Dedicated to the Health, Well-Being and Performance of Horses through Veterinary Research, Education and Service
Director’s Message

The purpose of this Equine Health Studies Program (EHSP) Research Report is to document the activities pertaining to scientific investigations involving equine health and disease conducted at the LSU School of Veterinary Medicine. The contents of this inaugural issue of the EHSP Research Report represent equine biomedical scientific investigations completed between 1998 and 2000. The second issue will follow soon hereafter and will contain information related to studies completed between 2001 and 2003. The report demonstrates the diverse and extensive equine biomedical research conducted by the multidisciplinary faculty, advanced studies students and technical staff that comprise the EHSP. The quantity and quality of work is especially noteworthy considering the limited intramural funds available during this period. These works demonstrate the program’s commitment to the health, well-being and performance of horses through scientific investigations. Additionally, the report highlights information pertaining to research facilities and equipment, scientific manuscripts and abstracts published, and grants and contracts awarded.

The state-of-the-art laboratory facilities and equipment highlighted in this report represent major advances made in the EHSP as a result of acquisition of funds from the Louisiana Governor’s Biotechnology Initiative, the Louisiana Board of Regents Enhancement Grants Program, and recurrent Legislative funding obtained in 2003. Our research facilities are becoming second-to-none and provide faculty, students and staff an enriching and stimulating atmosphere to conduct leading-edge equine biomedical research. This helps to facilitate a better understanding of equine diseases and to assist with finding methods for improved treatment of critically ill and injured horses. We are also indebted to numerous organizations and individuals for their support of the basic and applied equine research activities.

The important findings of the research studies outlined in this report would not have been possible without the horses utilized for these studies. It should be noted that all research activities are conducted following federal guidelines for the care and use of animals. We do not take them for granted; we value their life and dignity, and we treat them with the utmost humane care. Their availability and utilization is necessary for progress toward the future care and treatment of ill and injured horses.

We are proud of the accomplishments we have made thus far and look to the future with optimism and excitement! We are well on our way to achieving and maintaining our mission of becoming an elite equine biomedical program.

Sincerely,

Rustin M. Moore, DVM, Ph.D., DACVS
Director
Published by the Equine Health Studies Program, School of Veterinary Medicine, Louisiana State University, Baton Rouge, Louisiana.

Dean, School of Veterinary Medicine: Michael G. Groves, DVM, MPH, DACVM, DACVPM, DAVPM

Executive Associate Dean: Peter F. Haynes, DVM, MS, DACVS

Associate Dean for Research and Advanced Studies: Thomas R. Klei, Ph.D.

Director, Equine Health Studies Program: Rustin M. Moore, DVM, Ph.D., DACVS

On the Cover: “Blue”, a commissioned oil painting by Anita Lejeune, a local artist from Lakeland, Louisiana, of “So Blue Riva”, a Quarter Horse mare owned by Ms. Sydney L. Hines of Pass Christian, Mississippi. “Blue” was successfully treated for a severe injury and infection of her right front foot at the LSU Equine Clinic in 2000, and subsequently gave birth to her first foal on April 1st of this year. Mrs. Jeanne Hines McDaniel, Sydney’s mother, gave a major charitable gift in honor of “Blue” and on behalf of her daughter and granddaughter, Ms. Jeanne L. Pitre. The gift assisted with construction of a new, 10-stall state-of-the-art Equine Intensive Care Unit, which opened in September 2004 in the LSU Veterinary Hospital's Equine Clinic. To view more of the artist’s equine and other works or to inquire about commissioned works, visit her website, www.anitalejeune.com, and help support the LSU Equine Health Studies Program (EHSP). The artist donates 20% of all sales to the EHSP.

The Equine Health Studies Program is supported with funds provided by the Louisiana State University School of Veterinary Medicine, the State of Louisiana, and contributions from private donors.

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Our Mission: The LSU Equine Health Studies Program will become a premier equine biomedical center in the 21st century through leading-edge research of equine diseases, contemporary instruction of professional veterinary students and veterinarians in advanced studies programs, and enhanced continuing education of the horse-owning public and private equine practitioners with the ultimate goal of providing state-of-the-art diagnostic and therapeutic capabilities for critically ill and injured horses and optimal clinical service to horsemen in Louisiana and the surrounding region.
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The LSU School of Veterinary Medicine has enjoyed a long standing and close relationship with the state and regional equine industry through its commitment to clinical service, research, professional instruction and outreach. It was through this relationship and industry support that the original Equine Veterinary Research Program was established in 1985. Though well supported by dedicated funds for only three years initially, the School has made every effort to continue program support albeit with limited funding, to maintain program visibility and responsiveness to the State’s equine industry.

Today’s Equine Health Studies Program emerged in 2000, armed with strong support of the industry, a renewed commitment and a refocused mission. Underpinned with recurrent state funding in 2003, and with the continued success of faculty in competitive extramural grant support, this program has become the most productive multidisciplinary program in the School. The program is very well positioned for future research and clinical productivity with the addition of new facilities (e.g., Lameness and Performance Evaluation Unit, Intensive Care Unit), state-of-the-art equipment and instrumentation, research oriented faculty and support staff.

This Research Report reflects the remarkable scientific productivity of a then small group of basic and clinical scientists during a period (1998 – 2000) when the program was only modestly supported. With the renewed financial support for the program, there is little doubt that it will become one of the globally preeminent programs for equine health. We are all very excited about the future and about research and clinical developments that will improve the health and welfare of the horse.

Sincerely,

Dr. Michael G. Groves, Dean
School of Veterinary Medicine
Louisiana State University

Dr. Michael G. Groves, Dean
School of Veterinary Medicine
Faculty

Abolghasem Baghian, Assistant Professor, Veterinary Microbiology & Parasitology
Dr. Baghian received his M.S. in Microbiology from Southeastern Louisiana University in 1981, and he received his Ph.D. in 1985 from Arizona State University. Dr. Baghian was a postdoctoral researcher at the School of Veterinary Medicine at Louisiana State University, where he later became an instructor and is currently an assistant professor. Dr. Baghian's research focuses on investigating the structure and function of herpes simplex virus glycoprotein K and the structure and function of Kaposi’s sarcoma associated herpesvirus (KSHV) glycoproteins gH, gL, and gB to those other herpesviruses.

Steven A. Barker, Professor, Veterinary Physiology, Pharmacology & Toxicology
Steven A. Barker is a professor of veterinary physiology, pharmacology and toxicology at the School of Veterinary Medicine in the department of Comparative Biomedical Sciences. He received his B.S. in 1971, his M.S. in 1973, and his Ph.D. in 1978, all from the University of Alabama. Dr. Barker is also the director of the Analytical Systems Laboratory.

Ralph E. Beadle, Professor Emeritus, Equine Medicine
Dr. Beadle was born and raised in Montana. He completed his pre-veterinary and veterinary education at Colorado State University, where he was awarded a DVM degree in 1967. He spent the next five years at the University of Georgia, where he worked in the Equine Clinic and obtained a Ph.D. degree in Veterinary Physiology. After a period of two years spent at Michigan State University, he has been at Louisiana State University for the rest of his professional career. During the first seven years at LSU, he was in the Department of Veterinary Physiology, Pharmacology and Toxicology where he taught both physiology and pharmacology. From that time until September of 1999, he was in the Department of Veterinary Clinical Sciences where he worked in the Medicine Section of the Equine Clinic. He retired in September of 1999, but since that time has continued to be involved in the activities of the Department of Veterinary Clinical Sciences as a professor emeritus. His research interests involve non-sweating horses, horses with recurrent airway disease and horses affected with acute and chronic laminitis.

Aloisio C. D. Bueno, Clinical Instructor, Equine Surgery
Dr. Bueno, originally from Brazil, obtained his veterinary medical degree from Unioeste University and then completed two years of work in a private equine practice in Brazil. He then completed a one-year internship in large animal medicine and surgery followed by a two-year M.S. program at the LSU School of Veterinary Medicine. Dr. Bueno then completed a one-year fellowship in large animal surgery at Oregon State University before going to the University of California-Davis for a three-year residency in equine surgery. Upon completion of his residency, Dr. Bueno returned to LSU as a clinical instructor of equine surgery and provides the majority of the equine emergency surgery service. Dr. Bueno is investigating the pathophysiology, prevention and treatment of laminitis.

Daniel J. Burba, Professor, Equine Surgery
Dr. Daniel J. Burba was born near Punxsutawney, Pa., on a dairy farm. He and his parents moved to Grayson, a small town in eastern Kentucky, when he was 13. His family raised Quarter Horses, and he still owns Quarter Horses and competes in team penning in the southern regional organization with family who live in Florida. He completed his pre-veterinary studies at Morehead State University in Kentucky. He received his DVM from Auburn University in 1986, and then completed a large animal internship (1987) and equine surgical residency (1990) at Oklahoma State University. He is board certified by the American College of Veterinary Surgeons. His clinical interests include lameness and orthopedic surgery and laser surgery. His research interests include musculoskeletal injuries, such as joint disease.

Dr. Sharon Chirgwin, Assistant Professor, Research
Dr. Sharon Chirgwin was born and raised in Australia. She obtained a Bachelor of Science with Honors, majoring in Biochemistry and Zoology, from James Cook University, in Townsville, Queensland. Dr. Chirgwin then completed a Ph.D. in Molecular Parasitology at the Queensland Institute of Medical
Research, before joining the laboratory of Dr. Thomas Klei at LSU, where she works on both human and horse parasites. Her research interests include the molecular characterization of the early infection events of parasitism. Dr. Chirigwits’ current position in the EHSP involves teaching students, staff and faculty molecular biological techniques, and advising on the contribution this technology can make to equine research.

Doo Youn Cho, Professor, Veterinary Pathology
Dr. Doo Youn Cho is a professor of veterinary pathology in the department of Pathobiological Sciences at the School of Veterinary Medicine. Dr. Cho is also the section chief for necroscopy/surgical biopsy in the School’s Veterinary Teaching Hospital and Clinics. He received his DVM in 1966 and his M.V.Sc. in 1970, both from Seoul National University in Korea. In 1973, he received his M.S., and in 1978, he received his Ph.D., both from Kansas State University.

Susan C. Eades, Professor, Equine Medicine
Dr. Susan Eades graduated from the LSU School of Veterinary Medicine, then completed an internship in large animal medicine and surgery, and a residency in large animal internal medicine at the University of Pennsylvania’s New Bolton Center. She then moved to Athens, Ga., and completed a Ph.D. program in Veterinary Physiology at the University of Georgia. Her doctoral studies concentrated on intestinal vascular and nonvascular smooth muscle physiology and pharmacology. Upon completion of her Ph.D., Dr. Eades began as an assistant professor of large animal medicine at the University of Georgia College of Veterinary Medicine, where she remained through 1997. She returned to LSU in 1997 as an associate professor of equine medicine. Dr. Eades’ clinical interests include equine internal medicine; however, she has a special interest in cardiology and ultrasound. Her research interests include intestinal disease and laminitis.

Bruce E. Eilts, Professor, Theriogenology
Dr. Bruce E. Eilts is originally from the Minneapolis/St. Paul area in Minnesota. He graduated from high school in West St. Paul, Minn., and then attended the University of Minnesota as pre-veterinary medicine student. He obtained a B.S. in veterinary science in 1975 and his DVM in 1977, both from the University of Minnesota. He was in private practice for one year before returning to the University of Minnesota to obtain an M.S. in theriogenology in 1982. After two and one half years in private practice in southern California, he came to Louisiana State University as an assistant professor in 1984. He became board certified in the American College of Theriogenologists in 1986. His main clinical interest is basic reproduction management in the horse. His main research interest is intrafollicular insemination in the mare.

Timothy P. Foster, Research Assistant Professor, Molecular Virology and Cell Biology
Dr. Timothy Foster was born in San Francisco, CA and obtained a BS degree in Biochemistry and a BS degree in Microbiology/Zoology from Louisiana State University in 1995. In 1999, he received a PhD in Veterinary Medical Sciences with an emphasis in Biochemistry and Molecular Virology from the Louisiana State University Departments of Biochemistry and Veterinary Microbiology and Parasitology. Dr. Foster is currently a Research Assistant Professor in the Division of Biotechnology and Molecular Medicine at the LSU School of Veterinary Medicine. His primary interests are deciphering the molecular interplay between host cells and various pathogens, as well as translational investigations that transition primary bench work science rapidly into the clinical environment.

Dennis D. French, Professor, Veterinary Science
Dr. Dennis D. French, originally from Chatfield, Minn., obtained his B.S. and DVM degrees from the University of Minnesota in 1976 and 1979, respectively. He is a Diplomate of the American Board of Veterinary Practitioners, certified in equine practice. His clinical interests include equine herd health and sport horse medicine. His research interests include equine parasitology, immunology and exercise physiology in horses. Dr. French is currently a professor of veterinary science at the LSU School of Veterinary Medicine, and provides equine ambulatory services for the Veterinary Teaching Hospital & Clinics. Dr. French is a past president of the Louisiana Veterinary Medical Association. Dr. French and his family are active in many equine and equestrian activities throughout the state.
William G. Henk, Professor, Veterinary Anatomy and Cell Biology
Dr. William G. Henk is a professor of veterinary anatomy and cell biology in the department of Comparative Biomedical Sciences at the School of Veterinary Medicine. He is also the chief of the Electron Microscopy Laboratory. Dr. Henk received his B.S. in 1967, his M.Ed. in 1971, and his Ph.D. in 1977, all from the University of Georgia.

Jeremy D. Hubert, Assistant Professor, Equine Surgery
Dr. Jeremy D. Hubert was born in Wales, but grew up on a ranch in Zimbabwe, where he received his veterinary degree (BVSc.). After two years of mixed animal practice in Zimbabwe and the United Kingdom, he completed an internship in equine medicine and surgery at LSU. This was followed by a year in equine practice in the U.K. before embarking on a combined equine surgery residency and M.S. program, which he completed in July 1999. He became board certified by the American College of Veterinary Surgeons in 2000. He worked as a clinical instructor in Equine Surgery for one year and accepted a position as an assistant professor of equine surgery at LSU in October 2001. His clinical interests include upper respiratory tract disease, as well as lameness and orthopedics. He is currently involved in scientific investigations involving extracorporeal shockwave therapy, bone density, and the role of eosinophils in gastrointestinal tract disease.

Jill R. Johnson, Professor, Equine Medicine
Dr. Jill R. Johnson is a native of South Dakota. She graduated from veterinary school at the University of Minnesota, then stayed on and completed a M.S. degree in Veterinary Surgery and Radiology. She joined the faculty of the School of Veterinary Medicine at LSU in 1977. She is a specialist in internal medicine (Diplomate, American College of Veterinary Internal Medicine) and Equine Practice (Diplomate, American Board of Veterinary Practitioners). Past research activities have centered on immuogenetics and immunology. Current research activities include evaluation of methods of quantifying exercise training using the global positioning system (GPS) and development of tissue culture models to study laminitis and chronic obstructive pulmonary disease using microgravity methods.

Dae Y. Kim, Instructor, Pathobiological Sciences
Dr. Dae Y. Kim is an instructor in the department of Pathobiological Sciences at the School of Veterinary Medicine. He received his DVM from Seoul National University in Korea, and he received his Ph.D. from Louisiana State University.

Thomas R. Klei, Boyd Professor, Parasitology and Veterinary Science
Dr. Thomas Klei obtained his B.S. and Ph.D. degrees in biology and zoology from Northern Michigan University and Wayne State University in 1965 and 1971, respectively. He then completed postdoctoral training at the National Institute of Health. He joined the faculty at the LSU School of Veterinary Medicine in 1975. He became a Boyd Professor in Parasitology and Veterinary Science at LSU in 1992. Dr. Klei has conducted leading-edge investigations into and has contributed greatly to our current understanding of equine parasitology. Dr. Klei is currently serving as the associate dean for Research and Advanced Studies at the School of Veterinary Medicine.

Konstantin G. Kousoulas, Professor, Veterinary Virology
Dr. Konstantin G. Kousoulas is a professor of veterinary virology in the department of Pathobiological Sciences at the School of Veterinary Medicine. He is also a professor of poultry science and an adjunct professor of biological sciences. Dr. Kousoulas is the director of the School of Veterinary Medicine’s Division of Biotechnology & Molecular Medicine. He received his B.S. in 1975 from Fairleigh Dickinson. In 1977, he received his M.S. and in 1981, he received his Ph.D., both from Pennsylvania State University.

Mandi J. Lopez, Assistant Professor, Equine and Comparative Orthopedics
Dr. Mandi Lopez was born and raised in the Pacific Northwest. She attended veterinary school at the University of California, Davis and then completed an internship at Kansas State University prior to going to the University of Wisconsin, where she completed a residency in large animal surgery and obtained both an M.S. and Ph.D. degrees. Her area of interest and expertise is comparative orthopedic research and surgery. She is board-certified by the American College of Veterinary Surgeons. She came to LSU in January 2004 and heads the Laboratory for Equine and Comparative Orthopedic Research.
Sara K. Lyle, Clinical Instructor, Theriogenology
Dr. Sara K. Lyle was born and raised in Gainesville, Fla. She obtained her B.S. in Chemistry at Duke University and her DVM from the University of Florida. She completed a residency in theriogenology in 1989 and a M.S. in reproduction in 1991 at the University of Florida. She is board certified by the American College of Theriogenologists. Her clinical interests include mare infertility and assisted reproductive technologies. Her research interests include reproductive immunology (equine) and assisted reproductive technologies in horses.

Rebecca S. McConnico, Assistant Professor, Equine Medicine
Dr. Rebecca S. McConnico is originally from north central Ohio, where she lived for 18 years. She obtained her B.S. in Animal Science from the University of Arkansas, her DVM degree from Louisiana State University, and her Ph.D. and clinical residency in large animal internal medicine from North Carolina State University. She is board certified in Equine Internal Medicine and her clinical interests are in equine critical care and internal Medicine. Her research interests include inflammatory disease of the equine large intestine and infectious diseases and the effects on mucosal physiology and permeability.

Rustin M. Moore, Professor, Equine Surgery
Dr. Rustin M. Moore, professor of equine surgery, currently serves as director of the Equine Health Studies Program and service chief of Equine Medicine and Surgery. He is originally from West Virginia and earned his B.S. from West Virginia University. He obtained his DVM and Ph.D. from The Ohio State University and completed his equine surgical residency at the same institution. He is board certified by the American College of Veterinary Surgeons. Dr. Moore began at the LSU School of Veterinary Medicine in October 1994. Some of his clinical interests include lameness, surgery and colic and its associated complications. Dr. Moore's research focuses on vascular and nonvascular smooth muscle physiology and pharmacology and the pathophysiology and treatment of colic, laminitis, endotoxemia and heaves.

Claudio C. Natalini, Assistant Professor, Veterinary Anesthesiology
Dr. Claudio C. Natalini is originally from Rio de Janeiro, Brazil, where he attended the Universidade Federal Fluminense (UFF) and graduated in veterinary medicine in 1984. From 1985 to 1986, Dr. Natalini enrolled in a residency program in veterinary surgery and medicine at the Universidade Federal do Rio Grande do Sul (UFRGS), Brazil. He worked from 1986 to 1992 as a staff surgeon/anesthesiologist at UFRGS. In 1991, Dr. Natalini completed an M.S. program in veterinary anesthesiology at Universidade Federal de Santa Maria (UFSM), Brazil. In 1992 he became an assistant professor of veterinary anesthesiology at UFSM. In 1994 he obtained his board certification from the Brazilian College of Veterinary Surgeons and Anesthesiologists (CBCAV) and served as CBCAV secretary for one year. In 1996, Dr. Natalini enrolled in a Ph.D./residency program at the University of Minnesota, earning his degree in 2000 working with opioid spinal mediated analgesia in the equine. In 2002 Dr. Natalini joined the Department of Veterinary Clinical Sciences at the LSU SVM. Dr. Natalini clinical interests are small and large animal pain management with emphasis in spinal analgesia and local anesthesia. His research interest includes the pharmacology and physiology of spinal administration of analgesic in horses.

Marlene Orandle, Assistant Professor, Virology
Dr. Marlene Orandle was born and raised in Baltimore, MD. She obtained her B.A. in Biology from Saint Mary’s College of Maryland in 1987. Following four years of research experience at Johns Hopkins University School of Medicine, Dr. Orandle completed her DVM from Iowa State University in 1995 and her Ph.D. in Veterinary Pathology from the University of Florida in 1999. Since receiving her Ph.D., she has completed Postdoctoral Fellowships at both the New England and Tulane National Primate Research Centers where she studied the pathogenesis of simian immunodeficiency virus (SIV) infection in the brain as a model for AIDS dementia. Dr. Orandle joined the faculty within the Department of Pathobiological Sciences at the LSU School of Veterinary Medicine in 2004. Her research interest is in the study of comparative lentiviral pathogenesis with a specific focus on factors contributing to the development of neurological disease. Ongoing research in her laboratory is focused on understanding the mechanisms involved in trafficking of virus-infected cells across the blood-brain barrier in SIV-infected rhesus macaques and in EIAV-infected horses.
Dale L. Paccamonti, Professor, Theriogenology
Dr. Dale L. Paccamonti, originally from Kankakee, Ill., completed his undergraduate and veterinary education at Michigan State University, receiving his DVM in 1981. After four years in a mixed practice in Chestertown, Md., he pursued advanced training at the University of Florida, where he completed a residency in Theriogenology and received his M.S. degree in 1988. Dr. Paccamonti is a Diplomate in the American College of Theriogenologists. He joined the faculty at the LSU School of Veterinary Medicine in 1988, where he is currently a full professor of theriogenology in the Department of Veterinary Clinical Sciences. Dr. Paccamonti’s primary research interests include the study of infertility in mares, assisted reproduction techniques in horses, factors affecting sperm motility in stallions, semen cryopreservation in stallions and the process of fetal maturation and parturition in mares. He also collaborates in reproductive research in other domestic species. In addition to research endeavors, his duties include teaching theriogenology to third and fourth year veterinary students. He shares responsibility for clinical theriogenology cases in all species presented to the Veterinary Teaching Hospital & Clinics.

Daniel B. Paulsen, Professor, Veterinary Pathology
Dr. Paulsen received his B.S. in 1975, his D.V.M. in 1977, and his M.S. in 1978, all from Kansas State University. In 1989, he received his Ph.D. from Oklahoma State University. Dr. Paulsen major areas of research interest are bovine respiratory disease with emphasis on Mannheimia haemolytica, Pasteurella multocida, Haemophilus somnus, bovine virus diarrhea, and bovine respiratory coronavirus; pathogenesis, bacterial genetics, respiratory immunity and vaccinology; toxicologic pathology associated with inhaled toxins and effects of inhaled substances on the pathogenesis of asthma; and application of immunohistochemical techniques in equine respiratory disease and laminitis and in cancer biology.

Glenn R. Pettifer, Assistant Professor, Veterinary Anesthesiology
Dr. Pettifer was born and raised in Ontario, Canada. He received his DVM from the Ontario Veterinary College at the University of Guelph. Following this he worked with the Equine Ambulatory Service at the Ontario Veterinary College and then with Humber Equine Clinic in Toronto, Ontario. He then returned to the Ontario Veterinary College, where he completed residency training and DVSc in Veterinary Anesthesiology. Since that time he has held instructor positions at the University of Saskatchewan and the University of Georgia. Most recently, Dr. Pettifer was assistant professor of veterinary anesthesiology at the Ontario Veterinary College before coming to LSU in the fall of 1999. His current research interests include pain management in all species, particularly horses. Dr. Pettifer is the recipient of Morris Animal Foundation support to investigate the analgesic effects of transdermally administered fentanyl in horses.

Changaram S. Venugopal, Professor, Veterinary Physiology & Pharmacology
Dr. Changaram S. Venugopal is a veterinarian who graduated from Kerala Veterinary College and Research Institute of Kerala University. After practicing as a veterinarian on the Kamadhenu Dairy Farm for 5 years he pursued and received his M.Sc. degree in neuropharmacology from Calicut University, India. He received his M.S. degree in cardiovascular pharmacology and Ph.D. in pulmonary pharmacology from Massachusetts College of Pharmacy and Allied Health Sciences in a cooperative program with Harvard University in Boston. Then he worked as a postdoctoral fellow at Harvard Medical School before he joined the faculty at Louisiana State University School of Veterinary Medicine in 1981. Dr. Venugopal is currently a professor of Pharmacology in the Department of Comparative Biomedical Sciences. He received his New Investigator Award grant from NIH in 1983 and the Beecham Award for Research Excellence in 1985. His research interests include the physiology and pharmacology of vascular and nonvascular smooth muscle physiology and pharmacology, and the pathophysiology of summer pasture associated obstructive pulmonary disease.

Dr. Ashley M. Stokes, Research Assistant Professor
Dr. Ashley M. Stokes was born in Baton Rouge and moved to Tuscaloosa, Alabama, to complete her bachelor’s degree from the University of Alabama. She returned to Baton Rouge to work in Oceanography for LSU for three years before her veterinary training. She completed the DVM/PhD program at the LSU School of Veterinary Medicine in the Department of Comparative Biomedical Sciences in 2001 and 2003, respectively. She completed a one-year post-doctorate research fellowship in the summer of 2004 where she continued her doctoral work on the vascular pathophysiology of equine laminitis. As a research
assistant professor within the EHSP, Dr. Stokes will continue focusing her efforts in cardiovascular physiology with special emphasis on equine diseases.

**H. Wayne Taylor, Professor, Veterinary Pathology**
Dr. H. Wayne Taylor is a professor of veterinary pathology in the department of Pathobiological Sciences at the School of Veterinary Medicine. He is also a veterinary pathologist and the director of the Louisiana Veterinary Medical Diagnostic Laboratory. Dr. Taylor received his DVM from Auburn University in 1967. In 1969, he received his M.S. from the University of Missouri, where he also received his Ph.D. in 1971. Dr. Taylor is a Diplomate of the American College of Veterinary Pathologists.

**Honor Ame Walesby, Assistant Professor, Equine Surgery**
Dr. Honor Ame Walesby, originally from Baltimore, Maryland, attended Virginia Tech for her BS in Animal Science, Virginia Maryland Regional College of Veterinary Medicine for her DVM, and Iowa State University for her MS in Veterinary Surgery. She became board certified in Large Animal Surgery with an equine emphasis in 2000. Her clinical interests lie in the filed of soft tissue and reconstructive surgery, abdominal surgery, lameness, and ultrasonography. Her research interests include pharmacokinetics and endotoxin-related abortion in late gestation mares.

**R. Wayne Waguespack, Assistant Professor, Equine Surgery**
Dr. Wayne Waguespack, graduated from Tuskegee University, completed an internship in large animal medicine and surgery at the University of Georgia, and then completed an equine surgery residency and MS degree at Auburn University. Dr. Waguespack has clinical interests in soft tissue and orthopedic surgery. He also has several research interests, including studying laminitis and potential ways to prevent and treat this devastating disease, as well as the effects of extracorporeal shockwave therapy on tendon/ligament healing.
Interdepartmental Equine Health Studies Program

The Equine Health Studies Program (EHSP) is one of four recognized priority research programs in the LSU School of Veterinary Medicine. Horses and equestrian activities are an important economic and recreational commodity in Louisiana and the surrounding region. Approximately 200,000 horses were owned by an estimated 60,000 people in the state, with a total direct economic impact of the equine industry in Louisiana of 1.4 billion dollars annually. Scientific investigation into the prevention and treatment of equine disease is critical to maintaining the health, well-being and performance of horses, and thus, is important for sustaining the equine industry. Substantial resources, including multidisciplinary, interdepartmental faculty, technical staff, facilities, and equipment provide an excellent environment for either graduate or clinical advanced studies.

Graduate Programs
Students in the School of Veterinary Medicine’s interdepartmental Equine Health Studies Program can obtain Master of Science (M.S.) and Doctor of Philosophy (Ph.D.) degrees in Veterinary Medical Sciences through the school’s academic departments: Comparative Biomedical Sciences, Pathobiological Sciences and Veterinary Clinical Sciences.

Current Research Interests
- Gastrointestinal tract disease (colic)
  - Intestinal ischemia-reperfusion
  - Ulcerative disease
  - Intestinal motility disorders
  - Inflammatory bowel disease
- Effect of gastrointestinal tract inflammation on mucosal permeability
- Effect of NSAIDs on colonic mucosal permeability
- Summer pasture-associated obstructive pulmonary disease/COPD and other respiratory tract diseases
- Nonvascular smooth muscle physiology, pharmacology, and pathobiology
  - Gastrointestinal
  - Bronchial
  - Uterine
- Vascular smooth muscle physiology, pharmacology, and pathobiology
- Analgesia and pain management
- Medication surveillance
- Synovitis and arthritis
- Acupuncture
- Laminitis
- Parasitology
- Endotoxemia
- Virology
- Use of global positioning system technology for equine epidemiologic studies
- Inflammatory mediators, including nitric oxide, endothelin and cytokines
- Mare reproductive physiology, infertility and placentitis
- Improving freezing methods for stallion semen
- Advancing the onset of the breeding season in mares
- Intrafollicular insemination of mares
- Equine embryo biotechnology
- Assisted reproduction techniques in horses
- Endotoxin-induced late gestation abortion in mares
- Musculoskeletal injuries and other diseases causing poor performance
- Comparative orthopedics
- Effects of extracorporeal shockwave therapy on bone, tendon, ligament and nerve
EHSP Grants and Contracts

2000
Costa LRR, Gaunt S, O'Reilly KL, Horohov DW, Moore RM: In vitro identification of mold allergens and cytokines involved in neutrophil chemotaxis in horses affected with summer-pasture associated obstructive pulmonary disease. $4,000.00. Comparative Respiratory Society, August 2000.

Costa LRR, Blackmer JJM, Truax R, Horohov DW, Moore RM: In vitro effects of IL-4 on mucus and endothelin-1 production by equine bronchial epithelial cells. $2,000.00. LSU Equine Health Studies Program, June 2000.

Curtis LA, Moore RM, Eades SC, Truax R: $5,000.00. LSU School of Veterinary Medicine Merck-Merial Student Research Grant Program, March 2000.


Venugopal CS, KrishKumar, Moore RM: Comparative in vitro responses of airways of clinically healthy and SPAOPD-affected horses to endothelin-1 in the presence and absence of endothelin receptor antagonists. $4,000.00. LSU Equine Health Studies Program, June 2000.

1999


Pinto CRF, Eades SC, Barker SA, Moore RM: Pharmacokinetics and hemodynamics of N-\textsuperscript{\textendash}nitro arginine methyl ester (L-NAME) administered intravenously in horses. $3,000.00. LSU Veterinary Clinical Sciences Organized Research Fund, September 1999.


Tetens J, Moore RM, Henk WG, Hosgood G: Quantification of colonic epithelial cell tight junction damage secondary to ATP depletion. $3,000.00. LSU Veterinary Clinical Sciences Organized Research Fund, September 1999.


Tetens J, Moore RM, Eades SC: Characterization of contractile purinergic receptors in equine colonic vessels and their in vitro response to exogenously administered adenosine triphosphate. $3,000.00. LSU School of Veterinary Medicine Research Funds, February 1999.


1998


Eades SC, Moore RM, Holm AS: Role of endothelin and nitric oxide in equine laminitis. $5,822.00. Louisiana State University School of Veterinary Medicine USDA 1433 Formula Fund, November 1998.


Goad ME, Moore RM, Ramaswamy CM, Hosgood G: Endothelin-1 immunohistochemical staining in the gastrointestinal tract of clinically normal horses and those with naturally acquired intestinal ischemia. $1,950.00. Louisiana State University School of Veterinary Medicine USDA 1433 Formula Fund, November 1998.


Venugopalan CS, Moore RM: Pharmacologic evaluation of endothelin antagonists in blocking constriction of equine colonic vessels induced by endothelin-1. $4,000.00. Louisiana State University School of Veterinary Medicine USDA 1433 Formula Funds, January 1998.
EHSP Selected Scientific Publications

2000


1999


1998


Sedrish SA, Moore RM, Kelly K, Martin GS, Burba DJ. In-vitro pullout strength of screws inserted in adult equine third metacarpal bone after overdrilling a 4.5-mm threaded insertion hole. Vet Surg 1998;27(2)143-149.


EHSP Selected Published Scientific Abstracts

2000


1999


Hubert JD, Hardy J, Holcombe SJ, Moore RM. Cecal amputation within the right ventral colon for surgical treatment of nonreducible cecocolic intussusception in 8 horses. BEVA Congress 1999.


1998


Horohov DW, Vanderheyden C, Pourciau S, Chapman M, Klei TR. Immunity to equine nematodes involves a Type 2 cytokine response. 5th International Veterinary Immunology Symposium 1998.


Swiderski CE, Klei TR, Horohov DW. Type 1 and Type 2 cytokine responses in horses, in Proceedings. 16th ACVIM Forum 1998;189.


How You Can Support the EHSP and Enhance the Health, Well-Being and Performance of Horses

There are many ways individuals or companies can help support the Louisiana State University (LSU) School of Veterinary Medicine (SVM) Equine Health Studies Program (EHSP). The EHSP is “Dedicated to the health, well-being and performance of horses through veterinary research, education and service.” In order to fulfill our mission of becoming one of the premier equine biomedical centers in the country, we have initiated a capital campaign to generate funds to enhance all aspects of our program.

The LSU School of Veterinary Medicine is a relatively young institution, with only 28 years of graduates. Our endowment is comparatively small, so each gift is extremely special to us and will make an important and immediate impact on our programs. Our fund-raising efforts have been principally through private, charitable, tax-deductible gifts as well as some other special events and activities. All gifts are completely tax-deductible and can be pledged with a portion being given annually over a period of a few years. We hope that you will give consideration to assisting us with our fund raising efforts for facility enhancements, endowed/distinguished professorships and chairs, and/or scientific investigation.

An endowed gift is a permanent gift. The principal is invested and returns annual interest. Part of the annual interest is reinvested to increase the principal, and part is used for the purpose intended (such as a professorship/chair or research activities). Endowed funds are usually named for the benefactor or for a designated honoree. Some examples of how your endowed gifts can advance the EHSP and its research, education and service missions include professorships, chairs, research, and facility construction.

Professorships and Chairs: The state of Louisiana has a matching program for Endowed Professorships and Endowed Chairs. The school currently does not have any Endowed Chairs and only three Endowed Professorships, neither of which is in the area of equine clinical or biomedical science. An Endowed Chair in equine biomedical sciences would be distinguished by being the first and only endowed chair in the School of Veterinary Medicine. These endowed positions are vital to move our instructional and investigational programs forward. The individuals in these positions will serve as leaders of teams of equine clinicians and investigators that conduct leading-edge scientific investigations to improve prevention and treatment of equine diseases.

Equine Biomedical Research: Private gifts can provide funds for conducting leading-edge scientific investigation into the cause, prevention and treatment of illnesses and injuries afflicting horses. With the limited amount of state and federal funding available for equine scientific investigations, it is vital to the health, well-being and performance of horses that we provide funds through private, charitable gifts to investigate and improve our ability to successfully prevent and treat illnesses and injuries of horses that can be performance-limiting, career-ending and even life-threatening.

General EHSP Support Fund: Gifts can be made into the General EHSP Support Fund, a non-endowed account, that is used to purchase new or replacement equipment in the Equine Clinic for scientific investigations. Additionally, these funds are often used for continuing educational activities for the horse-owning public and private equine veterinarians who rely upon us for consultation and referral services. These funds also are used to assist with other educational, promotional and fundraising activities.

Memorial Gifts and Naming Opportunities: Your gift may be used to honor or memorialize a beloved horse, family member, or friend. Naming opportunities exist for endowed gifts such as scholarships, professorships, and chairs. Construction projects such as the Equine Intensive Care Unit, Equine Isolation Unit, Equine Reproduction Unit, Equine Lameness and Performance Evaluation Unit and research laboratories offer a wide variety of naming opportunities.

Again, any gift will be very special to the LSU School of Veterinary Medicine’s Equine Health Studies Program and will make a dramatic and immediate impact on our teaching, service and scientific investigation programs. We thank you in advance for your generosity and support. To learn more about how your gift will assist the EHSP with its mission, please visit our website (www.equine.vetmed.lsu.edu) or contact Dr. Rustin Moore via telephone (225-578-9500) or e-mail (equine@vetmed.lsu.edu).
The Equine Health Studies Program is an interdepartmental, multidisciplinary equine biomedical program within the Louisiana State University School of Veterinary Medicine that is dedicated to the health, well-being and performance of horses through veterinary research, education and service. A diverse group of faculty, advanced studies students and staff conduct leading-edge scientific investigations involving equine health and disease utilizing state-of-the-art facilities and equipment. The program maintains a herd of 90 horses and ponies for use in scientific studies. The EHSP research facilities include the Equine Physiology & Pharmacology Laboratory, the Equine Performance Evaluation Laboratory, the Equine Cell & Tissue Culture Laboratory and the Laboratory for Equine and Comparative Orthopedic Research. Additionally, the clinical facilities and equipment within the Veterinary Teaching Hospital and other core research facilities and resources within the School of Veterinary Medicine support the research activities of the EHSP.

**Equine Physiology & Pharmacology Laboratory**

Numerous faculty and graduate students conduct scientific investigations involving equine physiology, pharmacology and pathophysiology, including but not limited to vascular and nonvascular smooth muscle physiology/pathophysiology/pharmacology (digital and intestinal vasculature, bronchial, uterine and intestinal smooth muscle); the effects of intestinal ischemia-reperfusion injury, nonsteroidal antiinflammatory drugs and parasitism on mucosal physiology and permeability; effects of endotoxin, experimental laminitis and medications on systemic and local digital hemodynamics; reproductive physiology related to mare and stallion fertility; effects of medications on behavior and activity; effects of drugs and delivery systems for analgesia and pain management. The laboratory contains 24 organ baths integrated with force transducers and polygraphs to measure tension on tissues (vascular smooth muscle, nonvascular smooth muscle, cardiac muscle, skeletal muscle and tendon) in response to inflammatory mediators or pharmacologic agents. For studies of tissue permeability and effects of pharmacological agents on tissue integrity, we have 12 Ussing chambers mounted within the main laboratory. Dual channel Doppler flow and laser Doppler ultrasound flow meters and probes to measure blood flow and tissue perfusion in several species are available. The lab also contains three 8-channel and six 4-channel polygraphs to record data from both force and pressure transducers. Additional instrumentation is available, including electromyography, electrocardiography, and equipment for cutaneous analgesia nerve conduction velocity studies. Two motion chambers are available for assessing the effects of medications on activity and behavior of horses.

**Equine Performance Evaluation Laboratory**

The Equine Performance Evaluation Laboratory (EPEL) is equipped with a high-speed treadmill for exercising horses at speeds that mimic racing conditions. The EPEL is also equipped with a TekScan digital pressure system that incorporates pressure sensors in specially designed horseshoes to evaluate static and dynamic weight bearing to assess lameness. Equipment is available for dynamic endoscopic examination. Polygraphs and pressure transducers are available for measuring airway pressures and impedance. Equipment is available for electrocardiographic and echocardiographic evaluations of the heart before and after intense exercise on the treadmill. A speed of sound
ultrasound machine for assessing bone density is available and used to assess the effect of growth, training, injury, and treatment modalities on bone density of the third metacarpal bone of horses. A focused extracorporeal shock wave therapy (ESWT) unit is available and is used to evaluate the effects of ESWT on healing of tendon, ligament and bone and on its functional (analgesia) and morphologic effects on nerves. Funds have been recently obtained to acquire a kinematic gait analysis system and a force plate, which will be installed in the EPEL in the near future.

Equine Cell and Tissue Culture Laboratory
The Equine Cell and Tissue Culture Laboratory is well equipped to support tissue culture activities of the investigators of the Equine Health Studies Program. The laboratory has 3 laminar flow biohazard hoods, 4 CO₂ incubators, 9 Synthecon bioreactors, a phase contrast microscope with digital image capture capabilities, 2 centrifuges, refrigerators and ultra-low temperature freezers. In concert with other centralized facilities in the SVM including electron and confocal microscopy and molecular biology, investigators have a wide range of state-of-the-art equipment and facilities to employ tissue culture as a research tool. Current projects involving the laboratory include the growth of laminar cells for use as an in vitro model of laminitis, bone marrow stromal cells for use in experimental tendon healing, colonic and cecal epithelium for use in the study of bacterial factors in laminitis, bronchoepithelial cells for the study of summer pasture-associated obstructive pulmonary disease, endometrial cells for the study of endometritis and other conditions affecting mare fertility, and corneal epithelial cells for the study of herpes virus infections of the eye. Our laboratory pioneered the use of rotating wall vessels for growing cells under microgravity conditions, which yield three-dimensional tissue assemblies for the study of various equine diseases.

Laboratory for Equine and Comparative Orthopedic Research
The Laboratory for Equine and Comparative Orthopedic Research (LECOR) is the newest addition to the Equine Health Studies Program. The laboratory is specifically designed and equipped for translational orthopedic research from the molecular/genetic level to the structural biomechanical level. The laboratory is equipped with the most modern equipment for molecular/genetic work including an MJ Research Chromo4 Detector and DNA Engine 200 for DNA fragment amplification and Quantitative PCR, a UVP hybrilinker for blot analysis and a Synergy HT multi-detection microplate reader for ELISA assays. Housed within the laboratory is a Leica DM 4000 light microscope with fluorescent, polarizing and phase contrast capabilities. The microscope is equipped with the latest in digital image capture equipment and software. Additionally, there is a PathScan Enabler to obtain ultra high quality images from 1 x 3 inch glass slides. A custom-designed servohydraulic axial torsional Material Testing System with a Flex Test SE Controller and equipped with a Multiple Gage Length Axial Extensometer makes nearly any level of mechanical tissue testing possible from the tissue and bone level to joint and whole limb testing. Presently, several state-of-the-art diamond saws are available for both orthopedic hard and soft tissue research.
microscopic and ultrastructural sample preparation. A section of the laboratory is devoted to histologic preparation of both calcified and decalcified tissue samples requiring special processing for leading-edge orthopedic research. Areas of research focus include the pathophysiology of hip dysplasia, the development and implementation of novel orthopedic devices, cranial cruciate disease, synovial fluid prognostic markers for joint disease, the effects of shock wave therapy on bone, minimally invasive treatments for bone spavin, and genetic markers for orthopedic disease. The Laboratory for Equine and Comparative Orthopedic Research has been established and designed to facilitate a strong association between clinical and basic orthopedic research for advancement of orthopedic knowledge across species and disciplines.

**Veterinary Clinical Facilities & Equipment**

The LSU Veterinary Teaching Hospital & Clinic is staffed by nationally and internationally recognized veterinary specialists (internal medicine, surgery, anesthesiology, and radiology) and highly-skilled veterinary technicians, and is furnished with state-of-the-art equipment necessary to provide advanced diagnostic and therapeutic services to private referral veterinarians and the animal-owning public. The hospital facilities include two equine anesthesia induction/recovery rooms and surgical suites with modern equipment; a modern, centralized, climate-controlled 10-stall equine intensive care unit for critically ill and injured horses; an isolation unit for horses with infectious/contagious disease; and diagnostic/therapeutic procedure rooms.

Diagnostic imaging capabilities include modern radiography, ultrasonography, computed tomography and nuclear scintigraphy facilities and equipment. Plans are underway to acquire magnetic resonance imaging and digital radiography. Endoscopy equipment is available for assessment of the upper respiratory tract, urogenital and gastrointestinal systems. Laparoscopic equipment is available for diagnostic and therapeutic applications.

Orthopedic-related equipment available in the hospital includes 2 arthroscopy units, ASIF equipment and implants for fracture repair, surgical lasers (Nd:YAG, diode and CO\textsubscript{2}), and an extracorporeal shockwave therapy unit. A new Equine Lameness and Performance Evaluation (75' x 125' covered pavilion) is provides a modern facility for evaluation of gait, locomotion and lameness in equine clinical patients.

**Equine Reproduction/Theriogenology Laboratory**

The Equine Reproduction/Theriogenology Laboratory has complete facilities for the evaluation, chill-transport, and cryopreservation of spermatozoa, including light and phase-contrast microscopes and a computer-assisted spermatozoal analysis system (Spermvision\textsuperscript{®}). We have three ultrasound machines with 5-7.5 MHz linear array transducers and a 5-7.5 MHz sector array transvaginal transducer for oocyte collection by follicular aspiration or twin reduction by aspiration. A fourth ultrasound system equipped with a 3.5 sector and 5-7.5 microconvex array are available for transabdominal imaging. An Olympus endoscope is also available for hysteroscopic examination and for hysteroscopic low-dose insemination. Laparoscopy is available and used for oviductal insemination and for minimally invasive placement of intrauterine catheters. We maintain a close collaborative relationship with the Equine Biotechnology Laboratory, which is part of the LSU Agricultural Center. This facility has tissue culture laboratories and micromanipulators that make possible such advance assisted reproductive techniques as intracytoplasmic spermatozoal injection and nuclear transfer ("cloning").
BIOMMED – Biotechnology and Molecular Medicine
The Division of Biotechnology and Molecular Medicine (BIOMMED), a division within the LSU SVM, is organized into three different Laboratories: 1) GeneLab; 2) Viral Vectors Laboratory (VVL); 3) Protein and Antibody Production and Purification Laboratory (PAPPL). **GENELAB:** GeneLab produces synthetic oligonucleotides including biotinylated, fluoresceinated, phosphorylated and phosphorothioate (antisense) oligonucleotides up to 200-bases long. Additional molecular biology services include cloning and automated sequencing of genes, real-time quantitative PCR, automated preparation of chromosomal and plasmid DNAs, cDNA library construction, and microarray production and analysis. **VVL:** This laboratory provides custom baculovirus, adenovirus, vaccinia virus, herpes, and other recombinant virus construction for heterologous gene expression, and vaccine and gene therapy studies. **PAPPL:** This laboratory concentrates on the production and purification of proteins and antibodies. The laboratory produces monospecific antibodies in rabbits and mice using conventional immunization methodologies using purified protein immunogens as well as genetic immunization methods. Antibodies are concentrated and purified using standard methodologies. The laboratory also provides on a limited basis the production and characterization of monoclonal antibodies.

GeneLab operates a new bioinformatics module, which is equipped with three Macintosh G4 computers and two DELL PC computers. A new WEB-based system direct communication between researchers and GeneLab staff has been recently purchased. Available software include: Primer Express (PE Biosystems) for the design of TaqMan probes for real-time PCR, MacVector (Genetics Computer Group, Inc.) for analysis of DNA and protein sequences, Oligo (Molecular Biology Insights, Inc.) for the design of PCR primers, Quantity One (BioRad, Inc.) for the visualization and analysis of images such as those produced by ethidium bromide agarose electrophoresis of DNA fragments, SDS-PAGE analysis of proteins, immunoblots, etc. GeneLab has also additional software for assembly of large DNA sequences (Sequencher), analysis of blots (alpha Innotech) and microarrays (Alpha Innotech 6000). Automated ordering for synthetic DNA and other reagents is assisted via a WEB-based Information System (DNA LIMS). BIOMMED has 3 automated sequences (ABI377, 310, 310), three real-time PCR equipment (PE 7900, 7400), LightCycler (Roche), 2 MagnaPure automated nucleic acid extractors (Roche), microarray OmniGrid spotter (Gene Machines), microarray reader (AlphaReader 6000, Alpha Innotech), Imager station (Alpha Innotech Fluorochem 8000), 5 PCR (thermocyclers, a New Brunswick Fermentor, AktaExplorer Chromatography System, Cyclone phosphorimager, 2 four column DNA synthesizers (ABI), one Synergy peptide synthesizer (ABI), and other equipment.

Immunology Laboratory
Three laboratories (~1200 sq ft) located on the third floor of the School of Veterinary Medicine (SVM) are designated for the Immunology Laboratory. These labs contain necessary equipment for immunological assays and the in vitro cultivation of lymphocytes, including; laminar flow biosafety cabinets, CO₂ incubators, microscopes, water baths, a pH meter, low speed centrifuges, mixers, stir plates, refrigerators and freezers. One of the laboratories is dedicated specifically for molecular biology procedures and contains all of the equipment and materials for the isolation of and analysis of RNA, DNA and proteins. Separate refrigerators and freezers for molecular biology samples are found in this laboratory. Spectrophotometers, pH meters, electronic balances, refrigerators and freezer and other small equipment items are also available in this laboratory.

The Flow Cytometry Facility is a core laboratory located on the third floor of the School of Veterinary Medicine building on the campus of Louisiana State University. The facility features a Becton Dickinson FACScan flow cytometer capable of measuring two light scatter parameters and three fluorescence emissions. Immunophenotyping, cell cycle analysis, apoptosis studies, and measurements of cellular function are examples of applications, which are performed routinely in this laboratory. A newly acquired FACS Aria Dual Laser Flow Cytometer, which is capable of high performance cell sorting of up to 30,000 cells per second and separation of 1 to 4 distinct cell populations. Additionally, multicolor immunophenotyping and cell functional assays can expand to seven-color analysis. This unique centralized facility provides analytical capabilities for investigators throughout the Louisiana State University System, including the LSU Agricultural Center and LSU Pennington Biomedical Research Center. Both PC and Macintosh computers are utilized in data acquisition and analysis and are all connected to the Veterinary Medicine and LSU networks.

Microscopy Center
The SVM Microscopy Center has state-of-the-art equipment for light and electron microscopy, including: Light Microscopes: Zeiss Axiosplan, Zeiss Axiovert with heated stage, Zeiss Photomicroscope III, Olympus Vanox, PALM Laser Scanning Confocal Microscope.
The Center features three new powerful microscopes: The laser capture and microdissection microscope (PALM Zeiss MicroBeam-Axiovert 200 System) allows researchers to dissect out parts of tissue on a slide and transfer it to a container for genetic, gene expression and proteomic analysis, which enables researchers to determine what genes are present and what genes and proteins are being expressed. The scanning laser confocal microscope (LEICA TCS SP2 AOBS) provides excellent quality three-dimensional reconstructions from cells and relatively thick sections of tissues, which enable researchers to examine cells and cell components in three-dimension and allows researchers to conduct co-localization studies to mark multiple proteins within the cell simultaneously. The environmental scanning electron microscope (FEI Quanta 200) provides a detailed evaluation of the surfaces with or without dehydrating the samples. This microscope also has an energy dispersive x-ray spectrometer that allows researchers to determine elemental composition.

**Analytical Systems Laboratories**

The Analytical Systems Laboratories are central service, comprehensive analytical laboratories, consisting of The Laboratory for Drug Residue Studies, The Equine Medication Surveillance Laboratory and The Analytical Systems Laboratory. The Laboratory for Drug Residue Studies provides instrumentation and expertise for the performance of drug and biological molecule pharmacokinetics, metabolism, tissue distribution and analytical method development. The laboratory is also equipped to conduct complete drug profiling using radiolabeled test materials. These laboratories operate under Federal Good Laboratory Practices regulations and have generated data for the Food and Drug Administration (FDA) and private industry for submission for veterinary drug approvals by U.S. and foreign regulatory agencies. The Equine Medication Surveillance Laboratory has served as the official laboratory for the Louisiana State Racing Commission. The laboratory screens over 10,000 urine and blood samples per year and has developed sophisticated methodology for detection and confirmation of drugs and their metabolites. The laboratory also conducts illegal drug-use testing for the LSU Department of Athletics and serves as a source of information to the racing industry and the public regarding drug pharmacology, metabolism and clearance. The Analytical Systems Laboratory houses advanced mass spectrometry and other analytical equipment that is used to support other laboratories as well as the research of SVM faculty and graduate students. Equipment in these laboratories includes a Micromass Quattro II GC/LC/MS/MS (+/-) with Apcl and ESP interfaces, one HP 1090 II, low-flow HPLC (1 ul/min), three 1090 HPLCs equipped with UV-diode array, fluorescence, electrochemical and radio-monitor detectors, a HP 5973 GC/MS system and beta and gamma counters for radiolabel analyses. The laboratory is currently developing full capabilities to conduct low- and high-throughput proteomics analysis and is enhancing its abilities to conduct small and large-scale protein purification. Appropriate and modern computer equipment and software is available for data acquisition, storage, and analysis. ASL: Hewlett-Packard 5970 MSD GC/MS system (ChemStation data system), three (3) Hewlett-Packard HP 1090 HPLCs (UV diode array, electrochemical, fluorescence and radiochemical detectors); Fisons VG Quatro II GC/LC/MS/MS +/- ion, extended mass range system, electrospray and APcI interfaces, HP5980 GC, HP 1090 II LC. The ASL recently acquired a Micromass Two-Dimensional-Capillary-Liquid Chromatography/Quadrupole-Time of Flight Mass Spectrometer, which enables separation and comprehensive structural analysis of proteins. The instrument is capable of conducting de novo sequencing of proteins and peptides as well as identifying and locating post-translational modifications. This type of equipment is essential for the rapidly growing fields of proteomics and bioinformatics for comprehensive examination of molecular events occurring in tissues in health and disease. This equipment is available for collaboration across the LSU campus and supports the research efforts of the
SVM faculty and the Equine Medication Surveillance Laboratory, which will use it to identify illegal peptide and protein drug use in racehorses under its contract with the Louisiana State Racing Commission.

**Pathology, Histopathology & Immunohistochemistry**

The equipment, instrumentation and personnel for gross necropsy, histologic evaluation and immunohistochemical staining are available in this facility. Equipment and for processing cryopreserved tissues, automatic immunohistochemical staining, and the computers and software (ImagePro) for evaluation of staining distribution and intensity are available for use in this core facility.

**Division of Laboratory Animal Medicine (DLAM)**

DLAM is housed within the SVM, and serves as a central administrative division for operating research animal holding facilities, including the SVM Laboratory Animal Medicine and Life Sciences Animal Care facilities. DLAM acquires, maintains and cares for teaching and research animals housed in the facilities, and is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) International.

**SVM Library**

The LSU-SVM Library is the largest health science library in the greater Baton Rouge area, and is a member of the National Library of Medicine South Central Chapter Regional Library Program. It occupies approximately 7,400 sq. ft. and its current holdings include 42,300 volumes and approximately 670 current periodical titles, dealing with all aspects of veterinary medicine, selected materials from human and comparative medicine, public health, animal sciences and other related areas.

*Louisiana State University School of Veterinary Medicine.*
Foals

Plasma magnesium concentrations in neonatal foal admitted to the intensive care unit

Authors/Investigators:
Lais R.R. Costa, MV, MS, DACVIM; Thomas L. Seahorn, DVM, MS; Susan C. Eades, DVM, PhD, DACVIM; R.T. Tulley, PhD; Catherine E. Koch, BS, MS; Amy Polkes, DVM, MS, DACVIM; Robert J. McKay, DVM, PhD, DACVIM; Rustin M. Moore, DVM, PhD, DACVS

Description of the Problem:
Magnesium (Mg) deficiency has been implicated in the pathogenesis of several neonatal diseases, including necrotizing enterocolitis (NEC), neonatal respiratory distress syndrome (NRDS), bronchopulmonary dysplasia (BPD), neonatal asphyxia and neonatal neurological syndromes. Most of the body magnesium is transferred from the mother to the fetus during the last trimester of gestation. Prematurity and low birth weight appear to increase the risk for development of several of those diseases.

Study Objective:
The objective of this study was to survey a population of critically ill neonatal foals admitted to two intensive care units for the occurrence of plasma magnesium abnormalities. Although, plasma magnesium may not accurately reflect the magnesium status of the body, it has been used as an indicator of magnesium status.

Approach:
A total of 106 critically ill foals presented to the Intensive Care Unit (ICU) at two referral hospitals, Louisiana State University and University of Florida, were evaluated in this study. The data was collected at the time of admission of the foal to the ICU, and included signalment, clinical information and initial laboratory work. The final diagnosis of the each foal illnesses and the outcome of the case were obtained at a later date. Heparinized plasma samples collected at the time of admission to the hospital were stored at –70C until assayed for total plasma magnesium concentration (tpMg) using a direct colorimetric method (Beckman Synchron CX7 automated chemistry analyzer). Forty-eight percent of the foal were males and 52 were females. Most of the foals were Thoroughbred (65%) and Quarter horse (14%). The average age of the critically ill foals was 5.5 ± 0.6 days. A published reference range of normal biochemical, hematological and clinical values for foals of this age was used. A total of 20 healthy foals were used as controls for the serum biochemistry profile and plasma magnesium concentrations.

Accomplishments/Results/Conclusions:
Abnormal tpMg plasma magnesium concentrations were common electrolyte abnormalities found in critically ill neonatal foals admitted to the intensive care units. All clinically normal foals had biochemical and hematological values within the reference ranges. Abnormal tpMg concentrations were common electrolyte abnormalities found in 29% of critically ill neonatal foals admitted to the intensive care units. Hypomagnesemia was present in the 20% whereas hypermagnesemia was present in 9% of critically ill neonatal foals. A variety of disorders affected the critically ill foals and in most cases there were two or more diseases diagnosed in the same case. The most common diseases included enterocolitis (n=49), neonatal septicemia (n=47), hypoxic-ischemic encephalopathy from perinatal asphyxia (n=15), prematurity (n=12), omphalophlebitis (n=12), pneumonia (n=12). Hypomagnesemia was frequent in foals diagnosed with prematurity (42%), septicemia (n=32) and enterocolitis (24%). Hypomagnesemia frequently accompanied hypocalcemia (48%) and hematological abnormalities associated with sepsis. Our study suggests that magnesium abnormalities are important in the critically ill neonatal foals suffering from a variety of disorders.

Benefits to /Impact on the Equine Industry:
Our study suggests that magnesium status should be evaluated in the critically ill neonatal foals and appropriate magnesium supplementation be is likely to improve the overall clinical status of the neonatal equine patients.
**Take Home Message:**
Magnesium abnormalities should be part of the biochemical evaluation of neonatal foals and correction of magnesium abnormalities should be part of the treatment of these foals.

**Acknowledgments:**
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**Year Completed:** 2000

**Published Manuscripts/Abstracts:**

Gastrointestinal Tract

In vitro effects of oxytocin, acepromazine, detomidine, xylazine, butorphanol, terbutaline, isoproterenol, and dantrolene on smooth and skeletal muscles of the equine esophagus

Authors/Investigators:
Anne A. Wooldridge, DVM, MS, DACVIM; Susan C. Eades, DVM, PhD, DACVIM; Giselle Hosgood, BVSc, MS, PhD, DACVS; Rustin M. Moore, DVM, PhD, DACVS

Description of the Problem:
Obstruction of the esophagus (choke) is a common problem in horses that most often results from impaction of food material preventing passage of feed material and water through the esophagus to the stomach. This results in regurgitation of food, water, and saliva from the nose and pain manifest as stretching the neck and gagging. Affected horses may cough, develop fever, and exhibit colic. The food impaction distends the esophagus causing pain and spasm of the esophageal muscles. The primary goal of treatment is to relieve the obstruction by gentle pressure and warm water lavage via a nasogastric tube. Esophageal spasm impedes these efforts at removing the impacted food. Medications are often administered to relax the esophagus and reduce esophageal spasm; however, the efficacy of these medications has not been demonstrated.

Study Objective:
The objective of this study was to characterize the in vitro effects of oxytocin, acepromazine, xylazine, butorphanol, detomidine, dantrolene, isoproterenol, and terbutaline on skeletal and smooth muscle from the equine esophagus.

Approach:
Circular and longitudinal strips from the skeletal and smooth muscle of the esophagus were suspended in tissue baths, connected to force-displacement transducers interfaced with a physiograph, and electrical field stimulation was applied. Cumulative concentration-response curves were generated for oxytocin, acepromazine, xylazine, detomidine, butorphanol, isoproterenol, terbutaline, and dantrolene. Mean maximum twitch amplitude for 3 contractions/min was recorded and compared with predrug-vehicle values for the skeletal muscle segments, and area under the curve (AUC) for 3 contractions/min was compared with predrug-vehicle values for the smooth muscle segments.

Accomplishments/Results/Conclusions:
No drugs caused a significant change in skeletal muscle response. In smooth muscle, isoproterenol, terbutaline, and oxytocin significantly reduced AUC in a concentration-dependent manner.

Benefits to/Impact on the Equine Industry:
Isoproterenol, terbutaline, and oxytocin cause relaxation of the smooth muscle portion of the esophagus. However, none of the medications affected the skeletal muscle strips.

Take Home Message:
These medications cannot cause enough relaxation of the esophagus to be relied upon heavily for removal of esophageal obstruction in horses.

Acknowledgments: This study was supported by funds from the Equine Health Studies Program, School of Veterinary Medicine, Louisiana State University.

Year Completed: 2000

Published Manuscripts/Abstracts:

Effects of treatment with oxytocin, xylazine butorphanol, guaifenesin, acepromazine, and detomidine on esophageal manometric pressure in conscious horses

Authors/Investigators:
Anne A. Wooldridge, DVM, MS, DACVIM; Susan C. Eades, DVM, PhD, DACVIM; Giselle Hosgood, BVSc, MS, PhD, DACVS; Rustin M. Moore, DVM, PhD, DACVS

Description of the Problem:
Obstruction of the esophagus (choke) is a common problem in horses that most often results from impaction of food material preventing passage of feed material and water through the esophagus to the stomach. This results in regurgitation of food, water, and saliva from the nose and pain manifest as stretching the neck and gagging. Affected horses may cough, develop fever, and exhibit colic. The food impaction distends the esophagus causing pain and spasm of the esophageal muscles. The primary goal of treatment is to relieve the obstruction by gentle pressure and warm water lavage via a nasogastric tube. Esophageal spasm impedes these efforts at removing the impacted food. Medications are often administered to relax the esophagus and reduce esophageal spasm; however, the efficacy of these medications has not been demonstrated.

Study Objective:
The objective of this study was to compare the effects of medications commonly used to reduce esophageal contractions in healthy horses.

Approach:
Small polyethylene tubes were placed within an equine nasogastric tube and the end fitted at an opening in the distal segment in the nasogastric tube. The polyethylene tube was filled with water and attached to a transducer to measure changes in pressure created by contractions of the esophagus. Contractions of the esophagus were measure before and after administration of one the medications (oxytocin, xylazine butorphanol, guaifenesin, acepromazine, detomidine, and saline solution control) in eight healthy horses. The experiment was repeated on each horse at 2 week intervals until all medications were tested.

Accomplishments/Results/Conclusions:
Acepromazine, detomidine, and the combination of xylazine and butorphanol decreased the number of swallows by causing sedation. However, the strength of normal contractions was not reduced.

Benefits to/Impact on the Equine Industry:
The results of this study demonstrate that acepromazine, detomidine, and the combination of xylazine and butorphanol may facilitate removal of feed impactions of the esophagus by causing sedation, thereby reducing the stimulation of swallowing by the brain.

Take Home Message:
Oxytocin, xylazine butorphanol, guaifenesin, acepromazine, and detomidine do not directly relax the esophagus in healthy horses; but acepromazine, detomidine, and the combination of xylazine and butorphanol provide sufficient...
sedation to reduce the stimulation of swallows by the brain, thereby indirectly decreasing the contraction of the esophagus. This allows feed impactions to be flushed out with water via a nasogastric tube more easily.

Acknowledgments:
This study was supported by funds from the Equine Health Studies Program, School of Veterinary Medicine, Louisiana State University.

Year Completed: 2000

Published Manuscripts/Abstracts:


Plasma magnesium concentrations in critically ill horses affected with gastrointestinal tract disease

Authors/Investigators:
Lais R.R. Costa, MV, MS, DACVIM; Susan C. Eades, DVM, PhD, DACVIM; R.T. Tulley, PhD; Catherine E. Koch, BS, MS; Giselle Hosgood, DVM, PhD, DACVS; Rustin M. Moore, DVM, PhD, DACVS

Description of the Problem:
Horses affected with acute gastrointestinal tract diseases are often dehydrated and have a variety of severe electrolyte abnormalities. Plasma or serum magnesium (Mg) concentration is likely affected, because its homeostasis results principally from intestinal absorption and renal excretion. However, the incidence of hypomagnesemia in critically ill horses affected with gastrointestinal tract disorders remains unreported.

Study Objective:
The objective of this study was to survey a subpopulation of critically ill horses affected with gastrointestinal tract disease to determine the incidence of plasma magnesium abnormalities and concurrent plasma electrolyte derangements.

Approach:
A total of 155 critically ill horses with gastrointestinal tract (GIT) disease referred to the Intensive Care Unit (ICU) at Louisiana State University Veterinary Teaching Hospital and Clinics were evaluated. The data collected at the time of admission to the ICU included signalment, initial clinical information and laboratory work. The final diagnosis and the outcome of each case were obtained at a later date. The GIT disorders were categorized in 5 groups: (1) small intestinal strangulation (n=34) which included incarcerations in the epiploic foramen and in a mesenteric rent, strangulating lipoma and volvulus; (2) large intestinal strangulation (n=22) which included large colon volvulus; (3) small intestinal inflammatory disease (n=21) which included duodenitis-proximal jejunitis and all other causes of enteritis; (4) large intestinal inflammatory disease (n=27) which included colitis and enterocolitis; and (5) simple obstruction of the large and small intestines (n=51) which included cases of impactions, enteroliths, fecaliths, intraluminal foreign bodies and non-strangulating large colon displacements. The biochemical variables evaluated included: sodium, potassium, chloride, calcium, creatinine, BUN, and total protein. Heparinized plasma samples were collected and stored at –70°C at the time of hospital admission, and total plasma magnesium concentration (tpMg) was measured using a direct colorimetric method (Beckman Synchron CX7 automated chemistry analyzer). Twenty clinically healthy horses were used to validate the colorimetric test used for Mg quantification.

Accomplishments/Results/Conclusions:
Abnormalities of plasma magnesium concentration were the most frequent electrolyte changes in plasma of critically ill horses with GIT disease. The incidence of hypomagnesemia was greatest in horses with small intestinal strangulation, colitis and simple intestinal obstructions, respectively. Several electrolyte abnormalities occurred concurrently with abnormalities in plasma Mg concentration. Hypocalcemia and hypokalemia were the most common concurrent electrolyte abnormalities in hypomagnesemic ill horses. There were no statistical differences for the plasma Mg concentrations among groups. Plasma concentrations of chloride, BUN, and PCV were significantly associated with
In vitro effects of an electron transport inhibitor on resistance, permeability, and adenine nucleotide content of equine colonic mucosal tissue

Authors/Investigators:
Joanne Tetens, DVM, MS, PhD; DACVS; Giselle Hosgood, BVSc, MS, PhD, DACVS; Steven A. Barker, PhD; Catherine E. Koch, MA; Marian Waguespack, BS; Priti Juneja, MS

Description of the Problem:
Because gastrointestinal mucosal cells are highly metabolically active and require large quantities of ATP for maintenance of normal cellular function, the mucosal layer is principally affected during ischemia. Increased cellular permeability, which occurs during ischemia and hypoxia, leads to translocation of bacteria and endotoxin from the gastrointestinal tract lumen into the splanchnic and systemic circulation. One mechanism that may be involved in the increase in gastrointestinal mucosal permeability during ischemia is decreased endogenous synthesis of adenosine triphosphate (ATP). The epithelial intercellular permeability barrier is maintained largely by the tight junction (TJ). In vitro studies have demonstrated that a correlation exists between cellular ATP content and TJ integrity. During ascending colon volvulus in horses, mucosal ATP content diminishes and does not fully recover upon reperfusion. The depletion of mucosal ATP content may lead to rapid opening to TJs and increased passage of bacteria and endotoxin through the paracellular pathway into the splanchnic circulation. Early re-establishment of cellular ATP content following an intestinal ischemic event is essential for normal mucosal barrier function.

Benefits to/Impact on the Equine Industry:
The results of this study demonstrate that abnormalities in plasma Mg concentration are common in critically ill horses affected with GIT diseases, and that hypomagnesemia is frequently accompanied by other plasma electrolyte abnormalities, especially hypocalcemia and hypokalemia. We speculate that the treatment of hypomagnesemia is likely to improve the balance of other electrolytes and contribute to the improvement of overall clinical status of these patients.

Take Home Message:
The results of this study indicate that abnormalities in magnesium concentration are important in the critically ill horses affected with gastrointestinal tract disorders.

Acknowledgments:
This study was supported by funds from the Equine Health Studies Program, School of Veterinary Medicine, Louisiana State University. The authors thank Drs. M. Mirza and C. Ramaswamy for their contributions to this study.

Published Abstracts/Manuscripts:


In vitro effects of an electron transport inhibitor on resistance, permeability, and adenine nucleotide content of equine colonic mucosal tissue

Photomicrograph of a normal section of the left ventral colonic mucosa.
event may help minimize mucosal barrier disruption with subsequent passage of toxic mediators into the splanchnic circulation, thereby decreasing morbidity and mortality.

**Study Objective:**
The objective of the study was to evaluate the effect of antimycin A (an agent to produce chemical hypoxia) on equine colonic mucosal resistance, permeability and adenine nucleotide content, and secondly, to determine the stability of adenine nucleotides in an in vitro system.

**Approach:**
Part 1: Colonic mucosal segments were harvested and placed in organ baths. The segments were bathed in oxygenated Ringer’s solution. Four different organ bath conditions were used (control with glucose, control without glucose, AA-depleted, AA-depletion/glucose-repletion). All tissues were allowed an equilibration period of 15 minutes prior to collection of the baseline sample. The remainder of the samples were collected at 60, 120, 180 and 240 minutes. The samples were frozen and subsequently analyzed for adenine nucleotide content (ATP, ADP and AMP). Part 2: Colonic mucosal segments were harvested and mounted in Ussing chambers. Tissues were bathed with Ringer’s solution and oxygenated. Tissues were continually short-circuited except at 15-minute intervals, when the spontaneous potential difference (PD) was measured. Resistance was calculated from the spontaneous PD and short-circuit current. Baseline electrical readings were taken following a 15-minute equilibration period. In all tissues except the control tissues, AA was added. After a 60-minute incubation period, the AA was removed from all tissues except the depletion group and the Ringer’s solution replaced. The tissues were repleted with the following treatments: no glucose, 10 mM glucose, 10 mM glucose plus 10⁻⁴M ATP, and 10 mM glucose plus 10⁻⁴M ATP-MgCl₂. The tissues were maintained for an additional 180 minutes. Additionally, mannitol fluxes were performed on all tissue types to determine changes in permeability.

**Accomplishments/Results/Conclusions:**
Part 1: There were no changes across time for ATP in the substrate-containing Ringer’s solution group. However, both ADP and AMP decreased across time. In the substrate-free group, ATP decreased at 240 min and ADP decreased 60-240 min. There were no changes across time for AMP. Significant decreases in adenine nucleotides were observed in tissues exposed to AA across time. In tissues depleted with AA and then repleted with 50 mM glucose, adenine nucleotide content followed a similar pattern as the depleted group. However, unlike the depleted group, there was no further decline in ATP content after the 60-min depletion period. Part 2: There was no change in the control group for resistance across time. There was a significant decrease in resistance across time in all groups treated with AA. Improvement in resistance upon repletion was not observed. There was no change in mannitol fluxes for any group across time with the exception of the AA-depleted and the AA-depleted/glucose-repleted groups.

**Benefits to/Impact on the Equine Industry:**
Adenine nucleotides are important for maintenance of normal cellular function and they have been implicated in regulation of tight junctions. When tight junctions open, both bacteria and endotoxin can escape from the intestinal lumen and enter the splanchnic and systemic circulations. The ability to develop in vitro system to deplete adenine nucleotides in equine colonic tissue and evaluate the effects of ATP depletion on tight junction integrity will enable investigators to develop therapies to replenish ATP, which may minimize/prevent transmural migration of endotoxin and bacteria. Ultimately, both morbidity and mortality may be decreased in horses with gastrointestinal tract disease.

**Take Home Message:**
Adenine nucleotides (such as ATP) have been demonstrated to help regulate the passageways (tight junctions) that exist between cells. When these tight junctions are open, bacteria and toxins from the intestinal tract can enter the blood stream, which will make the horse sick. This study attempted to create a depletion followed by repletion of ATP to determine the affects on tissue permeability. However, we were able to deplete ATP but were unsuccessful with our repletion technique.

**Acknowledgments:**
This study was supported by a grant from the Comparative Gastroenterology Society.

**Year Completed:** 2000

**Published Manuscripts/Abstracts:**
None at this time. Manuscript in progress.
The effect of Black Walnut extract on equine colonic histopathology and in vitro ion transport

Authors/Investigators:
Rebecca S. McConnico DVM, PhD, DACVIM; Ashley M. Stokes DVM, PhD; Susan C. Eades DVM, PhD, DACVIM; Rustin M. Moore DVM, PhD, DACVS

Description of the Problem:
Laminitis is a serious crippling disease affecting the equine hoof and often occurs secondary to episodes of colic and other systemic diseases. Elucidation of the pathophysiologic mechanisms associated with colic and laminitis and gastrointestinal tract disease are major goals of the Louisiana State University Equine Health Studies program, and in a recent large-scale survey of equine practitioners these were the most important disease categories that respondents felt needed further research. As global research efforts continue to help explain specific theories on the pathogenesis of both of these separate causes of morbidity and mortality, the interconnection between the two diseases is well-accepted and justifies further examination.

Study Objective:
The objective of this study was to examine in vitro intestinal secretory response and structural alterations of the large colon in horses following intragastric administration of BWE to induce toxicity compared to tissues from untreated control horses in the presence and absence of prostaglandin inhibition.

Approach:
Intestinal tissue samples were obtained from right ventral, left ventral, and right dorsal colons (RVC, LVC, RDC) of seven healthy control and seven adult horses after intragastric administration of BWE. Colonic mucosal segments were mounted in Ussing chambers and spontaneous electrical potential differences (PD) and short circuit current (Isc) were recorded. Tissue segments were incubated in Ringer’s solution with or without prostaglandin-inhibitor. Duplicate samples were examined by light microscopy and graded for necrosis, hemorrhage, edema, and inflammation.

Accomplishments/Results/Conclusions:
Colonic tissues from BWE-treated horses had an overall greater Isc for 4 h incubation period, compared to untreated control tissues. Right ventral colonic tissue from BWE-treated horses incubated with or without prostaglandin blockade showed a gradual decrease in short-circuit current over 4 hrs. No significant differences in Isc and PD were observed from BWE-exposed colonic tissues incubated with or without prostaglandin blockade (RVC, LVC). A gradual and steady increase in tissue Isc was observed for BWE-treated RDC (+/- prostaglandin inhibition) compared with that of healthy control horse tissue (+/- prostaglandin inhibition). BWE-treated colonic mucosal tissues had greater histological evidence of inflammation, edema, and hemorrhage compared to controls.

Benefits to/Impact on the Equine Industry:
This research project provides groundwork information furthering our understanding of this crippling equine musculoskeletal disease which will allow development of interventional preventative or treatment modalities.

Take Home Message:
Equine colonic tissue exposed to BWE has increased secretory response compared with normal control tissue and prostaglandin inhibition does not appear to attenuate this response. Exposure to BWE results in substantial mucosal edema and marked inflammation.

Acknowledgments:
This study was supported in part by a grant from the Equine Health Studies Program, School of Veterinary Medicine, Louisiana State University. The authors thank Dr. Joanne Tetens for surgical assistance and Mike Keowen, Frank Garza, Catherine Koch, and Priti Juneja for technical assistance.

Year Completed: 2000

Published Manuscripts/Abstracts:
Parasitized equine colonic epithelium exhibits an increased chloride secretory response compared to non-parasitized epithelium tissue in vitro

Authors/Investigators:
Rebecca S. McConnico, DVM, PhD, DACVIM; Thomas R. Klei, PhD, DABVP

Description of the Problem:
Larval cyathostominiosis, the most common condition attributed to cyathostomins, is characterized by a variety of signs including, acute or chronic diarrhea, peripheral subcutaneous edema, weight loss, general ill-thrift and in some instances death. The condition is generally attributed to the seasonal emergence, in the winter or spring, of large numbers of larvae from the intestinal mucosa.

Study Objective:
The objective of this study was to determine if alterations in epithelial secretion are associated with cyathostomin migration out of the intestine.

Approach:
Eight ponies determined to be free of parasite infection, were challenged with 10⁴ mixed, cyathostome larvae administered orally. Animals were placed into two groups and were either treated with an avermectin/cestodicide product or were left untreated. Right ventral and right dorsal colonic tissue was collected from ponies within 45 minutes of euthanasia. Colonic mucosa (stripped of the underlying serosa and muscular layers) was mounted in Ussing chambers and the spontaneous electrical potential differences (PD) and short-circuit current: Isc (direct measure of active ion transport) were recorded for 3.5 hours in the presence or absence of flunixen meglumine (for basal control of prostaglandin tone). Tissue resistance was calculated based on the PD and Isc using Ohm’s law. Necropsy examinations were performed at the completion of the study and samples were gathered for additional analyses.

Accomplishments/Results/Conclusions:
Tissues from both treatment groups exposed to flunixin meglumine, showed a gradual decrease in Isc over 3.5 hrs. whereas tissues without flunixin meglumine treatment showed an increase in Isc and PD. Tissue conductance continued to steadily increase in colon from the untreated (no anthelmintic) group compared to that of control tissues, suggesting a tissue inflammatory response. The results from this study suggest that antigenic stimulation of intestinal epithelium and the subsequent inflammatory cascade may contribute to an electrogenic chloride secretory response in parasitized equine intestine. These events in vivo may result in gradient-driven secretory diarrhea, which may occur as a protective response to enteric-dwelling parasites, or as a feature of a local colonic mucosal inflammatory response.

Benefits to/Impact on the Equine Industry:
The goal of this study was to define the mechanisms of cyathostomin associated disease in the horse and to determine points of time for interventional therapy for prevention and treatment of this disease process.

Take Home Message:
Results of this study suggest that antigenic stimulation of intestinal epithelium following anthelmintic treatment and the subsequent inflammatory cascade may contribute to an electrogenic chloride secretory response in parasitized equine intestine.

Acknowledgments:
This study was supported in part by funds from the Equine Health Studies Program, School of Veterinary Medicine, Louisiana State University. The authors thank Ms Catherine Koch and Mrs. Priti Juneja for technical assistance.

Year Completed: 2000
**Published Manuscripts/Abstracts:**
McConnico RS, Klei TR. Parasitized equine colonic epithelium exhibits an increased chloride secretory response compared to non-parasitized epithelial tissue in vitro. 7th International Equine Colic Symposium, Manchester, Eng, UK, July 2002.

**Detection and comparison of nitric oxide in clinically healthy horses and those with naturally acquired large colon and small intestinal strangulating obstruction**

**Authors/Investigators:**
Mustajab H. Mirza, DVM, MS; Thomas L. Seahorn, DVM, MS, DACVIM; Julian L. Oliver, DVM, PhD, DACVP; Giselle Hosgood, BVSc, MS, PhD, DACVS; Rustin M. Moore, DVM, PhD, DACVS

**Description of the Problem:**
Strangulation obstruction involving the large colon and small intestine is a common cause of colic in horses. The high mortality associated with intestinal strangulating obstruction may be related to a sustained reduction of blood flow and hypoperfusion (attributable to increased vascular resistance) after surgical correction and continued ischemic injury. There is limited information available regarding the pathophysiology of intestinal ischemia and ischemia-reperfusion in horses. It is well accepted that nitric oxide (NO) plays a role in these processes, and therefore, it is reasonable to assume that NO is involved in the pathophysiologic cascade of ischemia and ischemia-reperfusion.

**Study Objective:**
The objective of this study was to determine whether nitric oxide (NO) is present in clinically normal horses under basal conditions, and if it increases secondary to naturally acquired large colon volvulus or small intestinal strangulation.

**Approach:**
Adult horses with naturally acquired strangulating large colon volvulus (n=11), small intestinal strangulation (n=20) and 10 clinically healthy horses with no signs of gastrointestinal tract disease were used. Jugular venous blood and abdominal fluid were collected in both heparinized and EDTA tubes for NO quantification, and CBC and abdominal fluid cytology, respectively. Urine was collected via urinary catheterization or during voiding. Creatinine concentrations were determined in all fluid samples. NO concentrations were standardized to the creatinine concentration in the respective samples. A biopsy specimen was collected from the pelvic flexure of the large colon or the affected segment of the jejunum at surgery or after euthanasia and divided into subsections for fixation in zinc formalin and cryopreservation in OCT gel. NADPH diaphorase histochemical stains were performed on cryopreserved tissues. Routine histology and inducible nitric synthase (iNOS) and nitrotyrosine (NT) immunohistochemical stains were performed on paraffin-embedded tissues.

**Accomplishments/Results/Conclusions:**
Large colon - Based upon localization of NADPH diaphorase staining in endothelium and neuronal tissue, it appears that eNOS and nNOS are present under basal conditions in the vasculature and neuronal networks of the large colon, where they likely are responsible for mediating physiologic or cytoprotective effects of NO. The increased iNOS staining in mucosal and submucosal leukocytes may represent potential detrimental effects of iNOS-derived NO production locally in the large colon subsequent to naturally acquired large colon volvulus. The absence of an increase in nitrotyrosine staining in affected horses may reflect an inadequate time frame for its production in these horses. Alternatively, the lack of an increase in plasma, abdominal fluid and urine NO concentrations may reflect venous occlusion of colonic vasculature and subsequent prevention of locally produced NO from entering systemic circulation, or alternatively a lack of systemic production. This study suggest that NO is present under physiologic conditions in the large colon and its role in disease deserves further study. Small intestine - This study demonstrated indirectly through NADPH diaphorase staining that eNOS and nNOS are present under basal conditions in the vasculature and neuronal networks of the small intestine of clinically healthy horses, which have been shown in other species to be associated with physiologic or cytoprotective effects of NO. The increased iNOS staining in mucosal and submucosal leukocytes of affected horses may represent potential detrimental effects of iNOS-derived NO production locally in the small intestine. The increased mucosal leukocyte nitrotyrosine staining score in affected horses likely represents increased peroxynitrite subsequent to increased iNOS-derived NO and increased superoxide production. Although the biologic and pathophysiologic relevance of these findings could not be determined, this study provides preliminary evidence that NO deserves further investigation in small intestinal strangulation obstruction in horses.
**Benefits to/Impact on the Equine Industry:**
Nitric oxide is present under basal conditions in the large and small intestine of horses and appears to be involved in the pathophysiologic cascade of ischemia in the small intestine. Further study of the role of NO in this process may be warranted.

**Take Home Message:**
This study suggests that NO is present under physiologic conditions in the large colon and small intestine of horses, and provides preliminary evidence that NO deserves further investigation in small intestinal strangulation obstruction in horses.

**Acknowledgments:**
This study was supported by grants from the American Association of Equine Practitioners, Comparative Gastroenterology Society, Veterinary Clinical Sciences Organized Research Fund, and the Equine Health Studies Program, School of Veterinary Medicine, Louisiana State University.

**Year Completed:**
1998

**Published Manuscripts/Abstracts:**


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**Quantitation of adenine nucleotides in equine colonic mucosal tissue using high performance liquid chromatography**

**Authors/Investigators:**
Joanne Tetens, DVM, MS, PhD, DACVS; Steven A. Barker, PhD; Marian Waguespack, MS (?); Giselle L. Hosgood, BVSc, MS, PhD, DACVS

**Description of the Problem:**
Gastrointestinal mucosal cells have a high metabolic and, therefore, require large quantities of adenosine triphosphate (ATP) for maintenance of normal cellular function. As a result, the mucosal layer is principally affected during ischemia. One proposed mechanism for the increase in cellular permeability observed during ischemia is decreased endogenous production of ATP. In an experimental model of ascending colon volvulus in ponies, mucosal ATP content diminished 92% during ischemia and recovered to only 44% of control values upon reperfusion. In cultured renal and intestinal epithelial cell lines, decreasing cellular ATP content by inducing chemical hypoxia results in rapid opening of tight junctions, which increases paracellular permeability. Therefore, mucosal ATP depletion, which occurs during gastrointestinal ischemia, may cause rapid opening of tight junctions, leading to enhanced absorption of bacteria and endotoxin into the splanchnic circulation. These findings suggest that increased metabolic support to this chemically injured colonic epithelium may be important in decreasing morbidity and improving survival.

**Study Objective:**
The objectives of this study were to validate an established method for adenine nucleotide separation in equine colonic mucosal tissue, to determine the inherent variability in the tissue and extraction method, and to determine the stability of ATP, ADP, and AMP in the tissue across time.

**Approach:**
Equine colonic mucosal tissue obtained from a single horse was immediately submerged in liquid nitrogen and stored at -70°C. Samples were lyophilized, extracted, and separated by high performance liquid chromatography (HPLC). In experiment 1, one tissue sample from 1 parent segment was processed for adenine nucleotide quantitation. The single sample was loaded into the instrument and quantified 18 consecutive times to determine the inherent variability of the
instrument. In experiment 2, 6 individual small samples were obtained from 1 parent segment and processed individually for adenine nucleotide quantitation. The samples were analyzed in duplicate to determine the variation inherent in the sample preparation and extraction method. In experiment 3, multiple parent segments of tissue were crushed and combined to improve the homogeneity of the individual samples. Three samples of ground tissue were analyzed in duplicate. The analyses were performed at time 2 (2 days after collection, 1 day after lyophilization) and again at 9, 16, 23, 34, 37, 48, and 56 days to determine stability of adenine nucleotides in lyophilized tissue across time.

**Accomplishments/Results/Conclusions:**
The limit of quantitation was 0.05 ?g/mL. The coefficient of variation for the instrument was less than 10% for all nucleotides measured. When the tissue was not homogenized prior to sampling, there were significant differences in adenine nucleotide content between samples. However, when the tissue was homogenized prior to analysis, these differences were no longer significant. There was no significant decrease in ATP, ADP, or AMP content over a 54-day analysis period.

**Benefits to/Impact on the Equine Industry:**
Quantitation using HPLC analysis of adenine nucleotide content in equine colonic mucosal tissue will provide information regarding the relationship between ATP content and its catabolites (ADP and AMP) and alterations in epithelial cell permeability that occur during ischemia. Since only small volumes of tissue are needed for the analysis, adenine nucleotide quantitation can be used in conjunction with other techniques that measure cell viability, membrane permeability, etc. Evaluating numerous variables used to access cell viability will enable investigators to develop correlations between these different variables and to develop criteria to assist in predicting tissue survivability (and ultimately patient survivability). Additionally, the efficacy of pharmacologic agents, such as ATP-MgCl\(_2\), to enhance mucosal adenine nucleotide content can be determined using this technique.

**Take Home Message:**
A technique that has been previously described in other tissues has been validated for use in the horse. We were able to measure ATP, ADP and AMP concentrations in mucosal tissue from the large intestine of a horse using HPLC. Tissue samples could be stored for up to 54 days without significantly affecting the results. Finally, combining multiple samples from a similar site increased the accuracy of the results.

**Acknowledgments:**
This study was supported by a grant from the Department of Veterinary Clinical Sciences Organized Research Fund, School of Veterinary Medicine, Louisiana State University.

**Year Completed:** 2000

**Published Manuscripts/Abstracts:**

Naturally acquired strangulating volvulus of the large colon.
Pharmacological evaluation of endothelin-antagonists in blocking constriction of equine colonic vessels induced by endothelin-1

Authors/Investigators:
Changaram S. Venugopal, BVSc, MSc, MS, PhD; Rustin M. Moore, DVM, PhD, DACVS

Description of the Problem:
Horses with strangulating large-colon volvulus die owing to severe mucosal injury despite surgical correction and intensive medical care. Mucosal injury has been attributed to sustained reduction in blood flow. Endothelium derived substances such as endothelin and nitric oxide regulate vasomotor tone and blood flow. Experimental studies have shown that altered vascular tone contributes to ischemia/perfusion injury. Endothelin is a potent vasoconstrictor agent that could drastically increase vascular resistance resulting in reduced blood flow.

Study Objective:
The objective of this study was to find a potential therapeutic agent that will block the contractile effects of ET-1 on vascular smooth muscle. This project determined the efficacy and affinity of two newly discovered endothelin-antagonists to block ET-1 induced responses and compared them as potential therapeutic agents in alleviating endothelin induced contractile response of colonic vessels in horses.

Approach:
Mesenteric colonic vessels were collected from the left ventral colon of horses under barbiturate anesthesia. From the segment, 4 mm wide vessel rings were cut and were fixed in a tissue bath containing tyrode's solution. The ring is also attached to a force transducer and measured the tension developed on the tissues induced by graded concentration of ET-1. The effectiveness of the antagonists were measured by incubating the tissues with the antagonists and then constructing a concentration response curves. The inhibition of the contractile response to ET-1 was considered as the efficacy of the antagonists.

Accomplishments/Results/Conclusions:
Both antagonists dose-dependently inhibited the contractile responses of the vessel rings to ET-1. Upon comparing the other properties of the compounds, B1, which was water-soluble was preferred to B2 for further studies.

Take Home Message:
The contractile effects of endothelin on equine colonic vessels can be inhibited by its receptor antagonists. Further studies are needed to evaluate the therapeutic use of these agents in disease conditions. Search for specific receptor antagonists are also needed.

Acknowledgments:
This study was supported by a grant from the Equine Health Studies Program, School of Veterinary Medicine, Louisiana State University. The authors thank Earnestine Holmes and Catherine Koch for technical assistance.

Year Completed: 1998

Published Manuscripts/Abstracts:


Hemodynamic

**Transient weakness, ataxia, and recumbency associated with catheterization of the right side of the heart in 3 horses**

**Authors/Investigators:**
Aloisio C. Bueno, MV, MS; Rustin M. Moore, DVM, PhD, DACVS; Thomas L. Seahorn, DVM, MS, DACVIM; Janyce Cornick-Seahorn, DVM, MS, DACVA; Catherine E. Koch, BS, MS

**Description of the Problem:**
Right-sided cardiac catheterization for measuring cardiac output via thermodilution is a standard method for assessing cardiovascular/hemodynamic physiological and pathophysiological function in horses.

**Study Objective:**
The objective of this study is to report clinical complications related to right-sided cardiac catheterization including weakness, ataxia, collapse and a concomitant rise in pulmonary artery pressure; clinical signs resolved in 2 to 24 hours.

**Approach:**
For measurement of cardiac output and pulmonary artery pressures, a balloon tipped, flow directed thermodilution catheter (Swan Ganz) was inserted into the right jugular vein and advanced until the distal port was positioned in the pulmonary artery. Polyethylene tubing (OD, 1.57 mm) was inserted through another portal into right jugular vein and advanced until the distal tip was positioned within the right ventricle for infusion of ice-cold dextrose for measurement of cardiac output (CO). A 50-ml volume of dextrose was infused quickly (5 sec) into the right ventricle and the cardiac output meter calculated the CO based upon thermodilution. All pressures (systemic and pulmonary artery) were measured with a pressure monitor; the transducer was placed at the level of the point of the shoulder.

**Accomplishments/Results/Conclusions:**
Differential diagnoses for the clinical signs in the horses of this report include air embolism, stimulation of mechano-receptors in the pulmonary artery (vasospasm/vasoconstriction), thromboembolism, stress, and sepsis or endotoxemia.

**Benefits to/Impact on the Equine Industry:**
Cardiac catheterization may become a more common procedure in horses in the near future for monitoring critically ill patients or for use in the diagnosis and treatment of various cardiovascular diseases.

**Take Home Message:**
Awareness of the potential risks of cardiac catheterization in horses may help researchers and clinicians to prevent or minimize some of the more serious complications inherent to the procedure.

**Acknowledgments:**
The authors thank Lee Ann Curtis for technical assistance.

**Year completed:** 1998

**Published Manuscripts/Abstracts:**
Cardiopulmonary and sedative effects of intravenous administration of low doses of medetomidine and xylazine to adult horses

Authors/Investigators:
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Description of the Problem:
Alpha-2 adrenoceptor agonists are commonly used in equine practice for sedation and analgesia for diverse reasons including diagnostic, dental and standing surgical procedures; preanesthesic medication, and to control abdominal pain (colic).

Study Objective:
The objective of this study is to determine the cardiopulmonary and sedative effects of medetomidine hydrochloride in adult horses and to compare those effects with effects of an equipotent dose of xylazine hydrochloride.

Approach:
Ten healthy adult female horses of various breeds were used. Horses were randomly assigned to 2 groups. Group-1 (n=5) received medetomidine (4?g/kg IV) and group-2 (n=5) received xylazine (0.4 mg/kg IV). Heart rate (HR), respiratory rate (RR), arterial blood pressures (ABP), pulmonary arterial blood pressures (PABP) and cardiac output (CO) were recorded at baseline and every 5 minutes for a total of 1 hour. Rectal body temperature and blood gas analyses were performed every 15 minutes, and sedation and ataxia scores were assigned every 5 minutes throughout the study period.

Accomplishments/Results/Conclusions:
Arterial blood pressure was significantly decreased throughout the study for G-1 horses (medetomidine) and was significantly decreased for 40 minutes for G-2 horses (xylazine). Compared with baseline values cardiac output was significantly decreased in G-1 at 10, 20, and 40 minutes and significantly increased in G-2 at 40 and 60 minutes. Despite the significant decreases in RR, blood gas values did not significantly change over time. Ataxia and sedation scores were of similar magnitude for both groups, and all horses resumed eating hay 10 to 55 minutes after drug administration. This study suggests that equipotent low-doses of medetomidine and xylazine induce comparable levels of ataxia and sedation and similar cardiopulmonary changes in adult horses.

Benefits to/Impact on Equine Industry:
Although not approved for use in horses, medetomidine could be used for a variety of reasons for more profound sedation/analgesia with similar physiological alterations compared with xylazine.

Take Home Message:
Medetomidine (sedative/analgesic) may provide more profound analgesia and sedation of longer duration for horses.

Acknowledgments:
This study was supported by grants from the the Equine Veterinary Research Program and the Department of Veterinary Clinical Sciences Organized Research Fund, School of Veterinary Medicine, Louisiana State University. The authors thank Catherine E. Koch and Dr. Lee Ann Fugler for technical assistance.

Year Completed: 1998

Published Manuscripts/Abstracts:

Hemodynamic and metabolic alterations associated with intravenous infusion of a combination of adenosine triphosphate and magnesium chloride in conscious horses

Authors/Investigators:
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Description of the Problem:
Shock can be defined as “inadequate blood flow to vital organs or the inability of the body cell mass to metabolize nutrients normally.” The predominant changes during shock develop in the microcirculation, affecting cell membrane transport and function, energy metabolism, and mitochondrial function. During hypoxic conditions, endogenous production of ATP is decreased. A major rate-limiting factor in shock and ischemia, and thus resuscitation, is resynthesis of ATP. Therefore, a logical therapeutic approach to increase tissue ATP concentrations is to infuse the substrate (ATP) directly, rather than administer agents that would lead to ATP synthesis. Low-flow conditions and organ ischemia develop commonly in horses subsequent to intestinal strangulation, enterocolitis and proximal enteritis, laminitis, endotoxemia, sepsis, and severe dehydration and exhaustion. Administration of a combination of ATP and MgCl₂ after shock and other adverse circulatory conditions in humans and laboratory animals improves mitochondrial function and tissue ATP content; restores organ function, blood flow and microcirculation; improves reticuloendothelial function, survival time, and survival rate; and down-regulates synthesis and release of inflammatory cytokines.

Study Objective:
The objectives of this study were to determine the hemodynamic and metabolic effects of IV infusion of ATP-MgCl₂ combination in clinically normal, conscious adult horses and to determine a maximal safe IV infusion rate.

Approach:
Six horses received an IV infusion of ATP-MgCl₂ combination, beginning at a rate of 0.05 mg of ATP/kg of body weight/min, which was increased by 0.05 mg/kg/min increments at 10-minute intervals until a rate of 1.0 mg/kg/min was achieved. Data were collected prior to the start of the infusion, at the end of each infusion rate, and at 15-minute intervals for the next hour after discontinuation of the infusion. Measured or calculated hemodynamic variables included cardiac output, cardiac index, heart rate, stroke volume, systemic and pulmonary arterial pressures, and systemic and pulmonary vascular resistances. Arterial blood gas tensions, CBC, plasma biochemical profiles, urine volume and specific gravity, and selected clinical signs of disease also were evaluated.

Accomplishments/Results/Conclusions:
Intravenous infusion of ATP-MgCl₂ significantly increased cardiac output, decreased systemic vascular resistance, and caused mild pulmonary hypertension. Magnitude of the hemodynamic alterations was dependent on rate of infusion. Maximal safe infusion rate for these horses was 0.3 mg/kg/min. All horses became lethargic, and their appetites diminished during the infusion; 5 horses had mild signs of abdominal discomfort. Flank sweating was observed in all horses as infusion rate increased. Urine volume and specific gravity and hematologic, biochemical, and arterial blood gas alterations were detected during and after the infusion. Intravenous administration of ATP-MgCl₂ in healthy, conscious, adult horses caused various metabolic and hemodynamic alterations that were without appreciable detrimental effects.

Benefits to/Impact on the Equine Industry:
Endotoxemia and gastrointestinal tract diseases in horses can be associated with high morbidity and mortality. During these disease processes, endogenous production of ATP is markedly decreased, which leads to significant alterations in cellular...
metabolic processes. Such alterations can be irreversible and lead to death of the affected animal. Since IV administration of an ATP-MgCl<sub>2</sub> combination in healthy, conscious, adult horses was without appreciable side effects, especially when administered at a rate of 0.3 mg of ATP/kg of body weight/min, this combination may be useful during endotoxemia or gastrointestinal tract disease by supplying ATP to help maintain cellular metabolic functions.

**Take Home Message:**
A combination of ATP and magnesium chloride can be safely administered to conscious, healthy, adult horses at a rate of 0.3 mg of ATP/kg of body weight/min. Since many disease processes result in decreased ATP production, administration of ATP-MgCl<sub>2</sub> may lessen the severity of illness and/or increase survival rate of sick horses. However, since ATP-MgCl<sub>2</sub> can have profound effects on blood pressure, further investigations will need to be performed before this combination can be recommended clinically as a therapeutic agent for conditions resulting in decreased blood flow to major tissues/organs.

**Acknowledgments:**
This study was supported by funds from a USDA Section 1433 grant, School of Veterinary Medicine, Louisiana State University.

**Year Completed:** 1998

**Published Manuscripts/Abstracts:**


**Pharmacokinetics, clinical signs and hemodynamic effects of an intravenous bolus dose of N<sup>ω</sup>-nitro-L-arginine methyl ester (L-NAME) in healthy conscious horses**

**Authors/Investigators:**
Carlos R. Pinto, MedVet, PhD, DACT; Susan C. Eades, DVM, PhD, DACVIM; Steve A. Barker, PhD; Charles R. Short, DVM, PhD; Rustin M. Moore, DVM, PhD, DACVS

**Description of the Problem:**
Nitric oxide is an endogenous mediator involved in numerous biologic function and pathophysiologic processes. Studies evaluating the role of NO have shown it plays an important role in several pathologic conditions in horses, including but not limited to diseases of joints, intestinal tract and reproduction. Because of its solubility, tissue distribution, and effects on NOS blockade, L-NAME is a potentially useful drug for experimental or clinical use in horses. Knowledge of its pharmacokinetics is crucial to pursue research investigating the role of NO in equine physiological and pathophysiological processes, and potentially for therapeutic purposes.

**Study Objective:**
The objective of this study is to determine the pharmacokinetics, clinical signs and hemodynamic effects, including systemic arterial blood pressure, pulmonary arterial blood pressure, right atrial pressure, heart rate and rhythm, of an intravenous bolus administration of L-NAME in clinically healthy conscious horses.
**Approach:**
Six clinically healthy adult horses. A bolus dose of L-NAME (40 mg/kg, iv, 10%, w/v) was infused into the left jugular vein. Blood was collected prior to injection and serially thereafter for 36 hours from the right jugular vein and the samples were processed for measurement of L-NAME and its metabolite, N\textsuperscript{\textdagger} -nitro-L-arginine (L-NNA), using HPLC. Arterial, right atrial and pulmonary arterial pressures, heart rate and rhythm, clinical signs, and intestinal sounds were monitored continuously 20 min before and for 6 hrs after intravenous administration of L-NAME.

**Accomplishments/Results/Conclusions:**
The overall biologic half-life of L-NAME was 3.7 minutes whereas the half-life was 19.5 hours for L-NNA. The mean residence time, the average time a compound remains in the body, was 14.4 minutes and 28.2 hours, respectively. There was an immediate increase in systemic arterial pressure that returned to normal baseline values by 6 hrs after L-NAME administration. Mean right atrial pressure was increased significantly between 20 and 30 min, whereas mean pulmonary arterial pressure was not significantly affected by L-NAME administration. Mean values for heart rates were not significantly different from the baseline values.

**Benefits to/Impact on the Equine Industry:**
Because of its solubility, tissue distribution, and effects on NOS blockade, L-NAME is a potentially useful drug for experimental or clinical use in horses. Knowledge of its pharmacokinetics and its effect on clinical signs and hemodynamic variables is crucial to pursue research investigating the role of NO in equine physiological and pathophysiological processes. This study provides useful information for the pharmacokinetics and hemodynamic effects of L-NAME administration in horses.

**Take Home Message:**
L-NAME has a short half-life and L-NNA is rapidly detectable in plasma after L-NAME administration. Hydrolysis of L-NAME does not correlate with plasma L-NNA concentrations. Transient L-NAME-induced systemic hypertension, lack of significant alterations in the pulmonary vascular system, heart rate or cardiac rhythm associated with a mild change in demeanor, depression and temporary anorexia that subsided within 12 hrs after L-NAME treatment indicated that L-NAME is relatively safe when administered to conscious, resting horses. The pharmacokinetics profile of L-NAME administration in horses documented in this study may be beneficial to developing pharmacologic regimens for experimental or therapeutic purposes.

**Acknowledgments:**
This study was supported by a grant from the Equine Health Studies Program, School of Veterinary Medicine, Louisiana State University.

**Year Completed:** 2000

**Published Manuscripts/Abstracts:**

**In vitro responses of equine colonic arterial and venous rings to adenosine triphosphate**

**Authors/Investigators:**
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**Description of the Problem:**
Strangulating volvulus of the ascending colon in horses is a disease characterized by colonic luminal obstruction and vascular occlusion that results in colonic ischemia, mucosal necrosis, and vascular thrombosis. This disease is associated with high mortality, which may be related to a sustained reduction of blood flow and hypoperfusion attributable to increased vascular resistance after surgical correction and continued ischemic injury. Endothelial damage in the colonic vasculature develops subsequent to ischemia-reperfusion injury, and this damage can be exacerbated by endotoxin. Therefore, the sustained decrease in colonic blood flow in horses with strangulating volvulus may be associated with damage to the endothelium of colonic vessels. Extracellular purines have important and diverse effects on many biological processes, including regulation of vascular tone. Adenosine triphosphate (ATP) is principally an endothelium-dependent vasodilator that is rapidly metabolized and has a brief duration of action. Because the endothelium is damaged during periods of colonic ischemia and reperfusion, the effects of ATP on
Hemodynamic

regulation of vascular tone may be diminished or abolished as a result of loss of endothelial-derived vasorelaxants, specifically nitric oxide.

**Study Objective:**
The objective of this study was to determine the effects of ATP on vasomotor tone (specifically the vasodilatory response) of isolated equine colonic arterial and venous rings with and without intact endothelium and after in vitro exposure to the nitric oxide synthase inhibitor N\textsubscript{\textgamma}-nitro-L-arginine methyl ester (L-NAME).

**Approach:**
Arteries and veins from the left ventral colon of 14 mixed-breed horses euthanatized for reasons unrelated to cardiovascular or gastrointestinal tract disease were used. Endothelium-intact and -denuded arterial and venous rings were precontracted with $10^{-7}$ and $1.8 \times 10^{-8}$M endothelin-1, respectively. In 1 trial, endothelium-intact rings were also incubated with $10^{-4}$M L-NAME to inhibit nitric oxide (NO) production. Adenosine triphosphate ($10^{-8}$ to $10^{-3}$M) was added in a noncumulative manner, and relaxation percentage versus time curves were generated. Areas in the curves (i.e., percentage of relaxation time) were calculated.

**Accomplishments/Results/Conclusions:**
Relaxation response of arterial and venous rings to ATP was dose-dependent. Percentage of relaxation time in response to $10^{-4}$ and $10^{-3}$M ATP was significantly greater, compared with that for rings not treated with ATP. Removal of endothelium attenuated but did not eliminate the relaxation response. Addition of L-NAME did not attenuate the relaxation response in arteries. At higher concentrations, the vascular response to ATP was biphasic. ATP applied to equine colonic arterial and venous rings with and without intact endothelium induced a biphasic response characterized by transient contraction followed by slow, substantial, and sustained relaxation. This ATP-induced response is possibly mediated by a mechanism other than NO. Adenosine triphosphate may be a useful treatment to modulate colonic vasomotor tone in horses with strangulating volvulus of the ascending colon.

**Benefits to/Impact on the Equine Industry:**
Since adenosine triphosphate caused a substantial and sustained relaxation response in both arteries and veins in this study, it may be useful as a treatment for ischemic conditions of the gastrointestinal tract by causing vasodilatation and improving blood flow. Additionally, ATP produced vasodilatation in the absence of endothelium. Improving blood flow to the damaged colon once a volvulus has been surgically corrected may decrease morbidity and lower the mortality rate associated with this disease process.

**Take Home Message:**
Adenosine triphosphate caused an initial constriction of blood vessels (both arteries and veins) followed by a slow, substantial, and sustained relaxation. In other animals, ATP causes relaxation of blood vessels by activating a substance called nitric oxide. However, this study suggests that in horses, nitric oxide may not be involved in the relaxation response produced by ATP.

**Acknowledgments:**
This study was supported by funds from the American Association of Equine Practitioners Foundation.

**Year Completed:** 2000

**Published Manuscripts/Abstracts:**
Inflammatory

Plasma and urine nitric oxide concentrations in horses given a low dose of endotoxin

Authors/Investigators:
Aloisio C. Bueno, MV, MS; Thomas L. Seahorn, DVM, MS, DACVIM; Janyce Cornick-Seahorn, DVM, MS, DACVA; David W. Horohov, PhD; Rustin M. Moore, DVM, PhD, DACVS

Description of the Problem:
Endotoxemia is often encountered in horses with a variety of diseases including those related to the gastrointestinal tract (colic). Endotoxemia develops in approximately 25% of horses with colic and high mortality rates have been reported. An increased production of nitric oxide (NO) has been reported in various species during endotoxemia. However, literature investigating the role of NO in endotoxemia in horses is lacking. Therefore, this experiment was conducted to determine if NO concentration in plasma and urine would be elevated in horses given a low-dose of endotoxin.

Study Objective:
The objective of this study is to determine if the induction of this low-dose model of endotoxemia would create an inflammatory response and to correlate the clinical signs, hemodynamic, metabolic, hematologic variables, and cytokine production with the measured nitric oxide concentrations in urine and plasma.

Approach:
All the horses were placed in stocks and instrumented in the same fashion. Eight horses received endotoxin (*E. coli*, 35 ng/kg, IV) over 30 min and 3 horses (sentinel group) received normal saline IV over the same time period. Clinical signs, hemodynamic variables were recorded, hemotologic, metabolic variables, serum for cytokine bioassays, and plasma and urine were collected prior to endotoxin or saline infusion (t=0) and then serially throughout the 24 hour study period.

Accomplishments/Results/Conclusions:
Despite the induction of an inflammatory response and characteristic alterations of endotoxemia, differences in plasma NO concentrations were not apparent. Moreover, urine NO concentrations significantly decreased at 4 and 20 to 24 hours in endotoxin treated horses. There were no differences in the sentinel group for any of the measured variables. Thus, more research is necessary to unravel and determine the complex role of NO in physiologic and pathologic processes especially in vivo.

Benefits to/Impact on the Equine Industry:
Determination of the role of NO in endotoxemia in horses may help define potential treatment options with NO agonist or antagonist depending on the stage of endotoxic shock in horses.

Take Home Message:
Endotoxemia is a complex and multifactorial condition, and it is very difficult to study in live animals owing to its variability. Although systemic NO concentrations were not increased in this study in horses exposed to a low-dose of endotoxin, additional studies are needed to better define its role in the pathophysiology of endotoxemia in order to try to increase survivability of this devastating condition.

Acknowledgments:
This study was supported by grants from the Equine Health Studies Program and the Department of Veterinary Clinical Sciences Organized Research Fund, School of Veterinary Medicine, Louisiana State University. The authors thank Catherine E. Koch, Dr. Lee Ann Fugler, and Dr. Britta S. Leise for technical assistance.

Year Completed: 1998

Published Manuscripts/Abstracts:


**Nitric oxide concentrations in cerebrospinal fluid of horses with or without neurologic disease**

**Authors/Investigators:**
Lais R.R. Costa, MV, MS, DACVIM; Thomas L. Seahorn, DVM, MS, DACVIM; Rustin M. Moore, DVM, PhD, DACVS

**Description of the Problem:**
Nitric oxide is a labile, soluble gas synthesized from L-arginine by nitric oxide synthase (NOS); it undergoes rapid spontaneous decomposition to the stable end-products, nitrate and nitrite. Nitric oxide has a myriad of local actions, most notably cytoprotective as well cytotoxic effects on the host tissue depending upon the source, duration and magnitude of its production.

**Study Objective:**
The objective of this study was to evaluate whether nitric oxide (NO) concentration increases in cerebrospinal fluid (CSF) and plasma of horses with neurologic disease, in particular equine protozoal myelitis (EPM). It was hypothesized that NO serves as an inflammatory mediator in the central nervous system.

**Approach:**
Horses admitted to the Louisiana State University and Kansas State University Veterinary Teaching Hospitals with complaint of neurologic disease were evaluated using a systematic approach to neurologic examination. CSF analysis, including cytology (erythrocytes and nucleated cell counts, and cell morphology), biochemistry (protein, creatine kinase), albumin quotient and IgG index were performed in all cases. Additional diagnostic tests, such as cervical radiographs and CSF serology, were performed as applicable in order to establish the diagnosis. Venous blood from jugular venipuncture and CSF samples from the lumbosacral or atlanto-occipital spaces were collected in heparinized tubes, and cell-free supernatants were stored at - 70°C until assayed for NO concentrations using an automated chemiluminescence NO analyzer. The controls for nitrate standards were used according to manufacturer’s instructions. The NO concentration was standardized to the creatinine concentration in respective samples, and expressed as µM NO/mg creatinine. The horses were grouped according to the final diagnosis, and the mean ± standard mean error (SEM) nitric oxide concentrations were calculated for each group.

**Accomplishments/Results/Conclusions:**
A wide variety of neurologic diseases were evaluated in this study. Those included 57 horses with a presenting complaint of neurologic problem and 12 horses that were completely normal. Among the horses evaluated for neurologic deficit, 6 had infectious neurologic diseases (1 verminous encephalitis, 1 bacterial meningitis, 4 viral encephalomyelitis by EEE, and 16 EPM), 13 had traumatic and degenerative diseases (8 CVM, 1 EDM, 1 EMND, 2 trauma/fracture and 2 idiopathic neuropathy), 10 horses had equivocal signs, 8 horses had musculoskeletal disorders and 4 had no neurologic deficits. The NO concentration in plasma and CSF varied among and within the different groups of diseases. The variation in plasma and CSF NO concentrations were not statistically significant between groups. The heterogeneity of the neurologic diseases presented and the small sample size for some of the categories are likely to have influenced the results. Moreover, the variation in duration and severity, thus accounting for different stages of the diseases, are also likely to affect the magnitude of NO concentrations in plasma and CSF. Concentrations of NO in the CSF samples did not correlate with protein concentration, nucleated cell count, creatine kinase or IgG concentration in CSF. Although the presence of nitrate has been shown to closely correspond to the levels of NO secretion. The concentrations of NO in CSF samples of horses affected with EPM were significantly higher than those of normal horses, and those with bacterial and viral encephalomyelitis, but lower than those from horses with degenerative diseases. Interestingly, NO synthesis by macrophages and neutrophils has been shown to markedly increase in response to protozoal infections in humans and animal models for human diseases, such as *Plasmodium, Leishmania* and *Trypanosoma*.

**Benefits to/Impact on the Equine Industry:**
The importance of NO as an inflammatory mediator in neurologic diseases of horses remains unclear. The present study is the first report of NO concentration in CSF of horses with neurologic diseases. Future investigations evaluating the
NO concentrations in CSF as well as the expression of the isoform of nitric oxide synthase in central nervous system tissues are warranted to further define if NO plays a role in the pathogenesis of specific neurologic diseases.

**Take Home Message:**
NO concentration in plasma and CSF varies in horses affected with different neurologic diseases, however the role of NO as an inflammatory mediator of the central nervous system in horses affected with specific diseases remains unclear.

**Acknowledgments:**
This study was supported by funds from the Equine Health Studies Program, School of Veterinary Medicine, Louisiana State University. The authors thank Drs. Bonnie Rush and Julia Flaminio for the samples collected at Kansas State University, and Ms. Leslie Talley, Ms. Catherine Koch and Mr. Frank Garza for their technical help.

**Year Completed:** 1998

**Published Manuscripts/Abstracts:**

**Cephalic venous plasma endothelin-1 concentrations and digital blood flow responses to ATP-MgCl₂ administration after low-dose endotoxin infusion in horses**

**Authors/Investigators:**
Joanne Tetens, DVM, MS, PhD, DACVS; Susan C. Eades, DVM, PhD, DACVIM; Giselle Hosgood, BVSc, MS, PhD, DACVS; Catherine E. Koch, MS; Frank Garza, MS; Rustin M. Moore, DVM, PhD, DACVS

**Description of the Problem:**
Endotoxemia affects all horses regardless of age, breed, gender, or geographic location. Horses with colic and subsequent endotoxemia are predisposed to develop numerous complications, the most important of which is acute laminitis. Although experimentally-induced endotoxemia does not consistently produce laminitis in horses, the incidence of laminitis is much greater in horses with illnesses associated with endotoxemia (intestinal ischemia, enterocolitis, anterior enteritis, pleuropneumonia and metritis), which provides empirical evidence that endotoxemia likely is involved in the pathogenesis of this condition. Administration of a low dose of endotoxin to horses causes profound decreases in digital blood flow. Increased systemic or local synthesis and release of endogenous vasoconstrictor substances (such as endothelin-1 (ET-1), one of the most potent and longest acting constrictor substances synthesized with the vasculature) may contribute to this reduction of perfusion of the equine digit. Adenine nucleotides (such as adenosine triphosphate [ATP] and its metabolites) have been demonstrated to alter vasomotor tone. A combination of ATP and magnesium chloride (MgCl₂) may improve survival and help preserve athletic function in horses with laminitis by causing vasodilatation and decreasing vascular resistance leading to improvement in tissue perfusion.

**Study Objective:**
The objective of the study was to evaluate the effects of IV ATP-MgCl₂ on digital hemodynamic variables and cephalic venous plasma ET-like immunoreactivity in horses administered low-dose endotoxin.
**Inflammatory Approach:**
Twelve adult horses were used in the study. Horses were administered endotoxin (LPS) or saline solution, IV, during a 30-minute period. Immediately thereafter, horses in each group (2 groups of 6 horses) were infused IV with ATP-MgCl₂ or saline solution. Two weeks later, horses were administered the opposite solution (LPS or saline solution), but it was followed by the same infusion as 2 weeks previously (i.e., ATP-MgCl₂ or saline solution). Digital arterial pressure, digital blood flow, and cephalic venous plasma ET-1 concentrations were measured.

**Accomplishments/Results/Conclusions:**
Digital arterial blood slow transiently increased and then decreased for 5 hours in horses administered endotoxin followed by ATP-MgCl₂. A similar, but shorter duration, decrease was observed in horses administered endotoxin followed by saline solution. In horses administered saline solution followed by ATP-MgCl₂, digital arterial blood flow decreased. Mean digital arterial pressure was decreased in horses administered endotoxin followed by either ATP-MgCl₂ or saline solution. In horses receiving saline solution followed by ATP-MgCl₂, mean digital arterial pressure transiently increased and then decreased until the ATP-MgCl₂ solution was discontinued. In horses receiving endotoxin followed by either saline solution or ATP-MgCl₂, cephalic venous plasma ET-like immunoreactivity increased. In horses receiving saline solution followed by ATP-MgCl₂, there was a transient increase in ET-like immunoreactivity.

**Benefits to/Impact on the Equine Industry:**
Low-dose endotoxin administration to clinically healthy horses resulted in a transient, significant increase in cephalic venous plasma ET-1 concentrations. Therefore, ET-1 may be responsible (via venoconstriction) for the decrease in digital blood flow that occurs during endotoxin administration. Additionally, ET-1 may play a pivotal role in the development of laminitis by contributing to poor laminar perfusion and subsequent rotation/distal displacement of the third phalanx. By blocking ET-1, we may be able to prevent laminitis during endotoxemic episodes. Finally, administration of ATP-MgCl₂ did not prevent the decrease in digital arterial pressure and blood flow or increase in cephalic venous plasma ET-1 concentrations during low-dose endotoxin administration.

**Take Home Message:**
Administration of a low-dose endotoxin (a component of bacteria commonly found in the gastrointestinal tract) significantly increased the amount of ET-1 (a potent constrictor of blood vessels) found in blood taken from a large vein which drains the equine foot. Therefore, ET-1 may be responsible for the decrease in blood flow and blood pressure to the foot of horses administered endotoxin. Since horses that suffer from endotoxia are prone to develop laminitis (inflammation of the soft tissues of the foot) and laminitis may be caused by constriction of blood vessels to the foot, ET-1 may be involved in the development of laminitis during disease processes that cause endotoxia. Finally, IV administration of ATP-MgCl₂ (which can dilate blood vessels) did not improve blood pressure or blood flow to the foot or lower the levels of ET-1 measured in the blood from horses given a low-dose of endotoxin.

**Acknowledgments:**
This study was supported by funds from the American Horse Show Association and the Morris Animal Foundation.

**Year Completed:** 2000

**Published Manuscripts/Abstracts:**


**Temporal effects of freezing on plasma nitric oxide concentrations in ponies**

**Authors/Investigators:**
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**Description of the Problem:**
The purpose of this study was to determine the effect of freezing upon plasma NO concentrations. Previous studies in other species have shown increases in NO concentrations subsequent to freezing and storage. Initial storage in
polypropylene containers revealed increased NO concentrations over time; this was also found in glass containers. These authors stated that fresh plasma samples should be analyzed. In this study nitric oxide (NO) in fresh plasma was compared to frozen plasma in order to determine the temporal effects of freezing. Nitric oxide (NO) is a known modulator of specific physiological roles namely NO derived from the enzyme constitutive nitric oxide synthase (NOS); this NO regulates vasomotor tone and neural pathways. However in certain situations the NO is stimulated by inducible NOS (iNOS) and released in greater concentrations and for a longer duration that may be pathophysiologic and damaging to tissues.

**Study Objective:**
The objective of this study is to compare concentrations of nitric oxide (NO) in fresh plasma versus frozen plasma and to determine the temporal effects of freezing on jugular venous plasma NO concentrations in clinically healthy ponies.

**Approach:**
Twenty-eight helminth-naive ponies, ranging in age from 4 to 6 months, were raised and maintained under parasite-free conditions. Blood was collected from the jugular vein into vacutainer tubes containing sodium heparin. These samples were centrifuged at 1,500 x g for 10 minutes, and the plasma supernatant was collected with glass pipettes. Samples were analyzed fresh for plasma NO concentrations, the remainder of the samples were divided into 1-mL aliquots which were stored in polypropylene vials at –70 C until analyzed for NO concentration. Plasma NO concentration was measured on fresh samples and on frozen samples at monthly intervals, using a chemiluminescent method. One aliquot from each pony was thawed in dark conditions for 20-30 minutes at one-month intervals, for a one-year period.

**Accomplishments/Results/Conclusions:**
There were significant differences in the plasma NO concentration across time compared with the baseline value. The NO concentrations were significantly increased above the baseline value at 1, 3, 4, 6, 7, and 11 months. Although the increases in plasma NO concentrations were statistically significant, compared with values in fresh samples, the values were not of a magnitude expected from diseased horses. Studies performed in our laboratory have indicated that if NO concentrations are increased in diseased states via pathophysiological mechanisms involving iNOS, the expected concentrations are considerably greater than controls.

Despite measurable changes in NO concentrations in frozen plasma samples, there was no loss of nitrate in our samples. Although plasma NO concentrations, compared with fresh samples, were increased at some sampling periods, the magnitude of the increase was not considered substantial compared with studies where there is evidence of increased NO production associated with disease conditions. To avoid any discrepancies, it may be preferable to analyze the fresh plasma samples for NO concentration. Alternatively, samples to be analyzed for NO and compared should be processed and stored frozen under similar conditions and for similar times.

**Benefits to/Impact on the Equine Industry:**
The roles of NO in many biological systems (gastrointestinal, respiratory, musculoskeletal, reproductive) in the horse are being studied by several researchers. This information is pertinent to other researchers to accurately elucidate NO concentrations in bodily fluids.

**Take Home Message:**
It may be pertinent to analyze fresh plasma when trying to elucidate NO concentrations.

**Acknowledgments:**
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**Year Completed:** 1999

**Published Manuscripts/Abstracts:**
**Effects of adenosine triphosphate-magnesium chloride on cardiopulmonary and clinicopathologic variables, cytokine activity, and endothelin concentration in horses administered a low dose of endotoxin**

*Authors/Investigators:*
Joanne Tetens, DVM, MS, PhD, DACVS; Rustin M. Moore, DVM, PhD, DACVS; Giselle Hosgood, BVSc, MS, PhD, DACVS; Susan C. Eades, DVM, PhD, DACVIM; Michael L. Keowen, BS; David W. Horohov, PhD

*Description of the Problem:*
Endotoxemia remains the leading cause of death in horses. Endotoxemia affects all horses regardless of age, breed, sex, or geographic location. The prevalence of endotoxia in horses with acute gastrointestinal tract disease (i.e., colic) admitted to referral veterinary hospitals is estimated to be approximately 25%, and it represents a major cause of death in horses with colic. The most common gastrointestinal tract diseases associated with endotoxia include enterocolitis-enteritis and intestinal strangulation-obstruction and are often associated with complications such as laminitis and gastrointestinal ileus. Bacteria attributable to gram-negative organisms or endotoxia causes decreases in cardiac output, systemic vascular resistance, and mean arterial pressure, whereas there is an increase in pulmonary vascular resistance. Some of the hemodynamic derangements during endotoxemia may be subsequent to release of endothelin-1 (ET-1), the most potent vasoconstrictor of mammalian vessels that has been identified. Concentrations of ET-1 increase during endotoxemia secondary to endotoxin-induced stimulation of endothelial cells. Endothelin-1 may act as a local vasoconstrictor during severe endotoxic shock and contribute to pulmonary hypertension and peripheral vasomotor disturbances (e.g., decreased blood flow to the gastrointestinal tract and digits) observed during this syndrome. Administration of ATP-MgCl₂ following hemorrhagic shock and other adverse circulatory conditions in laboratory animals can improve mitochondrial function and tissue ATP content; restore organ function, blood flow, and perfusion; improve reticuloendothelial function, survival time, and survival rate; and downregulate the synthesis and release of inflammatory cytokines.

*Study Objective:*
The objective of this study was to evaluate the systemic effects of IV infusion of ATP-MgCl₂ after low-dose endotoxin infusion in horses.

*Approach:*
Twelve adult horses were used in the study. Horses were administered endotoxin (LPS) or saline solution, IV, during a 30-minute period. Immediately thereafter, horses in each group (2 groups of 6 horses) were infused IV with ATP-MgCl₂ or saline solution. Two weeks later, horses were administered the opposite solution (LPS or saline solution), but it was followed by the same infusion as 2 weeks previously (i.e., ATP-MgCl₂ or saline solution). Cardiopulmonary and clinicopathologic variables, cytokine activity, and ET-1 concentrations were recorded.

*Accomplishments/Results/Conclusions:*
IV infusion of ATP-MgCl₂ after administration of a low dose of endotoxin failed to attenuate the cardiopulmonary, clinicopathologic, and cytokine alterations that develop secondary to endotoxin exposure. The combination of LPS and ATP-MgCl₂ potentiated pulmonary hypertension, leukopenia, and neutropenia when compared with the combination of LPS and saline solution. The combination of LPS and ATP-MgCl₂ resulted in thrombocytopenia. Endothelin concentration was increased in jugular venous and pulmonary arterial plasma in horses receiving LPS and ATP-MgCl₂. Similar increases were not observed with LPS and saline solution.

*Benefits to/Impact on the Equine Industry:*
Many biological agents are involved in the development of hemodynamic and metabolic derangements that occur during shock. Adenine nucleotides (which include ATP and its metabolites) can potentially have adverse effects on the cardiopulmonary and metabolic systems of diseased horses. Their role in hemodynamic derangements, leukocyte adherence, and coagulopathies that occur during endotoxemic episodes warrants further investigation. Gaining a better understanding of what roles (both positive and negative) adenine nucleotides play during shock could potentially result in decreased morbidity and mortality in equine patients by inhibiting the adverse effects and potentiating the positive effects of these substances.

*Take Home Message:*
In this study, administration of a combination of adenosine triphosphate and magnesium chloride did not protect horses from the adverse effects caused by administration of endotoxin (a toxin commonly absorbed into the blood stream from the intestinal tract of sick horses). The findings from this study also suggest that since adenosine triphosphate and
other compounds related to it are released from cells during shock, these agents could be partially responsible for some of the negative effects (i.e., poor blood pressure, clotting problems, etc.) that are observed both during experimentally-induced and naturally-acquired disease processes.

**Acknowledgments:**
This study was supported by funds from the Morris Animal Foundation.

**Year Completed:** 2000

**Published Manuscripts/Abstracts:**


Alterations of nitric oxide and endothelin-1 subsequent to experimental lipopolysaccharide induced acute synovitis in horses

Authors/Investigators:
Daniel J. Burba, DVM, DACVS; Jorge de la Calle, LVM, MS; Rustin M. Moore, DVM, PhD, DACVS; Julian Oliver, DVM, PhD; Casey J. LeBlanc DVM, PhD; Giselle Hosgood, BVSc, MS, PhD, DACVS; David W. Horohov, PhD

Description of the Problem:
Joint disease is a common clinical condition arising from numerous etiologies, and is a major cause of lameness and wastage in equine athletes. It is well documented that the concentration of certain inflammatory mediators increases locally in joints of horses with articular disease. Numerous investigations have revealed that nitric oxide (NO) and endothelin-1 (ET-1) are increased in serum and synovial fluid of humans with joint disease. Nitric oxide is a multi-functional intercellular and intracellular messenger molecule that plays a role in a variety of physiological processes. Some investigators believe that synthesis of NO in joints may play a major role in the pathophysiology of arthritis. Endothelin-1 is a 21-amino acid polypeptide with two intra-molecular disulfide bonds that induces potent and sustained vasoconstriction. Endothelin-1 may also play an important role in the pathogenesis of joint disease in horses. A model was used to induce acute synovitis by injecting lipopolysaccharide (LPS) into the joint cavity to measure the levels of NO and ET-1 in the synovial fluid.

Study Objective:
The objective of this study was to determine the effect of lipopolysaccharide (LPS) injection into the middle carpal joint on synovial fluid and cephalic venous nitric oxide (NO) and endothelin-1 (ET-1) concentrations and compare these to values obtained from the contralateral control limb, which was injected with saline solution.

Approach:
Ten clinically healthy horses were used in this study. Synovitis was induced in one randomly chosen middle carpal joint by intra-articular injection of E. Coli LPS. The contralateral joint was used as a saline injected control. Synovial fluid, cephalic and jugular venous blood samples were collected at 0, 2, 8, 12, 24, and 48 hrs after injection. Concentrations of ET-1 and NO were assessed by radioimmunoassay and by a chemiluminescence method, respectively.

Accomplishments/Results/Conclusions:
Comparisons of NO concentrations between treated and control joints did not reveal significant differences for venous plasma or synovial fluid samples. Comparisons of ET-1 concentrations between treated and control legs did not reveal significant differences for cephalic venous plasma. However, the concentrations for ET-1 in jugular venous plasma were significantly decreased at 8 hours after injection when compared with baseline. There was a significant increase in ET-1 concentrations of synovial fluid of treated and control joints at 8, 12, 24 and 48 hours after injection, compared with baseline. ET-1 appears to be locally synthesized in the joints of horses. Intra-articular injection of LPS did not significantly alter the NO concentrations in the synovial fluid in this study.

Benefits to/Impact on the Equine Industry:
Information gained from this study revealed that ET-1 (an enzyme) may play a part in degenerative joint disease in horses. Thus the next step is to determine if by blocking ET-1 will help with treating degenerative joint disease in horses.
**Take Home Message:**
ET-1 may play a role in the pathophysiology of joint disease in horses.

**Acknowledgments:**
This study was supported by a grant from the Houston Equine Research Organization. The authors thank Catherine Koch and Frank Garza for technical assistance.

**Year Completed:** 1999

**Published Manuscripts/Abstracts:**


**Synovial fluid and plasma nitric oxide and endothelin-1 concentrations in horses with and without joint disease**

**Authors/Investigators:**
Daniel J. Burba, DVM, DACVS; Jorge de la Calle, LVM, MS, DACVS; Rustin M. Moore, DVM, PhD, DACVS; Jamie Williams, DVM, MS; Casey J LeBlanc, DVM, PhD; Giselle Hosgood, BVSc, MS, PhD, DACVS

**Description of the Problem:**
Joint disease is a common clinical condition arising from numerous etiologies, and is a major cause of lameness and wastage in equine athletes. It is well documented that the concentration of certain inflammatory mediators increases locally in joints of horses with articular disease. Numerous investigations have revealed that nitric oxide (NO) and endothelin-1 (ET-1) are increased in serum and synovial fluid of humans with joint disease. Nitric oxide is a multi-functional intercellular and intracellular messenger molecule that plays a role in a variety of physiological processes. Some investigators believe that synthesis of NO in joints may play a major role in the pathophysiology of arthritis. Endothelin-1 is a 21-amino acid polypeptide with two intra-molecular disulfide bonds that induces potent and sustained vasoconstriction. Endothelin-1 may also play an important role in the pathogenesis of joint disease in horses.

**Study Objective:**
The objective of this study was to determine and compare the plasma and synovial fluid endothelin-1 (ET-1) and nitric oxide (NO) concentrations in normal joints from clinically healthy horses with values obtained from horses with joint disease affected with acute synovitis, degenerative joint disease (DJD) and joint sepsis.

**Approach:**
Thirty-six horses with joint disease and 15 clinically healthy horses with no evidence of joint disease were included in the study. Horses with joint disease were assigned to one of the three groups, including those with synovitis, DJD, or sepsis based upon clinical and radiographic exams and synovial fluid analysis. ET-1 and NO concentrations were measured in plasma from whole blood, collected from the jugular vein and cephalic/saphenous vein, respectively, of the limb with an affected or non-affected (normal) joint, as well as synovial fluid via arthrocentesis from the involved joint.

**Accomplishments/Results/Conclusions:**
Comparisons of ET-1 concentrations between affected and non affected groups did not reveal significant differences in venous plasma. However, concentrations of ET-1 in synovial fluid obtained from the joint sepsis group were significantly greater, compared with values from the synovitis, DJD and non-affected groups. Comparisons of NO concentrations between affected and non-affected groups did not reveal significant differences in venous plasma or synovial fluid. It appears from this study ET-1 is locally synthesized in the joints of horses with varying types of joint disease.

**Benefits to/Impact on the Equine Industry:**
Information gained from this study revealed that ET-1 (an enzyme) may play a part in degenerative joint disease in horses. Concentrations varied among different types of joint disease with concentrations significantly increased in synovial fluid of horses with joint sepsis. Thus the next step is to determine if by blocking ET-1 will help with treating degenerative joint disease in horses.


**Take Home Message:**
ET-1 may play a role in the pathophysiology of joint disease in horses.

**Acknowledgments:**
This study was supported by a grant from the Houston Equine Research Organization. The authors thank Catherine Koch and Frank Garza for technical assistance.

**Year Completed:**
1999

**Published Manuscripts/Abstracts:**


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**Pharmacological control of digital vasculature in the prevention and treatment of laminitis in horses**

**Authors/Investigators:**
Ashley M. Stokes, DVM, PhD; Changaram S. Venugopal, BVSc, PhD; Giselle Hosgood, BVSc, MS, PhD, DACVS; Susan C. Eades, DVM, PhD, DACVIM; Rustin M. Moore, DVM, PhD, DACVS

**Description of the Problem:**
Laminitis is a common, excruciatingly painful disease which forces the horse to lay in lateral recumbency for hours resulting in pressure sores, anorexia, and often leads to euthanasia. Although the etiology of laminitis is not fully understood, it has been hypothesized that ischemia, owing to vasoconstriction, plays a causative role in laminitis. During acute laminitis, vascular resistance increases 3.5 times above normal resulting in a significant reduction in blood flow to the foot. This period of reduced blood flow causes laminar inflammation, edema, and necrosis, which often leads to separation of the distal phalanx from the hoof wall. Endothelin (ET), a potent vasoconstrictor, is released by the endothelium and increases vascular resistance. ET receptors are found on vascular smooth muscle and endothelial cells. Stimulation of ET, and ETB, receptors result in sustained vasoconstriction and ETB1.

The synthesis of endothelin (ET-1) is stimulated by many inflammatory mediators and changes in blood flow. ET-1 is primarily synthesized and released from endothelial cells. The two important receptor types are ETα and ETβ. ET-1 effects on vascular smooth muscle are primarily through the ETα receptor, resulting in profound contraction.
receptors trigger the release of nitric oxide. Since the onset of laminitis is characterized by digital vasoconstriction and changes in systemic arterial and venous pressures, determining the role of ET in the pathogenesis of this disease may provide further important knowledge for the prevention and treatment of laminitis in horses. Availability of ET receptor antagonists has made it possible to study the individual effects of ET on equine palmar digital vessels.

**Study Objective:**
The objectives of this study were to determine the concentration-response relationship of endothelin-1 (ET-1), compare two ET receptor antagonists and determine the concentrations that effectively block the vasomotor effects of ET-1, and compare the effectiveness of ET-1 to previously studied vasoconstrictors in equine palmar digital arterial and venous rings in vitro.

**Approach:**
Palmar digital arterial and venous rings from 8 non-laminitic horses were used. Vessel rings were placed in Tyrode's solution, one side fixed to the floor of an organ bath and the other side to a force-displacement transducer. Two separate studies were conducted: (I) incubation with one ET receptor antagonist (PD142893 or PD145065) followed by an ET-1 concentration-response curve on medial vessel rings; and (II) comparison of ET-1 to norepinephrine (NE) and histamine (HST) and comparison of contractile responses of medial vs. lateral vessel rings.

**Accomplishments/Results/Conclusions:**
In study I, ET-1 administration caused pronounced and sustained concentration-dependent contraction of vessel rings. These contractile responses were effectively inhibited by the two ET antagonists in a concentration-dependent manner. Venous rings were significantly more sensitive to the contractile effects of ET-1 than arterial rings. In study II, ET-1 and NE were found to be significantly more potent vasoconstrictors than HST in both arteries and veins. No significant differences were found between the effects of ET and NE in arteries and veins.

**Benefits to/Impact on the Equine Industry:**
The pathophysiology of equine laminitis remains unclear; however, vascular alterations are known to be an important aspect of the earliest stages of the disease. The identification of a key vascular mediator, and the determination of an effective inhibitor of this mediator, may lead to the development of preventatives and therapeutics for this devastating disease.

**Take Home Message:**
Identification of ET-1 as a very potent vasoconstrictor of the equine digit is an important step in understanding the pathophysiology of equine acute laminitis, a disease characterized by altered digital hemodynamics. The receptor antagonist PD 145065 demonstrated potential therapeutic use in treating acute laminitis and may prove to be useful in preventing these known hemodynamic alterations.

**Acknowledgments:**
This study was supported by a grant from the Geraldine R. Dodge Foundation, the Kenneth A. Scott Trust, the Bernice Barbour Foundation, the Marilyn M. Simpson Charitable Trust, the Humane Society of the United States, and the Massachusetts Society for the Prevention of Cruelty to Animals. The authors would like to thank Earnestine P. Holmes, Catherine E. Koch, Dr. Lee Ann Curtis, and Dr. Chidambaram Ramaswamy for technical assistance.

**Year Completed:** 1998

**Published Manuscripts/Abstracts:**


The effects of endothelin-1 and nitric oxide on digital hemodynamics of normal horses

Authors/Investigators:
Ashley M. Stokes, DVM, PhD; Susan C. Eades, DVM, PhD, DACVIM; Changaram S. Venugopal, BVSc, MSc, PhD; Catherine E. Koch, MS; Rustin M. Moore, DVM, PhD, DACVS

Description of the Problem:
Acute laminitis is a severely debilitating and painful disease of the sensitive and insensitive laminae of the equine digit characterized by increased venous tone, increased pressure within the capillary bed, and edema formation leading to reduced delivery of oxygen and nutrients to the soft tissues of the digit. Ultimately, this leads to separation of the interdigitating soft tissue structures that suspend the distal phalanx within the hoof capsule. This separation leads to rotation and/or sinking of the distal phalanx, a very painful condition from which approximately 75% of horses require euthanasia. Endothelin-1 (ET-1), a potent vasoconstritor, is released by the endothelial cells that line the blood vessels. ET-1 production is increased by many of the inflammatory mediators known to be elevated during acute laminitis. Nitric oxide (NO) is also a substance produced by the endothelium, but its production leads to dilation of the vasculature and opposes the actions of ET-1. Knowledge of the effects of these substances on the digital vasculature would contribute to our understanding of the vascular pathophysiology of acute laminitis.

Study Objective:
Our global hypothesis is that the alterations in digital hemodynamics associated with the onset of acute laminitis are caused by the local imbalance of the endothelium-derived substances ET-1 (increased) and nitric oxide (decreased) which ultimately lead to laminar ischemia and necrosis. Our study hypothesis was the administration of an ET antagonist would prevent or reverse the decreased digital blood flow caused by ET-1 infusion into the digital artery. The purpose of these studies was to evaluate the effect of ET-1, an ET antagonist (PD145065), and nitroglycerine (NG) on digital hemodynamics in clinically healthy, conscious horses.

Approach:
Palmar digital blood flow, mean digital arterial (MDAP) and venous (MDVP) pressures, laminar capillary perfusion (CPU), and systemic mean arterial (MAP) pressure were evaluated in 3 studies. Study I - digital intra-arterial ET-1 infusion. Studies II and III - dose of ET-1 required to decrease digital blood flow by 75% (ET$_{75}$) administered followed by PD145065 or saline, and then NG administration.

Accomplishments/Results/Conclusions:
Endothelin-1 infused into the digital arterial vasculature resulted in a concentration-dependant reduction in blood flow, which could be prevented or reversed with administration of the ET antagonist. After administration of higher doses of ET-1, horses demonstrated lameness similar to that observed in laminitis that resolved as blood flow was restored to the digit. These results support a role for ET-1 as a vasoconstrictor of the equine digit, which indicates it may be involved in the pathogenesis of acute laminitis. NG administration improved ET-induced blood flow reduction in both the ET antagonist and saline-treated groups. The endothelial-derived substances ET-1 and nitric oxide have opposing and profound actions in the vasculature. Digital intra-arterial infusion of ET-1 reduced blood flow, which was reversible with ET antagonist administration and further improved with NG administration.

Benefits to/Impact on the Equine Industry:
Alterations in blood flow during the acute stages of laminitis have been well documented. Endothelin has been identified as an important mediator of atherosclerosis and hypertension. This study demonstrates the effectiveness of the ET antagonist and NG in restoring digital vasoconstriction due to the potent actions of ET-1. The identification of a key vascular mediator, and the determination of an effective inhibitor of this mediator, may lead to the development of preventatives and therapeutics for this devastating disease.

Take Home Message:
Future studies may demonstrate that an ET receptor antagonist and NG may prove useful for the correction of digital hemodynamic alterations characteristic of acute laminitis in horses.

Acknowledgments:
This study was supported by a grant from the Louisiana State University School of Veterinary Medicine USDA 1433 Formula Funds.

Year Completed: 1999
Published Manuscripts/Abstracts:


Characteristic stance of a horse with laminitis demonstrating shifting of the horse’s weight onto the rear limbs because of foot pain in the front limbs.
Parasitology

Alterations in histologic and immunohistochemical staining for nitric oxide alterations in parasite free ponies infected with Strongylus vulgaris

Authors/Investigators:
Jeremy D. Hubert, BVSc, MRCVS, MS, DACVS; Thomas L. Seahorn, DVM, MS; Thomas R. Klei, PhD; Julian L. Oliver, DVM, PhD; Giselle Hosgood BVSc, MS, PhD, DACVS; Rustin M. Moore DVM, PhD, DACVS

Description of the Problem:
This study utilizes a model of gastrointestinal inflammation created by the parasite Strongylus vulgaris to evaluate the role of nitric oxide in GI inflammation. Nitric oxide (NO) is a known modulator of specific physiological roles namely NO derived from the enzyme constitutive nitric oxide synthase (NOS); this NO regulates vasomotor tone and neural pathways. However in certain situations the NO is stimulated by inducible NOS (iNOS) and released in greater concentrations and for a longer duration that may be pathophysiologic and damaging to tissues. In this study the degree of NO release is determined by evaluating the tissues with special stains to determine if the parasite causes induction of iNOS and thus increased NO concentrations.

Study Objective:
The objective of this study is to determine the effect of infection with Strongylus vulgaris on helminth-naïve ponies and to determine the presence of tissue NO synthase (NOS) using NADPH diaphorase histochemical and inducible NOS and nitrotyrosine immunohistochemical staining.

Approach:
Twenty-eight ponies reared helminth-naïve were used. Group 1 ponies (n=7) were administered 500 third stage S. vulgaris larvae orally and given a placebo on day 56 postinoculation. Group 2 ponies (n=16) considered the control group, consisted of 13 ponies who were administered 500 S. vulgaris larvae orally on day 0 and were treated with a proprietary anthelminthic drug on day 56, and 3 ponies that did not receive any S. vulgaris larvae. The ponies were euthanatized on day 95. All group 2 ponies were determined to be free of helminths at necropsy. Specimens were collected after euthanasia for histologic evaluation, tissue NO synthase (NOS) as determined by NADPH diaphorase histochemical and inducible NOS and nitrotyrosine immunohistochemical staining.

Accomplishments/Results/Conclusions:
Evidence of increased NO synthesis in the intestinal tract of parasitized ponies is suggested by increased NADPH, iNOS and increased nitrotyrosine staining. These findings provide some evidence of the role of NO in parasite-induced intestinal inflammation in ponies.

Benefits to/Impact on the Equine Industry:
Gastrointestinal problems are the second most common problem after lameness or musculoskeletal related health issues due to the athletic nature of the horse. However; a significant component of gastrointestinal problems or colic are directly related to parasite burden and pathology due to parasites. Experimental infection with S. vulgaris larvae have been shown to cause significant inflammation and the mechanism of this inflammation needs to be elucidated so as to develop and institute relevant treatments methods and protocols.

Take Home Message:
This study reveals that there is a role of NO in gastrointestinal inflammation. Further studies are required to elucidate the exact role in the pathophysiological observed.

Acknowledgments:
This study was supported by grants from USDA 1433 funds and the Department of Veterinary Clinical Sciences Organized Research Fund, School of Veterinary Medicine, Louisiana State University. The authors thank Mae Lopez and Catherine Koch for technical assistance.

Year Completed: 1999
**Clinical signs, hematologic, cytokine, and plasma nitric oxide alterations in response to Strongylus vulgaris infection in ponies**

**Authors/Investigators:**
Jeremy D. Hubert, BVSc, MRCVS, MS, DACVS; Thomas L. Seahorn, DVM, MS; Thomas R. Klei, PhD; Giselle Hosgood BVSc, MS, PhD, DACVS; Rustin M. Moore DVM, PhD, DACVS

**Description of the Problem:**
This study utilizes a model of gastrointestinal inflammation created by the parasite *Strongylus vulgaris* to evaluate the role of nitric oxide in GI inflammation. Nitric oxide (NO) is a known modulator of specific physiological roles namely NO derived from the enzyme constitutive nitric oxide synthase (NOS); this NO regulates vasomotor tone and neural pathways. However in certain situations the NO is stimulated by inducible NOS (iNOS) and released in greater concentrations and for a longer duration that may be pathophysiologic and damaging to tissues. In this study the clinical signs of the ponies are observed as well as relevant hematological parameters measures to ascertain that there is inflammation caused by ingestion of the parasite and then the plasma levels of NO are measured to see if there is a correlation between NO and inflammation.

**Study Objective:**
The objective of this study is to illustrate the inflammatory response created by the parasite and correlate it with plasma nitric oxide concentrations.

**Approach:**
Twenty-eight ponies were reared parasite free. Group 1 (n=21) was administered 500 *S. vulgaris* L3 larvae via nasogastric tube and group 2 (n=7) received a saline control. Ponies were monitored for clinical signs daily, and blood was collected for CBC, serum cytokines (TNF, IL-1, IL-6), and plasma NO on day 0 and serially on days 3, 6, 9, 14, 21 and 45 days after infection.

**Accomplishments/Results/Conclusions:**
Group 1 ponies were depressed, anorexic and febrile for a variable period, but group 2 ponies remained free of these signs. Plasma NO was increased on day 21 in group-1 and on days 9 and 21 in group-2. Total WBC counts were increased on day 6, 9, 14, and 21 in group-1, but there were no changes in group-2. Segmented neutrophils were increased on day 6 in group-1 and were decreased on days 14, 21, and 45 in group-2; there were no differences in band neutrophils in either group. Lymphocytes were decreased on days 6 and 9 in group-1 and increased on days 14, 21, and 45 in group-2. The RBC count and PCV were decreased in group-1 on days 9, 14, 21, and 45 and in group-2 on day 45. Total plasma protein concentration was increased on days 14 and 45 in group-1; there were no differences across time in group-2. Fibrinogen concentration was increased on days 6, 9, 14, 21, and 45 in group-1, and on day 21 in group-2. There were no differences in serum cytokines across time in group 1 ponies.

**Benefits to/Impact on the Equine Industry:**
Gastrointestinal problems are the second most common problem after lameness or musculoskeletal related health issues due to the athletic nature of the horse. However; a significant component of gastrointestinal problems or colic are directly related to parasite burden and pathology due to parasites. Experimental infection with *S. vulgaris* larvae have been shown to cause significant inflammation and the mechanism of this inflammation needs to be elucidated so as to develop and institute relevant treatments methods and protocols.

**Take Home Message:**
The significance of the role of NO in GI inflammation in this model could not be determined. Further studies are required.
Acknowledgments:
This study was supported by grants from the USDA 1433 Formula Funds and the Department of Veterinary Clinical Sciences Organized Research Funds, School of Veterinary Medicine, Louisiana State University.

Year Completed: 1999

Published Manuscripts/Abstracts:


Hubert JD, Hardy J, Holcombe SJ, Moore RM. Cecal amputation within the right ventral colon for surgical treatment of nonreducible cecocolic intussusception in 8 horses. BEVA Congress 1999.


Epidemiology of small strongyle infections in southern Louisiana

Authors/Investigators:
Thomas R. Klei, PhD; Dennis D. French, DVM, DABVP; Melanie R. Chapman, BS

Description of the Problem:
The small strongyle nematodes, termed the cyathostomes or more recently the cyathostomins, are the most ubiquitous internal parasites of horses both in numbers of individuals per horse and in the diversity of species. Seasonal diarrhea, colic and poor growth and development of young animals have been attributed to the presence of these parasites. The potential subclinical effects of these worms on equine health are poorly understood. Horses on well managed farms and stables continue to be infected even in the face of regular anthelmintic treatments and resistance to all anthelmintics except ivermectin and moxidectin is wide spread. A better understanding the biology of these important parasites is necessary to define their relevance to equine health and the rational development of parasite control strategies which do not depend so heavily on the use of anthelmintic drugs which selects for resistant parasite populations.

Study Objective:
For more than ten years, efforts have been made by our laboratory to more clearly characterize the prevalence, transmission, developmental cycles and differences in the responses of horses to natural infections of these parasites when exposed to them on pastures in our region. A series of recently published papers presents these findings, which are briefly summarized below.

Approach:
We first published a survey of the prevalence of internal parasites of horses in our region more that 20 years ago prior to the release and use of ivermectin. Since then the broad-spectrum highly efficacious macrocyclic lactone (ML) anthelmintics ivermectin and moxidectin have seen wide and heavy use in horse populations. It is generally accepted that ML usage on well managed farms has eliminated parasites such as spirurid stomach worms which cause summer sores, large strongyles responsible for serious colic, and Onchocerca cervicalis a cause of seasonal dermatitis. A more recent survey of internal parasites was conducted on ponies necropsied for other purposes to determine if this new and more effective treatment had an effect on the diversity and intensity of parasites in a population of horses similar to...
those examined prior to the advent of ML use. The overall prevalence and intensity of strongyle parasites was reduced in the more recent survey. However, with the exception of the stomach worms, the diversity of parasites recovered was not altered. This suggests that while some species such as the very pathogenic large strongyles are less common and not found in the numbers per horse seen prior to ML use they still occur in horses not held in closed herds under heavy anthelmintic pressure. Twenty-six species of cyathostome including two species not found in the earlier survey were also found. The prevalence of round worms (Parascaris equorum) pinworms (Oxyuris equi) and tapeworms (Anoplocephala perfoliata) have not changed. Clearly while having a major positive impact on equine health, the use of ML anthelmintics has not modified the diversity of internal parasites in the horse population at large.

Accomplishments/Results/Conclusions:
The life cycle of the equine cyathostomin is typical of strongyle nematodes and is direct. Eggs released by female worms living in the large intestine and cecum pass on to pasture in the feces of the horse. These develop to infective third stage larvae (L3) which are ingested. These larvae enter the mucosa of the large intestine. At this point the developmental pattern can take one of two paths. L3 may encyst and go into an arrested phase of development (fig). These hypobiotic larvae or early L3 (EL3) live in this state for an undetermined period completing their development to the fourth stage when stimulated by unknown host factors. When this development occurs in a synchronous fashion clinical signs occur which are associated with the disruption of intestinal tissues and emergence of larvae into the lumen of the large intestine and cecum.

Benefits to/Impact on the Equine Industry:
Knowledge of the seasonal aspects of small strongyle parasitism is important in determining periods of maximal transmission and development of the parasites within the horse. While difficult, counting the numbers of L3 on pasture herbage has traditionally been used to indirectly determine seasonal transmission. Studies of this nature were conducted over a three-year period from 1986-1989 and again for another year in 1996-1997 in East Baton Rouge parish. The pastures used held a pony herd of mares and their foals. Fecal egg counts (FEC) from mares and foals and pasture L3/kg dry herbage were used to measure changes in parasite populations within the ponies and on the pasture. A FEC rise occurred during the late summer and early autumn, which preceded a peak of L3 on pasture during the winter season. The numbers of L3 were reduced during the hottest months of the year due mainly to minimum daily temperature above 18°C and in the winter due to brief periods of freezing (fig). However, L3 numbers quickly returned to peak levels when temperatures returned to normal winter ranges.

An alternate developmental path occurs when the entering L3 do not arrest but develop through the mucosal L4 stage to the luminal L4 and adult stages. A more accurate picture of the seasonal nature of cyathostomin transmission and development was seen in studies that counted parasites in ponies removed from pasture at different times of the year or others which utilized parasite free pony yearlings as sentinels. This allowed for the determination of the proportion of parasites in the different stages of development as well as seasonal transmission cycles. In studies of sentinels maximal transmission was seen during the winter and the lowest occurred during the summer, supporting the observations of L3 on pasture herbage. Adult parasite numbers were highest in the spring and mucosal parasite numbers were the lowest at this time as expected. This suggests that a seasonal development of EL3 and emergence of larvae occur in the winter-spring, which corresponds, to periods of larval cyatostominosis. Nonetheless, in these studies EL3 were the major portion of the parasite population within the horse at all seasons. The total parasite burdens, a sum of all stages, was constant in young ponies, <5yrs of age, removed from pasture. However, peak percentages of adult parasites occurred during the fall accounting for the pasture contamination and high L3 numbers seen on pasture during the winter. EL3 numbers increase during this period reaching a peak percentage during the summer. Increased age of ponies and presumably the development of acquired immunity resulting from increased parasite exposure resulted in an increased proportion of EL3 in the parasite population within the horse. This phenomenon appears to be separate from protective immunity in that increased induction of arrested development occurs rapidly in yearlings.

Take Home Message:
These experimental and field observations are the most extensive series of studies reported on seasonal and host factors effecting cyathostomin transmission and the accumulation of EL3 in a single region to date. The data collected indicate that treatment of horses in this region with anthelmintics which eliminate EL3 during the late summer or early fall will reduce the number of adults and concomitantly the contamination of fall and winter pastures with infective larvae. These treatments will also reduce the accumulation of potentially dangerous levels of EL3. This reduction in reinfection should make possible the development of parasite control programs less dependent on the extensive use of anthelmintics in mature horses. Other factors such as age, housing conditions and anthelmintic efficacy within a given population must also be taken into consideration in designing specific parasite control strategies.

Acknowledgments:
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**Immunity to strongyle nematodes in ponies**

**Authors/Investigators:**
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**Description of the Problem:**
Understanding the horse’s immune response to pathogens is an essential part of understanding diseases they cause. Detailed studies on acquired resistance to nematodes of horses are limited and pertain almost exclusively to strongyle infections. Based on their biology it is not surprising that the acquisition and character of immunity to infections of the large strongyle, specifically *Strongylus vulgaris* and the small strongyles or cyathostomes differ markedly. We have investigated both over the past 25 years and summarize our most recent studies below.

While *Strongylus vulgaris* is a rare parasite on well managed farms, infections of parasite free reared ponies with known numbers infective larvae (L3) has been a useful model to study equine immune responses to nematode parasites. The most detailed information on equine immunity to nematodes has come from this model system. In this system we have recently demonstrated that immunization of naive ponies with radiation attenuated L3 (IRRL3) induces a distinct Th2 immune response which is protective against reinfection. However, immunization with soluble adult worm antigen in Ribi adjuvant induces a marked Th1 response and is not protective. Cytokine, antibody and cellular responses were characterized to determine whether prior immunization with Ribi adjuvant would down regulate the

*A scanning electron micrograph of Strongylus vulgaris third stage infective larvae with attached eosinophils. Antibodies mediate the adherence of the cells and lead to killing in vitro.*
Th2 response induced by a subsequent IRRL3 immunization as well as the protection it induces. Similar to ponies vaccinated only with IRRL3, animals immunized with both Ribi and IRRL3 were still protected against challenge infections even though they demonstrated alterations in the factors associated with the protective immune responses. Protection against infection was associated with increases in IL-5 and IL-13 mRNA in colonic lymph nodes, as well as an eosinophilia. A prior immunization with Ribi and it Th1 response reduced levels of IL-5 and eosinophils it was not sufficient to reduce protective immunity. Unlike immune responses to small strongyles describe below elevate levels of II-4 and mastocytosis were not associated with protection against this large strongly parasite.

Unlike the large strongyles the small strongyles (cyathostomes or more recently cyatostomins) continue to be found in horses on well managed farms and have developed resistance to all classes of anthelmintic except the macrocyclic lactones. Data on field observations suggest that horses acquire immunity to cyathostome infections with age. When examining this resistance using strongyle fecal egg counts it is clear and not surprising that horses respond to strongyle nematode infections like other hosts. The frequencies of EPGs are evenly dispersed within the yearling population but over dispersed in the mature horse population. In this situation very few mature animals are infected with large numbers of parasites. It is likely this difference in acquired resistance is genetically regulated. Two experiments have been conducted recently to define more carefully the immunity to these most important worms. Parasite recovery data following challenge in these experiments showed that ponies with acquired resistance showed a reduction in the total numbers of parasites, including adults with in the lumen of the intestine, and developing larvae with in intestinal wall. The acquisition of resistance was also accompanied by an increase in hypobiotic larvae, intestinal mast cells, eosinophils and parasite specific antibody responses. These types of immune responses are the constant increases in Th2 cytokine mRNA seen in colonic lymph nodes, principally IL-4.

We have recently participated in studies with scientists at the University of Liverpool in their efforts to identify new diagnostic methods to determine the risk of larval cyathostomiasis. These studies utilize a unique antigen identified by the group from developing larvae within the mucosa of the horse. The titer of IgG(T) to these antigens corresponds to the number of larvae with in the intestinal wall of the horse. This assay when further developed will allow for the clinical assessment of cyathostomiasis and advance antemortum epidemiological studies.

The data recovered to date provide important information on immunity to nematode parasites. Further, these studies also help characterize basic aspects of equine immunology important to studies of pathogenesis and vaccination.

Because it is apparent that nematode parasites can significantly alter the horse’s cytokine profile, the question “do parasite infections alter the outcome of vaccinations with other proteins?” was asked. Antibody, lymphoproliferation and cytokine responses to single intra muscular inoculations of KLH (a large foreign protein) were measured in ponies with high, medium and low parasite burdens. The data indicated that heavily parasitized ponies had a uniformly marked reduction in immune responses directed toward the vaccination. This response was not polarized in a Th1/Th2 fashion. While necessary to confirm, the data suggest that nematode parasitism may detrimentally affect vaccination against other agents.

Acknowledgments:
These studies were supported in part by grants from the USDA-NRI-CGP, Fort-Dodge Animal Health, and the Agricultural Experiment Station, Louisiana State University.

Published Manuscripts/Abstracts:


Chapman, MR, French DD, Taylor HW, Klei TR. One season of pasture exposure fails to induce a protective resistance to cyathostomes but increases numbers of hypobiotic third stage larvae. *J Parasitol* 2002;88: 687-683.


Reproduction

Properly timed teasing can aid uterine clearance

Authors/Investigators:
Romana Stecco, DVM; Dale Paccamonti, DVM, MS, DACT; Stephan Gutjahr, DVM; Carlos Pinto, DVM, PhD, DACT; Bruce Eilts, DVM, MS, DACT

Description of the Problem:
Delayed uterine clearance results in persistent mating-induced endometritis and is a significant cause of infertility in mares. Exogenous oxytocin aids in improving uterine clearance and causes an increase in intrauterine pressure. In prior studies we have shown that this increase is correlated to the day of the estrous cycle. Oxytocin is released in response to teasing during both estrus and diestrus in mares, and at least during estrus, teasing results in an increase in uterine activity. We wanted to determine whether any positive effect on uterine activity, which would enhance uterine clearance, could result from teasing and whether this effect was influenced by the time in relation to ovulation.

Study Objective:
The objective of this study was to determine if teasing causes an increase in intrauterine pressure and if this response varies by day of the cycle.

Approach:
Intrauterine pressure was measured while mares were teased with a stallion 2 days before ovulation, on the day ovulation was detected and 2 days after ovulation.

Accomplishments/Results/Conclusions:
A significant increase in intrauterine pressure was observed in response to teasing both 2 days before ovulation and on the day of ovulation, when plasma concentrations of progesterone were low. No significant increase in intrauterine pressure was observed in response to teasing 2 days after ovulation when progesterone concentrations were elevated.

Benefits to/Impact on the Equine Industry:
Teasing or exposure to auditory and other stimuli from a stallion may be of benefit to some mares by enhancing uterine clearance.

Take Home Message:
Management practices that include teasing or stallion exposure may be beneficial in stimulating uterine clearance mechanisms in mares during the preovulatory period.

Acknowledgements:
This study was supported by funds from the Equine Health Studies Program, School of Veterinary Medicine, Louisiana State University.

Year Completed: 1999

Published Manuscripts/Abstracts:
Effect of dose and day of treatment on uterine response to oxytocin in mares

Authors/Investigators:
Stephan Gutjahr, DVM; Dale L. Paccamonti, DVM, MS, DACT; Jonathan Pycock, DVM; Marcel Taverne, PhD; Stef J. Dieleman, PhD; G.C. van der Weijden, DVM

Description of the Problem:
Delayed uterine clearance results in persistent mating-induced endometritis and is a significant cause of infertility in mares. Oxytocin is commonly used to treat this condition, however little is known about the effect elicited by different doses of oxytocin nor is there any information about the effect of treatment in relation to the time of ovulation.

Study Objective:
The objective of this study was to determine the effect of dose and day of oxytocin treatment on intrauterine pressure.

Approach:
Mares were treated with either a low (10 IU) or high (25 IU) dose of oxytocin 2 days before ovulation, on the day of ovulation and 2 days after ovulation.

Accomplishments/Results/Conclusions:
The administration of both 10 and 25 IU oxytocin induced a response. The intensity of response depended on the day of administration and the dose of oxytocin. The variation of response was significantly greater after 10 IU oxytocin compared with 25 IU oxytocin. The uterine response was greatest on Day 2 prior to ovulation and lowest on Day 2 after ovulation. The response was negatively correlated to increasing plasma progesterone.

Benefits to/Impact on the Equine Industry:
The results of this study show that oxytocin administration to mares before ovulation provides a greater response than after ovulation. A decline in the intensity of response after ovulation can be compensated for with a higher dose of oxytocin.

Take Home Message:
It may be beneficial to breed an infertile mare that has a uterine clearance problem earlier in relation to ovulation. We would then have a longer period before she ovulates when oxytocin treatment is most effective to treat the problem.

Acknowledgements:
University of Utecht, Netherlands.

Year Completed: 1999

Published Manuscripts/Abstracts:

Prostaglandin F2á metabolite (PGFM) response to exogenous oxytocin and determination of the half-life of oxytocin in nonpregnant mares

Authors/Investigators:
Dale L. Paccamonti, DVM, MS, DACT; Jonathan Pycock, DVM; Marcel Taverne, Ph.D.; Mart Bevers, Ph.D., G.C. van der Weijden, DVM; Stephan Gutjahr, DVM; D. Schams, Ph.D.; David Blouin, Ph.D.

Description of the Problem:
Prostaglandin and oxytocin are commonly used after breeding to treat infertility, specifically the problem of delayed uterine clearance. Both cause an increase in uterine contractions and improve the uterus' ability to clear itself of debris and infection. Some reports seemed to indicate that oxytocin caused a release of endogenous (the mare's own) prostaglandin yet the intensity of this effect was unclear. Furthermore, the half-life of oxytocin, an indication of how long it would remain active, was unknown.
**Study Objective:**
The objective of this study was to determine the half-life of oxytocin and to evaluate the release of endogenous prostaglandin after the administration of oxytocin to mares.

**Approach:**
Oxytocin was given intravenously and blood was then obtained at intervals to determine the amount of oxytocin and prostaglandin in the bloodstream.

**Accomplishments/Results/Conclusions:**
The half-life of oxytocin in the mare is 7 minutes. Administration of oxytocin results in a significant release of endogenous prostaglandin, further increasing uterine motility and enhancing uterine clearance.

**Benefits to/Impact on the Equine Industry:**
This study will help to design better protocols for the treatment of delayed uterine clearance.

**Take Home Message:**
Because of the short half-life of oxytocin, repeated small doses may be more beneficial than a single large dose.

**Year Completed:**
1998

**Published Manuscripts/Abstracts:**

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**Role of nitric oxide in ovulation in the mare**

**Authors/Investigators:**
Carlos Pinto, DVM, Ph.D., DACT; Dale L. Paccamonti, DVM, MS, DACT; Bruce E. Eilts, DVM, MS, DACT; C. S. Venugopal, DVM, Ph.D.; Charles R. Short, DVM, Ph.D.; Laura R. Gentry, Ph.D.; Donald L. Thompson, Ph.D.; Robert A. Godke, Ph.D.

**Description of the Problem:**
The process of ovulation in horses and other species is not completely understood. More detailed knowledge will enable us to better control ovulation and aid us in the breeding management of horses. Recent studies suggest that nitric oxide (NO) may have a role in regulating ovarian physiology.

**Study Objective:**
The objective of this study was to investigate the role of nitric oxide in ovulation in the mare.

**Approach:**
Nitric oxide synthesis in the pre-ovulatory follicle was inhibited to see if ovulation was delayed. Nitric oxide inhibitors (L-NAME or aminoguanidine) were injected into the pre-ovulatory follicle during estrus by a transvaginal ultrasound guided approach and follicular development and the time to ovulation were monitored. In another experiment, the level of nitric oxide in the follicle was evaluated before and after the administration of hCG (human chorionic gonadotropin), which stimulates follicular maturation and ovulation. Follicular fluid was obtained by transvaginal ultrasound guided aspiration for analysis. In a third experiment, the role that nitric oxide plays in the production of steroid hormones by the follicle was investigated in vitro. Granulosa cells were obtained from follicles and cultured in vitro. Nitric oxide donors and inhibitors were added to determine the effect on production of progesterone and estrogen.

**Accomplishments/Results/Conclusions:**
In the first experiment, the administration of nitric oxide inhibitors delayed ovulation, suggesting a role for nitric oxide in follicular growth and ovulation in horses. In the second experiment, increased intrafollicular concentrations of nitric oxide found after administration of hCG provide evidence for the presence of an nitric oxide-generating system in the equine preovulatory follicle. In the third experiment, addition of a nitric oxide donor to granulosa cells in vitro reduced estrogen production, while addition of a nitric oxide inhibitor increased estrogen production.

**Benefits to/Impact on the Equine Industry:**
These studies indicate a role of nitric oxide in the process of ovulation in the mare. Further studies may enable us to better control ovulation, either by inducing, or alternatively delaying, ovulation in a tightly controlled fashion.
**Take Home Message:**
Better methods to induce or delay ovulation, possibly with the aid of nitric oxide mediators may enable us to better control ovulation and enhance breeding management.

**Published Manuscripts/Abstracts:**


**“In-vitro” produced foals**

**Authors/Investigators:**
Richard Cochran, Ph.D.; Marius Meintjes, DVM, Ph.D.; Brett Reggio, Ph.D.; Darin Hylan, MS; Joel Carter; Carlos Pinto, DVM, Ph.D., DACT; Dale Paccamonti, DVM, MS, DACT; Robert Godke, Ph.D.

**Description of the Problem:**
In vitro fertilization is commonplace in other species. It has great economic benefits in the cattle industry and provides significant advantages in the treatment of infertility in humans. Successful in vitro fertilization in horses could provide similar benefits. However, success has proven elusive.

**Study Objective:**
The objective of this study was to develop a successful method for in vitro fertilization in horses and produce live foals.

**Approach:**
Evaluation of various methods including sub-zonal injection of sperm (SUZI) and eventually succeeded with intracytoplasmic sperm injection (ICSI).

**Accomplishments/Results/Conclusions:**
We were the first to report the birth of foals from oocytes obtained from live mares. Our first pregnancy failed to go to term. That same year, another laboratory announced the birth of a foal derived by the ICSI method, but the oocytes (‘eggs’) were obtained in the laboratory from ovaries acquired from a slaughterhouse. Foals developed from oocytes from live mares were still an elusive goal. The following year, our first live foal (a filly named Sweet Pea) was born, followed by another foal (another filly, Trixie) and then a third foal (this one only received a number). (Note: A group in Australia reported similar results the same year. We have developed a relationship with this group and currently have a joint equine practice residency program with them.)

**Benefits to/Impact on the Equine Industry:**
With these early achievements reported by our laboratory, other laboratories have been able to achieve improvements in the success rate of ICSI. This may prove to provide valuable benefits to the treatment of infertility in stallions and mares.

*Intracytoplasmic sperm injection into oocyte to yield in vitro-derived embryos.*
Take Home Message:
Research at LSU in assisted reproductive techniques continues to achieve important breakthroughs in a number of species. Continued research in this area will undoubtedly benefit the equine industry in numerous ways.

Acknowledgements:
This work was performed in collaboration with the Embryo Biotechnology Laboratory, LSU AgCenter.

Published Manuscripts/Abstracts:


Reproductive parameters of Miniature horse stallions

Authors/Investigators:
Dale L. Paccamonti, DVM, MS, DACT; Ab Buiten, Ph.D.; Joyce Parlevliet, DVM, Ph.D.; Ben Colenbrander, DVM, Ph.D.

Description of the Problem:
Standards for breeding soundness evaluation, including parameters used for semen evaluation, of ‘standard’ sized horses, such as Quarter Horses, Arabians and Thoroughbreds are well established. However, no such standards existed for Miniature Horses. The increasing popularity of Miniature horses, and the resulting increase in their economic value, resulted in an increased demand for the evaluation of breeding stallions. Veterinarians needed to know what could be considered normal and acceptable for a Miniature stallion.

Study Objective:
The objective of this study was to determine normal values for testicular measurements and semen parameters for Miniature stallions

Approach:
The results of 216 breeding soundness evaluations performed on Miniature stallions were analyzed.

Accomplishments/Results/Conclusions:
Miniature stallions differ significantly from ‘standard sized’ stallions in testicular size and numbers of sperm produced.

Benefits to/Impact on the Equine Industry:
Veterinarians now have reference values when evaluating a Miniature Horse stallion for breeding soundness.

Take Home Message:
Normal values have been established and can be used to assess the reproductive potential of Miniature horse stallions.

Acknowledgements:
University of Utrecht, Netherlands.

Year Completed: 1998

Published Manuscripts/Abstracts:
The effects of pH, osmolarity and urine contamination on equine spermatozoal motility

Authors/Investigators:
Stefanie Griggers, DVM; Dale L. Paccamonti, DVM, MS, DACT; R. Alex Thompson, DVM, Ph.D.; Bruce E. Eilts, DVM, MS, DACT

Description of the Problem:
Urospermia, the contamination of semen with urine, results in infertility in stallions. Development of techniques to treat semen of stallions experiencing urospermia thereby improving fertility would be beneficial.

Study Objective:
To determine whether it was the change in pH or the osmolarity resulting from urine contamination that was causing the most harm to the sperm, and to determine whether the addition of semen extender, either alone or in conjunction with centrifugation to remove the urine, could improve semen quality.

Approach:
Semen was collected and subjected to conditions of varying pH, of varying osmolarity, and various quantities of urine and effects on motility were recorded. Then semen contaminated with urine was extended with either of 2 semen extenders, followed by centrifugation or not centrifuged, in an attempt to alleviate the detrimental effect of urine on motility.

Accomplishments/Results/Conclusions:
The results of these experiments showed that alterations in pH and osmolarity negatively affected stallion sperm motility. Optimal pH and osmolarity appeared to be approximately 7.7 and 315, respectively. Contamination of the ejaculate with urine significantly decreased sperm motility. Smaller quantities of dilute urine were less detrimental than larger quantities of dilute urine, and dilute urine was less detrimental than more concentrated urine. The addition of semen extender restored the motility of urine contaminated semen to that of the uncontaminated control, however centrifugation to remove urine provided no significant advantage.

Benefit to/Impact on the Equine Industry:
Semen samples from stallions experiencing urospermia may be processed to restore fertility.

Take Home Message:
Addition of semen extender may improve fertility of semen contaminated with urine. Centrifuging the semen sample will not provide any additional benefit.

Year Completed: 1998

Published Manuscripts/Abstracts:

Inducing earlier cyclicity in mares

Authors/Investigators:
Angela Klump, MS; Abdul Aljarrah, DVM; Marina Sansinena, Ph.D.; Darin Hylan, MS; Carlos Pinto, DVM, Ph.D., DACT; Bruce Eilts, DVM, MS, DACT; Robert Godke, Ph.D.; Dale Paccamonti, DVM, MS, DACT

Description of the Problem:
Because many horse breed associations arbitrarily impose an artificial birthday of January 1 in the Northern hemisphere, horse breeders desire to get mares pregnant early in the year. However, mares are seasonal long-day breeders, and are usually in anestrus during the winter months. Before resuming normal estrous cycles, mares go through transition, a period of anovulatory estrual activity. During transition, estrous periods are irregular and ovulation unpredictable, frustrating reproductive management on the stud farm. Researchers have investigated many ways to advance the onset of cyclicity and shorten transition in order to expedite getting mares pregnant earlier in the year.
**Study Objective:**
We hypothesized that by aspirating the dominant follicle during the late transitional period, we could induce formation of luteal tissue and thereby hasten the onset of cyclicity. Our specific objective was to perform follicular aspiration and compare the date of first rise in progesterone between treated and control mares.

**Approach:**
In an attempt to shorten the period of transition after seasonal anestrus and hasten the onset of cyclicity in mares, ultrasound guided transvaginal follicular aspiration was performed on mares when a follicle >35 mm was present.

**Accomplishments/Results/Conclusions:**
Mares that underwent follicular aspiration had serum progesterone > 1 ng/ml sooner than untreated controls (80.5 +/- 7.3 d after January 1 vs. 104.3 +/- 8.8 d, mean +/- SE; P = 0.024). Nine of 11 treatment mares formed luteal tissue within 8 d after the first aspiration. The remaining two mares formed luteal tissue after a second aspiration procedure. When an aspiration procedure resulted in the formation of luteal tissue, progesterone rose to >1 ng/ml within one week (6.7 +/- 0.9 d).

**Benefits to/Impact on the Equine Industry:**
This technique, used to induce earlier cyclicity, resulted in mares cycling almost 3 weeks earlier than the control mares.

**Take Home Message:**
Follicular aspiration may be a useful tool to induce earlier cycling in mares.

**Acknowledgements:**
This work was performed in collaboration with the Embryo Biotechnology Laboratory, LSU Agricultural Center.

**Year Completed:** 2000

**Published Manuscripts/Abstracts:**

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**Using acupuncture to advance the onset of cyclicity**

**Authors/Investigators:**
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**Description of the Problem:**
Because many horse breed associations arbitrarily impose an artificial birthday of January 1 in the Northern hemisphere, horse breeders desire to get mares pregnant early in the year. However, mares are seasonal long-day breeders, and are usually in anestrus during the winter months. Before resuming normal estrous cycles, mares go through transition, a period of anovulatory estrual activity. During transition, estrous periods are irregular and ovulation unpredictable, frustrating reproductive management on the stud farm. Researchers have investigated many ways to advance the onset of cyclicity and shorten transition in order to expedite getting mares pregnant earlier in the year. Because a non-invasive method to advance cyclicity would be desirable, and there is currently interest in non-traditional medicine, acupuncture was investigated as a means to get mares to cycle earlier in the year.

Advancing the onset of cyclicity in mares will result in earlier pregnancy and subsequent foaling.
Study Objective:
The objective of this study was to use acupuncture to get mares cycling earlier in the year.

Approach:
In Year 1, traditional standard acupuncture at various sites (Rusty – can give you specific sites if you want them) was used beginning in January on a group of anestrous mares. In Year 2, modifications were made to include moxibustion and electro-acupuncture to increase the stimulation at the acupuncture sites.

Accomplishments/Results/Conclusions:
Treated mares and control mares began cycling at the same time each year. Acupuncture failed to induce earlier cyclicity.

Benefits to/Impact on the Equine Industry:
The use of acupuncture in equine medicine may have a place, for example in pain management. However, it is unlikely that acupuncture will be of use in inducing earlier cyclicity in mares.

Take Home Message:
Other methods, such as lighting schemes or follicular aspiration (see above) are more useful for inducing cyclicity in mares.

Acknowledgements:  This study was funded by a grant from the American Association of Equine Practitioners.

Year Completed:  2000

Published Manuscripts/Abstracts:  Manuscript in progress.

Using acupuncture to aid uterine clearance

Authors/Investigators:
Alexandra Niemantsverdriet Murton, DVM, Dip ACT; Paccamonti Dale L Dale DVM, MS, Dip ACT; Eilts Bruce E DVM, MS, Dip ACT; Carlos RF Pinto, DVM, PhD, Dip ACT; Glenn Pettifer, DVM, Dip ACVA

Description of the Problem:
Delayed uterine clearance has been identified as a significant cause of infertility in mares. Many treatment modalities have been investigated to aid in enhancing uterine clearance. Because of an interest in non-traditional methods and reports of the use of acupuncture to treat various reproductive problems in other species, yet a lack of scientifically controlled studies, we wanted to determine if acupuncture treatment could cause a change in intrauterine pressure. An increase in intrauterine pressure would indicate an increase in uterine motility and a positive effect on uterine clearance.

Study Objective:
The objective of this study was to determine if acupuncture at selected sites would stimulate uterine contractions and result in an increase in intrauterine pressure.

Approach:
When mares were in estrus, with a follicle > 35 mm, intrauterine pressure was measured for a period of time (as a control) and then acupuncture was performed while intrauterine pressure recordings were continued.

Accomplishments/Results/Conclusions:
Although final analysis of the data is still underway, preliminary analysis has not shown an effect of acupuncture on intrauterine pressure.

Year Completed:  2000

Published Manuscripts/Abstracts:  Manuscript in progress.
Respiratory

Cytokine gene expression: Is it altered in horses affected with summer pasture-associated obstructive pulmonary disease?

**Authors/Investigators:**
Ralph E. Beadle, DVM, PhD, David W. Horohov, BS, MS, PhD; Stephen D. Gaunt, DVM, MS, PhD, DACP

**Description of the Problem:**
Summer pasture-associated pulmonary disease (SPAOPD) is an inflammatory airway disease of horses that reside in the Southeast region of the United States, and it is estimated that up to 3% of the horses in this region may be affected. Affected horses show clinical signs of obstructive pulmonary disease and are unusable for any type of exercise. Additionally, severely affected mares are sometimes unable to carry a foal to term. Therefore, this disease has a large negative impact on the equine industry in Louisiana and other southeastern states. Presently, the etiology and pathogenesis of this disease are ill-defined. However, the disease has clinical signs that are similar to those shown by humans with extrinsic asthma which is characterized by altered cytokine gene expression by CD4+ lymphocytes.

**Study Objective:**
The objectives of this study were to:

1. Isolate lymphocytes from bronchoalveolar lavage (BAL) fluid and peripheral blood collected from SPAOPD-affected horses when they were in clinical remission from the disease (winter season) and determine the levels of interleukin-4 (IL-4) & interferon-gamma (INFγ) mRNA being expressed by these cells.

2. Isolate lymphocytes from BAL fluid and peripheral blood collected from SPAOPD-affected horses when they were displaying clinical signs of the disease (summer season) and determine the levels of IL-4 & INFγ mRNA being expressed by these cells.

3. Isolate lymphocytes from BAL fluid and peripheral blood collected from control horses coincident to the times that they were collected from SPAOPD-affected horses (summer season and winter season) and determine the levels of IL-4 & INFγ mRNA being expressed by these cells.

**Approach:**
Principal and control horses were maintained in the same pasture environment and were fed similarly. Data were collected from both principal and control horses during the summer when horses in the principal group were showing distinct signs of airway obstruction while on pasture and again in February when they were in remission. This allowed examination of data from both groups of horses during the zenith of the clinical syndrome in the principal group and again while the disease was in remission in the principal group. We attempted to eliminate diurnal variations in the data by studying the horses between 8:00 and 10:00 AM in both summer and the winter. No drugs were administered for at least two weeks prior to data collection and all data were collected from horses while they were being maintained on pasture.

**Accomplishments/Results/Conclusions:**
Principal horses had significantly increased levels of IL-4 mRNA in their summer samples as compared to those of control horses, whereas the levels of IL-4 mRNA were not significantly different in the winter samples taken from these two groups.
The levels of INFγ mRNA were significantly higher in principal horses than they were in control horses in the summer samples. Additionally, principal horses had significantly increased levels of INFγ mRNA in their summer samples as compared to their winter samples, whereas control horses did not.

These results are consistent with the hypothesis that SPAOPD horses are reacting to an antigen in their summer environment that is not producing a similar response in control horses. This finding indicates that there was not a TH1:TH2 isotype switching occurring in these horses but rather that both TH1 and TH2 lymphocyte populations were being simultaneously stimulated.

**Benefits to/Impact on the Equine Industry:**
The findings from this study that affected horses express higher levels of IL-4 mRNA and INFγ mRNA during disease exacerbations have important clinical implications. Such results indicate that it may be possible to manipulate the immune systems of affected horses to prevent them from expressing the higher levels of these cytokines or to prevent the resultant cytokines from interacting with their receptors. If such therapy were successful, a major step forward would have accomplished in the prevention and treatment of SPAOPD in horses.

**Take Home Message:**
The results of this study have provided insight into the pathogenesis of SPAOPD in horses. Further studies are needed to determine if it is possible to alter the cytokine gene expression of SPAOPD-affected horses with specific drug therapy and/or other management schemes. If appropriate management schemes can be devised, then it should be possible to more successfully treat or prevent this disease in the future.

**Acknowledgments:**
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The investigators gratefully acknowledge and thank Keiko Antoku, Kim Snedden, Chad Vanderheyden, Dr. Britta Leise, Susan Porciau, Paul Hollier and Frank Garza for their technical assistance.

**Year Completed:** 1998

**Published Manuscripts/Abstracts:**

**Correlation of clinical score, pleural pressure, bronchoalveolar lavage fluid cytology, and pulmonary histology in horses with summer pasture-associated obstructive pulmonary disease**

**Authors/Investigators:**
Lais R.R. Costa, MV, MS, DACVIM; Thomas L. Seahorn, DVM, MS, DACVIML; Rustin M. Moore, DVM, PhD, DACVS; H. Wayne Taylor, DVM, PhD, DACVP; Stephen D. Gaunt, DVM, MS, PhD, DACP; Ralph E. Beadle, DVM, PhD

**Description of the Problem:**
Summer pasture-associated obstructive pulmonary disease (SPAOPD) is a recurrent seasonal form of chronic obstructive pulmonary disease (COPD) observed in horses residing in pasture in the southeastern region of the United States. It is a naturally-acquired, progressive, obstructive disease of the small airways characterized by coughing and pronounced abdominal lift at the end of exhalation manifested during late spring, summer and early autumn, first described by R. Beadle in 1983. Nonetheless, very little has been published with respect to clinical and pathological features of this disease.

**Study Objective:**
The objectives of this study were: (1) to describe the clinical presentation (clinical score of respiratory effort) and pulmonary function test (pleural pressure), bronchoalveolar lavage fluid (BALF) cytology, pulmonary histopathology of percutaneous biopsy and of postmortem specimens from horses affected with SPAOPD, (2) to correlate the clinical and pathological changes, and (3) compare these findings with those in clinically normal horses.
Approach:
A total of 14 horses were evaluated in the study, 8 adult horses affected with SPAOPD and 6 adult horses without evidence of respiratory disease. Clinical scores of respiratory effort (CSRE), changes in pleural pressure during tidal breathing, cytology and bacterial culture of BALF and light microscopy of pulmonary parenchyma collected via percutaneous biopsy and at postmortem were evaluated.

Accomplishments/Results/Conclusions:
The CSRE for SPAOPD-affected horses (median 5.75; range 4.0-7.5) was significantly greater compared with normal horses (median 2.0; range 2.0-3.0). Cytology of BALF from SPAOPD-affected horses yielded predominantly non-degenerate neutrophils. Histopathologic lesions were identified throughout the lung lobes, and included marked accumulation of basophilic mucus and neutrophils within the small airways, bronchiolar goblet cell metaplasia and mild peribronchial inflammatory infiltrate. Histopathology of specimens collected via percutaneous biopsy was predictive of disease and corresponded to findings at postmortem. The CSRE was highly correlated to pulmonary function in SPAOPD-affected horses. The CSRE and pulmonary function were highly correlated with mucus accumulation in the airways in affected horses. Peribronchial inflammatory infiltrate correlated with percentage of neutrophils in BALF of affected horses. Both CRSE and pulmonary function provided valid estimation of disease severity. BALF cytology of SPAOPD-affected horses was variable, although in most cases diagnostically useful. Severe mucus accumulation in the airways was the most remarkable histopathologic finding in SPAOPD. Lung biopsy specimens were consistently useful in the diagnosis of SPAOPD. The lung biopsy procedure was safe as no complications were observed during a 24-hour period.

Benefits to /Impact on the Equine Industry:
This study reports in detail the clinical and pathologic findings in horses affected with SPAOPD providing a landmark description of the disease. The correlation of the clinical findings and severity of the clinical signs with the pathologic lesions indicated that the disease is characterized by reversible airway remodeling primarily associated with mucus hypersecretion.

Take Home Message:
The clinical and pathologic findings in horses affected with SPAOPD suggest that signs are likely to be reversible after instituting therapy that effectively yields clearance of airway mucus and bronchodilation.

Acknowledgments:
This study was supported by a grant from the USDA 1433 Formula Funds, School of Veterinary Medicine, Louisiana State University.

Year completed: 1998

Published Manuscripts/Abstracts:


Plasma and bronchoalveolar fluid concentrations of nitric oxide and histochemical and immunohistochemical localization of nitric oxide synthesis in lungs of horses with summer pasture-associated obstructive pulmonary disease

Authors/Investigators:
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Description of the Problem:
Summer pasture-associated obstructive pulmonary disease (SPAOPD) is a naturally-acquired, seasonal, obstructive disease of the small airways of horses residing in pasture. There are a number of potential inflammatory mediators incriminated in the pathogenesis of SPAOPD, which clinically resembles human asthma. Nitric oxide has been demonstrated to be involved in the pathogenesis of airway inflammation and hypersensitivity of asthma.

Study Objective:
The objective of this study was to determine the concentration of nitric oxide (NO) in plasma and bronchoalveolar lavage fluid and localization of nitric oxide synthesis in lungs of horses affected with summer pasture-associated obstructive pulmonary disease (SPAOPD), compared with non-affected horses.

Approach:
Seven adult horses affected with SPAOPD and 6 clinically normal (non-affected) adult horses were evaluated clinically and the severity of SPAOPD was determined by clinical scores, change in intrapleural pressure during tidal breathing, cytologic analysis of bronchoalveolar lavage fluid (BALF) and histopathologic evaluation by H&E staining. Nitric oxide concentrations in plasma, BALF and epithelial lining fluid (ELF) were determined via a chemiluminescent method. Immunohistochemical staining of formalin-fixed specimens of pulmonary tissue for inducible nitric oxide synthase (iNOS) and nitrotyrosine (NT), and histochemical staining for nicotinamide adenine dinucleotide phosphate diaphorase (NADPHd) activity in cryopreserved specimens were performed and evaluated with light microscopy. Sections from each lung lobe were evaluated and compared.

Accomplishments/Results/Conclusions:
There was a trend of higher plasma NO concentrations in SPAOPD-affected compared with clinically normal horses. Nitric oxide concentrations in BALF or ELF did not differ between SPAOPD-affected and non-affected horses. The iNOS staining of bronchial epithelial cells was greater in affected than non-affected horses in 3/5 lung sections. The NT staining and NADPHd activity of bronchial epithelial cells, as well as the NT and iNOS staining and NADPHd activity of peribronchial leukocytes in the pulmonary tissue did not differ between SPAOPD-affected and non-affected horses.

Benefits to/Impact on the Equine Industry:
We demonstrated that immunoreactivity to iNOS is increased in bronchial epithelial cells of SPAOPD-affected compared with non-affected horses, suggesting that NO plays a role in the airway inflammation and hypersensitivity seen in horses with this disease. Clinical Relevance is that NO may have cytotoxic effects in the airway of horses with SPAOPD.

Take Home Message:
Nitric oxide may play a role in the airway inflammation and hypersensitivity seen in horses with SPAOPD.

Acknowledgments:
This study was supported by a grant from the United States Department of Agriculture 1433 Formula Funds, School of Veterinary Medicine, Louisiana State University. The authors thank Mae Lopez for performing the immunohistochemical and histochemical staining, and Catherine Koch, Dr. Lee Ann Curtis and Frank Garza for their valuable technical assistance.

Year completed: 1999

Published Manuscripts/Abstracts:


Comparative responses of bronchial rings to mediators of airway hyper reactivity in healthy horses and those affected with summer pasture-associated obstructive pulmonary disease

Authors/Investigators: Changaram S. Venugopal, BVSc, MSc, MS, PhD; Rustin M. Moore, DVM, PhD, DACVS; Earnestine P. Holmes, BS; Catherine E. Koch MS; Thomas L. Seahorn, DVM, MS; Ralph E. Beadle, DVM, PhD

Description of the Problem:
Summer pasture-associated obstructive pulmonary disease (SPAOPD) is an inflammatory, obstructive airway disease similar to heaves (COPD) that occurs in subtropical regions of the US during late summer and early fall and is associated with grazing in lush pastures. It is a severely debilitating, devastating and often career ending or life threatening pulmonary disease of horses leading to morbidity and loss of performance. During periods of airway obstruction, horses develop airway hyper reactivity resulting in severe bronchoconstriction to inflammatory mediators released locally into the airway lumen and toward the bronchial smooth muscle. These mediators are responsible for bronchial smooth muscle contraction, increased vascular permeability, increased mucus secretion, and damage to the airway epithelium resulting in airway remodeling leading to airway hyper reactivity. Airway hyper reactivity is a condition characterized by increased sensitivity (hyper sensitivity) and increased maximal responsiveness (hyper responsiveness). Commonly accepted airway inflammatory mediators involved in airway disease include histamine, bradykinin, prostaglandins F_2alpha, serotonin, leukotrienes (LTD), substance P and platelet activating factor (PAF).

Study Objective:
The objective of this study was to compare responses of bronchial rings obtained from clinically healthy horses and those affected with SPAOPD to selected mediators known to cause airway hyper reactivity.

Approach:
4mm wide bronchial rings were cut from the 4th -7th generation branches of airways from clinically healthy and SPAOPD affected horses. They were mounted in organ baths containing Tyrode's solution and were attached to force transducers interfaced with a polygraph. Initial tension of 2 g was applied to the rings and an equilibration time of 45 minutes were given. Then concentration response relationships of the rings to graded concentrations of selected mediators (10^-8 to 10^-4M) were determined and analyzed for statistical significance at each concentration of the agents.

Accomplishments/Results/Conclusions:
Acetylcholine, histamine, serotonin and leukotriene D_4 (LTD) induced consistent concentration-dependent contractile responses in bronchial rings. PGF_2alpha induced week and inconsistent contractile response. The other two agents, nor epinephrine and substance P, did not induce concentration–dependent responses. Considering the overall group-drug effect, acetylcholine, histamine serotonin and LTD were effective in causing consistent concentration-dependent contractile responses in both groups of rings. Only serotonin and histamine induced significant responses between the groups. The responses to serotonin on SPAOPD rings were significantly greater that those of the clinically healthy horses whereas, histamine produced a significantly lower response in SPAOPD horses. Significant responses were evident at concentrations ranging from 10^-6 to 10^-4M.

Take Home Message:
Serotonin causes airway hyper reactivity in SPAOPD horses. Therefore, treatment modalities using serotonin antagonists should be investigated in SPAOPD horses. Antihistamines may not be useful.

Acknowledgments:
This study was supported by a grant from the Equine Health Studies Program, School of Veterinary Medicine, Louisiana State University.

Year Completed: 1999

Published Manuscripts/Abstracts:
Nd:YAG laser assisted modified Forssell’s procedure for treatment of cribbing (crib-biting) in horses

Authors/Investigators:
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Description of the Problem:
Cribbing (crib-biting) is an acquired stereotypic oral behavior, in which a horse grasps a horizontal solid object with its upper incisors, contracts its ventral neck muscles and arches its neck, retracts the larynx, and pulls backward. The reason for starting cribbing is unknown, however; commonly cited causes are boredom or frustration. Sequelae to cribbing include poor performance, weight loss, abnormal wear of the incisor teeth, and flatulent colic. Various surgical procedures have been described to treat cribbing in horses. However most have resulted in considerable disfigurement of the neck or poor success rate. Recurrence of cribbing behavior after surgery may be related to seroma or hematoma formation that could allow development of tendon-like fibrous tissue between the cut muscle ends. The wavelength of the neodymium:yttrium aluminum garnet (Nd:YAG) laser makes it ideal for coagulation of vascular tissues because of the relatively good absorption of the laser beam by hemoglobin. A reduction in the amount of muscle remaining ventral to the larynx may further minimize the development of the tendon-like fibrous tissue that is believed to facilitate retraction of the larynx and reoccurrence.

Study Objective:
The objective of this study was to report an Nd:YAG laser-assisted modified Forssell’s surgical technique and outcome for treatment of cribbing (crib-biting) in horses.

Approach:
This was a retrospective clinical study evaluating the outcome of 10 adult horses with stereotypic cribbing behavior post surgery. Data was obtained from medical records and telephone conversations with owners, trainers, and veterinarians. Surgical technique involved an approximately 34-cm ventral median skin incision starting rostral to the larynx and extending caudally. A 10-cm section of the ventral branch of the spinal accessory nerve was removed, using an Nd:YAG laser at 25 watts and continuous pulse with a contact, sculpted-fiber tip. After neurectomy, approximately 34-cm sections of the omohyoideus and sternothyrohyoideus muscles were removed starting 2 cm cranial to the ventral aspect of the larynx, at the basihyoid bone, using the Nd:YAG laser.

Accomplishments/Results/Conclusions:
None of the horses had cribbing behavior after surgery and all returned to their previous use. The successful outcome we obtained is better than reported previously using a modified Forssell’s technique.

Benefits to/Impact on the Equine Industry:
With this modification of the Forssell’s technique, successful elimination of cribbing in the 10 horses in this study was accomplished. This outcome may have been a result of transection of the sternothyrohyoideus and omohyoideus muscles rostral to the ventral aspect of the larynx, at the basihyoid bone, use of an Nd:YAG laser to perform the neurectomy and control hemorrhage during muscle transection, or a combination of these techniques.

Take Home Message:
Surgical treatment for cribbing by Nd:YAG laser assisted myectomy and neurectomy resulted in an excellent prognosis for resolution of the stereotypical behavior with minimal complications.
Acknowledgments:
This study was supported by funds from the Equine Health Studies Program, School of Veterinary Medicine, Louisiana State University.

Year Completed: 2000

Published Manuscripts/Abstracts:


**Cecal amputation within the right ventral colon for surgical treatment of nonreducible cecocolic intussusception in eight horses**

Authors/Investigators:
Jeremy D. Hubert, BVSc, MRCVS, MS, DACVS; Joanne Hardy, DVM, PhD, DACVS; Susan J. Holcombe, VMD, PhD, DACVS; Rustin M. Moore, DVM, PhD, DACVS

Description of the Problem:
Although relatively uncommon, a certain condition has been described in horses where their cecum will invert into their colon, this is known as ceco-colic intussusception. Initial reports of this condition have described the surgical difficulties in trying to reduce the intussusception in a routine manner and the poor success of such attempts. As such this retrospective study describes a technique to reduce those cases deemed non reducible and the follow up of eight horses who underwent this procedure.

Study Objective:
The objective of this study was to report a surgical technique for treatment of non-reducible cecocolic intussusception and outcome in eight horses.

**Approach:**
Data was obtained from medical records and telephone conversations, using a standardized questionnaire. The large colon was exteriorized and, if necessary, evacuated of its contents through a pelvic flexure enterotomy. A second colotomy was made on the ventral surface of the right ventral colon (RVC) centered over or immediately distal to the intussusceptum. Attempts made in most horses to manually reduce the intussusception by pushing the cecum from within the RVC through the cecocolic orifice were unsuccessful. Invaginated cecum was then pulled into the RVC and amputated; the cecum was either ligated with umbilical tape or sutured proximal to the site of amputation. After amputation, the remainder of the invaginated cecum was reduced, then after further resection to healthy tissue, the typhlectomy was closed using a double inverting suture pattern.
Surgery

Accomplishments/Results/Conclusions:
All horses survived to hospital discharge. One horse died 3 months postoperatively; however, the remainder survived (median survival 30 months; range, 6-96 months) and returned to or exceeded previous function. Despite some contamination during surgery, this method of surgical treatment for horses with nonreducible cecocolic intussusception had a good prognosis for survival.

Benefits to/Impact on the Equine Industry:
This reported technique will provide the equine surgeon with the ability to resolve what was initially considered a condition with an extremely poor prognosis.

Take Home Message:
Cecocolic intussusceptions do not have the extremely poor prognosis they were considered to have and surgical repair should be attempted in the manner described in this report.

Acknowledgments:
This study was supported by grants from USDA 1433 funds and the Department of Veterinary Clinical Sciences Organized Research Fund, School of Veterinary Medicine, Louisiana State University.

Year Completed: 1998

Published Manuscripts/Abstracts:

Hubert JD, Hardy J, Holcombe SJ, Moore RM. Cecal amputation within the right ventral colon for surgical treatment of nonreducible cecocolic intussusception in 8 horses. BEVA Congress 1999.


Factors associated with racing performance of Thoroughbreds undergoing lag screw repair of condylar fractures of the third metacarpal or metatarsal bone

Authors/Investigators: George S. Martin, DVM, MS, MBA, DACVS

Description of the Problem: Condylar fractures of the third metacarpal and metatarsal bones occur relatively commonly in racing Thoroughbreds. The prognosis for horses with these injuries depends upon the fracture type and configuration. The gender of the horse also affects decision regarding treatment of these fractures. There are conflicting opinions from surgeons regarding the best surgical treatment of horses with condylar fractures.

Study Objective: The objective of this study was to evaluate effects of sex, fracture configuration, affected limb, and screw placement on outcome of Thoroughbreds with condylar fractures involving the third metacarpal or metatarsal bone.

Approach: This cohort study involved 56 Thoroughbred racehorses that sustained a condylar fracture of the third metacarpal or metatarsal bone and underwent surgical repair. The age, sex, affected limb, fracture configuration, fracture length, fracture fragment width, and distance of the most distal screw from the articular surface were analyzed, using logistic regression models.

Accomplishments/Results/Conclusions: Females were more likely to have displaced fractures and race in fewer races after surgery than males. The sex and fracture configuration were associated with the number of postoperative races. Among horses that returned to racing, those with thicker fracture fragments were 11 times more likely as horses with thinner fracture fragments to win a race after surgery. Horses with longer fractures and older horses had fewer postoperative races. Horses in which the most distal screw had been placed further from the joint surface had more races.
Benefits to/Impact on the Equine Industry: This study provides objective data regarding the outcome of horses with surgically repaired condylar fractures and may offer veterinarians and horse owners and trainers objective information to base their decisions regarding surgical treatment. It appears that this group of horses was as competitive after surgery as they had been before injury.

Take Home Message: Results suggest that female horses with displaced condylar fractures and male horses with nondisplaced condylar fractures are more likely to be referred for treatment. The effect of sex on outcome for these horses cannot be clearly separated from the effect of fracture configuration. When adjusted for fracture configuration, males were 6 times as likely as females to race after surgery. When adjusted for sex, horses with nondisplaced fractures were 17 times as likely as horses with displaced fractures to race after surgery. Results suggest that the most distal screw should be placed above the epicondylar fossa. Earnings percentage is a useful method to adjust for the expected decline in purse value, and it adjusts for inflationary effects.

Year Completed: 1999

Published Manuscripts/Abstracts:


Authors/Investigators: Eric Strand, George S. Martin, Peter F. Haynes, DVM; Jill R. McClure, J. Donald Vice

Description of the Problem: Idiopathic laryngeal neuropathy is a relatively common cause of exercise intolerance, abnormal respiratory noise and poor performance. Despite several potential complications, prosthetic laryngeoplasty continues to be the most commonly performed surgical procedure for treatment of this condition.

Study Objective: The objective of this study was to compare racing performance before and after prosthetic laryngeoplasty for treatment of laryngeal neuropathy in inexperienced and experienced Thoroughbred racehorses. A technique developed and validated previously for performance evaluation was used to better understand how laryngeal neuropathy and its treatment affect racing ability.

Approach: This retrospective study assessed the racing performance of 52 Thoroughbred racehorses before and after treatment with a prosthetic laryngoplasty for laryngeal neuropathy. Lifetime race records were analyzed by use of a verified regression model. Individual race records and hospital records were also reviewed.

Accomplishments/Results/Conclusions: Experienced horses had a decline in performance, as measured by performance index, earnings percentage, and mean prediction error, during the 6-month period before prosthetic laryngoplasty. Performance improved after surgery, relative to performance in 1 to 4 races immediately before surgery, but did not attain previous baseline values for performance index and earnings percentage, although racing speed was restored to baseline values. Factors associated with failure to attain baseline levels of performance included other racing-related injuries and disorders, major complications of surgery, and age. Individually, however, many horses had long and successful careers after surgery. Performance of inexperienced horses after surgery was at least equal to that of experienced horses.
**Surgery**

**Take Home Message:** In addition to warning clients of the complications associated with prosthetic laryngeoplasty, it may be prudent to provide a guarded prognosis for full restoration of racing performance in older horses, unless they are especially talented and are free of other racing-related problems.

**Acknowledgments:** This study was supported by a grant from the Department of Veterinary Clinical Sciences Organized Research Fund.

**Year Completed:** 1999

**Published Manuscripts/Abstracts:**

Standing endoscopic image of a Thoroughbred racehorse with left laryngeal hemiplegia.