V_{\text{d,area}}$ (L/kg)</td>
<td>---</td>
<td>1.29 ± 0.08</td>
</tr>
<tr>
<td>$\text{Cl}$ (L/kg/hr)</td>
<td>---</td>
<td>0.11 ± 0.03</td>
</tr>
<tr>
<td>$\text{AUC}$ (hr*µg/mL)</td>
<td>50.81 ± 16.07</td>
<td>9.23 ± 2.01</td>
</tr>
<tr>
<td>$t_{1/2}$ (hr)</td>
<td>13.11 ± 2.85</td>
<td>8.89 ± 2.31</td>
</tr>
<tr>
<td>$F$ (%)</td>
<td>91.63 ± 16.55</td>
<td>---</td>
</tr>
</tbody>
</table>

- Plasma protein binding was lower in the horse than other species at approximately 31.68 ± 1.91%.
- Voriconazole is highly lipophilic with an octanol:water partition coefficient of 64.69 ± 0.38.
- No adverse side effects were noted during this study.
- Oral doses of 1.5-2 mg/kg are recommended for further study.

**Benefits to the Equine Industry:**

Voriconazole is well absorbed following oral administration in the horse. It has a long half-life making once daily dosing regimens possible. Currently, it is the only antifungal drug with activity against clinically relevant pathogens, such as *Aspergillus* sp., that has consistent oral absorption and is available in a convenient formulation.
Expression of Cyclooxygenase-1 and -2 in Equine Squamous Cell Carcinoma and Corresponding Normal Skin

Yvonne A Elce, DVM, Dip ACVS
Assistant Professor of Equine Surgery

Description of the Problem:

Squamous cell carcinoma (SCC) is the most common urogenital and ocular tumor in the horse. Long-term non-recurrence rates following surgical excision alone range from 28 to 71%. The use of chemotherapy and radiation therapy improves the prognosis but both of these adjunctive therapies are expensive and many facilities do not offer radiation therapy. In dogs and humans COX-2 inhibitors are used to treat SCC. In these species SCC has been found to express cyclooxygenase-2 (COX) and little to no COX-1. Alternatively, normal dermal tissue in those species expresses COX-1 but not COX-2. The use of oral COX inhibitors, such as piroxicam, are an attractive adjunctive therapy to treat SCC in the horse as they can be cheap and easy to administer.

Study Objectives:

- To assess the expression of COX-1 and COX-2 in naturally occurring equine SCC and the corresponding normal tissues.

Experimental Approach:

Tissue was harvested during surgical excisions from 3 conjunctival, 2 vulvar, 4 preputial and 5 penile SCC, snap frozen in liquid nitrogen and stored at -80°C until analysis. Tissue was also harvested from 5 normal horses euthanized for reasons unrelated to neoplasia from the conjunctiva (5 horses), vulva (2 horses), penis and prepuce (3 horses). Protein was extracted from the frozen tissue and Western blot analysis was performed.
Accomplishments/Results:

- All equine tissues, including both normal and SCC tissues expressed both COX-1 and COX-2 at similar levels. The expression of COX proteins in the horse is markedly different from other species studied.

Benefits to the Equine Industry:

Drugs that target COX proteins may still be of value in the treatment of equine cancer but further research needs to be performed to evaluate their efficacy. Further studies into the mechanisms behind equine cancer should be performed to increase understanding of this disease and how it differs from other species.
Cardiovascular effects of acute pulmonary obstruction (heaves) in the horse

Sarah Y. Gardner, DVM, PhD, DACVIM
Associate Professor of Equine Medicine

Anna M. Johansson, DVM, MS, DACVIM
Equine Medicine Resident

Clarke E. Atkins, DVM, DACVIM
Professor of Medicine and Cardiology

D. Heath LaFevers, BS
Equine Medicine Research Technician

Babetta A. Breuhaus, DVM, PhD
Associate Professor of Equine Medicine

Description of the Problem:

Heaves is commonly encountered in equine practice as up to 12% of horses have been reported to be affected. The disease is similar to human asthma in that it is caused by inflammation and narrowing of the lower airways of the lung, which leads to cough, shortness of breath, and decreased ability to exercise. As a result of the disease process, the blood vessels of the lung constrict (pulmonary hypertension), increasing the work of the heart to pump blood through them. For this reason in humans, heart disease often occurs as a complication of asthma, and theoretically could occur as a complication of heaves in horses, however there is little research in this area.

Study Objectives:

➢ To compare heart rate, heart size, heart function, and evidence of heart muscle damage in 6 horses when clinically healthy and during an acute episode of heaves.

Experimental Approach:

Five horses with heaves in the NCSU research herd participated in the study. The horses were studied initially during disease remission after a prolonged period of time on pasture with no access to allergen (hay). At this time, each horse had a pulmonary function test, echocardiogram, and measurement of blood troponin concentration (evidence of heart muscle damage). The horses were then brought indoors and exposed to allergen (hay). One week after the development of clinical signs of heaves, the tests listed above were performed again. Exit criteria were set prior to the study which would require
removal of a horse from the study prior to a point at which the horse would experience distress. Horses were returned to pasture with no access to allergen (hay). After 1 month on pasture when the horses were again in disease remission, the above tests were performed again.

**Accomplishments/Results:**

- Horses developed heart disease during the episode of heaves which was reversible when they were returned to pasture and their disease went into remission. Changes in the heart noted during the episode of heaves included: increased heart rate; decreased volume of blood pumped per heart beat; enlargement of the pulmonary artery and right ventricle; decrease in volume of the left ventricle; and abnormal movement of the septum between the left and right ventricle. There was no evidence of heart muscle damage (normal blood troponin concentrations).

**Benefits to the Equine Industry:**

The results of this study stress the importance of management and treatment efforts to maintain horses with heaves in disease remission.

Heath LaFevers performs a pulmonary function test on “Chance” during the study.
Acute renal failure as a consequence of other systemic problems in a referral equine hospital population (1990-2002): 13 cases

Sarah Y. Gardner, DVM, PhD, DACVIM
Associate Professor of Equine Medicine

Anna M. Johansson, DVM, MS, DACVIM
Equine Medicine Resident

Malcolm C. Roberts, BVSc, PhD, MPH, FRCVS, FACVSc, DACVPM
Professor of Equine Medicine

Description of the Problem:

Acute renal failure occurs primarily as a complication to other systemic disease processes in horses. Early recognition and intervention with judicious use of fluids, electrolytes, pressor agents, and diuretics while carefully monitoring hydration status and urine output is of importance in order to prevent further systemic deterioration and progression of renal disease.

Study Objectives:

This case series was performed in order to identify equine patients at particular risk for developing acute renal failure. The specific objectives were to identify clinical conditions associated with development of acute renal failure, to describe clinicopathologic abnormalities, and determine the effect of acute renal failure on outcome.

Experimental Approach:

Medical records from all horses greater than one year of age admitted to the Veterinary Teaching Hospital at North Carolina State University, College of Veterinary Medicine between 1990 and 2002 were included in a database search. Horses that had a clinical diagnosis of acute renal failure, azotemia (serum creatinine > 2.0 mg/dl), urinalysis compatible with renal failure (urine specific gravity < 1.025), and clinical signs of renal failure for less than 7 days duration were identified, and their records were reviewed.
Accomplishments/Results:

- Thirteen horses with acute renal failure were identified. All horses had concurrent problems; laminitis (7), colitis (5), rhabdomyolysis (3), Red Maple toxicosis (2), and hepatitis (2). Several horses had more than one coexisting problem. Eight horses had received nonsteroidal anti-inflammatory drugs (NSAIDs). The most common electrolyte abnormalities were low phosphorus, sodium, bicarbonate, and chloride concentrations. Urinalysis revealed increased amounts of blood, protein, casts, and white blood cells. Eight horses were euthanized and five were discharged. No horse was euthanized solely because of the renal problem.

Benefits to the Equine Industry:

Based on these results it appears that laminitis and treatment with NSAIDs in systemically ill horses are frequently associated with acute renal failure.
Molecular Detection of Bacteria in Pericardial Fluid from Horses with Pericarditis

Samuel Jones, DVM, PhD, DACVIM  
Associate Professor of Equine Medicine

Edward Breitschwerdt, DVM, DACVIM  
Director, Vector Borne Disease Laboratory

Amy Valenzisi, DVM  
Research Associate

Description of the Problem:

Effusive, fibrinous pericarditis is an uncommon disease in horses characterized by suppurative inflammation suggesting a bacterial etiology. However, bacteria are infrequently isolated from pericardial fluid from affected horses using conventional bacteriological culture techniques. In 2001, an outbreak of pericarditis accompanied reproductive losses and endophthalmitis in central Kentucky, collectively called Mare Reproductive Loss Syndrome (MRLS). While the cause of MRLS has yet to be specifically defined, the outbreak of MRLS syndrome has been associated with exposure to eastern tent caterpillars. One hypothesis for the link between eastern tent caterpillar exposure and MRLS is that contact or ingestion of the caterpillars either increased exposure to an unidentified pathogen or altered host defenses to increase susceptibility to bacterial infection. The purpose of this study was to test the hypothesis that MRLS is associated with exposure to a pathogenic bacteria or increased susceptibility to bacterial infection by identifying bacteria associated with equine effusive, fibrinous pericarditis.

Study Objectives:

- Test the hypothesis that MRLS is associated with exposure to a pathogenic bacteria or increased susceptibility to bacterial infection by identifying bacteria associated with equine effusive, fibrinous pericarditis
- Test the utility of a novel culture technique using DS2 liquid insect growth media to isolate bacteria from equine pericardial fluid.
Experimental Approach:

Pericardial fluid was collected from four horses with pericarditis. Pericardial fluid was cultured using conventional bacterial culture methods. In addition, DS2 media was inoculated with pericardial fluid. Isolated bacteria were identified if possible using standard morphological and biochemical methods and using molecular diagnostic techniques. Specifically, PCR was used to amplify the 16S RNA genes from each bacterial isolate. The DNA sequence of the PCR clones obtained for each 16S gene was determined and the sequences were compared to the 16S gene sequences for known bacterial species in Genbank.

Accomplishments/Results:

Different bacteria were isolated from DS2 cultures inoculated with the pericardial fluid from each horse. In contrast, inoculation of 10% blood agar plates yielded no growth for any pericardial fluid sample. 16S ribosomal gene sequencing identified the bacteria as Propionibacterium acnes, Staphylococcus equorum, a Streptococcus sp, and Pseudomonas rhodesiae. Electron microscopic examination of the cultured pericardial fluid samples revealed bacteria in two samples that had incomplete or absent cell walls. Our results suggest that the DS2 culture method is superior to conventional bacteriological culture methods for isolating bacteria from equine pericardial fluid. Moreover, our results suggest that the bacteria in the pericardial fluid have altered cell wall physiology, perhaps explaining the inability to grow these organisms using conventional culture techniques.

Publications:


This work was supported by a grant from the North Carolina Horse Council.
Novel Anti-inflammatory Targets for Treatment of Sepsis Associated with Endotoxemia in Horses

**Study Objective:**

- To determine if the signaling molecule p38 has an important role in the mechanism leading to pro-inflammatory gene expression in leukocytes stimulated by endotoxin.

**Experimental Approach:**

We have developed a system in which the expression of a number of pro-inflammatory genes can be determined at the protein and mRNA level in equine blood leukocytes stimulated with endotoxin. Currently, we are focusing on tumor necrosis factor-a, interleukin 1-ß, interleukin 6, interleukin-8, and cyclooxygenase-2 because they are key pro-inflammatory genes that account for much of the pathophysiology of sepsis associated with endotoxemia. The role for p38 will be determined by examining their effect of specific pharmacological p38 inhibitors.
inhibitors on inflammatory gene expression and function in our system.

**Accomplishments/Results:**

- Initial studies demonstrated that p38 is essential for endotoxin-induced expression and function of cyclooxygenase-2 in equine leukocytes.

**Benefits to the Equine Industry:**

New, efficacious drugs will improve the survival of horses with gastrointestinal diseases and infections of other tissues complicated by sepsis associated with endotoxemia.
Implementation and evaluation of a pilot practice-based surveillance program for equine infectious diseases in North Carolina

Malcolm C Roberts
BVSc, PhD, MPH,
FRCVS, FACVSc,
DACVPM

Richard A Mansmann
VMD, PhD

College of Veterinary
Medicine, NC State
University

Wilbur S Brannan BS

*Robert Dickens DVM,
MA

Emergency Programs
Division, NCDA&CS

*USDA APHIS VS

Description of the Problem:

Surveillance is a continuous, systematic collection, analysis, interpretation, and dissemination of health data used in the planning, implementation, and evaluation of public or animal health programs. There is no established surveillance system for equine diseases using organized clinical or laboratory input. Baseline data on the prevalence or incidence of equine disease is lacking, through absence of meaningful denominators and methods to collect incident case data. For example, information on West Nile virus infection in horses in NC and in the US was based on serological, viral, and pathological confirmation, although the proportion of affected horses the figures represent can only be estimated. Ongoing active surveillance can improve precision of the estimate.

Study Objectives:

The purpose of the study was to develop a user friendly, web-based surveillance system for selected equine syndromes and infectious diseases. The objectives were to test the system with a group of 15 sentinel practices in NC, and to obtain feedback during and after the period of data collection that could facilitate refining the system prior to recruiting a larger number of equine practitioners.

Experimental Approach:

A novel software program was devised enabling equine practitioners to contribute to a surveillance system in which participants would report cases by syndrome.
that could be further categorized as a suspected disease entity. Each week participants would log into a password-protected, online reporting page maintained by the NCDA&CS, to record the number of cases seen for each syndrome. Clicking on the syndrome name opened an adjacent field of 3 to 10 suspected diseases associated with that syndrome, including “unknown” and “other infectious agent” categories. Therefore, the potential existed to identify the case more specifically. An email was sent to the sentinel practices each week as a reminder to submit data even if their case total for that week was zero. Cumulative information was integrated, and was accessible on the web in “real time”. A seamless link to a GIS state map indicated case by syndrome totals for the practice. Monthly, an additional reporting page was available to record laboratory-confirmed diagnoses received that month. There was no attempt at this stage to match laboratory diagnosis to an already reported suspected disease. This was a marker of the likelihood of samples being submitted by practices to confirm a suspected diagnosis.

Accomplishments/Results:

An online surveillance pilot program was tested in 15 equine practices in NC for up to 8 months in 2004. Participants included single-handed to multi-person practices distributed throughout the state. All practices entered data; between 7 and 13 practices provided data each week for 18 weeks. The preferred method was recording syndromes, the highest ranked being upper respiratory +/- fever, fever of unknown origin, metritis, and enterocolitis +/- fever, particularly in the spring and early summer. Few confirmatory diagnoses were made. The most frequent were strangles, neonatal septicemia, chronic airway disease, and EPM. Thirteen of 15 practices (86.7%) provided survey feedback. Strengths of the program included ease and rapidity of use, a well-categorized logical layout, the syndrome diagnostic format, and the ability to collect and share important information. The major limitation was difficulty in continuing to enter data. Survey respondents provided input to improve the software program. All were positive about working online, collaborating on the project, and would participate long term. The model could provide a template for an expanded practice-based disease surveillance program.

Benefits to the Equine Industry:

Participation in an active surveillance program raised clinicians’ level of awareness of infectious and emerging diseases, and of the threat of introduced disease. The information has tangible benefits to the equine community by substantiating preventive health and management recommendations. The evidence supported implementation of such measures and client education in participating practices. Continuation and expansion of the program to include most veterinarians caring for horses in NC is dependent on identifying longer term funding.

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