Louisiana State University
School of Veterinary Medicine
Equine Health Studies Program

2005
Research Report

Dedicated to the Health, Well-Being and Performance of Horses through Veterinary Research, Education and Service
Welcome to the 2005 Equine Health Studies (EHSP) Research Report. The purpose of this publication is to document scientific investigations involving equine diseases and injuries. All studies represented in this report were conducted by scientists at the Louisiana State University School of Veterinary Medicine (LSU-SVM) between 2001 and 2003.

This report demonstrates the extensive and diverse equine biomedical research conducted at the LSU-SVM. The works contained herein are the collective efforts of our multidisciplinary faculty, advanced studies students, veterinary students, undergraduate students, and technical staff comprising the EHSP.

The quality and quantity of these studies is particularly impressive considering the limited intramural funds available during this time. Our ability to acquire extramural support for our program during this period is particularly noteworthy given the highly competitive nature of these funds and the relatively small number and amount of grants available for equine research. These works demonstrate the Program’s commitment to promoting the health, well-being and performance of horses.

The state-of-the-art facilities and equipment highlighted in this issue represent major advances made in our program since 2003. This progress was facilitated in part by the combination of the receipt of a Governor’s Biotechnology Initiative Grant awarded by the Louisiana Board of Regents, a Board of Regents Enhancement Grant, and recurrent funding through the State Legislature resulting from Louisiana racetrack slot machine revenue. The quality of these facilities provides our faculty, staff, and students with an enriching and stimulating environment in which to conduct leading-edge equine scientific investigations.

A 2005 study conducted by the American Horse Council demonstrated that Louisiana’s horse industry ranks fifth nationally in terms of its economic contribution to the Gross Domestic Product. We have a tremendous responsibility to the equine population and horse-owning community that we serve. It is a responsibility that we embrace and continually strive to fulfill through ongoing cutting-edge research and state-of-the-art clinical service.

The important findings resulting from the scientific investigations presented in this report would not have been possible without the horses that were utilized for these studies. The availability of horses to be carefully and compassionately used for research is necessary for advancing equine health, and progress toward the future prevention, care and treatment of ill and injured horses. All scientific works conducted at LSU-SVM involving horses are conducted following Federal Guidelines for the Humane Care and Use of Animals. We greatly value the lives of these horses and treat them with the utmost compassion and dignity.

We do not use client-owned horses for research; however, retrospective analysis of medical records are sometimes used to assemble information on a series of cases with a specific illness or injury. The information obtained from these records is utilized to determine factors that might prove useful to improve the outcome of horses in the future with similar conditions through enhanced treatments or management strategies.

We are extremely proud of the progress we have made and the scholarly findings we have been able to contribute toward promoting and improving equine health. We look to the future with excitement and optimism. We continue to set our sights on establishing the EHSP as one of the premier equine biomedical programs in the world.

Respectfully,

Rustin M. Moore

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

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On the Cover: “Wisdom and Anticipation,” an oil painting by Anita Lejeune, a local artist from Lakeland, Louisiana. 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 Louisiana State University School of Veterinary Medicine is again extremely proud to present this Research Report of the Equine Health Studies Program. This multidisciplinary program continues to bring recognition to the School for its outstanding achievements and remarkable scientific productivity in equine research, clinical service, instruction, and outreach.

We have been playing a little “catch-up” in getting the backlog of program information out to the local, regional and national industry, and external stakeholders who have supported our program over the years. This 2005 Report captures the scientific activities of the program from 2001 to 2003. It clearly demonstrates the depth and breadth of the faculty and their accomplishments, as the program moves to establish itself in the upper tier of programs nationally and internationally.

Currently, the School is in the process of a Comprehensive Facility Master Planning initiative that includes facilities to further strengthen the program’s mission. Key among these plans are new Equine Isolation and Reproduction Units, and the program is pursuing private funds to construct them.

Indeed, one of the best examples of the program’s commitment to the health and welfare of the horse was their extraordinary participation in the recovery efforts following the destruction caused by Hurricanes Katrina and Rita last fall. Faculty and staff spent countless hours recovering and transporting stranded horses, establishing a shelter and providing medical services, and reuniting horses with their owners. Their efforts and services truly represented the veterinary profession in an exemplary fashion during a time of greatest need.

Our commitment to improve the health and welfare of the horse through research and clinical developments is unwavering. Stay tuned—our Equine Health Studies Program is moving forward at a rapid pace. With continued facility enhancements, state-of-the-art equipment, and instrumentation underpinned with outstanding faculty and staff, there is little doubt that we are on a projection to become a preeminent program.

Sincerely,

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

Dr. Michael G. Groves, Dean
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 LSU School of Veterinary Medicine, 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
Dr. Barker is a professor of veterinary physiology, pharmacology and toxicology at the LSU 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 D.V.M. 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. in Veterinary Physiology. After a period of two years spent at Michigan State University, he has been at LSU 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 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. 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 D.V.M. 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.

Sharon Chirgwin, Assistant Professor, Research
Dr. Chirgwin was born and raised in Australia. She obtained a B.S. 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. Chirgwin’s 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. Cho is a professor of veterinary pathology in the Department of Pathobiological Sciences at the LSU School of Veterinary Medicine. Dr. Cho is also the section chief for necropsy/surgical biopsy in the School’s Veterinary Teaching
Laise Rosa Rodriques Costa, Visiting Assistant Professor, Equine Emergency and Critical Care

Dr. Costa was born and raised in the city of Sao Paulo, State of Sao Paulo, Brazil. She completed her veterinary degree at the School of Veterinary Medicine, FMVZ, Sao Paulo State University, UNESP-Botucatu in December 1987. After almost two years working as a Large Animal Clinician at UNESP-Botucatu in Brazil, Dr. Costa came to the LSU School of Veterinary Medicine for an Internship in Equine Medicine and Surgery. She then moved to Lexington, Ky. where she obtained her Master of Science degree at the University of Kentucky in May 1994 studying the immune response to equine infectious anemia virus. During the ensuing two years, Dr. Costa worked for the Veterinary Medical Teaching Hospital - Equine Medicine Service, at the University of California-Davis and obtained a grant to study genetic variability of Corynebacterium pseudotuberculosis isolates affecting horses, cows, sheep and goats. In 1996, Dr. Costa returned to LSU for an equine internal medicine residency, and she became Board Certified in Large Animal Internal Medicine in 1999. She worked for 6 months as a clinical instructor in Equine Medicine at LSU and then at the University of Georgia for one month. Dr. Costa initiated her Ph.D. program in the spring of 2000. While pursuing her Ph.D. in the Department of Pathobiological Sciences, Dr. Costa worked as a Clinical Fellow in Equine Medicine, through the Department of Veterinary Clinical Sciences. She obtained funding for several clinical studies and five research projects involving equine airway disease. Dr. Costa has also been involved in several activities concerning neonatology and intensive care.

Susan C. Eades, Professor, Equine Medicine

Dr. 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. 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 D.V.M. 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 a half years in private practice in southern California, he came to LSU 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. Foster was born in San Francisco, Calif., and obtained a B.S. degree in Biochemistry and a B.S. degree in Microbiology/Zoology from LSU in 1995. In 1999, he received a Ph.D. in Veterinary Medical Sciences with an emphasis in Biochemistry and Molecular Virology from the LSU 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. French, originally from Chatfield, Minn., obtained his B.S. and D.V.M. 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. Henk is a professor of veterinary anatomy and cell biology in the department of Comparative Biomedical Sciences at the LSU 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. 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. 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 LSU School of Veterinary Medicine 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 immunochemistry 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.

Thomas R. Klei, Boyd Professor, Parasitology and Veterinary Science
Dr. 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 LSU School of Veterinary Medicine.

Konstantin G. Kousoulas, Professor, Veterinary Virology
Dr. 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 LSU 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. 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 her M.S. and Ph.D. degrees. Her area of interest and expertise is comparative orthopedic research and surgery. Dr. Lopez 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. Lyle was born and raised in Gainesville, Fla. She obtained her B.S. in Chemistry at Duke University and her D.V.M. 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. 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 D.V.M. 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. 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 D.V.M. 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. Natalini is originally from Rio de Janeiro, Brazil, where he attended the Universidade Federal Fluminense 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 the 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 School of Veterinary Medicine. Dr. Natalini’s 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.

Kathy L. O’Reilly, Associate Professor, Veterinary Immunology

Dr. O’Reilly was born in Corona, Calif. She obtained her B.S. and M.S. in Microbiology from the University of Wyoming in 1977 and 1982, respectively. Following a year working as a research scientist at the Wyoming State Diagnostic Laboratory and then in the University of Wyoming Department of Biochemistry, Dr. O’Reilly completed her Ph.D. in Veterinary Science (Immunology) at the University of Wisconsin-Madison in 1989. After receiving her Ph.D., Dr. O’Reilly completed postdoctoral training at in the Colorado State University Department of Pathology, where she studied the immune response to feline immunodeficiency virus and feline leukemia virus. Dr. O’Reilly joined the faculty within the Department of Pathobiological Sciences (then the Department of Veterinary Microbiology and Parasitology) at the LSU School of Veterinary Medicine in 1992 and has an adjunct appointment in the Department of Biological Sciences. Her research interest is in immune responses to intracellular pathogens of animals; specifically mechanisms of cell-mediated immunity and immune evasion, including cellular interactions and control of cells responding during infection. Her current research focuses on the bacterial pathogen Bartonella hesealae in the feline reservoir, respiratory bovine coronavirus, and the development of in vitro models studying lung disease in cattle and horses.

Marlene Orandle, Assistant Professor, Virology

Dr. 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 D.V.M. 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. Paccamonti, originally from Kankakee, Ill., completed his undergraduate and veterinary education at Michigan State University, receiving his D.V.M. 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’s 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.

Gary A. Sod, Clinical Instructor, Farm Animal Health Management
Dr. Sod received his M.A. in Mathematics from the University of California at Berkeley in 1975. He earned his Ph.D. in Applied Mathematics from that same institution in 1976. The next 12 years were spent doing research in mathematical and computational physics resulting in the writing of a monograph on numerical methods in fluid dynamics and 42 journal publications. Dr. Sod served as an adjunct professor in the Department of Mechanical Engineering and a professor in the Department of Mathematics at Tulane University from 1985 through 1997. Dr. Sod then attended the LSU School of Veterinary Medicine and obtained his D.V.M. in 2001. He has since completed an equine internship and food animal medicine and surgery residency at LSU and is now a clinical instructor with the Farm Animal Health Management service. Dr. Sod received the American College of Veterinary Surgeons Resident Research Publication Award in 2004 and the Mark S. Bloomberg Memorial Resident Research Award from the Veterinary Orthopedic Society in 2004 and 2005. Dr. Sod is enrolled in a large animal surgery residency in conjunction with the Sawtooth Equine Service in Idaho. His research interests include biomechanics and the design of orthopedic implants specific to the equine patient.

Changaram S. Venugopal, Professor, Veterinary Physiology & Pharmacology
Dr. 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 five 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 his 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 joining the faculty at LSU 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 the National Institutes of Health 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.

Ashley M. Stokes, Research Assistant Professor
Dr. Stokes was born in Baton Rouge, La., and moved to Tuscaloosa, Ala., 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 D.V.M./Ph.D. 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. Taylor is a professor of veterinary pathology in the department of Pathobiological Sciences at the LSU School of Veterinary Medicine. He is also a veterinary pathologist and the director of the Louisiana Veterinary Medical Diagnostic Laboratory. Dr. Taylor received his D.V.M. 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.
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 are owned by an estimated 60,000 people in the state, with a total direct economic impact of the equine industry in Louisiana of 2.5 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 LSU 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
- Laminitis
- Nonvascular smooth muscle physiology, pharmacology, and pathobiology
  - Gastrointestinal
  - Bronchial
  - Uterine
- Vascular smooth muscle physiology, pharmacology, and pathobiology
- Analgesia and pain management
- Inflammatory mediators, including nitric oxide, endothelin and cytokines
- Medication surveillance
- Synovitis and arthritis
- Acupuncture
- Parasitology
- Endotoxemia
- Virology
- Use of global positioning system technology for equine epidemiologic studies
- 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 shock wave therapy on bone, tendon, ligament and nerve
EHSP Grants and Contracts

2003

Bueno ACD, Moore RM, Eades SC, Blackmer JM, Stokes AM, Curtis LA: Use of an extracorporeal pulsatile perfusion system (MOX-100R) to maintain viability of the disarticulated equine digit. $6,000. LSU Equine Health Studies Program, July 2003.


Lyle SK, Paccamonti DL, Hubert JD, Horohov DW, Eilts BE: The role of pro-inflammatory cytokines in pre-term pregnancy loss due to placentalitis in the mare. $3,000. LSU Equine Health Studies Program, July 2003.

Mitchell MA, Chapman AM, Eades SC: Characterizing the prevalence of Salmonella in equids at four Louisiana Racetracks. $6,000. LSU Equine Health Studies Program, July 2003.


2002
Moore RM, Venugopal CS, Horohov DW, Eades SC, Blackmer JM, Barker SA, McConnico RS, Pettifer GR, Burba DJ, Holm AS: Molecular, hemodynamic, pharmacologic and epidemiologic approaches to lameness and poor athletic performance in horses: Biomedical and technologic initiatives to strengthen Louisiana's equine industry and enhance the state's economy. $308,000 annually recurring and $167,000 one-time capital outlay. Governor's Biotechnology Initiative Grant Program, Louisiana Board of Regents, October 2002.

Wilke M, Blackmer JM, Moore RM: Development of a 3-dimensional culture technique for equine mesenchymal stem cells to develop a cell-based cartilage resurfacing technique. $4,000. Department of Veterinary Clinical Sciences Organized Research Fund, September 30, 2002.

Rumbaugh ML, Burba DJ, Natalini C, Moore RM: Evaluation of a vessel sealing device (LigaSureTM) for small intestinal resection and anastomosis in horses. $3,000. LSU Equine Health Studies Program, June 2002.


Gilhooly M, Eades SC, Moore RM: The ability of a nitroglycerine transdermal system to deliver nitric oxide to the equine digital vasculature. $5,000. LSU School of Veterinary Medicine Merck-Merial Student Research Program Grant Competition, April 2002.

Wilson S, Venugopal CS, Moore RM: In vitro characterization of bronchial ring responses to Neurokinin-A in normal horses and those with obstructive pulmonary disease. $5,000. LSU School of Veterinary Medicine Merck-Merial Student Research Program Grant Competition, April 2002.


2001

Costa LRR, Moore RM, Eades SC, Kousoulas KG: Quantification of endothelin-1 gene expression in lung specimens from SPAOPD-affected and non-affected horses. $4,500. LSU School of Veterinary Medicine, Veterinary Clinical Sciences Organized Research Funds, October 2001.


Pettifer, GR, McConnico RS, Burba DJ, Barker SA. The analgesic efficacy and side effects of transdermally administered fentanyl in horses. $30,155.00, Morris Animal Foundation, August 2001.


EHSP Selected Scientific Publications

2003


2002


Walesby HA, as expert opinion in the article by Marcia King called Babes in training: Do we ask too much of young horses? Horse Illustrated 28-30, January 2003.


2001


EHSP Selected Published Scientific Abstracts

2003


2002


2001


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 School of Veterinary Medicine Equine Health Studies Program. 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 campaign to generate funds to enhance all aspects of our program.

The LSU School of Veterinary Medicine is a relatively young institution, with only 29 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 fundraising efforts have been principally through private, charitable, tax-deductible gifts, as well as some other special events and activities. All gifts are 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, none of which are 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), which 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 for your generosity and support. To learn more about how your gift will assist the EHSP with its mission, please visit our website (www.LSUEquine.com) or contact Ky Mortensen via telephone (225-578-9590) or e-mail (kmortensen@vetmed.lsu.edu).
EHSP Facilities and Equipment

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 investigations and instructional activities. Three research barns collectively containing over 40 stalls and several pastures and paddocks are available for housing horses used 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 EHSP. The laboratory has three laminar flow biohazard hoods, four CO₂ incubators, nine Synthecon bioreactors, a phase contrast microscope with digital image capture capabilities, two centrifuges, refrigerators and ultra-low temperature freezers. In concert with other centralized faculties in the School of Veterinary Medicine, 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 is the newest addition to the EHSP. 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 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
Equine Health Studies Program 2005 Research Report

EHSP Facilities and Equipment

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 & Clinics are staffed by nationally and internationally recognized veterinary specialists (internal medicine, surgery, anesthesiology, and radiology) and highly-skilled veterinary technicians, and are 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 CO₂), and an extracorporeal shockwave therapy unit. A new Equine Lameness and Performance Evaluation Unit (75’ x 125’ covered pavilion) 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®). 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 advanced assisted reproductive techniques as intracytoplasmic spermatozoal injection and nuclear transfer (“cloning”).

Equine Molecular Biology Research Laboratory

The Equine Molecular Biology Research Laboratory is a new addition to the EHSP. The laboratory is equipped to support the molecular biology aspects of research conducted by the EHSP investigators. The missions of this laboratory are: to perform basic, cutting-edge research in molecular biology to elucidate normal equine physiology as well as the pathophysiology of equine disease, to train scientists, students and visitors at all levels, and to develop new

Nuclear scintigraphy (bone scan).

Equine Lameness and Performance Evaluation Unit.
instruments and methods in equine molecular biology. This laboratory provides refrigerated centrifuge and micro-centrifuges, electrophoresis/transilluminator equipment, refrigerators and ultralow temperature freezers, and PCR thermocycler equipment located in a molecular biology-dedicated laboratory space capable of multiple simultaneous studies. We also have direct access to the Division of Biotechnology and Molecular Medicine within the LSU School of Veterinary Medicine for quantitative Real-Time PCR, Primer Express primer/probe design, Quantity One for DNA fragment visualization and analysis, SDS-PAGE analysis, MagnaPure automated nucleic acid extraction, and microarray spotters and readers. Seminars and hands-on learning are key components of training provided by the laboratory to students, staff, faculty, and visiting scientists. Current investigations utilizing this laboratory include study of key mediators in equine laminitis, placentitis, gastrointestinal disease, summer pasture-associated obstructive pulmonary disease, bone healing, mechanisms of pain sensation and modulation, and stem cell biology. Plans are in place to expand the capabilities of this laboratory in light of the increasing importance of the molecular biological approach to the investigation of equine health and disease.

**BIOMMED – Biotechnology and Molecular Medicine**

The Division of Biotechnology and Molecular Medicine (BIOMMED), a division within the LSU School of Veterinary Medicine, 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 providing direct communication between researchers and GeneLab staff has recently been purchased. Available software includes: 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 also has 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 three automated sequences (ABI377, 310, 310), three real-time PCR equipment (PE 7900, 7400), LightCycler (Roche), two MagnaPure automated nucleic acid extractors (Roche), microarray OmniGrid spotter (Gene Machines), microarray reader (AlphaReader 6000, Alpha Innotech), Imager station (Alpha Innotech Fluorochem 8000), five PCR (thermocyclers), a New Brunswick Fermentor, AktaExplorer Chromatography System, Cyclone phosphorimagert, two 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 LSU School of Veterinary Medicine 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 freezers and other small equipment items are also available in this laboratory.

**Flow Cytometry Facility**

The Flow Cytometry Facility is a core laboratory located on the third floor of the LSU School of Veterinary Medicine. 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. There is also 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 LSU 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 School of Veterinary Medicine and LSU networks.

**Microscopy Center**


The Center features three new powerful microscopes. The laser capture and microdissection microscope (PALMZeiss 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 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.

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. The ASL also has a Hewlett-Packard 5970 MSD GC/MS system (ChemStation data system), three 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 School of Veterinary Medicine 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 LSU School of Veterinary Medicine and serves as a central administrative division for operating research animal holding facilities, including the LSU School of Veterinary Medicine 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 International.

SVM Library
The LSU School of Veterinary Medicine 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.
Anesthesia

Cardiopulmonary, behavioral, and analgesic effects of epidural administration of hydromorphone in standing and anesthetized horses

Authors/Investigators:
Claudio C. Natalini, DVM, MS, PhD; Glenn Pettifer, DVM, DVSC; Giselle Hosgood, DVM, PhD; Anderson F. Da Cunha, DVM, MS; Simone D. L. Alves, DVM, MS; Carlos A. Valadao, DVM, PhD; Andrew J. Lewis; Izane G. Fuchs.

Description of the Problem:
The use of opioids in horses is limited because they tend to cause marked central nervous system stimulation when injected intravenously. The advantage of epidural analgesic opioid administration in horse is that it allows for analgesia without causing these systemic side effects. Hydromorphone, an agonist mu-opioid eight times as potent as morphine is intermediate in lipid solubility between morphine and fentanyl. Intermediate lipid solubility may improve the ability to provide spinal analgesia, producing a faster onset of action. The biggest advantage of hydromorphone epidural administration would be a faster onset of action than morphine and longer-lasting analgesia compared with other epidural analgesics used in horses such as detomidine and ketamine.

Study Purpose/Objectives:
The purpose of this study was to observe the cardiopulmonary, behavioral, and analgesic effects of epidurally administered hydromorphone. The influence of epidurally administered hydromorphone on the quality of anesthetic induction and recovery was also assessed.

Approach:
Six healthy adult gelding horses received a pre-anesthetic epidural administration of either hydromorphone (0.04mg/kg) or sterile water in equal volume (20ml) as a control solution at a minimum 7-days interval between each treatment. Horses were sedated with intravenous xylazine (1mg/kg), for induction it was used intravenously, diazepam (0.05mg/kg), 5% guaifenesin, and a bolus dose of ketamine (1.5mg/kg), and the general inhalation anesthesia was maintained with isoflurane. Data were collected immediately before epidural administration and once under general anesthesia beginning 15 min after induction and every 15 min thereafter until a period of 90 min had passed, when inhalation anesthesia was discontinued. Arterial blood gases, arterial blood pressure, respiratory rate, cardiac output, and analgesia were measured.

Accomplishments/Results/Conclusion:
Mean arterial blood pressure and cardiac output were higher after epidural administration of hydromorphone and some respiratory depression was noted compared to the saline group. Neither sedation nor ataxia was observed with the epidural administration of hydromorphone. Under inhalation general anesthesia, mean arterial pressure decreased significantly from baseline in both groups, but no difference between groups was detected. It was concluded that epidural hydromorphone is not associated with negative side effects on cardiopulmonary performance in horses.

Take Home Message:
Epidural hydromorphone (0.04mg/kg) produces moderate analgesia that lasts approximately 120 minutes over the lumbar, sacral and perineal regions. When given to horses anesthetized with isoflurane, there is no cardiovascular or respiratory depression and the quality of recovery is improved.
Acknowledgments:
This study was supported by funds from the Department of Veterinary Clinical Science, School of Veterinary Medicine, Louisiana State University.

Year Completed: 2003

Published Manuscripts/Abstracts:

Epidural administration of tiletamine/zolazepam in horses

Authors/Investigators:
Claudio C. Natalini, DVM, MS, PhD; Simone D. L. Alves, DVM, MS; Alonso G. P. Guedes, DVM, MS; Alexandre S. Polydoro, DVM, MS; Juliana T. Brondani, DVM; Simone Bopp, DVM.

Description of the Problem:
In human and animal anesthesia, the epidural administration of drugs is used to provide surgical anesthesia and/or post-operative analgesia. In horses, caudal epidural anesthesia is used to desensitize the anus, rectum, perineum, vulva, vagina, urethra, and bladder. The goal is to produce surgical regional anesthesia without losing the motor function of the hind limbs. A combination of a local anesthetic drug with an alpha-2 adrenergic agonist or an opioid is the most popular option as this combination extends the period of action of the epidural anesthesia or analgesia in horses, humans, and small animals. The spinal analgesic effect of benzodiazepine is mediated by the activation of a benzodiazepine-GABA receptor complex within the spinal cord. Zolazepan is the only benzodiazepine derivative licensed for use, exclusively in combination with tiletamine in animals. Tiletamine is a phencyclidine derivative similar to ketamine, although more potent. Tiletamine/zolazepam has been used in horses to induce anesthesia in combination with potent sedatives such as xylazine. Epidural tiletamine has not been studied epidurally in horses.

Study Purpose/Objectives:
The objective of this study was to evaluate the analgesic, physiologic, and behavioral effects of the epidural administration of tiletamine/zolazepam in horses.

Approach:
Five horses were sedated with 1.0mg.kg⁻¹ intravenous xylazine, and an epidural catheter was placed into the first intercoccygeal intervertebral space using a 17-SWG 8.75-cm epidural Huber point needle. After 48-hour resting period, epidural tiletamine/zolazepam, 0.5mg.kg⁻¹ (treatment I) or 1.0mg.kg⁻¹ (treatment II), diluted up to 5mL in sterile water, was administered with a 1-week interval between the treatments. Heart rate, respiratory rate, arterial blood pressure, and sedation were evaluated. In order to evaluate the respiratory effects, blood from the carotid artery was withdrawn at time 0 (baseline), and then after 60 and 240 minutes. Analgesia was evaluated by applying a noxious stimulus with blunt-tipped forceps on the perineal region, and graded as complete, moderate, or absent. Data were collected before tiletamine/zolazepam administration and at 15-minute intervals for 120 minutes, and 4 hours after tiletamine/zolazepam administration. Data were analyzed with ANOVA and Bonferroni’s test with p<0.05.

Accomplishments/Results/Conclusion:
The results showed no significant difference between treatments in cardiovascular and respiratory measurements. Sedation was observed with both doses, and it was significantly different from baseline at 60, 75, and 90 minutes in treatment II. Moderate analgesia and locomotor ataxia were observed with both treatments. It was suggested that

Needle placement for caudal epidural anesthesia.
caudal epidural 0.5 and 1.0mg.kg\(^{-1}\) tiletamine/zolazepam increases the threshold to pressure stimulation in the perineal region in horses. The use of epidural tiletamine/zolazepam could be indicated for short-term moderate epidural analgesia.

**Benefits to/Impact on the Equine Industry:**
Analgesia is limited in horses due to the possibility of side effects occurrence when several drugs are intravenous and intramuscular administered. The systemic administration of tiletamine/zolazepam in horses produces apneustic respiratory pattern, hypoxemia, and hypercarbia, and decreases respiratory rate. Respiratory depression and other side effects were not response to the administration of epidural tiletamine/zolazepam. Epidural administration of dissociative drugs may be one more option in equine analgesia.

**Take Home Message:**
Short term moderate analgesia without adverse effects is obtained with 0.5mg/kg to 1.0mg/kg epidural tiletamine-zolazepam.

**Acknowledgments:**
This study was supported by funds from Fort Dodge Animal Health (Brazil).

**Year Completed:** 2003

**Published Manuscripts/Abstracts:**
Gastrointestinal Tract

Systemic and colonic hemodynamic alterations associated with intravenous administration of ATP-MgCl₂ in healthy anesthetized horses

Authors/Investigators:
Joanne Tetens, DVM, PhD, DACVS; Susan C. Eades, DVM, PhD, DAVCIM; Giselle Hosgood, BVSc, PhD, DACVS; Catherine E. Koch, BA; Rustin M. Moore, DVM, PhD, DACVS.

Description of the Problem:
Gastrointestinal tract disease (colic) is the most common natural cause of death in horses, especially those conditions resulting in strangulation obstruction or ischemia. Gastrointestinal ischemia commonly develops secondary to low-flow or no-flow conditions with volvulus or incarceration of the small intestine and volvulus of the large colon being common causes. Colonic mucosal ATP content decreases 92% during experimental ischemia and recovers to only 44% after reperfusion, thereby limiting substrate availability for cellular metabolic functions. Adenosine triphosphate (ATP) is principally an endothelium-dependent vasodilator that is rapidly metabolized and has a short duration of action. Use of ATP-MgCl₂ following shock in humans and laboratory animals has been shown to improve tissue ATP content; restore organ function, blood flow and perfusion; and improve survival time and survival rate. Previous work in our laboratory demonstrated that intravenous infusion of ATP-MgCl₂ to healthy conscious horses caused a dose-dependent increase in cardiac output and decrease in systemic vascular resistance without appreciable detrimental effects.

Study Purpose/Objectives:
The purpose of the study was to characterize alterations in systemic and local colonic hemodynamic variables associated with IV infusion of ATP-MgCl₂ in healthy anesthetized horses. We hypothesized that administration of ATP-MgCl₂ would cause a rate-dependent decrease in systemic and colonic vascular resistance, principally via vasodilation.

Approach:
Twelve adult horses were used. Six horses were given ATP-MgCl₂, IV, beginning at a rate of 0.1 mg of ATP/kg of body weight/min with incremental increases until a rate of 1.0 mg/kg/min was achieved. The remaining 6 horses were given an equivalent volume of saline (0.9% NaCl) solution over the same time period. Colonic and systemic hemodynamic variables and colonic plasma nitric oxide (NO) concentrations were determined before, during, and after infusion.

Accomplishments/Results/Conclusions:
Infusion of ATP-MgCl₂ caused a rate-dependent decrease in systemic and colonic vascular resistance, principally via its vasodilatory effects. A rate of 0.3 mg of ATP/kg/min caused a significant decrease in systemic and colonic arterial pressure and colonic vascular resistance without a significant corresponding decrease in colonic arterial blood flow. Consistent alterations in NO concentrations of plasma obtained from colonic vasculature were not detected, despite profound vasodilatation of the colonic arterial vasculature.

Benefits to/Impact on the Equine Industry:
This study demonstrated that intravenous administration of ATP-MgCl₂ to anesthetized horses causes a rate-dependent decrease in systemic and colonic vascular resistance. Additional studies are needed to determine the efficacy of ATP-MgCl₂ for use in the treatment of horses with intestinal ischemia. The reduction in blood flow and decreased mucosal ATP content that persists after correction of experimentally induced ischemia of the large colon may be attenuated by intravenous administration of ATP-MgCl₂ by improving blood flow and tissue perfusion, by supplying substrate (ATP) and cofactor (magnesium) to the highly metabolically active colonic mucosal layer.

Horses with a strangulating large colon volvulus, a relatively common cause of colic, often have a guarded to poor prognosis for survival.
Take Home Message:
Results revealed that IV infusion of ATP-MgCl_2 may be beneficial in maintaining colonic perfusion in horses with ischemia of the gastrointestinal tract, provided a sufficient pressure gradient exists to maintain blood flow.

Acknowledgments:
The study was funded by a grant from the LSU Equine Health Studies Program. The authors thank Joshua Austin, Lane Breaux, Lee Ann Fugler, Dr. Ashley Stokes and Dr. Ramswamy M. Chidambaram for technical assistance.

Year Completed: 2001

Published Manuscripts/Abstracts:


Distribution of endothelin-1 immunohistochemical staining in the intestinal tract of clinically healthy horses and in those with small intestinal and large colon strangulation obstruction

Authors/Investigators:
Ramaswamy M. Chidambaram, BVSc, MSc, PhD; Daniel Paulsen, DVM, PhD, DACVP; Ashley Stokes, DVM, PhD; Susan C. Eades, DVM, PhD, DACVIM; Changaram S. Venugopal, BVSc, PhD; Rustin M. Moore DVM, PhD, DACVS.

Description of the Problem: (Can show photos of a horse with joint disease)
Osteoarthritis is the most common joint disease in horses. Chondrocyte apoptosis has been implicated as a major pathological osteoarthritis change in humans and experimental animals, but no other studies have been performed on equine osteoarthritis. Nitric oxide (NO) has been impacted as an important biological mediator of osteoarthritis, and it has been shown that NO production from chondrocytes is significantly greater in osteoarthritis cartilage compared with controls and directly correlates with the severity of osteoarthritis. The major source of NO in articular cartilage is inducible NO synthase. NO reacts with superoxide radicals to form peroxynitrite, and in biological fluids peroxynitrite leads to nitration of aromatic amino acid residues; the presence of such nitration can be used as an in vivo marker of peroxynitrite-mediated NO activity.

Study Purpose/Objectives:
We hypothesized that the presence of ET-1 like immunoreactivity would be present in the gastrointestinal tract of clinically healthy horses, suggesting its role in normal physiological functions, whereas increased expression of ET-1 in blood vessels and intestinal tissues during strangulation obstruction may indicate its involvement in the pathogenesis of these disorders. The objectives of the study were to evaluate the regional distribution of ET-1 in different segments of the gastrointestinal tract of clinically healthy horses, and to determine and compare the expression of ET-1 like immunoreactivity in the intestinal segments of horses with naturally acquired small intestinal and large colon strangulating obstruction.

Approach:
Gastrointestinal tract biopsy specimens were collected from 6 clinically healthy adult horses with no history or clinical or laboratory evidence of disease involving the gastrointestinal tract or cardiovascular system (control, n =6) destined for euthanasia for other reasons. Samples were collected from the control horses immediately after euthanasia. Sections were collected from the greater curvature of stomach, duodenum (1 foot from pylorus), ileum (1 foot from ileocecal junction), cecal body (half way between apex and base), right ventral colon (1 foot from the cecocolic junction), left ventral colon (1 foot from pelvic flexure), left dorsal colon (1 foot from pelvic flexure), right dorsal colon (1 foot from pelvic flexure), and transverse colon (1 foot from pelvic flexure).
foot from diaphragmatic flexure), transverse colon (6 inches from duodenocolic ligament), and the small colon (4 feet from duodenocolic ligament) of control horses. Biopsy specimens were also collected from horses admitted to the LSU Veterinary Teaching Hospital with naturally acquired small intestinal (n=25) and large colon (n=13) strangulation obstruction at the time of exploratory surgery or after euthanasia. Tissues were fixed in 4% aqueous zinc buffered formalin for approximately 24 hours and embedded in paraffin for immunohistochemistry. Immunostaining was performed, using a modified three-step avidin-biotin complex (ABC) method with a Vector Elite ABC Rabbit IgG kit. Three sections from each slide were evaluated twice to minimize variation. A numerical value ranging from 0 to 3 was assigned to each slide based on the relative amount of staining, with control slides (blocked control). The intensity and distribution of the ET-1-like immunoreactivity for the following variables was evaluated: surface epithelium, crypt epithelium, mucosal vasculature, submucosal arterioles and venules (smooth muscle, endothelium), muscularis and serosa. A zero score was assigned if there was no staining present; Grade 1 indicated a mild increase brown staining compared with blocked antibody; Grade 2 indicated a moderate increase brown staining compared with blocked antibody; and Grade 3 indicated an intense increase in brown staining over that with blocked antibody. The affected segments (duodenum, jejunum, ileum, pelvic flexure) of horses with small intestinal and large colon strangulating obstruction were compared with the duodenum, jejunum, ileum, and pelvic flexure scores from control horses.

**Accomplishments/Results/Conclusions:**
In healthy horses, the intestinal surface epithelium and large vessels, mainly the veins and the muscular layer, were positive for ET-1-like immunoreactivity. No staining was observed in mucosal mucus glands and Brunner's glands in duodenum and jejunum, respectively. ET-1-like immunoreactivity was completely abolished by pre-absorption of antisera with an excess amount of ET-1. Under high magnification, ET-1-like immunoreactivity was observed in the cytoplasm of the surface epithelial cells and endothelial cells with moderate staining intensity. The staining was more intense in the apical region, compared with the base, of the surface epithelium. Staining of crypt epithelium and endothelial cells were variable and inconsistent. Veins stained more intensely than arteries. ET-1-like immunoreactivity was diffuse and variable in the endothelium of blood vessels. ET-1-like immunoreactivity staining in the muscularis was diffuse and variable, with the greatest staining intensity in the outer muscular and serosal layers.

Heavy hemorrhage, congestion and lack of structural details in affected tissue segments from horses with naturally acquired intestinal strangulation obstruction made it difficult to subjectively evaluate ET-1-like immunoreactivity. Overall, staining intensity was greater in the serosal and outer muscular layers of the affected intestinal segments. Diffuse staining was also noted in the mucosa and in blood vessels of affected intestinal segments. The staining was intense and diffusely distributed in affected intestinal segments from horses with large colon volvulus, compared with control tissues.

**Benefits to/Impact on the Equine Industry:**
Colic is the leading natural cause of death in adult horses. The gastrointestinal tract is the major target tissue of low-flow induced circulatory shock such as occlusion, volvulus, hemorrhage and or sepsis. Small intestinal and large colon strangulating obstruction account for approximately 10% off all horses with colic, and these conditions are universally fatal without surgical correction. Despite surgical correction and intensive medical care, many horses die owing to the rapidity with which the intestinal mucosa undergoes irreversible damage during ischemia, and subsequent endotoxemia and hypovolemic shock. This study provides evidence that ET-1 is present and likely plays an important role in the physiologic functions in the intestinal tract of normal horses and that it may play a role in the pathogenesis of ischemic-induced injury in horses with naturally acquired large and small intestinal strangulation obstruction. Further study is needed and warranted to determine whether the ET-1 pathway could be modulated to improve intestinal blood flow and perfusion and thus decrease morbidity and mortality of horses with strangulating intestinal lesions.

**Take Home Message:**
The most important findings of this study were the presence of ET-1 like immunoreactivity in the villi and surface epithelium of the small intestine and large intestine, respectively, in healthy horses, which suggest a role for ET-1 in normal gastrointestinal tract function. The ET-1-like immunoreactivity was predominantly confined to the cytoplasm of the surface epithelium with less staining in the crypts, and variable, diffuse ET-like staining in the muscularis. The endothelial lining of blood vessels in different segments of the gastrointestinal tract stained positive for ET-1, suggesting its involvement in the regulation of regional intestinal blood flow. The increased expression of ET-1...
observed in intestinal segments from naturally acquired small and large intestinal strangulation obstruction supports the hypothesis that ET-1 is involved in the pathogenesis of ischemic intestinal injury in horses.

**Acknowledgments:**
This study was funded by a grant from the LSU Equine Health Studies Program. The authors thank Dr. Lais Costa and Catherine Koch for technical assistance.

**Year Completed:** 2002

**Published Manuscripts/Abstracts:**
Chidambaram RM. Distribution of endothelin-1 immunohistochemical staining in the intestinal tract of clinically healthy horses and in those with small intestinal and large colon strangulation obstruction. Role of Endothelin-1 in the Gastrointestinal Tract of Horses in Health and Disease. Doctoral Dissertation, Louisiana State University Graduate School 80-101, May 2003.

**Characterization of endothelin-1 mediated responses of equine cecal smooth muscle**

**Authors/Investigators:**
Chidambaram M. Ramaswamy, BVSc, MS, PhD; Changaram S. Venugopal, BVSc, MSc, MS, PhD; Rustin M. Moore, DVM, PhD, DACVS; Susan C. Eades, DVM, PhD, DACVIM.

**Description of the Problem:**
This is a pilot study to evaluate the involvement of endothelin in gastrointestinal motility in horses. Colic is a common condition seen in horses. Large intestines of horses are very prone to twisting which leads to reduced blood supply and even after surgical correction to restore blood flow, majority of horse die of hemodynamic problems. Chemical mediators such as endothelin and nitric oxide play a major role in such conditions. The purpose the study was to investigate the role of ET and its receptors in such conditions.

**Study Purpose/Objectives:**
The objectives of the study were to (1) determine the responses of longitudinal smooth muscles of cecum to graded concentrations of endothelin-1 and (2) determine the effect of ET receptor antagonists on blocking the effect on the cecal strips due to field stimulation.

**Approach:**
Longitudinal cecal strips from normal horses were prepared (1.5 cm long 4mm wide). Initial tension of 2 g was applied to the strips and an equilibration time of 45 minutes was given. Then concentration response relationships of the strips to graded concentrations of ET-1 were determined. Field stimulations were performed on the tissue strips and the effects of ET receptor antagonists were investigated.

**Accomplishments/Results/Conclusions:**
ET-1 induced contractions were significantly less than those produced by carbachol, cholinomimetic agents. ET-1 may play a role in cecal contractions for movement of ingesta. We failed to document its role in spontaneous contractions of cecum.

**Take Home Message:**
Both ET$_B$ and ET$_A$ receptors are contractile in nature in the longitudinal smooth muscles cecum.

**Acknowledgments:**
Supported by a grant from the LSU Equine Health Studies Program.

**Year Completed:** 2002

**Published Manuscripts/Abstracts:**

**Plasma concentrations of endothelin-like immunoreactivity in healthy horses and horses with naturally acquired gastrointestinal tract disease**

**Authors/Investigators:**
Chidambaram M. Ramaswamy, BVSc, MSc, PhD; Rustin M. Moore, DVM, PhD, DACVS; Thomas L. Seahorn, DVM, MS, DACVIM; Changaram S. Venugopal, BVSc, MSc, MS, PhD; Susan C. Eades, DVM, PhD, DAVCIM.

**Description of the Problem:**
Gastrointestinal tract disease is the leading natural cause of death in horses, especially horses with intestinal strangulating obstruction. Endothelin (ETs) is a family of peptides that exert numerous biological and pathological effects by binding to ET receptors. Endothelins are potent vasoconstrictors and are produced by numerous cells throughout the body. Production of ET by endothelial cells increases during periods of reduced blood flow or ischemia. The sustained reduction in intestinal blood flow after complete arteriovenous occlusion could be due to increased endothelial release of ET-1, with a resulting vasonconstriction.

**Study Purpose/Objectives:**
The objective of the study was to determine and compare plasma endothelin (ET)-like immunoreactivity between healthy horses and those with naturally acquired gastrointestinal tract disorders, and to investigate the relationship between plasma ET-1 like immunoreactivity concentrations and clinical and clinicopathologic variables and survival in affected horses.

**Approach:**
Blood samples were collected via jugular venipuncture from healthy horses and from horses with naturally acquired gastrointestinal tract disorders prior to treatment. The magnitude and duration of abnormal clinical signs were recorded, and clinical variables were assessed via thorough physical examination. Plasma concentrations of ET-like immunoreactivity were measured by use of a radioimmunoassay for human endothelin-1, and CBC and plasma biochemical analyses were performed.

**Accomplishments/Results/Conclusions:**
Plasma ET-like immunoreactivity concentration was significantly increased in horses with gastrointestinal tract disorders, compared with healthy horses. Median plasma concentration of ET-like immunoreactivity was 1.80 pg/ml (range, 1.09 to 3.2 pg/ml) in healthy horses. Plasma ET-like immunoreactivity was greatest in horses with strangulating large-intestinal obstruction (median, 10.02 pg/ml; range, 3.8 to 22.62 pg/ml), peritonitis (9.19 pg/ml; 789 to 25.83 pg/ml), and enterocolitis (8.89 pg/ml; 6.30 to 18.36 pg/ml). Concentration of ET-like immunoreactivity was significantly associated with survival, PCV, and duration of signs of pain. However, correlations for associations with PCV and duration of pain were low.

**Benefits to/Impact on the Equine Industry:**
This study demonstrated that horses with naturally acquired gastrointestinal tract disease have increased circulating plasma ET-like immunoreactivity concentrations, which suggests ET-1 might be involved in the pathophysiology of these conditions. However, further work is needed to ascertain whether these measured increases occur as a result of the pathologic condition or contribute to the pathology. Use of ET-1 receptor antagonists may help clarify the role of ET-1 as a biologic marker of disease or a pathologic mediator involved with colic in horses.

**Take Home Message:**
Horses with gastrointestinal tract disorders have increased plasma concentrations of ET-like immunoreactivity, compared with healthy horses. The greatest values were detected in horses with large-intestinal strangulating obstructions, peritonitis, and enterocolitis. This suggests a potential involvement of ET in the pathogenesis of certain gastrointestinal tract disorders in horses.

**Acknowledgments:**
The study was funded by a grant from the LSU Equine Health Studies Program. The authors thank Catherine Koch, Earnestine Holmes and Marian Waguespack for technical assistance.

**Year Completed:** 2001
Published Manuscripts/Abstracts:

Exercise alters the immune response to equine influenza virus and increases susceptibility to infection

Authors/Investigators:
Robert W. Folsom, DVM; Martha A. Littlefield-Chaubaud, DVM; Dennis D. French, DVM, DABVP; Susan S. Pourciau; L. Mistric; David W. Horohov, PhD.

Description of the Problem:
Equine influenza virus remains the most common pathogen isolated from primary equine respiratory tract infections in North America and continues to be a major health concern for the equine industry in spite of ongoing vaccination programs. Young horses and those involved in competitive activities appear to be at particular risk of infection. Previous work has shown that the immune system of horses can be affected by strenuous exercise. Data was lacking on the possible adverse consequence of exercise-induced alterations in immune function and immunity to influenza virus infection.

Study Purpose/Objectives:
The purpose of this study was to investigate an association between exercise stress-induced inhibition of influenza virus-specific T cell responses and an increased susceptibility to equine influenza virus infection in vaccinated ponies.

Approach:
Twelve mixed breed ponies were reared under influenza virus-free conditions having no exposure either through vaccination or infection. Eight ponies were vaccinated with 2 doses of a commercial equine influenza virus vaccine 6 weeks prior to the study. One group (n=4) of ponies was subjected to a rigorous 5 day exercise program on a high-speed treadmill whereas the second group (n=4) of ponies served as rested controls. A third group of 4 nonvaccinated, rested ponies served as positive controls for the equine influenza virus challenge. Peripheral blood mononuclear cells (PBMC) were collected from jugular venous blood on days 1 and 5 for determination of lymphoproliferative responses to equine influenza virus and for quantitative reverse-transcriptase polymerase chain reaction (RT-PCR) for gamma interferon and interleukin-2 in virus stimulated PBMC cultures. Immediately after the final exercise period, all 12 ponies were fitted with a mask and infected intranasally with aerosolized equine influenza virus. Ponies were monitored daily and nasal secretions were collected daily for equine influenza virus antigen detection. Serum antibody titers to equine influenza virus were determined immediately prior to challenge and 3 weeks post-infection.

Accomplishments/Results/Conclusions:
Exercising ponies for 5 days resulted in a significant suppression of the lymphoproliferative response to equine influenza virus compared to their pre-exercise values and those of the non-exercised, rested ponies. Exercise for 5 days resulted in a significant decrease in gamma interferon, but not IL-2, mRNA levels compared with pre-exercise levels and time-matched samples from rested ponies. Challenge of the ponies led to detection of equine influenza virus antigen in the nasal secretions of exercised and naïve ponies, but not from rested controls. Although no difference was noted in the pre-challenge titers or post-challenge titers between exercised and rested ponies, there were significant increases from the pre-to-post challenge titers for both groups.

Benefits to/Impact on the Equine Industry:
This study demonstrates that rigorous exercise, as experienced by race horses and/or horses used for other strenuous activities are at greater risk for viral respiratory tract disease associated with suppression of their T cell-mediated immune response. Additional studies are warranted to further investigate this phenomenon with the hope of providing improved strategies for prevention of viral respiratory disease in horses involved in strenuous exercise activities.

Take Home Message:
Subjecting vaccinated ponies to a 5 day strenuous exercise program resulted in a significant suppression of their T cell-mediated immune response to equine influenza virus as measured by decreased lymphoproliferation and gamma interferon production measured in vitro. These same ponies also demonstrated increased susceptibility to influenza disease following a challenge exposure to the same strain of virus. Rested ponies that had received the same vaccine and challenge were completely protected from disease. Our results demonstrate that exercise-induced suppression of the equine immune response to influenza virus can be associated with an increased susceptibility to disease.
Immunology

Acknowledgments:
This work was supported by grants from the Grayson-Jockey Club Research Foundation, Inc. and the United States Department of Agriculture National Research Competitive Grants Program.

Year Completed: 2001

Published Manuscripts/Abstracts:

Effect of aging on T cell responses in horses

Authors/Investigators:
David W. Horohov, PhD; J. H. Kydd; D. Hannat.

Description of the Problem:
Immunosenescence is characterized by an age-related decline of the immune function leading to increased risk of infection, tumor development and autoimmune disease. Changes in T lymphocyte function underlie much of the age-related decrease in the protective immune response. Several studies have shown that immune dysfunction in aged mice and humans may be due to a defect in the production of IL-2 by helper T cells. Horses over 20 years of age comprise approximately 15% of the horse population, and many of these are actively involved in various equestrian sports, recreational and breeding activities. Advancing age in horses is associated with declining body condition, muscle tone and their general well-being. Aging in horses has previously been shown to be characterized by reduced immunocompetency.

Study Purpose/Objectives:
The purpose of this study was to provide an initial description and characterization of immunosenescence in horses.

Approach:
Two groups of ponies were used in this study: Group 1 included 11 mixed breed animals of at least 20 years of age and Group 2 consisted of 10 ponies aged 2 to 3 years of age. All ponies were determined to be healthy at the start of the study and were maintained on grass pasture. Blood samples were collected while the ponies were on pasture except for the cortisol study whereby baseline samples were collected at 4-hour intervals for 20 hours. Cortisol was measured by a competitive immunoassay. Blood was collected from the jugular vein for complete blood count and differential. Blood was also collected for peripheral blood mononuclear cell isolation, which was subsequently used for lymphoproliferation assays, in vitro bulk mitogen stimulation and two-color immunocytochemical labeling. Flow cytometric analyses were performed using a FACScan where gates based on forward and side scatter were focused on lymphocytes.

Accomplishments/Results/Conclusions:
There was a significant decrease in lymphocytes, monocytes and eosinophils in aged ponies compared with young ponies. There were no significant differences in CD4:CD8 T cell ratios between aged and younger ponies. All other hematologic variables including plasma glucose were within normal limits and not different between the two groups of ponies. Aged ponies had increased levels of plasma cortisol compared with younger ponies and there was also a diurnal effect noted. Lymphocytes from older ponies had a reduced proliferative response to the mitogens at all doses tested. Although there was no difference in the overall IL-2 receptor (IL-2R) expression between young and aged ponies, there was a greater percentage of IL-2R⁺ CD8⁺ in aged ponies compared with younger ponies.

Benefits to/Impact on the Equine Industry:
The results of this preliminary study indicate that age-related decrease in the proliferative response to mitogens is not due to a failure to produce or respond to IL-2 but probably involves some other process.

Take Home Message:
Advancing age in horses is often associated with declining body condition, muscle tone and general well-being. This study demonstrated that peripheral blood mononuclear cells from aged ponies exhibit decreased proliferative responses to mitogens, which is consistent with other reports of aging on T-cell mediated proliferative responsiveness in horses and other species. While the precise mechanism responsible for this decreased proliferative response is unknown, altered patterns of cytokine production have been implicated and deserve further investigation.
Acknowledgments:
The authors acknowledge the Home of Rest for Horses for financial support of this study and Burroughs-Wellcome Trust which supported Dr. Horohov’s sabbatical work. The authors thank Dr. Eve Pleydell, Mr. Paul Wilton, and staff of the Animal Health Trust and Dr. Mark Holmes of Cambridge University for technical assistance.

Year Completed:  2001

Published Manuscripts/Abstracts:
Laminitis

Effects of an endothelin receptor antagonist on digital hemodynamics and Starling forces in horses administered carbohydrate overload

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

Description of the Problem:
Acute laminitis is a severely debilitating, excruciatingly painful, and often life-threatening or career-ending disease of the sensitive and insensitive laminae of the equine digit. Numerous studies have suggested that hemodynamic alterations, especially venoconstriction, occur in the equine digit during the developmental stages of laminitis, and that these contribute to the development of acute laminitis. Endothelin-1 (ET-1) is a potent vasoconstrictor peptide produced by endothelial cells, vascular smooth muscle cells, and macrophages. It not only induces prolonged vasoconstriction in arteries and arterioles, but also causes intense profound vasoconstriction in both the systemic and pulmonary circulations. We have measured high ET-1 plasma concentrations systemically in horses with acute gastrointestinal tract disease, and in the digital venous plasma of horses with experimental endotoxemia and black walnut extract-induced laminitis. Additionally, we have previously shown that ET-1 causes a concentration-dependent, profound and long-lasting in vitro contraction of colonic and palmar digital arterial and venous rings in horses and that treatment with an ET receptor antagonist will prevent the ET-1 induced contraction. Nitroglycerine (NG) causes vasodilation by releasing nitric oxide (NO); we have previously shown in vitro and in vivo that NG causes digital arterial and venous relaxation. Thus, we believe that administration of an ET receptor antagonist and nitroglycerine will improve the hemodynamic and Starling force alterations that occur with carbohydrate overload induced laminitis

Study Purpose/Objectives:
We hypothesized that local infusion of an endothelin receptor antagonist and nitroglycerine would improve digital hemodynamics and Starling forces in horses administered CHO overload. The studies reported herein were performed to evaluate serial changes in digital hemodynamics and Starling forces in horses with carbohydrate overload (CHO)-induced laminitis and to determine the effects of an ET receptor antagonist on the vascular dysfunction in laminitis

Approach:
Horses (n=20) were administered a CHO ration by nasogastric tube. Right atrial pressure, body temperature, heart rate, respiratory rate, coronary band skin temperature, and hoof wall surface temperature were recorded, and digital arterial blood flow, digital arterial pressure, and digital vein pressure were recorded at baseline and hourly after the CHO. Digital and jugular venous blood was collected for measurement of ET-1 concentrations. Horses were evaluated in 4 groups of 5 each in which horses were: (1) treated with saline and anesthetized for microvascular assessment 8 hours after CHO; (2) treated with saline and anesthetized for microvascular assessment 16 hours after CHO; (3) treated with an ET receptor antagonist and anesthetized for microvascular assessment 8 hours after CHO; and (4) treated with an ET receptor antagonist and anesthetized for microvascular assessment 16 hours after CHO. Under general anesthesia, an isolated perfused digit was established to measure digital blood flow, digital arterial pressure, digital venous pressure, digital capillary pressure, total resistance, precapillary resistance, and postcapillary resistance. Then, NG was infused in an amount that resulted in a 10^{-5} M in the digital blood based, and then digital microvascular assessment was repeated.

Accomplishments/Results/Conclusions:
Administration of CHO did not alter digital arterial pressure, digital venous pressure, digital arterial blood flow, or hoof wall surface temperature, but did cause a significant decrease in right atrial pressure by 14 hours. Administration of saline or an ET receptor antagonist had no effect on any of these variables. In the isolated digit in the anesthetized horses, CHO resulted in a significant decrease in digital blood flow associated with a significant increase in total
resistance. Postcapillary resistance was significantly increased at 16 hours after CHO. Treatment with the ET receptor antagonist and NG caused significant decreases in total resistance with the greatest component of the decrease resulting from combined treatment with both the ET antagonist and NG. Treatment with the ET antagonist caused a significant decrease in postcapillary resistance at 16 hours. There were no significant changes in jugular or digital venous ET-1 concentrations after CHO.

**Benefits to/Impact on the Equine Industry:**
Treatment with an ET receptor antagonist resulted in significant improvement in vascular resistance in the isolated perfused digit of anesthetized horses after CHO. The ET antagonist significantly decreased postcapillary resistance at 16 hours after CHO overload. The greatest improvement in vascular resistance and blood flow occurred after treatment with both the ET antagonist and NG. Laminitis caused by CHO was not accompanied by significant increases in vascular endothelin concentration.

**Take Home Message:**
Although the treatment with the ET antagonist improved vascular function in horses after CHO, further study is needed to document that endogenous ET-1 production is responsible for the vascular dysfunction in equine laminitis.

Acknowledgments:
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**Year Completed:** 2002

**Published Manuscripts/Abstracts:**


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**Role of endothelin-1 in naturally-acquired equine laminitis**

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

**Description of the Problem:**
The laminae of the equine hoof are soft tissues that suspend the most distal bone (distal phalanx; P₃) within the hoof capsule and support the tremendous weight of the horse. Acute laminitis (inflammation and disruption of the laminae) is a severely debilitating, potentially career-ending and often life-threatening disease that is often associated with other diseases such as colic, retained fetal membranes and subsequent metritis, pleuropneumonia, and other diseases. Endothelin-1 (ET-1; a very potent vasoconstrictor) blood concentrations are elevated in many diseases, such as these, and laminar tissue expressions of ET-1 are elevated in horses with laminitis. The fundamental pathophysiologic mechanisms of acute laminitis are believed to be vasoconstriction followed by edema, ischemia, and necrosis of the interdigitating laminae ultimately leading to mechanical failure with rotation or sinking of the distal phalanx. The study of ET-1 as a potential key mediator of acute laminitis will aid in our understanding of this devastating disease.

**Study Purpose/Objectives:**
The purposes of the initial in vitro studies were: (1) utilizing palmar digital vessel rings from non-laminitic horses, compare the effectiveness of two ET receptor antagonists, determine the concentrations that effectively block the vascular effects of ET-1, and compare the effectiveness of ET-1 to previously studied potent vasoconstrictors; and (2) compare these responses to palmar digital vessel rings from horses with naturally-acquired laminitis.

A lateral radiograph of the equine digit. Rotation of the distal phalanx (P₃) from its normal position (parallel to the dorsal hoof wall) is a common sequella of laminar tissue injury due to decreased delivery of oxygen and nutrients.
The purpose of the subsequent studies of blood acquired from horses with and without naturally-acquired laminitis was to quantify jugular (systemic) venous (JV) and cephalic (forelimb) venous (CV) plasma ET-like immunoreactivity.

**Approach:**
In addition to vessel rings collected from a previous study of normal horses, palmar digital arteries and veins were collected from 9 laminitic adult horses. Vessels were placed in an organ bath solution, cut into rings, and one side fixed to the floor of an organ bath and the other to a force-displacement transducer to measure vessel contraction. The first study was conducted using vessel rings from non-laminitic horses to compare two ET receptor antagonists (PD 142893 and PD 145065) and to examine the effects of ET-1. The second study utilized vessel rings from laminitic horses and examined the effects of ET-1, the effects of the ET antagonist, and contractile effects were determined for the known vasoconstrictors norepinephrine (NE) and histamine (HST). During the subsequent study, systemic and digital blood samples were collected from 46 horses (34 with laminitis; 12 normal) and plasma ET-like immunoreactivities were evaluated.

**Accomplishments/Results/Conclusions:**
ET-1 is a potent vasoconstrictor and has similar efficacy to other known potent vasoconstrictors, such as NE. In response to ET-1, veins were more sensitive and responded with a greater maximal contraction than arteries. In vessel rings from non-laminitic and laminitic horses, ET antagonist PD145065 at the [10-5 M] inhibited the contractile effects of ET-1. Vessel rings from non-laminitic horses responded in the same manner as those from laminitic horses to the contractile effects of ET-1 and the antagonist was effective in both groups. This suggests that this ET receptor antagonist may have potential use in horses before and after development of laminitis. Cephalic venous plasma ET-like immunoreactivity had a trend for higher values in horses with laminitis compared with normal horses.

**Benefits to/Impact on the Equine Industry:**
During a number of diseases characterized by altered circulating levels of mediators, receptor numbers and function can differ compared with the normal physiologic state. The effectiveness of the ET antagonist in the digital vasculature of horses with laminitis, demonstrated during this study, supports its potential benefit when administered as a preventative and as a treatment of this devastating disease. Additional studies are required to further define the effectiveness of this ET antagonist for equine laminitis.

**Take Home Message:**
Endothelin, a potent vasoconstrictor produced by the lining of blood vessels, appears to play a role in the development of naturally-acquired laminitis in horses. In addition, the ET antagonist that is the subject of this in vitro study proved to be effective in horses already affected by this disease. These findings together demonstrate the need for further research of this drug in treating laminitis.

**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 P. Holmes; Catherine E. Koch, MS; and Lee Ann Curtis, DVM for technical assistance.

**Year Completed:** 2002

**Published Manuscripts/Abstracts:**


Quantification of laminar microcirculatory perfusion in normal horses using isotopic-labeled microspheres

Authors/Investigators:
Ashley M. Stokes, DVM, PhD; Diane Savois; Susan C. Eades, DVM, PhD, DACVIM; Mike Keowen; Frank Garza, Jr. MS; Rustin M. Moore, DVM, PhD, DACVS.

Description of the Problem:
Although the pathogenesis of equine laminitis is not fully understood, the fundamental mechanisms are believed to be decreased blood flow to the soft tissues of the digit due to venoconstriction, edema, collapse of the capillaries, lack of oxygen and nutrient delivery to the laminae, and ultimately breakdown of the interdigitating laminae and structural failure of the foot. Arteriovenous shunts are connections directly between arteries (blood delivery to the foot) and veins (blood return toward the heart). Previous studies have identified numerous shunts in the digit of the horse although their role remains unclear. Opening of these shunts would divert blood (oxygen and nutrients) away from the soft tissues of the digit; this is thought to occur during the development of acute laminitis. Direct measurement of blood flow to the tissues of the digit has been very limited since these soft tissues are located between the hoof wall and bone. Availability of microspheres allows for us to measure laminar perfusion as an indication of blood flow distribution in the foot.

Study Purpose/Objectives:
Our hypothesis was that within the digit of normal healthy horses, blood delivery would be equal to the three regions (proximal, middle, and distal) of the laminae and arteriovenous shunts would be closed. The specific objectives of this study were to inject colored isotopic-labeled microspheres intra-arterially into the digital circulation of normal laminitis-free horses. We would measure microsphere distribution in the three regions of the laminae and measure the amount returning in the venous circulation.

Approach:
Six horses free of laminitis were instrumented with palmar digital arterial and venous catheters. Two million colored isotopic-labeled 15 um microspheres were injected into the digital arterial circulation. A reference venous sample was collected during microsphere injection. Laminar tissues were collected and divided into proximal, middle, and distal samples. Blood and tissue samples were assayed by the manufacturer using neutron activation for quantification of microspheres.

Accomplishments/Results/Conclusions:
Microsphere activity was significantly (p < 0.001) higher in the digital venous blood compared to each of the regions of laminae sampled. There were no significant differences (p > 0.05) between any of the regions of laminae. As hypothesized, microsphere distribution was equal in the three regions of laminae. A vast majority of microspheres were found in the digital venous circulation following digital arterial injection in normal laminitis-free horses, strongly suggestive of open arteriovenous shunts. This finding did not support our hypothesis that during normal physiologic states arteriovenous shunts would be closed and suggests that during the development of laminitis, altered function (closing) of the arteriovenous shunts may inhibit a protective mechanism within the digit. Further study of the normal states of the arteriovenous shunts in the equine digit, and their role in diseases, is currently being studied in our laboratory.

Benefits to/Impact on the Equine Industry:
In addition to the extreme pain and debilitation of affected horses, there is substantial emotional distress and frustration for owners, trainers and veterinarians. Annual monetary losses

Photomicrograph of the distal laminae in the digit of normal horses after injection of colored isotopic-labeled microspheres of 15 um diameter. (10x magnification) These microspheres lodge in the capillaries and allow for us to measure blood flow to tissues. A lack (or shunting) of blood flow would result in a reduction of microsphere present in the tissue sample.
related to laminitis have been estimated at greater than $13 million associated with its diagnosis, treatment and loss of horses subsequent to complications. Understanding the basic mechanisms of blood flow to the foot will enable better investigation of potential therapeutics for the prevention and treatment of numerous diseases of the equine digit.

Take Home Message:
Results of this study suggest that perfusion to the laminar tissues is evenly distributed in normal horses, and arteriovenous shunts are present and open during normal physiologic states. The finding of equal perfusion of the regions of the laminae serves as a comparison for studies examining perfusion of the laminar tissues during the developmental stages of acute laminitis. Further research is needed to define the role and function of these arteriovenous shunts in physiologic and pathophysiologic states.

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

Year Completed: 2003

Published Manuscripts/Abstracts:

Assessment of apoptosis in epidermal laminar cells in clinically healthy horses and those with naturally acquired and experimentally induced laminitis

Authors/Investigators:
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Description of the Problem:
Despite extensive research and advances made in our knowledge of this complex disease, the pathophysiology of acute laminitis remains poorly understood. The term apoptosis was first used to describe a distinct form of cell death with features different from necrosis. These dying cells that undergo apoptosis develop an active process of fragmentation, chromatin condensation, membrane blebbing and cell shrinkage, resulting in membrane-enclosed fragments (apoptotic bodies) that are phagocytized by macrophages and other adjacent cells. Apoptosis can also occur physiologically to assist with the body’s removal of senescent cells. Apoptosis is triggered by numerous agents such as cytokines, hormones and free radicals; these agents can also activate caspases, a family of proteases that initiate a cascade that results in apoptosis.

Study Purpose/Objectives:
The hypothesis was that apoptosis would be detectable in digital laminae of clinically healthy horses, and compared with those findings, the number of apoptotic cells would be increased in horses with laminitis. The objective of the study was to determine and compare the number, type, location, and distribution of apoptotic epidermal cells in the laminae of clinically healthy horses and those with naturally acquired and experimentally induced laminitis.

Approach:
Formalin-fixed samples of digital lamellar tissue were used from 47 horses, including 7 clinically normal control horses, 4 with acute and 7 with chronic naturally acquired laminitis, and 11 with black walnut extract-induced and 18 with carbohydrate overload-induced experimental laminitis. Paraffin-embedded tissues were processed and stained for DNA fragmentation with the terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL) technique. Differential immunohistochemical staining for caspase 3 and 14 were used to confirm apoptosis.

Accomplishments/Results/Conclusions:
The number of TUNEL-positive epidermal cells per 0.1mm of primary laminae was significantly greater in the acute naturally acquired laminitis group than in the other groups. In the acute laminitis group, there were 17- and 1,025-times as many TUNEL positive basal layer cells and keratinocytes, respectively, compared with the clinically normal control horses. Apoptosis of TUNEL-positive basal layer cells was confirmed by results of caspase 3 immunohistochemical
staining. The TUNEL-positive keratinocytes did not stain for caspase 3 or 14. The large number of apoptotic basal layer cells detected in the lamellar tissue of horses with acute naturally acquired laminitis suggests that apoptosis may be important in the development of acute laminitis. The role of the large number of TUNEL-positive keratinocytes detected in the interface of primary and secondary epidermal laminae of horses with acute laminitis remains to elucidated.

**Benefits to/Impact on the Equine Industry:**
Laminitis is considered to be one of the most important diseases in the equine industry. It has been estimated that approximately 15% of horses in the United States develop laminitis during their lifetime and that 75% of horses admitted to referral hospitals with laminitis are eventually euthanatized because of the extreme pain and debilitation associated with this disease. Additionally, there is considerable emotional distress and frustration for owners, trainers and veterinarians. This disease has a substantial economic impact on the equine industry because of the costs associated with diagnosis and treatment, and the resulting morbidity and mortality. The results of this study advance our understanding of this complex disease and may help to unravel the mysteries regarding the pathogenesis of the initiating and propagating events involved with laminitis.

**Take Home Message:**
The number of apoptotic cells in the equine hoof during the normal physiologic state is low. The increased number of apoptotic basal layer cells detected in horses with laminitis may indicate that apoptosis is an important factor in the pathogenesis of this disease. Moreover, detection of cells with DNA fragmentation in the interface of primary and secondary epidermal laminae in horses with acute naturally acquired laminitis may indicate that necrosis of these keratinocytes leads to laminitis-associated structural failure of the lamellae. Further study of the apoptotic pathway may provide support to one of the existing theories regarding the pathogenesis of laminitis or may suggest the involvement of novel mechanisms.

**Acknowledgments:**
This study was funded by grants from the Grayson-Jockey Club Research Foundation, Inc., United States Department of Agriculture National Research Initiative, LSU Equine Health Studies Program, and the Coordenancao de Aperfeicoamento de Pessoal de Nivel Superior do Ministerio da Educacao e Cultura.

**Year Completed:** 2003

**Published Manuscripts/Abstracts:**


Effect of topically applied nitroglycerine on digital venous plasma nitric oxide concentrations and digital blood flow in healthy conscious horses

Authors/Investigators: Meaghan H. Gilhooly, DVM; Susan C. Eades, DVM, PhD, DACVIM; Ashley M. Stokes, DVM, PhD; Rustin M. Moore, DVM, PhD, DACVS.

Description of the Problem: Laminitis is a debilitating, excruciatingly painful, and often career-ending or life-threatening disease of adult horses. Although the pathogenesis of laminitis is still unclear, the current hypothesis suggests that vasoconstriction of the digital vasculature arises from an imbalance of Starling forces and two endogenous vasoactive substances, nitric oxide (NO; decreased amounts leading to vasoconstriction) and endothelin-1 (ET-1; increased amounts leading to vasoconstriction), thereby causing laminar ischemic necrosis. Nitric oxide has been identified as a potent vasodilator. Currently, nitroglycerine (NTG) patches are applied to the skin over the palmar digital vessels as a treatment of laminitis. The general belief is that NO diffuses transdermally from the patches into the digital blood and causes relaxation of digital vascular smooth muscle with subsequent vasodilatation and improved blood flow and laminar perfusion. Further, this is believed to possibly reverse some of the ischemic effects of laminitis.

Study Purpose/Objectives: The study hypothesis was that transdermally applied NTG patches would increase digital venous plasma NO concentrations and increase digital blood flow in horses. The objective of the study was to document that NTG patches result in increased NO concentrations in the digital vasculature with a subsequent increase in digital blood flow.

Approach: Eight healthy adult horses with ultrasonic Doppler flow probes placed around their palmar digital arteries had the medial digital vein catheterized for blood collection. Two horses received an intra-arterial infusion of a NTG solution into the medial palmar digital artery in a pilot study. Either NTG patches (4 horses) or 2% NTG ointment (2 horses) were applied to the palmar digital vessels for 120 minutes. Digital blood flow and digital venous plasma NO concentrations were measured serially before, during and after application of transdermal NTG.

Accomplishments/Results/Conclusions: Digital blood flow and digital venous plasma NO concentrations were measured serially before, during and after application of transdermal NTG. Transdermal NTG did not cause a significant increase in the digital venous plasma NO concentration or digital blood flow.

Benefits to/Impact on the Equine Industry: This study demonstrated that topically applied NTG does not increase digital venous plasma NO concentrations or digital blood flow in clinically healthy horses. Although it has not been evaluated in horses with acute laminitis or in horses predisposed to developing laminitis, it is unlikely that topically applied NTG would improve digital blood flow in these horses either.

Take Home Message: Transdermal application of NTG did not increase digital blood flow or digital venous plasma NO concentration in healthy, conscious horses. Further studies are warranted to further determine the effect of...
transdermal NTG application on nitric oxide concentrations and blood flow in laminitic horses.

Acknowledgments:
The study was funded by a grant from the LSU-SVM Merck-Merial Summer Student Research Program and the LSU Equine Health Studies Program. The authors thank Angelica Veitch, Catherine Koch, Michael Keowen, Frank Garza and Michael Broussard for technical assistance.

Year Completed: 2003

Published Manuscripts/Abstracts:


Role of endothelin and nitric oxide in equine laminitis

Authors/Investigators:
Ashley M. Stokes, DVM, PhD; Susan C. Eades, DVM, PhD, DACVIM; Catherine Koch, MS; Frank Garza, Jr., MS; Mike Keowen; Rustin M. Moore, DVM, PhD, DACVS.

Description of the Problem:
Ingestion of black walnut shavings by horses has historically led to the development of laminitis. Laminitis induced by black walnut extract (BWE) administration is associated with decreased digital venous blood flow, increased drive of fluid into the laminar tissues from the arterial side, and increased pressure within the laminar tissues pressing on the capillary bed. These events lead to a “compartment-like syndrome” characterized by collapse of the digital capillaries, which result in laminar ischemia and necrosis, and eventual devastating separation of the distal phalanx from the hoof wall. Based on our previous research, endothelin-1 (ET-1) has been shown to be particularly effective in causing vasoconstriction in equine digital vessels in vitro, and in reducing in vivo digital blood flow in normal horses when infused into the palmar digital artery.

Study Purpose/Objectives:
Our global hypothesis is that the initiating factor in the onset of acute laminitis in horses is a disruption in the balance between endothelium-derived nitric oxide and ET-1, which leads to digital vasoconstriction and subsequent laminar ischemic necrosis. Our objectives were to examine digital hemodynamics, plasma ET levels, hematologic parameters, and physical examination parameters before and after induction of acute laminitis using BWE and evaluate the usefulness of the ET antagonist PD145065 in attenuating the blood flow alterations. Another specific objective of this study was to determine the effects of an ET antagonist and nitroglycerine (NG), a NO donor, on Starling forces at the conclusion of the BWE study.

Approach:
Fourteen adult horses free of laminitis and gastrointestinal disease were administered BWE for induction of acute laminitis. PD145065 was administered into the digital circulation in 7 horses and the remaining 7 were administered saline as control. Baseline blood flow (BF), palmar digital venous pressure (MDVP), palmar arterial pressure (MPAP), and systemic arterial pressure (MAP), ET-like plasma immunoreactivity, CBC parameters, jugular and digital venous platelet/neutrophil aggregate counts, TPR, and clinical signs of laminitis were evaluated at three intervals starting 8 hours before and then hourly after BWE administration. The study was concluded once Obel stage 1 was reached and Starling forces were measured.
Laminitis

Accomplishments/Results/Conclusions:
Eleven horses developed Obel grade 1 laminitis. An initial period of reduced blood flow occurred early in the development of laminitis followed by a period of increased flow that corresponded with the demonstration of clinical signs of laminitis. Equine digital venous plasma ET-1 concentrations increased after BWE administration and remained elevated as Obel stage 1 was reached. Other variables followed similar patterns to previously published studies using this model. Measurements obtained with the Starling force preparation revealed that treatment of horses with experimentally-induced laminitis with the ET receptor antagonist decreased vascular resistance thereby improving digital perfusion. Horses receiving nitroglycerine had further improvements in Starling force values. Together with our current knowledge of the pathogenesis of laminitis, ET-1 may be an important factor in the cascade of events that occur during the development of acute laminitis. The use of the endothelin antagonist still remains a possibility for the prevention and treatment of acute laminitis.

Benefits to/Impact on the Equine Industry:
Laminitis is considered one of the most important diseases in the equine industry with an estimated 15% of horses developing laminitis during their lifetime, and 75% of those admitted to referral hospitals eventually requiring euthanasia. The identification of a key mediator in this disease will allow for the development of effective preventatives and therapeutics; thus, we could substantially decrease suffering and loss of horses and decrease monetary loss within the equine industry. We believe this study has greatly advanced our knowledge regarding the hemodynamic alterations of this disease and support continued research in this area.

Take Home Message:
Results of this study suggest that alterations in digital blood flow are the initial events in the development of laminitis and ET-1 is a likely mediator of the vasoconstrictive components. Therefore, correction of these vascular abnormalities should remain the target of therapeutic investigations.

Acknowledgments:
This study was funded by a grant from the Grayson-Jockey Club Research Foundation, Inc.

Year Completed: 2001

Published Manuscripts/Abstracts:


Equine Health Studies Program 2005 Research Report


Stokes Holm AM, Eades SC, Moore RM. Effects of an endothelin-1 receptor antagonist and nitroglycerine on digital Starling forces in horses with black walnut extract induced laminitis. FASEB J 16 (4) A436, 2002.

Nitric oxide and endothelin-1 synthesis by cultured equine digital endothelial cells exposed to endotoxin and cytokines

Authors/Investigators:
Lee Ann Fugler, DVM; Susan C. Eades, DVM, PhD, DACVIM; Robert E. Truax, PhD; Ashley M. Stokes, DVM, PhD; Frank Garza, MS; Rustin M. Moore, DVM, PhD, DACVS.

Description of Problem:
Laminitis is a painful and often life-threatening disease of the equine foot. Although the pathogenesis of laminitis remains unknown, digital hypoperfusion due to vasoconstriction is the predominant hypothesis. Endothelium controls vascular relaxation and constriction through release of nitric oxide (NO) and endothelin-1 (ET-1), respectively. Many diseases of horses lead to endotoxemia, which has been closely associated with laminitis, but the link between the two has not been identified. It is possible that the unidentified central mediator could be ET-1 because of its potent and long-lasting vasoconstricting action. Endotoxin (LPS) also induces synthesis of tumor necrosis factor-α (TNF-α) and interleukin-1α (IL-1α), cytokines shown to affect endothelial homeostasis. We hypothesized that laminitis occurring secondary to endotoxemia may be due to an LPS-, TNF-α-, and/or IL-1α-mediated decrease in NO synthesis and increase in ET-1 synthesis by endothelial cells resulting in vasoconstriction. Therefore, the purpose of this study was to determine the effects of LPS, TNF-α, and IL-1α on cultured digital arterial and venous endothelial cell synthesis of NO and ET-1.

Cannulation of palmar digital artery and vein of a fresh cadaver limb for collection of endothelial cells.
**Study Purpose/Objectives:**
The objectives of this study were to (1) successfully culture equine palmar digital arterial and venous endothelial cells; (2) measure NO synthesis in the endothelial cell culture medium of non-stimulated cells and cells exposed to LPS and cytokines; and (3) measure ET-like immunoreactivity in the endothelial cell culture medium of non-stimulated cells and cells exposed to LPS and cytokines.

**Approach:**
Immediately following euthanasia, endothelial cells were harvested from the medial and lateral palmar digital arteries and veins of both forelimbs of 4 adult horses. After establishment of digital arterial and venous endothelial cell cultures, cells were incubated with media alone (non-stimulated), IL-1α, TNF-α, LPS, IL-1α + LPS, and TNF-α + LPS for 4 and 24 hours. After incubation, immunohistochemical staining for von Willebrand factor was performed on the endothelial cells. The culture supernatant was collected for analyses of NO and ET-1 using an amperometric method and ELISA, respectively.

**Accomplishments/Results/Conclusions:**
All cells stained positively for von Willebrand factor and exhibited the "cobblestone" morphology typical of endothelial cells. Nitric oxide concentrations were significantly greater in arterial samples exposed to LPS, LPS + IL-1α, and LPS + TNF-α at both 4 and 24 hour incubations when compared with non-stimulated cells or those exposed to IL-1α and TNF-α. Nitric oxide concentrations were significantly greater in venous samples exposed to LPS, LPS + IL-1α, and LPS + TNF-α at 4 hours and in LPS and LPS + TNF-α treated samples at 24 hours compared with non-stimulated cells or those exposed to IL-1α and TNF-α. There were no significant differences in ET-like immunoreactivity between treatment groups at 4 hours for either arterial or venous samples. At 24 hours, ET-like immunoreactivity was significantly decreased in arterial and venous samples exposed to LPS and LPS + IL-1α, and in venous samples exposed to LPS + TNF-α, compared with non-stimulated samples or those exposed to IL-1α and TNF-α. In vitro administration of LPS results in altered NO and ET-1 concentrations in cultured digital endothelial cells. Further study is needed to determine the implications of these results in the role of endotoxin and cytokines in the pathophysiology of laminitis.

**Benefits to/Impact on the Equine Industry:**
The results of this study may act as a building block for future research into the association between equine laminitis and endotoxemia. This study, in combination with past and future studies, will hopefully provide further insight into the pathophysiology of equine laminitis and the alleviation of this crippling disease.

**Take Home Message:**
Equine laminitis is a painful and crippling disease which is not fully understood. This study investigated the role of bacterial toxins and inflammatory mediators on the synthesis of vascular mediators within the foot. The results indicate that alterations in these vascular mediators do occur when exposed to bacterial toxins and could play an important role in laminitis. Further research is needed to determine how these mediators are involved in this devastating disease.

**Acknowledgments:**
This study was funded by grants from the Merck-Merial Student Scholars Program and the LSU-SVM Equine Health Studies Program. The authors thank Dr. Britta Leise, Ms. Catherine Koch, and Ms. Mae Lopez for technical assistance.

**Year Completed:** 2002

**Published Manuscripts/Abstracts:**

**Characterization of the role of endothelin-1 in platelet-neutrophil aggregation and its association with acute laminitis in horses**

**Authors/Investigators:**
Ashley M. Stokes, DVM, PhD; Susan C. Eades, DVM, PhD, DACVIM; Casey J. LeBlanc, DVM PhD; Julie Millard; Rustin M. Moore, DVM, PhD, DACVS.

**Description of the Problem:**
Laminitis is a debilitating, excruciatingly painful, and often life-threatening or career-ending disease of the sensitive and insensitive laminae of the equine digit. Although the pathogenesis is not fully understood, the fundamental
mechanisms are believed to be hypoperfusion due to vasoconstriction and microthrombi formation. In normal physiologic states, the endothelium is responsible for production of vasoactive substances, such as nitric oxide (vasodilator) and endothelin-1 (vasoconstrictor), which regulate vascular tone. These substances also influence platelet activation and white blood cell adherence (especially platelets adhered with neutrophils).

**Study Purpose/Objectives:**
Our global hypothesis is that the initiating factor in the onset of acute laminitis in horses is a disruption in the balance between nitric oxide and endothelin-1 which leads to digital vasoconstriction, increased platelet-neutrophil aggregation, and subsequent laminar ischemic necrosis. The specific aim of the proposed study is to determine the effects of an endothelin antagonist (PD145065) on platelet-neutrophil aggregation in horses using an acute laminitis model.

**Approach:**
Fourteen adult horses free of laminitis and gastrointestinal disease were administered black walnut extract (BWE) as part of a larger study for induction of acute laminitis. PD145065 was administered into the digital circulation in 7 horses and the remaining 7 were administered saline as control. Systemic (jugular) and digital venous platelet/neutrophil aggregate counts were evaluated at three intervals starting 8 hours before and then hourly after BWE administration (2g shavings/kg body weight). The study was concluded once horses demonstrated early signs of laminitis (Obel stage 1).

**Accomplishments/Results/Conclusions:**
Eleven horses developed Obel grade 1 laminitis. Jugular venous platelet-neutrophil aggregates did not significantly change over time and were not significantly different from palmar digital venous platelet/neutrophil aggregate counts. Palmar digital venous platelet/neutrophil aggregate counts significantly changed over time and were significantly less at 4, 7, and 9 hours post-BWE. ET antagonist administration did not alter palmar digital venous platelet/neutrophil aggregate counts. The formation of platelet/neutrophil aggregates did not appear to be a significant contributor to the development of the vascular alterations observed with the administration of BWE in this study. Causes of the decreases in P/N aggregate formation observed at three time points are unclear and warrant further investigation into the formation of these aggregates.

**Benefits to/Impact on the Equine Industry:**
The lack of complete understanding regarding the pathophysiology of laminitis has hindered the ability to develop effective preventatives and therapeutics for this devastating and common disease. This study aids our knowledge concerning the role of cellular aggregates in decreasing blood flow to the laminar tissues during BWE-induced laminitis. Our data suggests vascular mediators (i.e. endothelin-1) play a larger role than cellular aggregates in causing the observed alterations in oxygen and nutrient delivery to the laminar tissues that leads to the development of laminitis.

**Take Home Message:**
Decreased digital perfusion is considered an important component of the development of acute laminitis; however, during this study the formation of cellular aggregates did not appear to be responsible for the altered hemodynamics observed. Other factors, such as increases in vasoconstrictive substances, likely contribute to these vascular alterations and warrant further study.

**Acknowledgments:**
This study was funded by grants from the Merck-Merial Student Scholars Program and the LSU-SVM Equine Health Studies Program.

**Year Completed:** 2001

**Published Manuscripts/Abstracts:** None yet.
Laminitis most commonly occurs in the front feet since horses bear about 60% of their weight in their front limbs but laminitis in all four feet is not uncommon, such as this horse is experiencing.

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, 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 vasoconstrictor, 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 Purpose/Objectives:
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 funded by a grant from the LSU School of Veterinary Medicine USDA 1433 Formula Funds.

**Year Completed:** 1999

**Published Manuscripts/Abstracts:**


**Presence and distribution of matrix metalloproteinase-2 and -9 immunohistochemical staining in laminar tissue of clinically healthy and laminitic horses**

**Authors/Investigators:**
Erica L. Wallace; Ashley M. Stokes, DVM, PhD; Daniel B. Paulsen, DVM, PhD, DACVP; Giselle Hosgood, BVSc, PhD, DAVCS; Susan C. Eades, DVM, PhD, DACVIM; Rustin M. Moore, DVM, PhD, DACVS.

**Description of the Problem:**
Laminitis is an extremely painful, debilitating disease of the laminae of the equine hoof. It can be career-ending and even life-threatening. The pathogenesis of the disease is not completely known. The three main theories for laminitis are the vascular theory, the enzymatic theory, and the mechanical theory. This project explores the enzymatic theory in relation to how over-activation of matrix metalloproteinases (MMPs)-2 and -9 leads to the degradation and failure of the laminar basement membrane. Matrix metalloproteinases are zinc-containing proteolytic enzymes that play a role in normal remodeling.

**Immunohistochemistry of equine laminar tissue using anti-MMP-9 with Image-Pro Plus analysis overlay showing location of various intensities of staining.**
Study Purpose/Objectives:
We hypothesized that there would be an increase in immunohistochemical staining of matrix metalloproteinases-2 and -9 in the laminae of horses with experimentally-induced laminitis, compared with clinically healthy horses and naturally-acquired laminitis horses, and will be less in laminae treated with an endothelin antagonist. The objectives were to (1) validate the immunohistochemistry staining technique for use in equine laminar tissues to determine the presence of MMP-2 and -9; (2) determine the effect of the receptor antagonist on the presence and location of MMP-2 and -9; and (3) determine the difference in MMP synthesis and location between two experimentally-induced acute laminitis models.

Approach:
Immunohistochemistry was performed on laminae collected from horses used in two experimentally-induced acute laminitis models [Black Walnut Extract study (BWE) and Carbohydrate Overload study (CHO)] and compared to those from clinically healthy horses (LAMNEG) and naturally-acquired laminitis horses (LAMPOS). In these studies, a receptor antagonist of the potent vasoconstrictor, endothelin, was used in half of the experimentally-induced laminitic horses.

Accomplishments/Results/Conclusions:
Validation of the immunohistochemistry staining technique and use of the MMP-2 and -9 antibodies in equine laminae was achieved. Significant differences were noted in staining intensity of the CHOB (CHO group with endothelin antagonist administered) and LAMPOS groups. There were significant differences between the CHOB and BWETX (BWE group with endothelin antagonist administered) MMP-2 groups and their opposite leg controls from the same horses. Non-statistical observations show that BWE and CHO study models have a greater staining of MMP-2 and -9 in the tips of the laminae. Both MMP-2 and -9 activity is confined to the secondary epidermal laminae. The ET antagonist may have an effect on MMP-9 staining intensity in CHO-laminitis induced horses.

Benefits to/Impact on the Equine Industry:
Discoveries made during this study should lead to a better understanding of the involvement of MMP-2 and -9 in the pathophysiologic cascade of laminitis and should also stimulate future studies in the prevention of the disease through inhibition of these destructive enzymes.

Take Home Message:
It appears that MMP-2 and MMP-9 are increased in the epidermal laminae of horses with naturally acquired and experimentally induced laminitis, and these enzymes may be involved in the laminar damage/destruction associated with laminitis.

Acknowledgments:
The study was funded by a grant from the LSU Merck-Merial Summer Student Research Program and the LSU Equine Health Studies Program. The authors thank the Equine Health Studies Program for support and thank Julie Millard, Pat Triche, Catherine Koch, Mike Keowen, Frank Garza, Dr. Lais Costa, Dr. Rafael Falerios, Dr. Jill Blackmer, Michael Broussard, Diane Savois, Aimie Hunt, Ben Granger, Ben McMath, and Jeffery Cardinale for technical assistance.

Year Completed: 2003

Published Manuscripts/Abstracts:

Effects of insulin infusion on digital hemodynamics and digital venous plasma endothelin-like immunoreactivity and nitric oxide in healthy conscious horses

Authors/Investigators:
Angelica Veitch, DVM; Susan C. Eades, DVM, PhD, DACVIM; Rustin M. Moore, DVM, PhD, DACVS; Ashley Stokes, DVM, PhD; Frank Garza, Jr., MS.

Description of the Problem:
Endothelin-1 is a potent vasoconstrictor peptide produced by endothelial cells, vascular smooth muscle cells, and macrophages. It induces prolonged vasoconstriction in arteries and arterioles, and also systemic and pulmonary
venoconstriction. Insulin is a two-chain polypeptide hormone produced by the b-cells of pancreatic islets. It regulates the cellular uptake, utilization, and storage of glucose, amino acids, and fatty acids, and inhibits the breakdown of glycogen, protein and fat. Research into the vasculotoxic effects of insulin, as it relates to peripheral blood flow in humans with diabetes mellitus, has also pointed to the role of ET-1. In pathological hyperinsulinemic states such as hypertension, obesity, and aging, the NO/ET-1 balance is disturbed due to reduced NO, allowing ET-1 to predominate, leading to increased vasoconstriction.

**Study Purpose/Objectives:**
The purpose of the study reported here is to determine the effects of intra-arterial infusion of regular insulin at 0.1mU per kg of body weight per minute (1 ml/min infusion rate) on digital arterial blood flow and pressure and digital venous plasma concentrations of ET-like immunoreactivity and NO in horses. The objectives of this study were to determine the effects of hyperinsulinemia in vivo, during local infusion of insulin in the digit and during infusion of insulin with concurrent blockade of ET-1 production, on arterial blood flow, arterial and venous pressures, and serum concentrations of endothelin-1 and nitric oxide.

**Approach:**
Six healthy horses, having no physical or radiographic evidence of laminitis, between the ages of 4 and 15 years, and of different breeds, were chosen for this study; each horse acting as its own control. A Doppler ultrasonic blood flow probe was surgically implanted on the right, front, medial digital artery. After baseline measurements, intra-arterial insulin (0.1mU/kg of body weight per minute, 1 ml/min infusion rate) was infused. At 120 min post insulin, an intra-arterial infusion of ET-1 antagonist (10^-5M) was administered. Insulin infusion was continues for an additional 2 hours. Digital arterial blood flow, and digital arterial and venous blood pressures were recorded during the study, and blood samples for ET-1 and NO serum concentrations were collected.

**Accomplishments/Results/Conclusions:**
Insulin infusion caused an increase in digital blood flow, digital venous blood pressure and NO concentration but no change in ET-1 concentration.

**Benefits to/Impact on the Equine Industry:**
The study suggests insulin has hemodynamic properties in horses via stimulation of NO release, and may be involved in the pathophysiology of laminitis caused by carbohydrate overload and/or that observed in horses with peripheral insulin resistance due to Cushing’s disease.

**Take Home Message:**
This study suggests that insulin exerts local hemodynamic effects in the digital vasculature of horses and may be involved in the pathophysiological cascade of acute laminitis and further studies investigating the role of hyperinsulinemia and its relation to endothelin, nitric oxide and laminitis are warranted.

**Acknowledgments:**
The study was funded by a grant from the LSU Equine Health Studies Program. The authors thank Catherine Koch, Michael Keowen and Dr. Meaghan H. Gilhooly for technical assistance.

**Year Completed:** 2002

**Published Manuscripts/Abstracts:** None yet.
Musculoskeletal

Analgesic effect of percutaneous extracorporeal shock wave application to the third metacarpus bone in horses

Authors/Investigators:
Daniel J. Burba, DVM, DACVS; David M. Bolt, Dr. med. Vet., MS, DACVS; Jeremy D. Hubert, BVSc, MRCVS, DACVS; Glen R. Pettifer, DVM, DACVA; Giselle Hosgood, BVSc, PhD, DACVS; George Strain, PhD; William G. Henk, BS, M. Ed., PhD; Do Youn Cho, DVM, PhD.

Description of the Problem:
Musculoskeletal disease is the number one cause of attrition in Thoroughbred racehorses and horses that perform in other athletic events. Extracorporeal shock wave therapy (ESWT) represents a new treatment modality for numerous orthopedic injuries in performance horses, including dorsal metacarpal disease. It appears from limited clinical data and anecdotal information, that ESWT may induce local analgesia when used for musculoskeletal diseases in the horse. This analgesic effect may have a rapid onset following treatment and may be independent of any beneficial effect on tissue healing. This could place numerous equine athletes with a support structure injury at risk of a career-ending or life-threatening injury if exercised strenuously after application of ESWT to the distal extremity.

Study Purpose/Objectives:
The objective was to document the onset, duration, and degree of cutaneous analgesia after application of ESWT to the dorsal metacarpal region in horses by means of a standardized pain model using a focused light source.

Approach:
Twelve mature horses free of musculoskeletal diseases of the metacarpal bones and having no clinical signs of forelimb lameness were used in the study. Two investigators without knowledge of treatment regimen performed all clinical evaluations. The dorsal, dorsolateral, and dorsomedial surface of the middle aspect of one metacarpus (MCIII) was randomly selected and treated with 1,000 cycles from the non-focused shock wave therapeutic unit. The treated areas were evaluated using a focused light source prior to treatment and every 2 hours post ESWT for the first 24 hours and every 8 hours thereafter to determine if any analgesic effect was present. The untreated contralateral limb served as a control. Evaluation continued until the degree of response to a superficial stimulus on the treated area was similar to the response on the untreated limb.

Accomplishments/Results/Conclusions:
The limb withdrawal reflex latency (LWRL) responses were similar in all treated and control areas over time with a significant decrease noted at most areas and time points compared with baseline values. The findings of the present study suggest that a single application of non-focused ESW had no noticeable cutaneous analgesic effect in the equine metacarpal region.

Benefits to/Impact on the Equine Industry:
The findings of this study indicate the there is no cutaneous analgesic affect of ESWT on the dorsal metacarpal region and that this mode of therapy should be safe for use in racehorses with dorsal metacarpal disease.
Take Home Message:
Extracorporeal shock wave therapy (ESWT) represents a new treatment modality for numerous orthopedic injuries in performance horses, including dorsal metacarpal disease. Extracorporeal shock wave therapy applied to the front of cannon bone of horses did not produce a numbing affect after treatment in this study. Thus according to this study, ESWT applied to the cannon bone should not mask pain to predispose the horse to a more serious injury.

Acknowledgments:
This study was funded by a grant of the Louisiana State University Equine Health Studies Program. The non-focused shock wave generator was kindly provided by EMS Electro Medical Systems, Nylon, Switzerland. The authors thank Catherine Koch, Jessica Carey and Misty Gray for technical assistance.

Year Completed: 2002

Published Manuscripts/Abstracts:

Functional and morphological changes in palmar digital nerves after extracorporeal shock wave application in horses

Authors/Investigators:
Daniel J. Burba, DVM, DACVS; David M. Bolt, Dr. med. Vet., MS, DACVS; Jeremy D. Hubert, BVSc, MRCVS, DCVS; Glen R. Pettifer, DVM, DACVA; Giselle L. Hosgood, BVSc, PhD, DACVS; George Strain, PhD; Do Youn Cho, DVM, PhD; William G. Henk, BS, M. Ed., PhD.

Description of the Problem:
Extracorporeal shock wave therapy (ESWT) is a newly adapted treatment modality used to treat musculoskeletal disorders in horses. Treatment with both focused and non-focused extracorporeal shock waves has been reported to cause analgesia. This analgesic effect is most likely independent of any other potential beneficial effects on tissue healing and is observed rapidly after treatment. Analgesia of an injured limb represents a concern in an equine athlete because it disables protective limiting mechanisms and may place horses with predisposing lesions at an increased risk of sustaining a catastrophic injury when exercised with altered peripheral pain perception. This has been recognized by the equine industry and has led to the development of regulations concerning the use of ESWT prior to competition by racing jurisdictions and the Federation Equine International (FEI).

Study Purpose/Objectives:
The objective was to document functional and morphological changes in palmar digital nerves of horses after extracorporeal shock wave application to the pastern area.

Approach:
Six adult horses were used in the study. The medial and lateral palmar digital nerves of the left forelimb were treated with non-focused extracorporeal shock wave therapy (ESWT). The right medial palmar digital nerve served as an untreated control. At 3, 7, and 35 days after treatment, respectively, two horses were anesthetized and nerves were exposed. Sensory nerve conduction velocities (SNCV) of treated and control nerves were recorded, followed by palmar digital neurectomy. Changes in nerves were assessed by histology and transmission electron microscopy (TEM).

Accomplishments/Results/Conclusions:
ESWT resulted in significantly decreased SNCV in treated medial and lateral nerves at 3 and 7 days after ESWT. A significantly decreased SNCV was observed in treated medial, but not in lateral, nerves at 35 days. Swelling and dissolution of the axonal cytoplasm with a granular texture was observed.
histologically in treated and control nerves at 3, 7 and 35 days after ESWT. TEM revealed disruption of the myelin sheath without evidence of damage to Schwann cell bodies or axons in treated nerves at 3, 7 and 35 days following ESWT. Non-focused ESWT results in decreased SNCV in treated palmar digital nerves. This is likely to contribute to the observed post-treatment analgesic effect in horses and may cause altered peripheral pain perception.

**Benefits to/Impact on the Equine Industry:**
Extracorporeal shock wave therapy (ESWT) is a newly adapted treatment modality used to treat musculoskeletal disorders in horses. Our study supports the premise that extracorporeal shock waves cause damage to peripheral nerves resulting in slower conduction velocities and potentially impaired perception of peripheral pain. Morphological and functional changes associated with neuropraxia are reversible over time; however, repeated application of ESWT may cause more extensive damage to exposed peripheral nerves and may result in prolonged or permanent alterations in nerve conduction. Horses with predisposing lesions may be at increased risk for sustaining catastrophic injuries when exercised immediately after ESWT.

**Take Home Message:**
This provides evidence direct treatment of subcutaneous nerves in horses with a single treatment of non-focused extracorporeal shock waves resulted in a decrease of nerve transmission. Although these findings do not provide a conclusive explanation for the numbing effect observed in a clinical situation, we recommend careful use of this treatment regimen in equine athletes before training or competition.

**Acknowledgments:**
This study was funded by a grant from the Louisiana State University Equine Health Studies Program. The non-focused shock wave generator was kindly provided by EMS Electro Medical Systems, Nylon, Switzerland. The authors thank Catherine Koch, Jessica Carey and Misty Gray for technical assistance.

**Year Completed:** 2002

**Published Manuscripts/Abstracts:**


Effect of extracorporeal shock wave energy on mineral apposition rate in healing equine third metacarpal bone

Authors/Investigators:
Jeremy D. Hubert BVSc, MRCVS, MS, DACVS; Daniel J. Burba DVM, DACVS; Julia Coutin; Susan M. Stover DVM, PhD, DACVS.

Description of the Problem:
Recently the use of Extracorporeal Shock Wave Energy (ESWE) has become extremely popular for the treatment of numerous equine orthopedic disorders, with many anecdotal reports of success. However, a mechanism for a treatment effect has not been elucidated. The effect of ESWE on normal equine third metacarpal bone is unclear. Microfractures were observed after ESWE treatment of the third metacarpal bone in an ex-vivo study, but not after treatment in an in-vivo study. Studies have shown that ESWE may result in activation of osteonal remodeling, however it is unclear if this response subsequently enhances the rate of bone formation.

Study Purpose/Objectives:
The goal of this study was to determine if ESWE treatment enhances mineral apposition rate in a model that induces healing of equine cortical bone tissue.

Approach:
Eight adult horses were studied. Under general anesthesia two 4.5 mm diameter holes (lesions) were drilled in the dorsal cortex of bilateral third metacarpal bones, one in the proximal diaphyseal region and one in the distal diaphyseal region. The left or right third metacarpal bone was randomly chosen for ESWE therapy, and the contralateral limb served as an untreated control. ESWE treatment was administered twice, 3 weeks and 6 weeks postoperatively, using a focused unit (HMT Versatron). Fluorochrome bone labels (oxytetracycline, calcein green, and oxytetracycline) were administered intravenously at the time of lesion creation, first ESWE treatment, and second ESWE treatment, respectively. Horses were humanely euthanized at nine weeks after lesion creation. Undecalcified metacarpal bone samples were embedded and sectioned for determination of mineral apposition rate (interlabel distance / interlabel time interval). Interlabel distance was measured in 4 orthogonal directions for labeled osteons by an evaluator blinded to the treatment status of the specimens.

Accomplishments/Results/Conclusions:
The results of this study indicate that the rate at which bone is deposited within a lesion made in the diaphysis of the equine third metacarpal bone is not affected by ESWE. Further data is required before inferences can be made regarding the effect of ESWE treatment on the amount of bone formed.

Benefits to/Impact on the Equine Industry:
Although anecdotal results have favored use of this treatment modality the effect upon healing bone will need to be studied further to objectively assess the benefits.

Take Home Message:
ESWE will make horses more comfortable for certain selected musculoskeletal diseases but more work needs to be done to determine the effect upon bone physiology.

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

Year Completed: 2003

Published Manuscripts/Abstracts: None yet.
Changes in bone properties after extracorporeal shock wave application to the third metacarpus of horses

Authors/Investigators:
Jeremy Hubert, BVSc, MS, MRCVS, DACVS; Daniel Burba DVM, DACVS; David Bolt DVM, MS, DACVS; Jill Johnson DVM, MS, DACVIM, DABVP; Giselle Hosgood, BVSc, MS, PhD, DACVS.

Description of the Problem:
Dorsal metacarpal disease (DMD) is a significant cause of musculoskeletal disease in young racehorses that can result in stress fractures or catastrophic fractures of the third metacarpus. Present treatment methods involve rest and a change in training protocol so as to allow sufficient remodeling of the dorsal cortical bone to occur. Recently the use of extracorporeal shockwave therapy (ESWT) has been advocated as a form of treatment for DMD. There are anecdotal reports and case reports of successful treatment of DMD with ESWT but few controlled studies for the use of ESWT have been conducted in veterinary medicine. Quantitative ultrasonography has been shown to precisely measure superficial cortical bone properties by evaluating the speed of sound (SOS) through the bone. SOS measurements provide information about factors that affect bone strength including bone mineral density, bone size, mass distribution, elasticity and stiffness.

Study Purpose/Objectives:
Our objective in this study is to document the changes in SOS measurements of the dorsal third metacarpal bone in adult horses after application of extracorporeal shock waves. We hypothesized that extracorporeal shock waves applied to the third metacarpal bone would result in an increase in the SOS measurements of the bone over time.

Approach:
Twelve adult horses, kept on pasture, clinically and radiographically free of any signs of disease to the dorsal metacarpus, were used. ESWT (Dolorclast Vet, EMS Electro Medical Systems, Dallas, Texas) was applied to standing horses using a non-focused EWST unit. One limb was randomly selected and treated with 1,000 cycles at 0.9mJ/mm², the other serving as a control. SOS measurements were obtained in standing sedated horses using quantitative ultrasonography with a multisite QUS device (Sunlight Omnisense, Sunlight Ultrasound Technologies, Rehovot, Israel). The SOS was measured over three sites, (medial, dorsal and lateral aspects) of the mid-third metacarpus of the treated and control limbs. Group 1 (n=6) had one treatment only, the SOS was measured before treatment and then weekly for three weeks and at 7 weeks after treatment. Group 2 (n=6) had three treatments at two week intervals. SOS was measured before the initial treatment and then weekly for three weeks and at 7 weeks after treatment. Group 2 (n=6) had three treatments at two week intervals. SOS was measured before the initial treatment and then every week for nine weeks.

Accomplishments/Results/Conclusions:
The results from this study suggest that non-focused ESWT does not have any appreciable effect on SOS in non exercised, normal horses. Both groups showed no differences between treated and control limbs at any site but showed similar significant differences between sites. The SOS was significantly greater on the lateral region than medial, and medial than dorsal. This reveals that the ESWT application did not have any effect upon bone SOS and thus the density of the bone.

Benefits to/Impact on the Equine Industry:
This treatment modality is currently extremely popular. Anecdotally, horses with dorsal metacarpal disease appear to be more comfortable after treatment with ESWT; however, the ability of the shock waves to induce changes in the bone itself remains to be shown.

Take Home Message:
This treatment modality does not change the SOS and thus the density of bone in normal horses with no bony abnormalities.

Acknowledgments:
This study was funded by a grant from the Organized Research Fund, Department of Veterinary Clinical Sciences, School of Veterinary Medicine, Louisiana State University. We would also like to acknowledge Sunlight Omnisense for the use of their SOS equipment.
Year Completed: 2001

Published Manuscripts/Abstracts:


A technique for quantification of exercise in horses in race training using the global positioning system (GPS)

Authors/Investigators:
Jill R. Johnson (McClure, Blackmer), DVM, MS, DACVIM, DABVP; George S. Martin, DVM, MS, DACVS; Daniel J. Burba, DVM, DACVS; Daniel Scholl, DVM, MPVM, PhD; James “Sonny” Corley, DVM.

Description of the Problem:
A paucity of quantitative information exists about how horses are trained and conditioned. Statistics for actual races are readily available, but the vast majority of a horse’s time is spent in training, not in racing. Prior to this time, only estimates were available for actual distance traveled, speeds attained and time actually exercising.

Study Purpose/Objectives:
The goal of the study was to develop an objective method of quantifying training exercise for use as a tool for studying the effect of training methods on the occurrence of musculoskeletal injuries. The objectives of this study were to determine whether a hand-held GPS data recorder could satisfactorily collect positions of exercising horses for calculation of time and distance information and to develop methods of quantifying and displaying exercise data collected by GPS on exercising horses.

Approach:
Position and time data were collected using Trimble Geo-Explorer™ II GPS data recorders in auto 2D/3D mode set to record all points. Acceptable PDOP mask, a measure of optimal satellite positioning, was set at 6.0. Positions were collected and stored in dated files in the data recorder until downloading to a computer for post-processing. The GPS data was post-processed for differential correction to remove the effect of selective availability (SA) using Trimble Pathfinder® Office 2.51 software. Base files for correction were obtained over the internet from continuously operating reference stations (CORS) within 300 km of Lafayette, LA. Positions were reported as northings and eastings using the Universal Transverse Mercator (UTM) coordinate system, NAD 1983 HPGN (Louisiana) datum, Zone 15 north. Coordinate units were meters. All exercise under saddle of fifteen 2 year old Thoroughbred horses in race training at Evangeline Downs in Lafayette, Louisiana was documented over a period of 11
Exercise on the walking wheel was not logged or recorded. GPS data recorders were mounted in a pocket on the back of exercise flack jackets positioned on the upper torso of the exercise riders. The GPS data recorder was turned on just before the first horse was taken to the track and turned off after the last ride of the morning. All of the morning rides for an instrumented rider were recorded in a single data file. The order in which the horses were ridden was recorded in a logbook.

The daily log file from the GPS recorder was separated into files containing data on individual horses based on examination of the data for time spans of > 2 minutes at a specific geographic location (the training barn). Each day’s tracings were examined manually for instances where the horse appeared to ‘cut corners’ on the track. These observations were used to flag spurious decreases in speed as being artifactual. Data for each point were imported into a spreadsheet for analysis. Distance between consecutive points was calculated using the Pythagorean theorem. Velocities between points were defined as < 3, 3-8, >8-11, >11-14.5 and >14.5 m/sec, respectively. Velocity data was smoothed using a 10 period moving average. Daily and cumulative parameters obtained from the data included total distance traveled, time and distance trained at each gait, mean velocity of each training day, and percentage of work at each gait.

Accomplishments /Results/ Conclusions:
Seventy-eight percent of the training days were successfully captured by GPS, and following an initial period of adjustment, collection of data became routine. Days trained (under saddle on the track) ranged from 26 to 79 for individual horses in this group, whereas days of training ‘captured’ with GPS ranged from 10 to 62 per horse.

The Trimble GPS data recorders used were durable and tolerated the exercising regime well. The data recorders operate through a system of scroll-through menus and require several steps to initiate data collection. In this regard they were not user-friendly at the level of the exercise riders. Battery life and data capacity of the GPS unit limited the use of a single recording session to 2 to 3 hours or 3 to 5 horses. This was generally sufficient to record training of all of the horses ridden by an individual instrumented-rider during the morning. Rechargeable batteries were not satisfactory for powering the data recorders due to sudden and unpredictable loss of power. Intermittent gaps in data points occasionally occurred due to unavailability of at least 3 satellites from which to triangulate and/or multi-path interference from adjacent buildings. Use of a 10-point moving average minimized the effects of the lost data points. Differential correction of the data was necessary prior to data analysis to remove the effect of selective availability (SA), a form of signal scrambling. The collection of data in 2D/3D mode (which will register a point with only 3 satellites) was a compromise to allow collection of data on the turns on the racetrack without ‘cutting corners’. 3D mode (which requires clear view of 4 satellites) resulted in more accurate points but took longer to register a position. When the horses worked at high speeds around turns this resulted in missed information. We could identify these ‘short cuts’ by manually tracing the path with the Pathfinder mapping software. These events resulted in spurious transient decreases in velocity because the horse appeared to travel less distance (direct from point to point) than it actually traveled around the turn.

A variety of types of training information were harvested from the GPS data including daily and cumulative total distance traveled, time and distance trained at each gait and percentage of work at each gait. Speed vs. time graphs were generated to graphically depict a day’s training pattern. A time line was constructed for each horse showing the daily distance and proportion of distance at each gait. Cumulative distance and percentage of distance at each gait were calculated and displayed graphically.

The day-to-day time line of training varied considerably among horses however, the ‘average daily training’ pattern varied little among horses at similar stages of training. The average daily
distance trained was relatively constant among horses and varied surprisingly little over the course of training. Horses on average walked 357 m, trotted 1,021 m, galloped 612 m, breezed 178 m, and worked 22 m a day. The cumulative totals were directly related to the number of days in training. As an example, horse 17 trained on the track 36 days and walked a total of 12,096 m, trotted 37,718 m, galloped 28,848 m, breezed 7,827 m, and worked 1,041 m. Horses spent less than 10 minutes under saddle (from leaving the barn until returning) on a training day. Three horses raced during the period of observation. Days in training prior to first start for these 3 horses were 118, 119 and 157. The number of training sessions (under saddle on the track) prior to first start was 58, 60 and 79 respectively. The cumulative total distance traveled prior to the first start was 53, 67 and 75 km, respectively.

**Benefits to/Impact on the Equine Industry:**
For the first time, an accurate practical method of quantifying the amount of exercise horses in training perform is available. We have demonstrated a proof of concept, with which if hardware and software were customized, would be a simple useful tool to evaluate training methods, and to evaluate the influence of training distance and speed on injuries.

**Take Home Message:**
GPS is a useful utility for quantification of exercise of horses in race training. This tool will be useful for evaluating the effectiveness of training programs as well as for studying the training conditions that lead to musculoskeletal injuries by objectively recording the amount of exercise performed.

**Acknowledgments:**
Funding for this project was provided by grants from the Louisiana Veterinary Medical Association Equine Committee and the Louisiana Thoroughbred Breeders Association. The authors would also like to thank the personnel involved in data collection, including Will Meaux (trainer), Glenn Miller and Brian Bernard (exercise riders), and Tony Cavell and Navigation Electronics Incorporated (Trimble dealer in Lafayette).

**Year Completed:** 2002

**Published Manuscripts/Abstracts:**


Articular chondrocyte apoptosis in normal and osteoarthritic joints in horses

Authors/Investigators:
Dae-Young Kim, DVM, PhD, DACVP; H. Wayne Taylor, DVM, PhD; Rustin M. Moore, DVM, PhD, DACVS; Daniel B. Paulsen, DVM, PhD, DACVP; Dae-Young Cho, DVM, PhD.

Description of the Problem: (Can show photos of a horse with joint disease)
Osteoarthritis is the most common joint disease in horses. Chondrocyte apoptosis has been implicated as a major pathological osteoarthritis change in humans and experimental animals, but no other studies have been performed on equine osteoarthritis. Nitric oxide (NO) has been implicated as an important biological mediator of osteoarthritis, and it has been shown that NO production from chondrocytes is significantly greater in osteoarthritis cartilage compared with controls and directly correlates with the severity of osteoarthritis. The major source of NO in articular cartilage is inducible NO synthase. NO reacts with superoxide radicals to form peroxynitrite, and in biological fluids peroxynitrite leads to nitration of aromatic amino acid residues; the presence of such nitration can be used as an in vivo marker of peroxynitrite-mediated NO activity.

Study Purpose/Objectives:
The purpose of the study was to investigate and compare chondrocyte apoptosis in the articular cartilage of horses with and without naturally acquired osteoarthritis and examine the relationship between chondrocyte apoptosis and cartilage degeneration.

Approach:
Articular cartilage was collected from the joints of three normal and five osteoarthritic horses. Histopathological changes were scored by a modified Mankin grading system. A terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL) assay was used to identify chondrocyte apoptosis. Nitric oxide (NO) production from chondrocytes was indirectly evaluated by immunohistochemical staining with polyclonal antibodies to nitrotyrosine.

Accomplishments/Results/Conclusions: (Can show photos here)
The histopathological score and percentage of chondrocyte apoptosis from the osteoarthritic cartilage were significantly greater than from normal cartilage. There was a significant correlation between histopathological grade and the percentage of chondrocyte apoptosis. Osteoarthritis cartilages exhibited stronger immunoreactivity to nitrotyrosine than normal cartilage. Topographical distributions of chondrocyte apoptosis, cartilage matrix degeneration, and NO production overlapped in equine osteoarthritic cartilages, suggesting that these pathological phenomena are closely interrelated.

Benefits to/Impact on the Equine Industry:
Joint disease caused by osteoarthritis leading to pain and reduced mobility is one of the most common causes of poor performance and retirement in horses involved with racing and other competitive activities. This study demonstrates a potential link between the production of NO and chondrocyte apoptosis, which is supported by work in other species demonstrating excessive NO production is linked to cartilage catabolism. Future work in this area may lead to discoveries that will aid in the prevention and treatment of osteoarthritis and joint disease in equine athletes.

Take Home Message:
The topographical distribution of chondrocyte apoptosis, cartilage matrix degeneration, and NO production nearly completely overlapped with the lesional areas of osteoarthritic cartilage in equine joints, which suggests that these pathological phenomena are closely interrelated. Further study is needed to confirm our findings from this preliminary study and to determine the role of NO and apoptosis in articular cartilage degeneration in equine osteoarthritis.

Year Completed: 2002

Published Manuscripts/Abstracts:
Effects of intra-articular silicone polymer on synovial fluid, synovial tissue, and articular cartilage in the equine middle carpal joint

Authors/Investigators:
Marilyn Rumbaugh, DVM, MS; Daniel J. Burba, DVM, DACVS; Joanne Tetens, DVM, MS, PhD; Julian Oliver, DVM, PhD; Jamie Williams, DVM, MS; Giselle Hosgood, BVSc, PhD, DACVS; Casey J. LeBlanc DVM, PhD.

Description of the Problem:
Joint disease is a major cause of unsoundness in horses. The conventional therapeutic options for performance horses with joint disease include intra-articular administration of corticosteroids, systemic administration of polysulfated glycosaminoglycans (PSGAGs), systemic and/or intra-articular administration of hyaluronic acid, systemic administration of non-steroidal anti-inflammatory drugs (NSAIDs), and prolonged periods of rest. There is anecdotal documentation that intra-articular administration of liquid silicone is used occasionally as an intra-articular lubricant for equine joints. It is used in Standardbred racehorses, often in conjunction with a corticosteroid, to treat osteoarthritis that has become refractory to more conventional treatments. Controlled studies evaluating its efficacy have not been published. The effects of liquid silicone polymer on synovial fluid and articular cartilage in the equine middle carpal joint are not known.

Study Purpose/Objectives:
The objective was to characterize the effects of intra-articular administration of liquid silicone polymer on clinical, synovial fluid, and microscopic features of the synovial membrane and articular cartilage of normal middle carpal joints of horses.

Approach:
Eight clinically healthy horses with no evidence of joint disease in the middle carpal joints were used in the study. A liquid silicone polymer or 0.9% NaCl was injected into the middle carpal joints. Clinical signs, synovial fluid analyses, radiographic examination and histologic examination of synovial membrane, and articular cartilage.

Accomplishments/Results/Conclusions:
A single injection of two milliliters of liquid silicone causes acute synovitis in the equine middle carpal joint. Silicone is phagocytized by macrophages and incorporated into the subsynovium with minimal inflammatory response; however, clinical signs of joint disease persist. Silicone did not result in appreciable articular cartilage damage six weeks after administration.

Benefits to/Impact on the Equine Industry:
There were no adverse systemic signs associated with intra-articular silicone administration despite the development of local signs of acute synovitis in the treated joint associated with effusion and lameness. Silicone appears to be phagocytized by macrophages and incorporated into the subsynovium with minimal inflammatory response; however, clinical signs of joint disease (effusion, lameness) persist. A single injection of liquid silicone did not result in appreciable articular cartilage damage six weeks after administration.

Take Home Message:
Due to the degree of acute synovitis, liquid silicone is not recommended for intra-articular use in the horse.
Muskuloskeletal

Acknowledgments:
This study was funded by a grant from the LSU SVM VCS Competitive Organized Research Program. The authors thank Catherine Koch and Frank Garza for their technical assistance.

Year Completed: 2001

Published Manuscripts/Abstracts:


An in vitro biomechanical comparison of a prototype intramedullary pin-plate with a dynamic compression plate for equine metacarpophalangeal arthrodesis

Authors/Investigators:
Gary A. Sod DVM, PhD; George S. Martin, DVM, MS, MBA, DACVS.

Description of the Problem:
The traumatic disruption of the suspensory apparatus is not an uncommon injury among Thoroughbred racehorses (Fig. 1). Affected horses do not have an athletic future, and the goal of treatment is salvage for breeding. Surgical management of traumatic disruption of the suspensory apparatus generally involves stabilizing the joint through metacarpophalangeal arthrodesis. Currently, a dynamic compression plate construct is the most extensively used technique for fetlock arthrodesis. The important features of the technique are: application of a 14-16 hole broad dynamic compression plate (DCP) on the dorsal surface of the third metacarpal (McIII) and proximal phalanx (P1) to maintain the limb in approximately 10° of extension, and use of a tension band wire on the palmar surface to absorb some of the cyclic load. Implant failure and support limb laminitis are common complications associated with this technique that develop during the convalescence and result in an unsuccessful outcome. In one study, 19 out of 43 cases were euthanatized.

Study Purpose/Objectives:
The purpose of this in vitro study is to evaluate the biomechanical properties of a prototype intramedullary pin-plate (IMPP) implant in adult equine cadaver limbs (Fig. 2). It is hypothesized that the IMPP implant, specifically designed for equine metacarpophalangeal arthrodesis, will provide significantly better stability under axial (single cycle and cycle to fatigue) loading and torsional loading. To provide clinical relevancy, the IMPP implant technique was compared with the DCP construct.

IMPP Implant:
The IMPP implant is a 316L stainless steel single fixator consisting of: i) a 3 hole, 13mm diameter interlocking, intramedullary nail, 150.0 mm in length, inserted into a hole drilled within the medullary cavity of distal aspect of McIII, and ii) a 4- hole wedge-shaped broad plate, at a 50° angle to the nail, which attaches to the palmar surface of the proximal phalanx. The angular transition from the intramedullary nail to the plate, as well as the wedge shape of the plate, provides greater strength at the level of the metacarpophalangeal joint where stresses may be large. The IMPP maintains the metacarpophalangeal joint in a degree of extension that is more physiologically correct (Fig. 3). A drill guide, attached to the plate portion of the IMPP, was used to locate and drill the holes in the interlocking, intramedullary nail portion of the IMPP without the need for imaging devices.

Approach:
Twenty-one pairs of forelimbs intact from midradius distally were collected from adult Thoroughbred horses that had been euthanatized for non-orthopedic reasons. The ages of the horses ranged from 3 to 5 years.
Limb preparations were chosen to simulate an in vivo biomechanical loading environment. The distal sesamoidean ligaments were severed in order to create a disrupted suspensory apparatus. This simulated a typical traumatic breakdown injury. The cadaver forelimbs were divided into three groups (seven pairs each) for: i) single cycle to failure testing under axial compression, ii) cyclic fatigue testing under axial compression, and iii) single cycle to failure testing under torsional loading. Mean values for each fixation method were compared using a paired \( t \)– test within each group. Significance was set at \( P < 0.05 \).

**Accomplishments/Results/Conclusions:**
The mean yield load, yield stiffness, and failure load, under axial compression was 10, 14, and 10 times greater, respectively, for the IMPP compared with the DCP construct. The mean yield load, yield stiffness, and failure load, under torsional loading was 7, 3, and 7 times greater, respectively, for the IMPP compared with the DCP construct. The mean number of cycles to failure under axial compression was 12 times greater for the IMPP compared with the DCP construct. The significance level in all cases was \( P < 0.0001 \).

In the single cycle to failure tests, the loading rates for axial compression and torsional loading were chosen to represent high-rate/high-energy forces that might occur during recovery from anesthesia. In cyclic fatigue testing, under axial compression, the loading rates were chosen to represent loads placed on the third metacarpal bone due to walking in a stall during convalescence. Under this loading rate, the IMPP should not undergo cyclic fatigue failure for at least six months, compared with the DCP construct, which should undergo cyclic fatigue failure is less than one month.

**Benefits to/Impact on the Equine Industry:**
The specific design of the IMPP implant may facilitate equine metacarpophalangeal arthrodesis and may obviate the complications encountered during convalescence due to cyclic instability, as the IMPP implant is stable and resistant to cyclic fatigue.

**Take Home Message:**
The results of this study support the hypothesis that the IMPP is superior to the DCP construct with regard to resisting the biomechanical forces that are most likely to cause failure with the fixation types used for metacarpophalangeal joint arthrodesis.

**Year Completed:** 2002

**Published Manuscripts/Abstracts:**


An in vitro biomechanical comparison of a limited-contact dynamic compression plate fixation with a dynamic compression plate fixation of osteotomized equine third metacarpal bones

Authors/Investigators:
Gary A. Sod, DVM, PhD; Jeremy D. Hubert, BVSc, MS, MRCVS, DACVS; George S. Martin, DVM, MS, MBA, DACVS; Marjorie S. Gill, DVM, MS, DABVP.

Description of the Problem:
The concept of biological plating has lead to the development of the limited-contact dynamic compression plate (LCD-CP). The goal of the LC-DCP is to reduce trauma to bone, preserve the blood supply, and to avoid producing stress risers at implant removal. The evenly distributed undercuts of the LC-DCP reduces the contact between the bone and plate to a minimum (Figure 1). However, compressive or bending forces in the plate and shear or bending forces in the screws may be magnified if there is any micromovement between the plate and bone. The bone-plate interface is a very important factor in the initial and continued stability of the fracture fixation. Plate luting, using polymethylmethacrylate (PMMA) interposed between the plate and the bone, as well as between the screw heads and the plate, allows greater contact between the implant and the bone, and thereby, improves the stability of the internal fixation and increases the fatigue life of cyclically loaded plates. It is common in large animal long bone fractures to lute dynamic compression plates (DCP) in order to overcome the limited contact observed in the DCP and reduced fracture stability. This seemingly defeats the purpose of the minimal bone contact design of the LC-DCP.

Study Purpose/Objectives:
The objective of this study is to compare the monotonic biomechanical properties of a broad LC-DCP fixation with a broad DCP fixation to repair osteotomized equine third metacarpal (MC3) bones. It is hypothesized that osteotomized equine MC3-LC-DCP composites have significantly greater single cycle caudocranial bending and torsional strength compared to osteotomized MC3-DCP composites. It is further hypothesized that the number of cycles to failure will be significantly greater for the osteotomized equine MC3-DCP composites compared to that for the osteotomized MC3-LC-DCP.

Approach:
Paired MC3 bones from twelve adult Thoroughbred horses, each having mid-diaphyseal osteotomies, were randomly chosen to receive the LC-DCP (8-hole, 4.5 mm broad) or the DCP (8-hole, 4.5 mm broad) applied to the dorsal surface. Each plate was secured in place with four 5.5 mm cortical screws (proximal and distal holes in the plate and the holes immediately adjacent to the osteotomies) and four 4.5 mm cortical screws (Figure 2). Four matching pairs of constructs were tested in caudocranial four-point bending in a single cycle to failure, four pairs of constructs were tested for cyclic fatigue under caudocranial four-point bending, and four pairs of constructs were tested in torsion. Mean test variable values for each method were compared using a paired t – test within each group. Significance was set at P < 0.05.

Accomplishments/Results/Conclusions:
The mean values for the yield bending moment and the failure bending moment were 1.3 times and 1.6 times greater, respectively for the MC3-LC-DCP composite compared to the MC3-DCP composite. In cyclic fatigue, the mean number of cycles to failure was 2.2 times greater for the MC3-DCP composite compared to the MC3-LC-DCP composite. In torsion, the mean stiffness was 1.6 times greater for the MC3-LC-DCP composite compared to the MC3-DCP composite.

The yield bending moment and failure bending moment for the LC-DCP fixation were significantly greater (P < 0.01) compared with the DCP fixation in caudocranial four-point bending. This can be understood since the area moment of inertia of the LC-DCP is greater than the area moment of inertia of the DCP, for any cross section of LC-DCP and DCP. The number of cycles to failure under four-point bending for the DCP fixation was significantly greater (P < 0.01) compared with the LC-DCP fixation. The footprint of the LC-DCP on the bone surface is, by design, smaller than that of the DCP, as such, micromovements between the LC-DCP and the bone are magnified during cycling, resulting in a significantly lower number of cycles to
failure compared to the DCP-bone construct. The yield load, stiffness, and failure load for the LC-DCP fixation were significantly greater (P < 0.05) compared with the DCP fixation in torsion. This can be understood since the polar moment of inertia of the LC-DCP is greater than the polar moment of inertia of the DCP, for any cross section of LC-DCP and DCP.

**Benefits to/Impact on the Equine Industry:**
The LC-DCP offers increased stability in static overload testing, however, the LC-DCP offers significantly less stability (46% of that of the DCP) in cyclic fatigue testing. Implants that have an increased cyclic fatigue life are necessary. However, practical limits to increasing the size of fixation devices do exist. Merely increasing the number of plates and screw or increasing the plate or screw size is not feasible. Large animal specific orthopedic implants are necessary to facilitate equine fracture repair.

**Take Home Message:**
The concept of biological plating is difficult to apply in equine orthopedic surgery.

**Year Completed:** 2003

**Published Manuscripts/Abstracts:**


**An in vitro evaluation of plate luting using ostotomized equine third metacarpal bones with a limited contact-dynamic compression plate**

**Authors/Investigators:**
Gary A. Sod, DVM, PhD; Jeremy D. Hubert, BVSc, MS, MRCVS, DACVS; George S. Martin, DVM, MS, MBA, DACVS; Marjorie S. Gill, DVM, MS, DABVP.

**Description of the Problem:**
Internal fixation with plates and screws is the most commonly practiced method of successful repair of long bone fractures in large animals. Long bones are rarely straight and its surface usually has different radius of curvature at different levels along its length. Because of this mismatch between the bone and plate, the plate may contact the bone only over short distances, and contouring the plate does not completely solve this problem. The interfaces between the bone and plate and between the screwhead and plate are important in the maintaining of fracture fixation. The frictional force generated by the screws between the bone and the plate, as well as between the fracture fragments with the use of lag screws, is responsible for the strength of the fixation. Plate luting, a technique that uses polymethymethacrylate (PMMA) interposed between the plate and the bone, as well as between the screw heads and the plate, has been used in large animal long bone fractures. Plate luting allows greater contact between the implant and the bone, and is used to improve the stability of internal fixation and thereby, increase the fatigue life of cyclically loaded plates. It is common to lute dynamic compression plates (DCP) in order to overcome the limited contact observed in the DCP and reduced fracture stability. This seemingly defeats the purpose of the minimal bone contact design of the LC-DCP.

**Study Purpose/Objectives:**
The objective of this *in vitro* study was to evaluate the effects of plate luting in a broad LC-DCP fixation to repair ostotomized equine third metacarpal bones. The hypothesis of this study was that there will be no significant differences measured in static overload tests between luted and nonluted plates, and that there will be a significant increase in the number of cycles to failure noted with the luted specimens.

**Approach:**
Ten pairs of adult equine cadaveric MC3 bones had LC-DCP (8-hole, 4.5 mm broad) applied to the dorsal surface across mid-diaphyseal ostotomies. Each plate was secured in place with four 5.5 mm cortical screws (proximal and distal holes in the plate and the holes immediately adjacent to the ostotomies) and four 4.5 mm cortical screws
One of each of the matched pairs of LC-DCP-MC3 constructs was randomly chosen to be luted with polymethylmethacrylate (PMMA). Five matching pairs of constructs were tested in caudocranial four-point bending in a single cycle to failure. Five pairs of constructs were tested for cyclic fatigue under caudocranial four-point bending. Mean test variable values for each method were compared using a paired t-test within each group. Significance was set at P < 0.05.

Accomplishments/Results/Conclusions:
There was no significant difference (P > 0.05) in mean yield bending moment and mean failure bending moment in caudocranial four-point bending in single cycle to failure between the luted LC-DCP-MC3 fixation and the non-luted LC-DCP-MC3 fixation. The mean number of cycles to failure was 7.2 times greater for luted LC-DCP-MC3 fixation compared to the non-luted LC-DCP-MC3 fixation. For the non-luted LC-DCP-MC3 construct the most common result of cyclic fatigue, was screw loosening. With plate luting the character of implant failure changes, the screws did not loosen, but rather, cyclic fatigue caused plate failure.

Benefits to/Impact on the Equine Industry:
The lack of performance of the LC-DCP in this and the previous study support the deviation from the current use of biological plating. Biological plating is effective in human, small animal, and possibly foal patients. However, in the case of large animals, where full weight bearing is the usual practice after the clinical use of plates and screws for fractures, catastrophic implant failure often results. Implants that can better resist cyclic fatigue are necessary. Practical limits to increasing the size of fixation devices do exist. Merely increasing the number of plates and screw or increasing the plate or screw size is not feasible. Implants specific to large animals need to be designed.

Take Home Message:
A new direction in equine long bone fracture repair is being followed with the development of the equine MC3 dynamic compression plate designed for equine third metacarpal or metatarsal fractures (Figure 2).

Year Completed: 2003

Published Manuscripts/Abstracts:

Pullout strength of orthopedic screws in polymethylmethacrylate filled medullary cavities of foal third metacarpal bones

Authors/Investigators:
Gary A. Sod, DVM, PhD; George S. Martin, DVM, MS, MBA, DACVS; Marjorie S. Gill, DVM, MS, DABVP.

Description of the Problem:
The ability of a screw to generate compression between the plate and the bone or between bone fragments to produce a stable osteosynthesis is largely dependent on the pullout strength of the screw. This pullout strength, which is one measure of screw performance, is the maximal uniaxial tensile force required to produce failure in bone. The pullout strength of a screw is a function of the outer thread diameter of the screw, the diameter of the screw shank, the diameter of the pilot hole, and the shear strength of the column of bone equal to the diameter of the outer thread diameter of the screw. If polymethylmethacrylate (PMMA) is used to fill the medullary cavity segmentally at sites of increased stress, the ends of the plates and nearest the fracture site, more stable internal fixation will result with minimal disruption of the medullary circulation.

Study Purpose/Objectives:
The purpose of this in vitro study is to determine and compare the pullout strength of 4.5 mm and 5.5 mm cortical screws in the mid-diaphysis of paired third metacarpal bones of foals, with and without the medullary cavity filled with PMMA. The hypothesis of this study is that the mean cortical screw pullout force (N) and mean cortical pullout strength per mm of bone (N/mm) will be significantly greater for foal metacarpal bones with the medullary cavities filled with PMMA than in metacarpal bones without PMMA. It is further hypothesized that the mean pullout strength per mm of PMMA will not be significantly different from the mean pullout strength per mm of metacarpal bone.
Approach:
Matching pairs of left and right McIII bones were harvested from 9 foals that had been euthanized for reasons unrelated to the bones used in this study. For each matched pair of McIII, one was selected at random to have the medullary cavity filled with PMMA (Figure 1). For each pair, the metaphyses and mid-diaphysis were cut, leaving two 4.0 cm diaphyseal sections, one proximal and the other distal to the mid-diaphysis. One of the paired (proximal and distal) sections would receive the 4.5 mm cortical screw while the other would receive the 5.5 mm cortical screw. The bone-screw constructs were subjected to a tensile loading using servo-hydraulic biaxial material testing system (Figure 2). Tensile force (N) as a function of displacement (mm) was recorded for each test. Mean values for each fixation method were compared while using a paired $t$-test within each group. Significance was set at $P < 0.01$.

Accomplishments/Results/Conclusions:
The mean pullout force and mean pullout strength per mm of bone with PMMA filling the medullary cavity were significantly greater ($P<0.01$) than in bone without PMMA. There was no significant difference ($P > 0.10$) between the pullout strength per mm of PMMA and the pullout strength per mm of bone. Thus each mm of PMMA filling the medullary cavity was equivalent to adding the pullout strength (for 4.5 mm or 5.5 mm cortical screws) of an additional 1.0 mm of cortical bone in the foal third metacarpal bone.

The results of this study support the hypotheses that the mean cortical screw pullout force and mean cortical screw pullout strength per mm of bone will be significantly greater for foal McIII bones with the medullary cavities filled with PMMA than in McIII bones without PMMA. A 2.0–fold increase in mean cortical screw pullout force for 4.5 mm cortical screws and a 2.2-fold increase in mean cortical screw pullout force for 5.5 mm cortical screws were achieved when filling the medullary cavity with PMMA.

Benefits to/Impact on the Equine Industry:
When long plates are used, the screws at the ends of the plates and nearest the fracture site are most common sites of increased stress. If PMMA is used to fill the medullary cavity segmentally at these sites of increased stress, resulting in increased pullout strength of the screws at these sites, more stable internal fixation will result. Additionally, in the case where a 4.5 mm cortical screw is stripped, after tapping for a 5.5 mm screw, injection of the PMMA into the medullary cavity through the screw hole may increase the pullout strength of the 5.5 mm cortical screw replacement.

Take Home Message:
The results of this study support the hypothesis that the mean cortical screw pullout strength per mm of bone will be significantly greater for foal metacarpal bones with the medullary cavities filled with PMMA than in third metacarpal bones without PMMA.

Year Completed: 2003

Published Manuscripts/Abstracts:

Prevalence of anthelmintic resistance on horse farms in the southern United States

Authors/Investigators:
Ray M. Kaplan, DVM, PhD; Thomas R. Klei, PhD; Dennis D. French, DVM, DABVP; Eugene T. Lyons, PhD; Guy Lester, DVM, PhD; Charles H. Courtney, DVM, PhD; Sharon C. Tolliver, M.S.; Anand N. Vidyashankar, Ph.D.; and Ying Zhao, M.S.

Description of the Problem:
The concept of strategic parasite control for horses was introduced almost 40 years ago in a program that became known as the interval dose system. Horse owners were advised to deworm all horses every 6 to 8 weeks to prevent parasite maturation and subsequent contamination of the environment with infective stages. This strategy was novel in that it not only addressed the treatment of current infections, but was designed also to prevent future infection and disease. These recommendations were adopted widely and dramatic reductions in clinical cases of colic caused by Strongylus vulgaris were noted by equine veterinarians whose practice spanned this period of transition. Consequently, the prevalence of S. vulgaris became reduced markedly, and by the early 1980s it was recognized that cyathostomes (small strongyles) frequently accounted for virtually 100% of the worm egg output of grazing horses. This major change in species prevalence has caused an important shift in the relative importance of these nematodes; cyathostomes are now recognized as the principal parasitic pathogen of horses. Larval cyathostomes encyst in the cecal and colonic mucosa where they produce a mild inflammatory enteropathy causing a subclinical alteration in gastrointestinal function. However, synchronous emergence of larval cyathostomes from the colonic mucosa may cause a life-threatening disease, known as larval cyathostomosis, which is characterized by severe weight loss, chronic diarrhea, and edema. More common clinical signs of cyathostome infection include decreased level of performance, decreased rates of growth, weight loss, colic, rough hair coat, and debilitation. Because immunity to cyathostomes is slow to develop and is incomplete, in the absence of other parasite control measures, most horses require regular anthelmintic treatment throughout their lives.

Currently, there are 3 major classes of anthelmintics used to control nematodes in horses: benzimidazoles (fenbendazole, oxfendazole, oxibendazole, others), tetrahydropyrimidines (pyrantel salts), and avermectin/milbemycins (macrocyclic lactones; ivermectin and moxidectin). When first introduced, all of these drugs had very good to excellent efficacy against cyathostomes. However, reports of drug-resistant cyathostomes are becoming increasingly common, and this is gaining recognition as a serious concern in the health management of horses. The earliest documented cases of anthelmintic resistance in cyathostomes of horses were to phenothiazine. Resistance to thiabendazole was reported shortly thereafter, subsequent to only a few years of use, and resistance to benzimidazole (BZ) anthelmintics is now highly prevalent and widespread throughout the world. The prevalence of resistance to pyrantel has remained fairly low in comparison to the BZ drugs; nevertheless, pyrantel-resistance has been reported throughout the southeastern United States (US), in Canada, and in northern Europe. Ivermectin (IVM) was first introduced as an equine anthelmintic in 1983 and remained the only avermectin/milbemycin (AM) anthelmintic used in horses until the recent introduction of moxidectin (MOX) in 1997. Interestingly, there are still no reports of cyathostome-resistance to IVM despite over 20 years of use as the most commonly administered anthelmintic drug. It is not known why IVM resistance has not appeared yet, although several hypotheses have been proposed. However, considering the growing reliance upon these drugs, and the fact that IVM resistance is becoming increasingly common in gastrointestinal nematode parasites of small ruminants and cattle, many parasitologists suspect that resistance in cyathostomes is inevitable.

It is well-established that anthelmintic resistance is a growing problem for the control of cyathostome parasites throughout the world. Yet, few studies investigating the prevalence of this resistance in the US have been published, and these were performed within 2 small geographic areas in Florida (FL) and Georgia (GA), and on relatively few farms. Thus, confidence limits for estimations of resistance prevalence were large, and it remained unknown whether these results were representative of the larger region. The purpose of this study was to establish an accurate estimation for the prevalence of cyathostome resistance on horse farms throughout the southern US.

Study Purpose/Objectives:
The purpose of the study was to determine the prevalence of anthelmintic resistance in cyathostome (small strongyle) nematode parasites of horses in the southern United States.
Approach:
786 horses of various breeds on 44 farms/stables in Georgia, South Carolina, Florida, Kentucky, and Louisiana were examined to determine the level of small strongyle resistance in the southern US. Fecal egg count (FEC) reduction tests were performed on these 44 large farms/stables (24 or more horses). Horses on each farm were assigned to 1 of 4 different treatment groups. Within each group, horses were administered an oral paste formulation of fenbendazole (5.0 mg/kg), oxibendazole (10 mg/kg), pyrantel pamoate (6.6 mg base/kg), or ivermectin (200 ug/kg). FEC reduction was calculated by comparing pretreatment FEC to posttreatment (10-14 days) FEC and then means of percent reductions were calculated for each treatment group on a particular farm. A mixed linear model was fitted to the percent reduction in FEC accounting for differences between farms, states, age, treatment and treatment by state interactions. Anthelmintic efficacy for each treatment group was evaluated using the following criteria: < 80% reduction = ineffective/worms resistant; 80-90% reduction = equivocal/worms suspected resistant; > 90% reduction = effective/worms sensitive.

Accomplishments/Results/Conclusions:
Using a conservative measure for declaring resistance (<80% reduction), the percent of farms demonstrating the presence of anthelmintic-resistant cyathostomes was 97.7%, 0%, 53.5%, and 40.5% for fenbendazole, ivermectin, oxibendazole, and pyrantel pamoate, respectively. Mean percent reductions in fecal egg counts for all farms were 24.8%, 99.9%, 73.8% and 78.6% for fenbendazole, ivermectin, oxibendazole, and pyrantel pamoate, respectively. Pairwise contrasts between states for each treatment using a mixed linear model revealed that in almost all instances there were no statistical differences in results between states.

Benefits to/Impact on the Equine Industry:
The prevalence of resistance found in this study is higher than that reported in any previously published work, suggesting that the problem of anthelmintic resistance is worsening rapidly. Furthermore, data from these 5 southern states, which are geographically and physiographically distinct, were remarkably similar.

Take Home Message:
This suggests that drug resistance in cyathostomes is highly prevalent throughout the entire southern United States and probably nationwide. The high prevalence of multiple-drug resistance emphasizes the need for a re-examination of current cyathostome control practices and greater involvement by veterinarians in designing, implementing, and monitoring the effectiveness of parasite control programs.

Acknowledgments:
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Year Completed: 2002

Published Manuscripts/Abstracts:


Cyathostomin associated pathophysiologic changes in the equine large intestine

Authors/Investigators:
Rebecca S. McConnico, DVM, PhD, DACVIM; Sharon R. Chirgwin, PhD; Jeremy D. Hubert, BVSc, MRCVS, MS, DACVS; Thomas R. Klei, PhD.
Description of the Problem:
Larval cyathostomiasis, 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 Purpose/Objectives:
The objective of the study was to determine if alterations in colonic epithelial secretion are associated with cyathostomin infection compared to helminth-free tissues.

Approach:
The effect of naturally acquired cyathostomin infections on colonic epithelial secretory function was measured in two experiments. Chloride secretion as reflected by short-circuit current of colonic epithelium was measured by Ussing chamber analysis. In the first experiment 12 yearling ponies reared under helminth free conditions were divided into two groups of 6 each. One group was exposed to cyathostomin infested pastures for the winter and spring grazing season and the second group of 6 remained in box stalls (helminth-free). At the time of necropsy and tissue collection animals removed from pasture had a mean of 4043 EL3 and 3,599 DL. No differences were observed between treatment groups, with or without the addition of exogenous secretagogues. Significant differences in the numbers of colonic mast cells or eosinophils were also not seen between groups. These data suggest that a higher “steady state” of infection is necessary to induce the expected alterations of secretory function, or that an induction of excystation/activation of EL3 is necessary.

Accomplishments/Results/Conclusions:
No differences were observed between treatment groups, with or without the addition of exogenous secretagogues. Significant differences in the numbers of colonic mast cells or eosinophils were also not seen between groups. These data suggest that a higher “steady state” of infection is necessary to induce the expected alterations of secretory function, or that an induction of excystation/activation of EL3 is necessary.

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 what level of helminth infection affects epithelial cell secretion.

Take Home Message:
Low levels of helminth infection may not affect colonic epithelial cell secretion in vitro.

Acknowledgments:
This study was funded by a grant from the LSU Equine Health Studies Program.

Year Completed: 2003.

Published Manuscripts/Abstracts:

Equine cyathostome populations: Accuracy of species composition estimations

Authors/Investigators:
Melanie R. Chapman; Michael T. Kearney, PhD; Thomas R. Klei, PhD.

Description of the Problem:
The small strongyle nematodes, termed the cyathostomes 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 identification and enumeration of cyathostome species is important to surveys, population biology studies, anthelmintic efficacy determinations, and other experimental studies. However, there is no standard or definitive guideline for making
species determinations. Historically, surveys of equine parasites have provided information regarding the composition of cyathostome species but have not been quantitative in regard to prevalence and intensities of cyathostomes. Those studies that have reported quantitative data are usually estimates based on the identification of a very small sample of the parasite population. Due to the magnitude of cyathostome infections, most parasite surveys and anthelmintic efficacy studies in the past have followed a relatively standard protocol which has involved the identification of only 100-200 specimens randomly selected from a larger aliquot. While this procedure appears to allow for accurate identification and estimation of high-prevalence cyathostome species, many other species that occur in small numbers may be easily overlooked. A comprehensive study examining the mathematical probability of accurately reporting cyathostome species composition has not been previously reported. This study strives to provide sufficient information allowing researchers to present species data obtained from future studies with a reasonable (95%) level of confidence.

**Study Purpose/Objectives:**
Our hypothesis is that the identification of an increased number of cyathostomes from naturally-infected ponies would result in the increased identification of low-prevalence species and therefore, provide data that would more accurately reflect the cyathostome population dynamics within the infected ponies. The purpose of the study was to develop a more accurate method of determining the cyathostome population dynamics in equids.

**Approach:**
In the current study, 5% aliquots were collected from total washings of the dorsal colon (DC), ventral colon (VC) and cecum of 10 naturally-infected ponies maintained under the same management regime. From this collection, random sub-samples of 200 worms were examined and identified to species. This procedure was continued in aliquots of 200 until all collected worms were identified. Data were collated and a statistical model was used to determine the number of worms required to give a 95% confidence level that all species present had been identified.

**Accomplishments/Results/Conclusions:**
When one subset of 200 worms from each of the 10 ponies was examined we found an average of 10.9 ± 4.3 cyathostome species. However, when the entire 5% aliquots were examined from each pony, the mean number of species increased to 25.2+/-2.6, indicating that prevalence rates from species with low intensities are probably much greater than previous survey data indicate. Indeed, of the 29 species identified using this latter technique, 11 are considered rare or very rare in other studies. An intercept model was developed which predicted that for each 2000 worms counted in a study, at least 1300 should be identified to provide an accurate representation of all species present.

**Benefits to/Impact on Equine Industry:**
This study has produced important data and provides critical guidelines that will aid researchers in generating both more accurate biological, epidemiological and anthelmintic data regarding the cyathostome parasites of horses.

**Take Home Message:**
The current cyathostome enumeration and identification techniques may not be producing data that accurately reflects existing parasite population dynamics. The relative importance or pathogenicity of individual cyathostome species is unknown at this time, and it cannot be assumed that the more prevalent species are the most pathogenic. For biological, epidemiological and drug resistance studies, it is critical to have a complete picture of the species diversity within a population and the data generated during this study will help contribute to this.

**Year Completed:** 2001

**Published Manuscripts/Abstracts:**

**Equine cyathostome populations in Louisiana: Seasonal transmission and species prevalence**

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

**Description of the Problem:**
Cyathostomes are currently considered to be the major pathogenic nematode parasites infecting well-managed equids. The seasonal nature of cyathostome transmission in livestock is central to the development of strategic parasite control programs. Transmission cycles are affected by climatic conditions. A severe clinical syndrome, larval cyathostomosis,
Parasitology

is seasonal in onset, occurring most typically in younger animals in late winter or early spring. In addition, the seasonal recovery of adult cyathostomes has been reported worldwide. These studies generally suggest a seasonal cycle of cyathostome infections, with most adults being found in the spring and fall months, an inhibition of larval development in winter and a resumption of larval development in spring associated with larval cyathostomosis. Studies were conducted to investigate seasonal-associated transmission of gastrointestinal parasites in ponies in Louisiana in order to provide further information regarding targeted anthelmintic treatment for large and small strongyle control.

**Study Purpose/Objectives:**

Our hypothesis is that characterization of seasonal transmission patterns and prevalence of cyathostome populations will contribute to the development of better treatment regimes for the control of cyathostomes in equids. The purpose of the studies was to determine seasonal variation and prevalence of cyathostomes in equids residing in Louisiana.

**Approach:**

Two studies were conducted in order to investigate the seasonal effect of transmission on (1) parasite-naïve, and (2) naturally-infected ponies in southern Louisiana. In the first study, 20 parasite-free pony foals were grazed on a 6-acre pasture in groups of 2 or 3, for each grazing season between 1988-1989 and 1992-1993. The pasture contained resident parasitized pony mares which had limited history of anthelmintic treatment. During grazing, fecal egg counts were determined and pasture conditions and larval counts were recorded. Following grazing, ponies were held in box stalls for 6 weeks. At necropsy, cyathostomes adults and larvae were enumerated in each pony. The second study utilized data collected from 117 naturally-infected ponies known to have grazed within southern Louisiana for at least a year. Ponies <5 years were classified as young while ponies >8 years of age were classified as old. Following necropsy, adult and larval cyathostomes were enumerated and identified for each animal.

**Accomplishments/Results/Conclusions:**

Study 1: Data recovered on this study indicate that transmission of stronglye parasites is maximal in cooler seasons of the year in Louisiana and minimal during the summer months. This correlates with larval number on pasture, with cyathostome infective larvae numbers on pasture peaking during cooler months and lowest during summer months. The larvae recovered from the pasture were > 99% cyathostomes, although transmission of the Strongylinae also followed a similar pattern. Total numbers of cyathostomes recovered from ponies were highest during the winter months and lowest during the summer months, correlating with pasture larval infectivity. As expected, hypobiotic early L3 (EL3) were also highest during the winter months, although EL3 comprised the highest percentage of the fall population, suggesting increased hypobiosis during this season. Total numbers of luminal L4 were lowest during the summer, as reported by others in the southern United States. Highest numbers of adult worms were recovered during the spring and fall. This study represents the only survey of seasonal transmission of internal parasites of equines in non-immune ponies and, consequently, furthers our understanding of the transmission of these parasites.

Study 2: The data collected during this study suggests a seasonal pattern of infection and development for equine cyathostomes in southern Louisiana. In the young animals studied, adult worms were most abundant in the fall, when conditions are favorable for egg and larval development on pasture. In addition, most species diversity was also observed in the fall. Fecal egg count and pasture L3 numbers were also highest in fall and early winter in correlation with adult burdens. Numbers of hypobiotic EL3 were lowest at this time and peak during the summer months, when conditions are unfavorable for larval development on pasture. At all time, the data suggests the luminal L4 developmental stage is one which cyathostomes pass through rapidly. Interestingly, older, non-immune animals also showed highest adult numbers in the fall but otherwise showed a different seasonal distribution pattern. EL3 remain high at all times and directly reflect larval numbers on pasture, while number of L4 are highest in spring. It is likely that the dynamics of cyathostome infections in older, immune animals involve some regulatory feedback mechanisms not apparent in naïve animals.

**Benefits to/Impact on Equine Industry:**

The data suggest treatment of equids in Louisiana to eliminate the EL3 population during late summer to September will decrease the number of EL3 developing to adults and therefore reduce larval burdens on pasture during fall and winter. Further treatments would depend on egg reappearance periods following initial anthelmintic usage.
Take Home Message:
The dynamics of cyathostome infections in equids is complex and appears to be affected by host-dependant factors such as age and immune status, as well as host-independent factors such as climate. These studies provide in depth analysis of seasonal transmission on both young (naïve) and older (immune) ponies. The data suggests seasonal transmission does occur, although it affects different aged ponies in different ways. It is likely that the dynamics of cyathostome infections in older, immune animals involve some regulatory feedback mechanisms not apparent in naïve animals.

Acknowledgments:
The authors would like to thank J. Johnson, N. French, C. Monahan, J. Woody, H. Neumann, K. Romagosa and C. Walker for technical assistance.

Year Completed: 2001

Published Manuscripts/Abstracts:


Gastrointestinal helminths of ponies in Louisiana: Species composition analyses following twenty years of macrocyclic lactone treatment

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

Description of the Problem:
Cyathostome and other gastrointestinal (GI) parasites of equids have been targeted through the use of antiparasitic compounds for more than 100 years. However, the past 2 decades has seen the emergence of macrocyclic lactones (ML), specifically ivermectin (IVM) and moxidectin (MOX) and the broad application of these to parasite control in equids. It is now generally accepted that the continued use of ML on well-managed farms has nearly eliminated some parasites, including some spirurids, large strongyles and filarial parasites. The effect of these drugs on other parasite populations, however, is less clear. This survey examines the changes that have occurred in the prevalence and intensities of species that have parasitized ponies in Louisiana over the past 2 decades by recording the parasites present in 117 ponies from southern Louisiana which have had minimal exposure to anthelmintic compounds and comparing these data with a prior study conducted 20 years earlier.

Study Purpose/Objectives:
Our hypothesis is that the species composition of gastrointestinal parasites recovered from the 117 Louisiana ponies will be different to that observed in ponies from the same geographic region 20 years earlier, and that this difference may be due to the use of broad-spectrum anthelmintics in the last two decades. The purpose of the study was to compare the composition of gastrointestinal parasite species from ponies now and twenty years earlier.

Approach:
Ponies (117, 67 males, 50 females) ranging in age from 4 months to 20 years and known to have grazed for at least 1 year in southern Louisiana were used in this study. All ponies had little or no previous exposure to anthelmintic treatment. Following necropsy, 5% aliquots were taken of the dorsal colon, ventral colon and cecum and parasites recovered, enumerated and identified. Smaller parasites were counted by aliquot with an average of 519 small worms identified per animal. Larger worms were counted in toto. Data collected were compared to that obtained from an earlier survey published in 1986 (Torbert et al. 1986).
Accomplishments/Results/Conclusions:
In this study, we identified 26 species of cyathostomes and 8 species of strongyles from infected ponies, including 2 species of cyathostomes, Cylicostephanus asymmetricus and C. bidentatus, 1 large strongyle Oesophagodontus robustus and 1 cestode, Paranoplocephala mamillana, not found in the 1986 survey. Otherwise, the diversity of identified species has remained the same, and there has been no dramatic change in the prevalence ranking when compared with the 1986 survey. The only species that appears to have been eliminated over this time, Draschia megastoma. Cylicostephanus longibursatus was the most prevalent species found, both in the current study and in 1986. It occurred with almost the same mean intensity in both surveys. Both prevalence and intensity of all other species, however, were significantly reduced in the current survey. The reasons for this decrease are unknown, although it may be linked to the development and intense use of ML parasiticides. While the ponies used in this study were not exposed to heavy ML use, they would have had some exposure to ML over their life. In addition, the prevalence and intensity of parasite species could have been indirectly affected by the use of ML in equids other than those examined, but which were maintained on the same pastures. It should be noted that the intensity and prevalence of tapeworms did not change between the 2 surveys. Cestodes are not susceptible to ML, and this data supports the speculation that the changes in strongyle species may be due to the action of ML parasiticides. It is also noteworthy, however, that in spite of much anthelminthic pressure, there has been little change in the past 20 years in the distribution and relative rankings of the most populous cyathostome species. It is possible that the harboring of cyathostomes in refugia within the mucosa has contributed to this lack of change. Adults and L4 are efficiently removed from the lumen by IVM treatment. Larvae in refugia then emerge to repopulate the lumen. All cyathostome species are likely equally involved in this emergence and therefore contribute equally to the new infection.

Benefits to/Impact on Equine Industry:
The prevalence and species composition of cyathostomes, the causative agent of larval cyathostomosis in equids, has not changed much in Louisiana ponies over the past 20 years, despite the introduction and broad application of ML for GI parasite control. The cyathostome life cycle and it’s refugia stage are likely responsible for this lack of change in species prevalence composition data over 20 years. Species rated as important more than 2 decades ago can still be ranked as primary targets for control today.

Take Home Message:
The widespread use of ML in equids is thought to have almost eliminated some parasites. However, this survey shows that those cyathostome species that were most abundant 20 years ago, prior to the introduction of extensive ML use, still remain most prevalent today in Louisiana.

Acknowledgments:
The authors thank J. Johnson, M. Johnson, M.A. Baudena, J. Woody, C. Walker and W. Wiles for technical assistance.

Year Completed: 2001

Published Manuscripts/Abstracts:

Evaluation of ivermectin efficacy against equine gastrointestinal parasites

Authors/Investigators:
Thomas R. Klei, PhD; S. Rehbein; M. Visser; W. K. Langhoff; Melanie R. Chapman; Dennis D. French, DVM, DABVP; P. Hanson.

Description of the Problem:
Ivermectin (IVM), first introduced for use against internal equid parasites in 1981, has a broad spectrum of activity against both adult and migrating larval stages of various nematode parasites. It is not believed to be effective against larval stages of encysted cyathostomes, found in the mucosa of the large intestine. Ivermectin has now been available for more 20 years. During this time, parasite recovery techniques and identification have improved considerably.

Study Purpose/Objectives:
Our hypothesis is that the administration of ivermectin paste will continue to prove to be highly effective for treatment and control of a broad range of small and large strongyle species, as well as other species of gastrointestinal (GI) parasites. The purpose of the study was to reevaluate the efficacy of ivermectin in controlled studies, using more sensitive procedures that previously reported.
Approach:
Two trials using 20 ponies each were conducted in Rohrdorf-Lauterbach, Germany and in Baton Rouge, USA. Ponies (22 male, 18 female) had naturally-acquired GI parasite infections and unknown history of anthelmintic exposure. For each study, 10 animals were randomly allocated to an untreated control group and 10 were allocated to receive ivermectin paste at 200 mcg kg⁻¹, orally, once, on Day 0. Necropsies were carried out on Day 14 or 15. Aliquots (5% and 10%) were taken from total washings of the stomach, small intestine, ventral colon (vc), dorsal colon (dc) and cecum. Worms were isolated from aliquots, enumerated and identified. All intestinal sections were examined grossly for bots (stomach), large strongyles (rectum), ascarids and Strongyloides (cecum, dc, vc).

Accomplishments/Results/Conclusions:
Ponies treated with IVM had significantly fewer adult small strongyles and adult large strongyle than did control animals. IVM was also highly effective against non-strongyle species. Efficacy was observed against a wide range of large strongyles and 24 species of small strongyles. The levels of efficacy observed were comparable to those reported 20 years ago.

Benefits to/Impact on Equine Industry:
The efficacy of IVM was shown to be comparable to that exhibited 20 years ago at its release. Although this is not surprising, these observations are important in that they document the continued high degree of efficacy of this drug against randomly selected populations of these parasites in both the USA and in Europe.

Take Home Message:
IVM remains as effective against a broad range of large and small strongyles today as it was 20 years ago. This monitoring of efficacy is important, particularly with drugs such as IVM, with which there has been documented reports of developing resistance in GI nematodes of sheep and goats.

Year Completed: 2001

Published Manuscripts/Abstracts:

Effect of gastrointestinal worm burdens on immune response of ponies to immunization

Authors/Investigators:
Jennifer. D. Edmonds, DVM, PhD; David W. Horohov, PhD; Melanie R. Chapman; Thomas R. Klei, PhD.

Description of the Problem:
Helminth parasites are common in horses, even with the highly effective anthelmintics available today. These parasites have been shown to alter the outcome of heterologous immunization in other host species. Typically, gastrointestinal (GI) helminthes induce a dominant type 2 cytokine response, which can down regulate or alter protective type 1 cytokine responses required for protection against bacterial and viral challenge. In addition, parasite loads can result in multiple deleterious effects, both clinical and subclinical, that may alter the outcome of vaccination. These include inappetence, metabolism changes and decreased absorption of minerals. There are currently no studies reporting on the effect of parasitism on subsequent antigenic challenge.
Study Purpose/Objectives:
Our hypothesis is that horses with heavy GI parasite burdens will not develop the same immune responses to heterologous immunization as those with no or light GI parasite burdens. The purpose of the study was to evaluate the effect of GI parasites on the immune response to heterologous immunization by analyzing the immune response of horses with low, medium and high parasite burdens to vaccination with the protein keyhole limpet haemocyanin (KLH).

Approach:
12 naturally infected ponies were randomly allocated to 1 of 3 groups, based upon fecal egg count. Group 1 received moxidectin and were designated the low parasite group. Group 2 received pyrantel pamoate and were designated the medium parasite group. Group 3 remained untreated and were designated the high parasite group. All ponies were immuned 24 days post treatment with a single i.m. injection of KLH. Serum samples and PBMC samples were collected throughout the experiment for analyses of antibody titer and cytokine mRNA profile. At necropsy, the stomach, small intestine, cecum and large intestine contents were collected and a 1% aliquot taken. Adult and larval strongyles were enumerated.

Accomplishments/Results/Conclusions:
All protein immunized ponies displayed a weak proliferative response to KLH, regardless of parasite burden, although ponies with light burdens displayed slightly stronger responses than those with heavy burdens. Only lightly parasitized ponies exhibited a dominant type 2 cytokine profile. However, these animals also showed higher levels of the type 1 cytokine, IFN-g, than did ponies with medium or heavy parasite burdens. This may reflect the increased ability of the lightly parasitized animals to respond to antigen stimulation, compared to animals with larger parasite burdens. Ponies with low parasite burdens also showed increased total immunoglobulin production in response to immunization, compared with ponies with medium or heavy parasite burdens. These data suggest that heterologous immunization of heavily parasitized ponies results in decreased cellular and humoral immune response.

Benefits to/Impact on Equine Industry:
Ponies with large parasite burdens showed decreased immune responsiveness to immunization with a heterologous protein. This suggests that removing parasite burdens from equids through anthelmintic treatment may result in better overall immunity. This study illuminates a hidden problem which can be easily attended to by the use of regular monitoring of parasite burden and treatment when necessary.

Take Home Message:
This study has examined an important subclinical impact of parasitism in equids. It shows that high parasite burdens can result in the generation of sub-optimal immune responses to immunization/vaccination.

Acknowledgments:
The study was funded in part by Fort Dodge Animal Health. The authors acknowledge Dr. Paul Lunn for his contributions, and they thank S. S. Pourciau, K. Antoku, K. Snedden for technical assistance.

Year Completed: 2001

Published Manuscripts/Abstracts:

Effect of grazing on development of immunity to equine cyathostomes

Authors/Investigators:
Melanie R. Chapman, Dennis D. French, DVM, DABVP; Thomas R. Klei, PhD.
Description of the Problem:
Cyathostomes remain the most common helminthes in terms of both numbers of species and total parasite numbers per equid in the face of highly efficacious, broad-spectrum antiparasiticides. These nematodes have been shown to have a detrimental effect on foal growth and development and they induce a seasonal diarrheal syndrome in these young animals, termed larval cyathostomosis. It has been assumed that immunity to cyathostomes develops over time with exposure, based upon survey data comparing adult parasite numbers and fecal egg counts in young and old animals. More recently, we have shown that exposing foals to cyathostome larvae on pasture induces an immunity on a subsequent challenge, resulting in a self-cure phenomenon that is not parasite species-specific. While it is now clear that maximal acquired resistance to cyathostome challenge is slow to develop, there have been no in-depth studies investigating the degree to which immunity controls infection following natural exposure to pastures with different levels of cyathostome contamination.

Study Purpose/Objectives:
Our hypothesis is that ponies previously exposed to pastures heavily contaminated with cyathostomes will show increased resistance to a challenge infection of cyathostome larvae. The purpose of the study was to further define the effect of pasture exposure on acquired cyathostome infection in southern Louisiana.

Approach:
Ponies (17) aged 1- 4 years were used for this study. Thirteen of the ponies were divided into 2 groups. One group was moved to a highly contaminated pasture for 4 months with no anthelmintic treatment. The second group remained on a clean pasture and received ivermectin (IVM) every 8 weeks, for 4 months. Following this grazing period, all ponies were treated with IVM and oxibendazole and stalled for 3 weeks. Four foals were raised parasite free and used as controls. Each pony was given a total of 100,000 L3 and necropsied 7 weeks post challenge. All animals were monitored for daily health observations, including temperature and hematology. Fecal egg counts were collected throughout the entire study. At necropsy, aliquots of cecum, dorsal colon and ventral colon collected and parasite recovery was conducted. Intestinal biopsies were also taken.

Accomplishments/Results/Conclusions:
Five of the 7 exposed ponies developed positive fecal egg counts throughout the study. The total number of parasites recovered from ponies challenged with 100,000 cyathostome larvae was not different between groups, regardless of whether ponies had previously been exposed to a heavily contaminated pasture or a clean pasture. Previously exposed animals did have significantly higher numbers of early L3 and developing larvae in the mucosa, than did non-exposed and challenged ponies. Furthermore, the animals raise parasite-free and challenged showed a significantly higher percentage of adult cyathostomes than those animals raised either on clean pasture or heavily contaminated pasture. Animals exposed to heavily contaminated pasture and then challenged also showed larger weights of mucosal biopsies of the cecum and ventral colon, where significantly more encysted larvae were found. However, there were no histological changes in these animals, compared with the other two groups.

Benefits to/Impact on Equine Industry:
The natural acquisition of acquired resistance to cyathostome infection is an important consideration in the design of equine helminth control programs. This study shows that regular treatment with IVM allows only minimal infection that does not provide any levels of acquired immunity. Importantly, a single season exposure also fails to provide protective level of resistance to infection.

Take Home Message:
This study provides important information regarding the development of immunity in equid to cyathostome infections with exposure. The data suggest that a demonstrable resistance to infection is not acquired after 4 month exposure to a heavily contaminated pasture.

Acknowledgments:
The authors thank C. Walker, A. Hood, J. Edmonds and R. Klei for technical assistance.

Year Completed: 2001

Published Manuscripts/Abstracts:

Developing cyathostome larvae in the mucosa of the large intestine.
Pharmacology

In vivo distribution and elimination of 20% phenylbutazone solution administered orally to fasted horses

Authors/Investigators:
Diana M. Watson, BS; Honor Ame Walesby, DVM, MS, DACVS; Steve Barker, MS, PhD; Charles R. Short, DVM, MS, PhD, DACVCP.

Description of the Problem:
Proven efficacy and ease of administration make phenylbutazone (PBZ) one of the most popular non-steroidal anti-inflammatory (NSAID) drugs used in the horse. Although efficacious and widely used, the margin of safety between effective dose and toxic dose is small, and can vary among individuals and disease states. PBZ is available in many forms: 1) one gram oral tablets; 2) oral paste or gel; 3) 20% intravenous solution; and 4) micro-encapsulated oral powder. Accurate dosage of PBZ in horses weighing 150 kg is difficult and inaccurate. The standard dosage for PBZ is 2.2 mg/kg BWT. In a 150-kg horse, this translates to 330 mg or 1/3 of a tablet, a small glob of paste, or 1.65 ml of 20% the solution. Intravenous administration of PBZ solution is accurate, but requires a catheter or veterinary administration making PBZ solution impractical for horse owners. Metered syringes for delivering paste and gel are divided into one gram increments which are not designed for partial dose delivery. The tablet is scored for partial dose delivery; however, doses other than ¼ to ½ gram are inaccurate. The low margin of safety, coupled with individual variation, places horses weighing < 150 kg at high risk for phenylbutazone toxicosis associated with inaccurate phenylbutazone administration. Oral use of PBZ solution allows for exact dosage of horses weighing less than 150 kg. Increased accuracy decreases risk for over-dosage and toxicosis that exists with common oral formulations. The pharmacokinetics of phenylbutazone (20%) solution administered orally has not been described.

Study Purpose/Objectives:
We hypothesized that oral administration of 2.2 mg/kg BWT to fasted horses would yield statistically equivalent maximum concentration (Cmax), time to maximum concentration (Tmax), elimination half life (T1/2), and area under the curve (AUC) values for oral PBZ paste and PBZ 20% solution. The aims of the study were to: (1) determine the Cmax, Tmax, T1/2, and AUC for PBZ (20%) solution given orally; (2) determine Cmax, Tmax, T1/2, and AUC for PBZ paste given orally; and (3) compare pharmacokinetic data for the solution and paste formulations of PBZ. The specific objective was to verify the oral use of PBZ (20%) solution as a legitimate route of administration for this formulation in the horse.

Approach:
Horses (n=6) were weighed, fasted, and orally dosed with solution or paste (control) at 2.2 mg/kg BWT. Blood was collected at time 0, 0.5, 2, 4, 8, 12, and 24 hours post-administration (HPA). After a 14 day washout period, horses were fasted, dosed with the opposite formulation, and sampled as described. Serum samples underwent solid-phase acid extraction and quantification by high performance liquid chromatography. The Cmax values for paste (7.35 μg/mL) differed significantly (P<0.05) from solution (5.65 μg/mL). The Tmax values for paste (4.97 HPA) and solution (3.03 HPA) did not differ significantly. The AUC for paste (101.75) was 30% greater than AUC for solution (67.09). The T1/2 for both formulations was 12 hours.

Accomplishments/Results/Conclusions:
The results indicate that oral absorption of both PBZ paste and PBZ (20%) solution is highly variable when compared to the literature. Oral administration of PBZ solution is an accurate route of administration for horses weighing less than 150 kg; more work needs to be done to determine the dose that will yield Cmax and AUC values equal to PBZ paste. This data will provide a baseline pharmacokinetic data to support the oral administration of PBZ (20%) solution to horses. Oral use of the PBZ (20%) solution allows for more exact dosage of horses weighing less than 150 kg. Increased dose accuracy will decrease the risk for over-dosage and potential toxicosis that exists in this group of animals when using the other common formulations of PBZ such as paste, powder, gel, or tablet.

Phenybutazone is often administered to horses with lameness caused by musculoskeletal injuries.
Benefits to/Impact on Equine Industry:
This study demonstrates that oral administration of phenylbutazone solution (20%) can be a useful alternative to paste or tablet formulations due to more accurate dosing.

Take Home Message:
Oral administration of phenylbutazone solution allows more accurate dosing of horses weighing less than 150 kg and could be helpful to decrease the likelihood of toxicity in this group of horses.

Acknowledgments:
This study was funded by the Merck-Merial Animal Health Grants Program in support of student elective summer research scholarships. Special acknowledgment must be extended to Michael Keowen, Brigette Williams, Brenna Hanly, and Marian Waguespack for their contribution to data collection and analysis.

Year Completed: 2003

Published Manuscripts/Abstracts:

Interaction of cimetidine with equine hemoglobin

Authors/Investigators:
Robert P. Hunter, PhD; Charles R. Short, DVM, MS, PhD, DACVCP; A. A. Dees

Description of the Problem:
Cimetidine is a known inhibitor of cytochrome P-450, the inhibition of which is mediated through this heme containing protein by binding as a sixth axial ligand with iron. Previous reports from our laboratory demonstrate that cimetidine decreases both local and systemic NO₃⁻ production in an equine soft tissue inflammation model; however, there is no information available regarding the mechanism by which cimetidine exerts this effect.

Study Purpose/Objectives:
The purpose of these studies was to determine the mechanism by which cimetidine decreases nitrate in this model. The objective was to determine if cimetidine would bind to equine hemoglobin and if cimetidine binding to equine hemoglobin would alter nitrate formation.

Approach:
The first study was conducted to determine if spectral changes occurred with cimetidine in the presence of equine hemoglobin. Hemoglobin is necessary for the conversion of nitric oxide (NO) to nitrate (NO₃⁻). Equine hemoglobin was used at concentrations of 0.1 and 1 mg/ml in phosphate buffered saline (PBS). Cimetidine was added to the sample cuvette to yield final concentrations of 0, 0.1, 0.5 and 1.0 mM. Zaltidine, a guanidine derivative H₂-receptor antagonist, interaction was also evaluated at concentrations of 0, 1, 5 and 10 mM. Equal volumes of PBS were added to the reference cuvette when spectra were obtained. The second study was conducted in an attempt to determine where in the metabolism of L-arginine to NO₃⁻ cimetidine inhibits the formation of NO₃⁻. The effect of cimetidine on the conversion of NO to NO₃⁻ catalyzed by hemoglobin was investigated using a carbon fiber microsensor to detect NO.

Accomplishments/Results/Conclusions:
Cimetidine at a concentration of 1.0 mM produced a spectra different from that observed at lower concentrations of cimetidine, hemoglobin or at any concentration of Zaltidine, which indicates that cimetidine interacts with equine hemoglobin in some manner to induce spectral change, but there is no indication of direct interaction of cimetidine with the iron-heme of equine hemoglobin.

Benefits to/Impact on the Equine Industry:
This study demonstrates that cimetidine, an H₂-antagonist that is used for treatment and prevention of gastric ulcers in horses, has the capacity to reduce inflammation-induced production of NO₃⁻. What impact administration has on inflammatory processes in horses being treated with cimetidine remains to be determined.

Take Home Message:
Cimetidine is effective in reducing inflammation-generated NO₃⁻ concentrations. This could occur via inhibition of iNOS by the same mechanism of heme-iron binding that cimetidine employs to inhibit cytochrome P-450 mediated metabolism or its binding to equine hemoglobin.
These findings suggest further investigation is needed to address a number of questions, including (1) the potential effect of similar H2-receptor antagonists on inflammation related NO production; (2) the possibility that other guanidine moiety substances may affect NO production, and (3) the questions of whether cimetidine or other H2-anatoniants inhibit constitutive NOS isoforms and therefore affect physiologic processes other than inflammation.

**Year Completed:** 2001

**Published Manuscripts/Abstracts:**

Reproduction

**Improvement of sperm recovery rates after centrifugation of stallion semen**

**Authors/Investigators:**
Maria Soledad Ferrer, DVM, MS, DACT; Dale L. Paccamonti, DVM, MS, DACT; Bruce E. Eilts, DVM, MS, DACT; Sara K. Lyle, DVM, MS, DACT; Abdul H. Aljarrah, DVM, MS, DACT; R. Devireddy, PhD.

**Description of the Problem:**
Centrifugation is routinely performed during semen cryopreservation or preparation for semen shipment. Centrifugation is done with the objective of removing seminal plasma and achieving the optimum sperm concentration for packaging. Maximum sperm recovery with minimal sperm damage is one of the goals when centrifuging stallion semen. While gravitational force and time are parameters of concern, the height of the fluid column may be an important aspect that is commonly overlooked. Reducing the volume (column height) contained in a tube should increase sedimentation, thereby minimizing sperm loss in the supernatant.

**Study Purpose/Objectives:**
The objectives were to compare sperm yield and viability immediately after centrifugation and after 24 hours of storage at 4 °C. We hypothesized that an increase in gravitational force would increase sperm recovery rate without inducing immediate or long-term damage to the cells, and that a reduction in the height of the column would increase recovery rate.

**Approach:**
Ejaculates were collected from 8 stallions. Each ejaculate was extended to 25 x 10^6 cells/mL in a skim-milk extender and divided into 9 aliquots. Aliquots were centrifuged in 50-mL conical tubes under the conditions in Table 1. After centrifugation, sperm concentration in the supernatant was determined and sperm recovery rate was calculated. Each aliquot was resuspended to 25 x 10^6 cells/mL and stored at 4 °C for 24 h. Motility patterns were analyzed with a computer assisted semen analyzer and sperm viability was evaluated with SYBR14/Propidium Iodide. These parameters were assessed before and after centrifugation, and after 24 h of cooled storage.

<table>
<thead>
<tr>
<th>Table 1: Centrifugation conditions for the different treatments</th>
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<tr>
<td>Force (x g)</td>
<td>0</td>
<td>400</td>
<td>400</td>
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<td>400</td>
<td>900</td>
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<tr>
<td>Time (min)</td>
<td>0</td>
<td>10</td>
<td>10</td>
<td>5</td>
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<td>10</td>
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<tr>
<td>Volume (mL)</td>
<td>40</td>
<td>40</td>
<td>20</td>
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**Accomplishments/Results/Conclusions:**
There were no differences among treatments in total motility, progressive motility and percentage of live cells immediately after centrifugation and after 24 h of storage. Sperm recovery rate was higher for treatments 1 (100%), 3 (95.3±5.6), 6 (91±4.6), 7 (99.1±0.8) and 9 (93.5±10) compared to treatments 2 (74.3±9.2), 5 (72±11.3) and 8 (72.4±11) (mean ± SD, p<0.05). Treatment 4 provided the lowest recovery rate (44.1±8) (p<0.05).

The results supported our hypotheses. When using 400 x g for 10 min or 900 x g for 5 min, decreasing the height of the column of fluid increased the recovery rate. When dealing with a large volume of semen, recovery rate can be improved by centrifuging at 900 x g for 10 minutes without inducing immediate or long-term damage to spermatozoa.

**Benefits to/Impact on the Equine Industry:**
It has been estimated that around 20% of the spermatozoa initially present in the ejaculate are lost in the supernatant after centrifugation. When using a routine centrifugation force and time of 400 x g for 10 min, decreasing the height of the column of fluid allowed the recovery of 95% of the spermatozoa. When dealing with a large volume of semen, recovery rate can be improved by centrifuging at 900 x g for 10 minutes without inducing immediate or long-term damage to spermatozoa.

**Take Home Message:**
While gravitational force and time are parameters of concern when centrifuging stallion semen, the height of the fluid column is also an important aspect to consider. Reducing the volume (column height) contained in a tube increases...
sedimentation, thereby minimizing sperm loss in the supernatant. Recovery rate can also be improved by centrifuging at 900 x g for 10 minutes without inducing immediate or long-term damage to spermatozoa.

**Year Completed:** 2003

**Published Manuscripts/Abstracts:**

### Persistent mating induced endometritis after low dose hysteroscopic insemination in the mare

**Authors/Investigators:**
Maria Soledad Ferrer, DVM, MS, DACT; Sara K. Lyle, DVM, MS, DACT; Bruce E. Eilts, DVM, MS, DACT; Robert A. Godke, PhD; Dale L. Paccamonti, DVM, MS, DACT.

**Description of the Problem:**
Endometritis is the third most common disease in adult horses and is a major cause of economic losses in the equine industry. A transient sperm-induced endometritis normally follows mating in the mare. While reproductively normal mares are able to resolve the endometritis within 12 to 48 h after mating, the condition may develop into a persistent mating-induced endometritis (PMIE) in mares with delayed uterine clearance (DUC). Since the equine embryo enters the uterus 5 to 6 d after ovulation, any delay in the elimination of bacteria and inflammatory products after mating may create a uterine environment that is incompatible with embryonic survival.

The effect of low-dose hysteroscopic insemination on the occurrence of PMIE in the mare is controversial. It has been suggested that placement of a low volume and small number of spermatozoa at the uterotubal junction (UTJ) could minimize sperm-uterine interactions and neutrophil chemotaxis, possibly resulting in a reduced inflammatory response compared with routine uterine body insemination. If this is true, hysteroscopic insemination could become a valuable management option for mares with DUC. Conversely, other authors have postulated that the hysteroscopic procedure itself is inflammatory by nature and should not be used on mares susceptible to developing PMIE. Fertility was reported to be lower after hysteroscopic insemination in problem mares. If hysteroscopy induces an exaggerated inflammatory response, the method may either need to be avoided in mares with DUC, or the mares may need to be treated aggressively after insemination to maximize chances for conception.

**Study Purpose/Objectives:**
The objectives of this study were to evaluate the occurrence and severity of PMIE in reproductively normal mares and mares with DUC after hysteroscopic insemination and to determine whether hysteroscopic insemination could be used in the management of mares with DUC.

**Approach:**
Reproductively normal and DUC mares received three treatments: uterine body insemination (UB, 1 x 10^9 spermatozoa, 20 mL), hysteroscopic insemination (HYST, 5 x 10^6 spermatozoa, 0.5 mL) and sham hysteroscopic insemination (SHAM, semen extender, 0.5 mL). Endometritis was evaluated 24 h after treatment. The inflammatory response was assessed based on the presence of intrauterine fluid, and concentration and percentage of leukocytes present in uterine secretions obtained via a small volume flushing technique.

**Accomplishments/Results/Conclusions:**
There was no effect of treatment or mare classification on intrauterine fluid accumulation or leukocyte numbers 24 h after the procedures. Low dose insemination by hysteroscopy did not result in increased inflammation compared to uterine body insemination.

**Hysteroscopic insemination at the uterotubal junction of the mare.**
in a reduced inflammatory response compared with standard AI, nor was the inflammatory response after insemination by hysteroscopy worse than after standard AI.

**Benefits to/Impact on the Equine Industry:**
We have characterized the inflammatory response to hysteroscopic insemination in the mare. Our results do not support the use of the technique with the intention of minimizing PMIE in mares with DUC. However, because the endometritis was comparable to that induced by routine uterine body artificial insemination, hysteroscopic insemination can be used in DUC mares without a higher risk of inducing PMIE.

**Take Home Message:**
Uterine endoscopy induces an inflammatory response that is associated with the procedure itself so it should not be used with the intention of minimizing PMIE in mares with DUC. However, hysteroscopic insemination did not result in a more intense cellular response. Based on these results there is no contraindication to its use in mares with DUC.

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

**Year Completed:** 2003

**Published Manuscripts/Abstracts:**

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**Transvaginal intrafollicular sperm cell injection with concomitant artificial insemination in the cycling mare**

**Authors/Investigators:**
Bruce E. Eilts, DVM, MS, DACT; Carlos R. F. Pinto, Med Vet, PhD, DACT; Dale L. Paccamonti DVM, MS, DACT; A.S. Niemanstverdreit, DVM, MS, DACT; Elena Garcia, DVM, MS; Robert A. Godke, PhD.

**Description of the Problem:**
Intrafollicular insemination (IFI) is an assisted reproductive technique that has produced offspring in humans, and has potential applications for circumventing the uterine inflammation in mares with persistent mating induced endometritis, breeding mares with reduced numbers of sperm cells from oligospermic ejaculates or frozen semen, and research applications to further understand the fertilization process. However, there is only one published report in the veterinary literature of equine IFI, and it was unsuccessful at producing a pregnancy. Other researchers also have been unable to attain pregnancies using IFI in the mare. Unanswered questions as to the possible reasons that IFI has failed in the mare include: Was the sperm number per follicle adequate?, Did the sperm remain unbound in the follicular fluid?, Did the sperm cells survive in the follicular environment long enough to achieve in vivo fertilization in the follicle or oviduct?, or Is there a detrimental effect of the IFI procedure?

Normal pregnancy rates have been obtained when a portion of the follicular fluid was aspirated from equine ovulatory follicle; however a lower pregnancy rate was found when all the follicular fluid was aspirated. Regardless, the removal of follicular fluid did not fully inhibit fertilization or pregnancy. Therefore, even if the IFI insemination dose is too small, if sperm cells do not survive in follicular fluid, or if sperm cells bind to the granulosa cells after IFI, then AI with concomitant IFI should still result in normal pregnancy rates. However, if there are other factors in the IFI procedure that are detrimental to fertilization and pregnancy, then AI with concomitant IFI would yield lower pregnancy rates. This study was designed to determine if the IFI procedure was detrimental in establishing a viable equine pregnancy when AI was also performed. The successful outcome of the study was to be a single pregnancy that could be attributed to either the AI breeding or to the IFI procedure when the ovulatory follicle had IFI performed.

**Study Purpose/Objectives:**
The successful outcome of the study was to be a single pregnancy that could be attributed to either the AI breeding or to the IFI procedure when the ovulatory follicle had IFI performed.

**Approach:**
Nine mares were used and the study was done in May and June. Of the nine mares, three were mated either before or after the IFI cycle and became pregnant, one was pregnant two years before, and two of the remaining five had embryos recovered the following year. During each estrus, the uterus and ovaries were palpated per rectum and had transrectal ultrasound examinations performed daily, until 2 days after ovulation. When a mare had visible signs of
Reproduction

estrus, and a 35 mm follicle was identified on the ovary, the mare was mated using AI. Mares had intrauterine AI performed with 0.9-13 x 10^9 (average 6.7+ 4.4 x 10^9) motile sperm cells from the same proven stallion. An aliquot of the semen was then extended to a concentration of 50 million cells/mL in a commercial skim milk extender and cooled in a commercial equine chilled semen shipping container to be used for the IFI. Each mare was also administered 2,500 IU hCG iv at the time of AI to induce ovulation within the next 48 hours. Eight mares ovulated within 48 h and one mare ovulated within 24 h after hCG administration. For the IFI procedure each mare was administered 0.5 mg/kg xylazine iv, 0.1 mg/kg butorphanol iv, and 0.05 mg/kg atropine iv. After administration of the medications an ultrasound guided transvaginal IFI was performed with 2 ml of the previously extended, cooled, semen. Seven of the nine mares had IFI performed 30-34 h after AI, and two of the nine mares had IFI performed on the same day as AI because the follicle was so soft on the day of AI. The IFI insemination was performed using a 6.5 MHz transvaginal probe. Ovulation was confirmed using ultrasonographic examination every 24 h after intrauterine insemination. Three mares had double ovulations, but only one of the three had IFI performed on both follicles. One mare had IFI performed on 2 follicles, however only one ovulated. At 18 d after ovulation, the mares were examined for pregnancy by transrectal ultrasonography.

Accomplishments/Results/Conclusions:
One of the nine mares became pregnant, but that mare had a double ovulation and only one follicle had IFI performed. Therefore, it cannot be established if the pregnancy was from the follicle that had the IFI or the follicle that was not injected with semen. The pregnancy was terminated, so no foal was born. The poor results obtained after IFI in mares and in humans may be attributed to an unknown aspect in the IFI procedure itself and not necessarily follicular puncture, sperm dose, sperm binding, or sperm longevity. Since follicular puncture and sampling or follicular puncture and total fluid removal did not eliminate pregnancy in mares, the puncture procedures can be considered safe. The dose of semen placed into the follicle with IFI and concomitant AI should have no bearing on the pregnancy rates in the present study, since AI is generally sufficient to attain pregnancies in normal circumstances. However, if there is a detrimental effect of the sperm cells or seminal plasma on the oocyte or ovulation, the poor results of IFI in the mare may be explained. The mare fertility in the present study may be questioned, but four of the nine had been pregnant or were pregnant after the IFI and two more had embryos recovered the following year.

Although a mare became pregnant in this study, it cannot be ascertained whether the pregnancy was the result of fertilization from the IFI procedure, fertilization by AI of the oocyte that had IFI, or fertilization by AI of the oocyte that did not have IFI. It is also not certain whether ovulation and luteinization occurred after IFI, although it appeared so by ultrasound. It is known that ovulation and luteinization occur normally after follicular puncture or aspiration, however the effect on sperm or semen deposition into the ovulatory follicle is not known. Perhaps a component of the sperm cell or seminal plasma inhibits ovulation or the normal maturation of the equine oocyte. More work is required to determine why pregnancy using the assisted reproductive technique of IFI in the mare has not been attained.

Benefits to/Impact on the Equine Industry:
IFI can not be ensured as the means of attaining pregnancy in the mare. In fact, it appears as though IFI may be detrimental to establishing pregnancy for an as yet, unknown reason.

Take Home Message:
The IFI procedure may be detrimental to establishing pregnancy in the mare for an as yet, unknown reason.

Year Completed: 2001

Published Manuscripts/Abstracts:

Is the failure of embryo development after intrafollicular insemination in the horse due to sperm viability?

Authors/Investigators:
Joyce M. Parlevliet, DVM, PhD, ECAR; John W. Lynn, PhD; Bruce E. Eilts, DVM, MS, DACT; Sara K. Lyle, DVM, MS, DACT; Maria S Ferrer, DVM, MS, DACT; Abdul H. Aljarrah, DVM, MS DACT; Dale L. Paccamonti, DVM, MS, DACT.

Description of the Problem:
Intrafollicular insemination (IFI) may be an attractive and not technically complex approach to achieve a pregnancy by assisted reproduction. Intrafollicular insemination has successfully been used to obtain pregnancies in humans. To date, no pregnancies have resulted via IFI in the horse. In prior studies, we treated mares with or without hCG before
IFI and injected spermatozoa treated with or without calcium ionophore for induction of capacitation, into the pre-ovulatory follicle. However, none of the mares became pregnant. In a subsequent study, we performed IFI with concomitant artificial insemination (AI) in cyclic mares yet were unsuccessful in obtaining pregnancies. The effect of many factors, such as the IFI procedure itself, sperm dose, sperm oocyte binding, sperm longevity and influence of the procedure on oocyte maturation are unknown. The role of spermatozoa in the failure of IFI has not been investigated.

**Study Purpose/Objectives:**
The objective of this study was to evaluate sperm recovery rate (%), motility, the percent of live/dead and the acrosomal status of spermatozoa after IFI and in vivo incubation.

**Approach:**
Ten reproductively sound mares in estrus, with a follicle > 35mm, had IFI performed with either 50 million (n=5) or 500 million (n=5) motile spermatozoa 36 hours after hCG administration. After 4 hours the pre-ovulatory follicular fluid was aspirated. The recovered spermatozoa were evaluated for motility using light microscopy and the recovery rate (%) of spermatozoa was calculated using a hemocytometer. Recovered spermatozoa were stained for live/dead status with SYBR 14 and propidium iodide and the number of live dead cells were counted using fluorescent microscopy. Acrosomal status was evaluated after staining an air dried smear with FITC-PNA using a fluorescent microscope with a FITC-filter.

**Accomplishments/Results/Conclusions:**
In 7 of 10 samples a few motile spermatozoa were detected, however when stained for live/dead only 5 of 10 samples had live spermatozoa. Sperm recovery was 0.53% ± 0.38 (mean ± SEM) and 9.02% ± 4.41 for the 50 and 500 million IFI groups, respectively, and varied from 0.0022 to 24.8%, which was independent of initial sperm concentration (Student’s t test, P>0.05). All spermatozoa were acrosome reacting or reacted. These data suggest that the intrafollicular environment in the pre-ovulatory follicle might be unfavorable for sperm viability or fertilization. Further research is necessary to detect incongruous maturation of the oocyte and spermatozoa.

**Benefits to/Impact on the Equine Industry:**
The data from our experiment suggest that the intrafollicular environment in the pre-ovulatory follicle might be unfavorable for sperm viability or fertilization; however, the reasons for this are unclear. One possibility is asynchronous maturation of the spermatozoa and oocyte. Further research is necessary to detect incongruous maturation of the oocyte and spermatozoa.

**Take Home Message:**
The reasons intrafollicular sperm cell injection does not have fertility are the small number of sperm cells available to fertilize the oocyte and the functional status of the sperm in follicular fluid.

**Year Completed:** 2003

**Published Manuscripts/Abstracts:**
The in vitro contractile response of non-gravid equine circular and longitudinal myometrial smooth muscle from the uterine horn to endothelin-1

Authors/Investigators:
Honor Ame Walesby DVM, MS, DACVS; Changaram S. Venugopal BVSc, MSc, MS, PhD; Giselle Hosgood BVSc, PhD, DACVS; Susan C. Eades DVM, PhD, DACVIM; Rustin M. Moore DVM, PhD, DACVS.

Description of the Problem:
Horses develop endotoxemia secondary to gastrointestinal disorders, pleuropneumonia, retained placenta, and metritis. Mares exposed to endotoxin during gestation have a 32 to 60% pregnancy loss rate secondary to embryonic resorption or preterm fetal expulsion. This rate is four times the 8 to 15% annual rate of pregnancy loss reported for the industry, and represents a source of considerable financial liability. Although a correlation between endotoxia and pregnancy loss exists, the exact role of endotoxin and its mechanism of action relative to equine myometrial contractility has not been defined. Plasma concentrations of endothelin-1 (ET-1) are significantly increased in horses with naturally acquired gastrointestinal tract disease, and was greatest in horses with conditions typically associated with signs of endotoxia such as strangulating obstruction, inflammatory bowel disease, and peritonitis. The presence of mRNA for ET-1 has been detected in human endometrial stroma and rat uterus. ET-1 has been shown to cause receptor-mediated vasoconstriction of uterine blood vessels and tonic contraction of the myometrium in vitro. Pharmacological studies reveal that there are at least three distinct endothelin receptors. Both ET$_{	ext{A}}$ and ET$_{	ext{B}}$ receptors have been demonstrated in non-gravid myometrium of humans, guinea pigs, rabbits, and rats; cultured human myometrial cells; and gravid human, rat, guinea pig, and monkey myometrium. A third endothelin receptor, designated receptor ET$_{	ext{C}}$, has recently been described, but little is known about this receptor with regard to uterine contraction and parturition. ET-1 effectively increases concentrations of intracellular calcium, increases myosin phosphorylation, and stimulates the force and frequency of contractions for human myometrial cells, isolated non-gravid and late-gravid rat uterus, and isolated non-gravid and late-gravid human myometrium in vitro. However, the contractile response has been shown to vary between the circular and longitudinal muscle layers of the uterus in gravid humans, gravid and non-gravid rats, and non-gravid rabbits.

Study Purpose/Objectives:
The purpose of this study was to characterize the physiologic and pharmacologic response of circular and longitudinal myometrial smooth muscle from the horn of non-gravid equine uterus to ET-1. Based on the literature, we hypothesized that ET-1 would cause a concentration-dependent contractile response in equine myometrial smooth muscle, and that incubation with BQ-123 and IRL-1038 separately, or in combination, would inhibit the contractile response to ET-1.

Approach:
Muscle strips from the circular and longitudinal smooth muscle layers of the uterine horn of ten non-gravid female horses were suspended in tissue baths and connected to force-displacement transducers interfaced with a physiograph. The muscle strips were incubated for 45-minutes with no antagonist (control), BQ-123 (endothelin receptor subtype A antagonist), IRL-1038 (endothelin receptor subtype B antagonist), or BQ-123 + IRL-1038 (combined in the bath) at three different final bath concentrations (10$^{-9}$, 10$^{-7}$, and 10$^{-5}$ M) before concentration-response curves to ET-1 were generated. The contractile response to cumulative concentrations of ET-1 (10$^{-9}$ to 10$^{-6}$ M) was quantified by measuring change in area under the curve (AUC) for the 3-minute period following each ET-1 dose. The EC$_{50}$ of ET-1 for the control, BQ-123, IRL-1038, and BQ-123 + IRL-1038 were determined for circular and longitudinal muscle layers of the equine horn at each blocker concentration.

Accomplishments/Results/Conclusions:
The findings of the present study reveal important physiological and pharmacological information regarding the contractile response of adult equine uterine horn to ET-1 and the role of ET receptors in mediating the response in these tissues. These findings include: 1) ET-1 is a potent contractile agent of both circular and longitudinal smooth muscles of the equine uterine horn; 2) ET-1 yields a significantly greater concentration-dependent contractile response in the circular smooth muscle layer than the longitudinal smooth muscle layer of the equine uterine horn; 3) BQ-123 and IRL-1038 receptor antagonists confirmed the presence of both endothelin subtype A and B receptors within the circular and longitudinal smooth muscle layers of the equine uterine horn; and 4) BQ-123 and IRL-1038 receptor antagonists do not inhibit the contractile response of uterine smooth muscle to ET-1 in a concentration-dependent manner.

Benefits to/Impact on Equine Industry:
The findings of this study help to identify chemical factors that influence uterine contractility in health and disease. Identification of these factors contributes to the understanding of disease processes affecting the horse and may help
guide veterinarians in the clinical and pharmacologic management of high risk cases to reduce the incidence of fetal loss associated with endotoxemia.

**Take Home Message:**
The inability of these receptor antagonists to completely block the contractile response to ET-1 when incubated together suggests the existence of another type of receptor or another mechanism which also mediates the contractile response of uterine smooth muscle to ET-1.

**Acknowledgments:**
This study was funded by the Louisiana State University School of Veterinary Medicine Equine Health Studies Program. Special acknowledgment must be extended to Michael Keowen, Catherine Koch, Diana Watson, Brigette Williams, and Brenna Hanly for their contribution to data collection and analysis.

**Year Completed:** 2002

**Published Manuscripts/Abstracts:**


Temporal distribution of clinical exacerbation of equine summer pasture-associated obstructive pulmonary disease in south Louisiana and its relationship with environmental factors

Authors/Investigators:
Lais R. R. Costa, MS, PhD, DACVIM; Jill R. Johnson, DVM, MS, DACVIM, DABVP; Matthew E. Baur, BA, PhD; Ralph E. Beadle, DVM, PhD.

Description of the Problem:
Summer pasture-associated obstructive pulmonary disease (SPAOPD) is a naturally occurring, recurrent condition that affects horses residing on pasture. Clinical signs of SPAOPD can vary from mild to life-threatening episodes of wheezing and paroxysmal cough, and chronic debilitating breathlessness, manifested most profoundly during the summer among horses maintained on pasture. The disease is considered to be an airway hyperresponsiveness to inhaled aeroallergens that differs from the barn-associated recurrent airway obstruction (RAO), primarily with respect to environmental conditions associated with the onset of clinical exacerbation. Even though the etiology of SPAOPD is not completely understood, it appears that seasonal changes affect the exacerbation-remission cycle of the disease. Identification of environmental factors, particularly those that precede the onset of clinical signs, would prompt the removal of horses from the offending environment during high risk times even if the triggering agents or their mechanism are not completely understood.

Study Purpose/Objectives:
The principal goals of this study were to document the seasonal pattern of clinical exacerbation of SPAOPD, and to identify the environmental factors (climate and aeroallergens) associated with exacerbation of the disease.

Approach:
A total of 29 adult horses, 19 affected with SPAOPD and 10 non-affected horses were included in this study. Daily examinations were performed on all 29 horses while maintained on pasture conditions at the Louisiana State University farm for a period of three years. The onset and progression of clinical exacerbation based on the clinical score of respiratory effort were evaluated in relation to daily changes in selected environmental factors including maximum temperature ($T_{max}$), minimum temperature ($T_{min}$), mean temperature ($T_{mean}$), maximum dew-point temperature ($DPT_{max}$), minimum dew-point temperature ($DPT_{min}$), delta dew-point temperature ($DPT_{delta} = DPT_{max} - DPT_{min}$), and normalized difference vegetation index (NDVI) over a three-year period. The seasonal pattern of the clinical exacerbation-remission of SPAOPD was evaluated temporally and in parallel with the variations of the environmental factors. Additionally, for one year (1994), the aeroallergen counts, including 20 specific types of mold/fungal spores and 28 types of pollen, were evaluated in relation to the onset of clinical exacerbation.

Temporal distribution (1992 to 1994) of clinical exacerbation of SPAOPD in relation to environmental variables. Julian date is shown on the X-axis, temperature [maximum ($T_{max}$), mean ($T_{mean}$), minimum ($T_{min}$)] or dew-point temperature [maximum ($DPT_{max}$), minimum ($DPT_{min}$), delta ($DPT_{delta} = DPT_{max} - DPT_{min}$)] are shown on the Y1-axis (solid line), and the proportion of horses showing signs of clinical exacerbation of SPAOPD (CSRE $\geq$ 4.5) while kept on pasture is shown on the Y2-axis (grey fill area).
Accomplishments/ Results/Conclusions:
The seasonal pattern of the clinical exacerbation of SPAOPD was demonstrated, and its relationship to increases in environmental variables of temperature (heat) and dew-point temperature (humidity). Increases in temperature ($T_{\text{max}}$, $T_{\text{min}}$, and $T_{\text{mean}}$) and dew-point temperature ($DPT_{\text{max}}$, $DPT_{\text{min}}$) measurements closely paralleled increases in clinical score of respiratory effort. For each of the horses evaluated in this study, the date of onset of clinical signs was fairly consistent from year to year, for all three years. The seasonal pattern of clinical exacerbation paralleled increases in mold/fungal spore counts and grass pollen counts. Specific types of mold spores were identified.

Benefit to/Impact on the Equine Industry:
Clinical exacerbation of SPAOPD in south Louisiana is temporally associated with hot and humid conditions, and with increases in exposure to grass pollen grains and mold spores, suggesting that these are potential aeroallergens triggering clinical exacerbation of the disease. The results of this study benefits horse owners and veterinarians because it indicates the environmental conditions associated with clinical exacerbation of SPAOPD allowing the removal of affected horses from the offending environment and preventing clinical exacerbation and progression of the disease.

Take Home Message:
The climate, especially hot and humid conditions, and aeroallergens, specifically certain mold spores and grass pollen grains, are associated with clinical exacerbation of SPAOPD. Future studies may allow us to further dissect the factors involved in the pathogenesis of this seasonal hyperresponsive airway disease of horses.

Acknowledgments:
We thank Kevin Robbins and Jay Grimes from the Southern Regional Climate Center and Dr. O. Huh from the Earth Scan Laboratory of the Coastal Studies Institute, for their assistance in obtaining the climate data used in this study. We especially thank Susan Sturm and Dr. Powlin V. Manuel from the Acadian Allergy and Asthma Center, Lafayette, LA for the pollen and mold/fungal reports. This study was support by a grant from the Equine Health Studies Program, Louisiana State University. The authors thank Paul Hollier, Frank Garza, Kimberly Cousey and Jesse McClure for their technical assistance.

Year completed: 2001

Published Manuscripts/Abstracts:


Quantification of immunoreactive endothelin in plasma and pulmonary fluid during remission and clinical exacerbation of summer pasture-associated obstructive pulmonary disease in horses

Authors/Investigators:
Lais R. R. Costa, MS, PhD, DACVIM; Susan E. Eades, DVM, PhD, DACVIM; Frank Garza, MS; Catherine E. Koch, MA; Gizelle Hosgood, BVSC, PhD, DACVS; Rustin M. Moore, DVM, PhD, DACVS.

Description of the Problem:
Summer pasture-associated obstructive pulmonary disease (SPAOPD), a naturally occurring recurrent airway obstructive disease of horses residing on pasture in the southern United States, is characterized by airway...
hyperresponsiveness and airflow obstruction. Endothelin (ET) -1, a potent bronchoconstrictor, mitogen, secretagogue and pro-inflammatory mediator, has been implicated in the pathogenesis of airway hyperresponsive diseases such as human asthma and equine recurrent airway obstruction (RAO) formerly called equine chronic obstructive pulmonary disease.

**Study Purpose/Objectives:**
The overall objective of this study was to evaluate the concentration of immunoreactive ET in circulating plasma and pulmonary secretion during clinical exacerbation and remission of SPAOPD. The specific aim was to determine and compare the concentrations of ET in arterial and venous plasma, and bronchoalveolar lavage and pulmonary epithelial lining fluid samples obtained from SPAOPD-affected and non-affected horses, during different times of the year.

**Approach:**
 Twelve adult horses, six with SPAOPD and six healthy horses, were used. The diagnosis of SPAOPD was based on history of airway disease during summer following exposure to pasture, physical examination findings, clinical scores of respiratory effort (CSRE), cytologic analysis of bronchoalveolar lavage fluid (BALF) and pulmonary function testing. Samples (BALF, arterial and venous plasma) and clinical evaluations (physical examination, CSRE, cytologic analysis of BALF and pulmonary function testing determined by indirect measurement of intrapleural pressures) were obtained from each affected horse during the season of clinical exacerbation (mid-spring to mid-fall) and during clinical remission (winter). In addition, samples and clinical evaluations of non-affected control horses were collected during summer to mid-fall and during winter. The plasma and BALF concentrations of immunoreactive ET were determined, using a commercial ELISA kit (BIOMEDICA).

**Accomplishments/Results/Conclusions:**
The ET-1 concentrations in arterial and venous plasma samples were greater in SPAOPD-affected horses during clinical exacerbation (mid-spring to mid-fall) than those during remission (winter). There were no significant variations of ET-1 concentrations in plasma samples from non-affected control horses during summer and winter. During remission, all SPAOPD-affected horses evaluated in this study had intrapleural pressure differences within the normal range, similar to those present in the non-affected horses, which indicated complete reversibility of the airway obstruction. The increase in venous and arterial ET-1 concentration during clinical exacerbation of SPAOPD resembles the findings in human asthma and equine RAO.

**Benefits to/Impact on the Equine Industry:**
Increases in circulating ET-1 during clinical exacerbation of SPAOPD suggest that ET-1 contributes to the bronchoconstriction, hypersecretion and inflammation observed in this disease. Intervention with ET-1 synthesis and metabolism may prove to be an important therapeutic target.

**Take Home Message:**
The overall increase in circulating ET-1 concentrations during clinical exacerbation of SPAOPD suggests that ET may be involved in the pathophysiology of this disease.

**Acknowledgments:**
The study was funded by a grant from the American Association of Equine Practitioners and the LSU Equine Health Studies Program. The authors would like to thank Michael Keowen, Danielle Vallotton and Leslie Talley, Drs. C. S. Venugopal and S. Polikepahad, and the student workers Aimee Hunt, Diane Savois, Jenny Liford, Erica Wallace, Elizabeth Dequeant, Jeffrey Cardinale, Hilary French and Brenna Hanly for their assistance in collecting samples, entering the data, and in caring for the animals.

**Year Completed:** 2003
Published Manuscripts/Abstracts:


Comparative in vitro response of airways of clinically healthy horses and those affected with summer pasture-associated obstructive pulmonary disease to endothelin-1 in the presence and absence of endothelin receptor antagonists

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

Description of the Problem:
This in vitro project is to initiate the investigation of the role of endothelin also in SPAOPD affected horse and to compare the responses to that of the clinically healthy horses. Since endothelin mediates its responses via the actions on ETA and ETB receptors in other species, we decided to examine the responsiveness of bronchial rings to endothelin. The involvement of each receptor was examined by the use of specific ETA and ETB antagonists.

Study Purpose/Objectives:
The objectives were to determine and compare bronchial ring responses between clinically healthy horses and those affected with SPAOPD to graded concentrations of ET-1 in the presence and absence of ETA and ETB receptor antagonists. The purpose of the study was to collect preliminary data on endothelin-induced responses in bronchial rings.

Approach:
Bronchial rings (4 mm wide) were cut from the 4th - 7th generation branches of airways from clinically healthy and SPAOPD affected horses. Initial tension of 2 g was applied to the rings and an equilibration time of 45 minutes was given. Then concentration response relationships of the rings to graded concentrations of ET-1 were determined.

Accomplishments/Results/Conclusions:
Characterizing the responsiveness was the primary objective of this study. ET-1 produced a concentration-dependent response on bronchial rings from both healthy and affected horses. Bronchial rings from affected horse responded with significantly greater magnitude than those of the normal horses.

Benefits to/Impact on the Equine Industry:
Developing a better understanding of the pathophysiology of summer pasture-associated obstructive pulmonary disease may help to develop better prevention and treatment strategies for horses with reactive airway disease.

Take Home Message:
ET-1 is a potent contractile agent of the equine bronchial smooth muscle.

Acknowledgments:
The study was funded by a grant from the LSU Equine Health Studies Program.

Year completed: 2002
Bronchial smooth muscle response to neurokinin-A in clinically healthy horses and those affected with summer pasture-associated obstructive pulmonary disease

Authors/Investigators:
Shawn Wilson, BS, DVM; Changaram S. Venugopal, BVSc, MSc, MS, PhD; Rustin R. Moore, DVM, PhD, DACVS.

Description of the Problem:
This in vitro project is to initiate the investigation of the role of neurokinin-A (NK-A), a member of the tachykinin family, in clinically healthy and SPAOPD-affected horse. Since neurokinin-A is reported to a mediator in airway hyperreactivity in human asthma, we decided to determine and compare the response of the bronchial rings collected from SPAOPD-affected and unaffected horses to graded concentrations of NK-A. SPAOPD is a form of recurrent airway obstruction (RAO) in horses which has many features common to human asthma.

Study Purpose/Objectives:
The purpose of the study was to collect preliminary information on NK-A-induced responses in bronchial rings. The objectives of the study were to determine and compare bronchial ring responses between clinically healthy horses and those affected with SPAOPD to graded concentrations of NK-A.

Approach:
Bronchial rings (4 mm wide) were cut from the 4th - 7th generation branches of airways from clinically healthy and SPAOPD affected horses. Initial tension of 2 g was applied to the rings and an equilibration time of 45 minutes was given. Then concentration response relationships of the rings to graded concentrations of NK-A were determined.

Accomplishments/Results/Conclusions:
Characterizing the responsiveness of bronchial rings to graded concentrations of NK-A (10^-8M to 10^-5.5M) was the primary objective of this study. Bronchial rings from clinically healthy horses did not respond until it reached higher concentrations of 10^-5M and 10^-5.5M. In fact, these rings showed a tendency to relax at lower concentration. On the contrary, the rings from the affected horses showed a concentration-dependent contraction. The rings showed slight contractions even at the lowest concentration of 10^-8M of NKA. The bronchial rings of the SPAOPD-affected horses responded with significantly greater magnitude than those of the unaffected horses. The findings of the study suggested that the airways of SPAOPD-affected horses become hypersensitive and hyperresponsive to NK-A.

Benefits to/Impact on the Equine Industry:
Developing a better understanding of the pathophysiology of summer pasture-associated obstructive pulmonary disease may help to develop better prevention and treatment strategies for horses with reactive airway disease.

Take Home Message:
NK-A is a potent contractile agent of the equine bronchial smooth muscle of SPAOPD-affected horses.

Acknowledgments:
This study was funded by a grant from the LSU Merck-Merial Summer Student Research Program and the LSU Equine Health Studies Program. The authors thank Catherine Koch and Earnestine Holmes for technical assistance.

Year Completed: 2002

Published Manuscripts/Abstracts:
A novel model for equine recurrent airway obstruction

Authors/Investigators:
K. S. Bowles; Ralph E. Beadle, DVM, PhD; S. Mouch; Susan S. Pourciau; Martha A. Littlefield-Chaubaud, DVM; Casey Le Blanc, DVM, PhD, DACVCP; L. Mistric; David W. Horohov, PhD.

Description of the Problem:
Recurrent airway obstruction (RAO) or heaves is one of the most commonly diagnosed conditions affecting the equine lung. Two forms of the disease have been identified based on seasonal occurrences, including chronic obstructive pulmonary disease (COPD) occurring principally in the winter months in stabled horses and summer pasture-associated obstructive pulmonary disease (SPAOPD) occurring principally in horses on pasture in the summer months. Similar clinical signs and cellular composition of bronchoalveolar lavage fluid suggest a common mechanism. The etiopathogenesis of RAO is unknown; however, pulmonary hypersensitivity to inhaled mold antigens may be involved. The reversible and anamnestic nature of RAO is similar to human asthma, suggesting an immunological basis. Based upon similarities between human asthma and equine RAO, it is likely that a T-helper 2 (Th2) cells and their cytokines play a role in the disease in horses. Recent work in our laboratory has demonstrated increased levels of IL-4 and IL-13 mRNA levels in the airway and peripheral blood of horses with RAO, which is consistent with the idea of a Th2-mediated response involved in the pathogenesis of equine RAO.

Study Purpose/Objectives:
The purpose of this study was to develop a novel model for equine RAO utilizing ovalbumin-coated polystyrene beads for airway sensitization and challenge.

Approach:
Ponies were sensitized by intramuscular administration of 10 mg ovalbumin in 225 mg alum. Control ponies were not injected. Two weeks following priming, ponies were exposed to aerosolized ovalbumin-coated polystyrene beads. Each pony underwent 60 minutes of inhalation of 14 ml daily for 5 days each week for 2 weeks. Ponies were then rested for 2 weeks and challenged with the same dose of ovalbumin-coated polystyrene beads. Pulmonary function testing was performed 48-72 hours post-challenge via forced airway oscillation. Bronchoalveolar fluid samples were collected afterward. The bronchoalveolar lavage fluid was stored at -20 C for future cytokine profile analysis and bronchoalveolar cells were harvested for mRNA analysis. Cytokine (IL-4 and IL-13) message was quantified using real time PCR using appropriate standard curves, and was normalized to house keeping genes.

Accomplishments/Results/Conclusions:
Aerosol challenge of sensitized ponies with ovalbumin-coated microbeads resulted in decreased airway compliance, an increase in lymphocytes and neutrophils in the bronchoalveolar lavage fluids, and increased IL-4, IL-5 and IL-13 mRNA levels, which provides evidence of a Th2 cytokine response in the bronchoalveolar cells in aerosol-challenged ponies compared to a predominantly Th1 response in control ponies.

Benefits to/Impact on the Equine Industry:
Development of this model of aerosol challenge with ovalbumin-coated microbeads of ovalbumin-sensitized ponies provides a potentially useful method for studying the pathogenesis of early phases of initiation and progression of equine RAO.

Take Home Message:
The results of this study demonstrate that this model may be a useful technique for studying and describing the initial stages of RAO development in horses. Further development and refinement of this model should enable further characterization of the early induction stages and progression of immune processes leading to the development of equine RAO.

Acknowledgments:
This study was funded by a grant from the Grayson-Jockey Club Research Foundation, Inc. and the United States Department of Agriculture National Research Competitive Grants Program. The authors thank the generous contributions of Dr. Thomas R. Klei and Dr. Dennis French.

Year Completed: 2001

Published Manuscripts/Abstracts:
**Characterization of equine airway endothelin receptors in health and disease**

**Authors/Investigators:**
Changaram S. Venugopal, BVSc, MSc, MS, PhD; Rustin M. Moore, DVM, PhD, DAVCS; Julian L. Oliver, DVM, PhD; John Vanden Heuvel, PhD.

**Description of the Problem:**
Chronic Obstructive Pulmonary Disease (COPD) is a common condition affecting the respiratory system in horses worldwide. Airway hyper responsiveness and mucus hyper-secretions are important characteristics in the pathogenesis of summer pasture associated obstructive pulmonary disease (SPAOPD), a form of COPD in the temperate regions. This disease is prevalent during late summer and early fall and is associated with grazing in lust pastures. Currently only symptomatic treatments are available. A clear understanding of the disease is still lacking. Several inflammatory mediators are believed to be responsible for the pathogenesis. Recently, attention has been given to endothelin-1 (ET-1), a 21 amino acid peptide mediator, for its involvement in airway hyperactivity diseases. It is the most potent vasoconstrictor known to date and is also a potent constrictor of non-vascular smooth muscles. ET-1 exerts its effects on airways mainly through two types of receptors, namely ET_A and ET_B receptors. We hypothesize that alteration of ET receptors is the primary cause for the pathogenesis. Therefore, the overall goal of the project was to characterize the ET receptors in clinically healthy horses and those affected with SPAOPD.

**Study Purpose/Objectives:**
The objectives of the study were to (1) compare bronchial ring responses of the two groups of horses to graded log molar concentrations of ET-1 with and without denudation of bronchial epithelium, (2) determine pA2 values of ET_A and ET_B receptor antagonists on bronchial rings from the two groups of horses to assess alterations in ET receptor affinity, (3) determine density and distribution of ET receptors in bronchi of these groups using immunohistochemical method, and (4) compare the mRNA and protein expressions for the receptors on these groups. This was to determine the alterations at the transcriptional and post-transcriptional levels, respectively.

**Approach:**
Bronchial rings (4 mm side) were cut from the 4th-7th generation branches of airways from clinically healthy and SPAOPD affected horses. Initial tension of 2 g was applied to the rings and an equilibration time of 45 minutes was given. Then concentration response relationships of the rings to graded concentrations of ET-1 were determined in presence and absence of 3 concentrations of ETA and ETB receptor antagonists separately. The pA2 values were calculated from these determinations using the Schild plot method, a modified linear regression analysis. Immunohistochemical staining was performed using commercially available polyclonal antibodies against either the ETA or ETB receptor following the manufacturers’ recommendations regarding antibody concentrations. Western blot and mRNA determinations were also performed.

**Accomplishments/Results/Conclusions:**
Endothelin-1 is a potent contractile agent of equine airways. It produced contractions in a dose-dependent manner. The contractile responses were significantly greater in rings from SPAOPD horses. In normal animals, ET_B receptor affinity was very low where as ET_A receptor affinity was high. On the other hand in affected horses a significant increase in ET_B receptor was noticed. ET_A receptor affinity remained the same in those animals. Western blot and mRNA studies showed ET_B receptor up-regulation in SPAOPD-affected horses. The study shows pharmacological and molecular evidence for active role of ETB receptor in SPAOPD.

**Take Home Message:**
Endothelin and it receptors, particularly ET_B receptors play an important role in the SPAOPD. The antagonists of ET_B receptors have potential use as therapeutic agents for the treatment of airway hyperactivity.

**Acknowledgments:**
This study was funded by a grant from the United States Department of Agriculture NRICGP.

**Year Completed:**
2003

**Published Manuscripts/Abstracts:**


Culture of equine primary bronchial epithelial cells and transformed equine airway cells under air-liquid interface conditions

Authors/Investigators:
Lais R. R. Costa, DVM, MS, PhD, DACVIM; Robert E. Truax, PhD; William G. Henk, PhD; Jill R. Johnson, DVM, MS, DACVIM, DABVP; Kathy O’Reilly, PhD; Rustin M. Moore, DVM, PhD, DACVS.

Description of the problem:
Airway epithelial cells serve as a barrier interface between the environment and the host, therefore representing an important site of injury by a number of damaging agents. Moreover, several respiratory diseases are characterized by epithelial hyperplasia, changes in mucus production and in cellular differentiation. The ability to culture differentiated equine bronchial epithelial cells would allow the evaluation of a variety of cellular mechanisms involved in the pathogenesis of equine respiratory diseases. Culture of airway cells can be challenging due to the loss of differentiation. The use of an air-liquid interface mimics the conditions that the airway epithelial cells are subjected to in vivo, and it has been a successful technique to establish differentiated epithelial cell culture in various species. There are no reports of culture of equine airway epithelial cells and their growth conditions requirements.

Study Purpose/Objective:
The overall goal of this study was to establish cultures of differentiated normal equine bronchial epithelial cells. Our specific objectives are to: (1) determine the optimal conditions for culture of primary normal equine bronchial epithelial cells and a spontaneously transformed equine tracheal cell line under submerged and air-liquid interface conditions; and (2) evaluate differentiation of these cells by light and electron microscopy.

Approach:
Fresh post-mortem specimens of lung tissue were obtained from adult horses without signs of respiratory disease. The bronchial epithelium was dissected and subjected to cold trypsinization overnight. Cells initially were cultured in Dulbecco’s modified Eagle’s medium: Ham’s F12 (1:1 v/v) containing 10% fetal bovine serum and epithelial growth factor in tissue culture flasks or plates coated with either collagen or fibronectin-collagen (FNC). The cells were subcultured into either collagen or FNC-coated Transwell culture inserts and maintained in submerged conditions until they reached 90 to 100% confluency. After which the cultures were either maintained as submerged monolayers or subjected to the air-liquid interface conditions, which was established by removal of media from the top of the cell monolayers. The cells were maintained in a serum-free media containing low concentration of epithelial growth factor. A spontaneously transformed equine fetal tracheal cell line was culture under the same conditions as the primary bronchial epithelial cells. After 6 to 20 days, the Transwell culture inserts were fixed and embedded in Historesin for histologic evaluation; sections of 1.5 µm thickness were stained with Toluene Blue, periodic acid-Schiff stain (PAS), vimentin or cytokeratin to evaluate cell differentiation and mucus production using light microscopy. Or, after osmication, the cultures were embedded for transmission electron microscopy (TEM). Additionally whole-mounts of the membranes were stained with PAS to evaluate cell differentiation and mucus production using light microscopy.

Accomplishments/Results/Conclusions:
Primary bronchial epithelial cell culture was successfully established from cells harvested from all eight horses, however only in three of them subculture was successfully maintained. Apical extracellular mucus secretion was evident in ALI cultures and the apical location of PAS vesicles were also noted in light microscopy of plastic embedded cross-sections. Mucus vesicles were identified upon TEM evaluation of cells grown submerged or under air-liquid interface conditions. Although the amount of mucus produced was not quantified, based on the PAS staining of the whole-mounts, it appears that cells grown under ALI conditions produced more mucus than those grown submerged. Light microscopy of the primary cultures revealed strong positive staining for cytokeratin. Incidentally, the presence of
intra-nuclear viral nucleocapsids, as well as intracytoplasmic vesicles containing enveloped viral particles in cultures from two horses. Morphologically the viral particles appear to be of herpevirus, however, attempts to isolate the virus from frozen culture supernatants were not successful.

The equine tracheal cell line grew successfully under ALI conditions, however it tended to overgrow forming multiple layers of poorly differentiated cells; mucus differentiation was not evident. Light microscopy of the equine fetal tracheal cell line revealed strong positive staining for vimentin, and weak staining for cytokeratin.

**Benefits to/Impact on the Equine Industry:**
Culture of equine primary airway epithelial cells under ALI conditions yields cellular differentiation that resembles those of the airway epithelial cells in vivo, whereas the spontaneously transformed equine fetal tracheal cells do not. It appears that the equine primary airway epithelial cells are suitable for studies of airway physiology and pathology, but the spontaneously transformed equine fetal tracheal cell line is not.

**Take Home Message:**
This is the first report of successful establishment of differentiated equine bronchial epithelial cell cultures. The primary equine airway epithelial cells are suitable for studies of airway physiology and pathology.

**Acknowledgments:**
This study was supported by funds from the Equine Health Studies Program, and Sigma Xi Grant-in Aid from Louisiana State University. The authors acknowledge the skilled technical help from Olga Borkhsenious from the Electron Microscopy Laboratory, Cheryl Crowder and the Histology Laboratory, and Catherine Koch and the Equine Health Studies Program at School of Veterinary Medicine, Louisiana State University. The authors thank the contributions of Dr. Lance Buoen from the Cytogenetics, Minnesota Veterinary Diagnostic Laboratory, Dr. E. G. Cothran from University of Kentucky, and Sandra Norton from the Histology Laboratory, North Carolina State University. Finally, the authors thank the insights from Dr. Kenneth Adler and his Laboratory, especially Nancy Akley and Dr. Linda Martin, from the School of Veterinary Medicine, North Carolina State University.

**Year Completed:** 2002

**Published Abstracts/Manuscripts:**
Costa LRR, O'Reilly K, Truax R, Blackmer JM, Moore RM. Culture of primary equine bronchial epithelial cells. 8th International Veterinary Emergency and Critical Care Symposium, San Antonio, TX, September 2002.

Costa LRR, O'Reilly K, Truax R, Blackmer JM, Moore RM. Culture of primary epithelial and transformed equine airway cells. 20th Veterinary and Comparative Respiratory Society, Boston, MA, October 2002.

Recipient of the 2002 Joan A. O'Brien Award for the best scientific poster presentation. Culture of primary and transformed equine airway cells. 20th Symposium of the Veterinary & Comparative Respiratory Society, Boston, MA.


**Localization and quantification of immunoreactive endothelin-1 in lungs of horses affected with summer pasture-associated obstructive pulmonary disease**

**Authors/Investigators:**
Lais R. R. Costa, DVM, MS, PhD, DACVIM; Mary E. P. Goad, DVM, PhD, DACVP; Thomas L. Seahorn, DVM, MS, DACVIM; DACVECC; Mae Lopez; H. Wayne Taylor, DVM, PhD, DACVP; Giselle Hosgood, BVSc, MS, PhD, DACVS; Rustin M. Moore, DVM, PhD, DACVS.

**Description of the Problem:**
The airway inflammation and hyperresponsiveness in equine recurrent airway obstruction (RAO) resemble those of asthma in people. Summer pasture-associated obstructive pulmonary disease (SPAOPD) is a form of RAO affecting horses residing on pasture during the summer. Because of effects of endothelin (ET)-1 in the lung, i.e., potent bronchoconstrictor, pro-inflammatory and secretory actions, ET-1 has been implicated as an important mediator in human asthma as well as barn-associated RAO.
**Study Purpose/Objective:**
The goal of this study was to quantify and localize ET-1 immunoreactivity in lung specimens of horses affected with summer pasture-associated obstructive pulmonary disease (SPAOPD) and compare to control horses.

**Approach:**
Clinical information and post-mortem specimens from 12 horses, eight SPAOPD-affected horses and four non-affected controls, were collected. The diagnosis of SPAOPD or control was based on history, physical examination and evaluation of bronchoalveolar lavage fluid collected during clinical exacerbation of recurrent airway obstruction. Sections of lung specimen stained by hematoxilin and eosin were evaluated. Immunoreactive ET-1 was quantified in filtrates from tissue lysates of frozen lung specimens using a commercial enzyme immunoassay kit for ET-1. Immunohistochemical staining of formalin-fixed lung specimens using anti-endothelin-1 antibody was performed and evaluated under light microscopy.

**Accomplishments/Results/Conclusions:**
The amount of immunoreactive ET-1 per mg of protein in tissue lysates was greater, although not statistically significant, in lungs from SPAOPD-affected horses compared to controls. The distribution of ET-1 immunoreactivity in the lung specimens differed between the SPAOPD-affected and control horses. The ET-1 immunoreactivity was present in lung tissues of non-affected horses at low intensity. The ET-1 immunoreactivity was greater in airway epithelium, in airway connective tissue and in airway and vascular smooth muscle of SPAOPD-affected horses, compared with controls. The greater ET-1 immunoreactivity in bronchial epithelial cells of SPAOPD-affected, compared with non-affected horses, suggests that ET-1 may be one of the mediators involved in the pathogenesis of this seasonal equine airway disease.

**Benefits to/Impact on the Equine Industry:**
Endothelin-1 appears to be a mediator of the airway hyperresponsiveness and airway inflammation in SPAOPD. Intervention with ET-1 metabolism may be an important therapeutic target.

**Take Home Message:**
Endothelin-1 appears to be overexpressed in airway structures of horses affected with SPAOPD, suggesting that ET-1 is a mediator of the airway hyperresponsiveness and inflammation. Further studies are necessary to determine if ET-1 plays a role in the functional and morphological abnormalities in the airways of horses affected with SPAOPD.

**Acknowledgments:**
This study was supported in part by a grant from the Equine Health Studies Program, School of Veterinary Medicine, Louisiana State University, Baton Rouge, LA, and by funds provided from Hagyard-Davidson-McGee Associates, Internal Medicine Group. The authors thank Catherine Koch, Frank Garza, Dr. Lee Ann Fugler and Drs. C. S. Venugopal and C. M. Ramaswamy for their valuable assistance. This report represents a portion of a dissertation submitted by the first author to the graduate school as partial fulfillment of the requirements for the PhD degree.

**Year Completed:** 2001

**Published Abstract:**
Costa LRR, Goad MEP, Seahorn TL, Moore RM. Endothelin-1 expression in horses affected with summer-pasture-associated obstructive pulmonary disease. 15th Annual Meeting of the American College of Veterinary Pathology, Chicago, IL, November 1999.


Surgery

Cardiopulmonary, blood and peritoneal fluid alterations associated with abdominal insufflation of carbon dioxide in conscious horses

Authors/Investigators:
Federico G. Latimer, DVM, MS; Susan C. Eades, DVM, PhD, DACVIM; Glenn Pettifer, DVM, DVSc, DACVA; Joanne Tetens, DVM, PhD; Giselle Hosgood, BVSc, MS, DACVS; Rustin M. Moore, DVM, PhD, DACVS.

Description of the Problem:
Abdominal insufflation is performed routinely during laparoscopy in horses to improve visualization and facilitate instrument and visceral manipulations during surgery. It has been shown that high-pressure pneumoperitoneum with carbon dioxide (CO₂) has deleterious cardiopulmonary effects in dorsally recumbent, mechanically ventilated, halothane-anesthetized horses. There is no information on the effects of CO₂ pneumoperitoneum on cardiopulmonary function and hematology, plasma chemistry and peritoneal fluid variables in standing sedated horses during laparoscopic surgery.

Study Purpose/Objectives:
The hypotheses were that CO₂ pneumoperitoneum flank laparoscopy in healthy, conscious, sedated mature horses would have no significant effects on cardiopulmonary function, blood gas values and hematological and serum chemistry variables, and that CO₂ pneumoperitoneum would induce peritoneal inflammation. The purpose of the study was to determine the effects of high pressure CO₂ pneumoperitoneum in standing sedated horses on cardiopulmonary function, blood gas, hematology, plasma chemistry and peritoneal fluid variables.

Approach:
Six healthy, mature horses were sedated with an intravenous bolus of detomidine (0.02 mg/kg) and butorphanol (0.02 mg/kg) and instrumented to determine the changes in cardiopulmonary function, hematology, serum chemistry and peritoneal fluid values during and after pneumoperitoneum with CO₂ to 15 mm of Hg pressure for standing laparoscopy. Each horse was assigned at random to either a standing left flank exploratory laparoscopy (LFL) with CO₂ pneumoperitoneum or sham procedure (SLFL) without insufflation, and instrumented for measurement of cardiopulmonary variables. Each horse underwent a second procedure in crossover fashion one month later so that all 6 horses had both an LFL and SLFL performed. Cardiopulmonary variables and blood gas analyses were obtained 5 minutes after sedation and every 15 minutes during 60 minutes of baseline (BL) monitoring, insufflation (15 mm of Hg) and desufflation. Hematology, serum chemistry analysis and peritoneal fluid analysis were performed at BL, insufflation and desufflation, and 24 hours after the conclusion of each procedure.

Accomplishments/Results/
Conclusions:
Significant decreases in heart rate, cardiac output and cardiac index and significant increases in mean right atrial pressure, systemic vascular resistance and pulmonary vascular resistance were recorded immediately after and during sedation in both groups of horses. Pneumoperitoneum with CO₂ at 15 mm of Hg had no significant effect on cardiopulmonary function during surgery. There were no significant differences in blood gas, hematology or plasma chemistry values within or between groups at any time interval during the study. There was a significant increase in the PF total nucleated cell count 24 hours following LFL compared with baseline values for LFL or SLFL at 24 hours. There were no differences in standing sedated horse undergoing laparoscopic removal of a cryptorchid testicle.
peritoneal fluid protein concentrations within or between groups at any time interval.

Benefits to/Impact on the Equine Industry:
Laparoscopy provides a minimally invasive approach for evaluation and treatment of diseases or conditions involving the abdominal cavity. Recent advances in the instrumentation and techniques have facilitated its usefulness in horses for a variety of diagnostic and therapeutic purposes. This study demonstrates that the use of CO\textsubscript{2} pneumoperitoneum for abdominal insufflation in standing horses is safe and does not cause any short-term or cumulative observable adverse effects.

Take Home Message:
Pneumoperitoneum with CO\textsubscript{2} during standing laparoscopy in healthy horses does not cause adverse alterations in cardiopulmonary, hematology or plasma chemistry variables, but does induce a mild inflammatory response within the peritoneal cavity. High pressure (15 mmHg) pneumoperitoneum in standing sedated mature horses for laparoscopic surgery can be performed safely without any short-term or cumulative adverse effects on hemodynamic or cardiopulmonary function.

Acknowledgments:
This study was funded by a grant from the LSU Equine Health Studies Program.

Year Completed: 2001

Published Manuscripts/Abstracts:

Evaluation of a vessel sealing device (LigaSure) for small intestinal resection and anastomosis in horses

Authors/Investigators:
Marilyn L. Rumbaugh, DVM, MS; Daniel J. Burba, DVM, DACVS; Claudio Natalini, MV, MS, PhD; Giselle Hosgood, BVSc, MS, DACVS; Rustin M. Moore, DVM, PhD, DACVS.

Description of the Problem:
Small intestinal disease requiring surgical intervention is not uncommon in horses. If a strangulating lesion has been of sufficient duration to compromise intestinal viability, a resection of the affected bowel is indicated. Ligation of mesenteric vessels with suture can be a time consuming portion of an intestinal resection. Disadvantages of ligature placement include the possibility of ligature slippage, time involved in the dissection of the mesentery to isolate vessels prior to ligature placement, and increased operative time. LigaSure™ is a feedback-controlled, bipolar vessel-sealing system used to establish hemostasis. The LigaSure™ applies high current and low voltage to denature collagen and elastin within the vessel wall and surrounding connective tissue. The addition of extreme pressure applied by the instrument causes the denatured protein to reform with the vessel walls in apposition. The use of the LigaSure™ to seal and divide vessels in equine intestinal surgery could greatly minimize operative time.

Study Purpose/Objectives:
The objectives of this study were: (1) compare the arterial bursting pressure between the LigaSure Atlas™, a commercially available ligate-and-divide stapling device (LDS®), and 2-0 polydioxanone suture in jejunal segments occluded using three methods, and (2) evaluate the LigaSure Atlas™ for sealing and dividing of jejunal mesenteric vessels during small intestinal resection in horses.
Approach:
Study A: Sixty jejunal arteries were harvested and used to compare the arterial bursting pressure between the LigaSure Atlas™, the LDS®, and 2-0 polydioxanone suture.

Study B: Six horses underwent a jejunal resection and anastomosis using a vessel-sealing device (LigaSure Atlas™) to provide hemostasis of the mesenteric vasculature.

Accomplishments/Results/Conclusions:
Study A: The mean + SEM bursting pressure attained by 2-0 polydioxanone ligation (1,014.50 + 279.05 mm Hg) was significantly greater than the mean bursting pressure attained with the LigaSure™ (554.25 + 228.79 mm Hg) which was significantly greater than the mean bursting pressure attained using the LDS® (373.25 + 183.69).

Study B: No major operative or post-operative hemorrhage occurred. The LigaSure™ appears to be a safe method for hemostasis of the mesenteric vasculature during small intestinal resection in horses.

Benefits to/Impact on the Equine Industry:
Benefits of the LigaSure Atlas™ include reduced time required to provide hemostasis, acceptable arterial bursting pressure, no remaining foreign material, and no risk for ligature slippage. The LigaSure Atlas™ can provide a means for equine surgeons to remove portions of intestines in horses with strangulated lesion much more efficiently thus reducing the anesthesia time for the patient.

Take Home Message:
The LigaSure Atlas™ is a blood vessel sealing instrument that uses an electrical current to clamp, seal, and divide vessels of the intestines. This study revealed that it is safe for use on intestinal vessels in horses, which will make is easier and more efficient for equine surgeons removed portions of intestines in horses colicking from strangulated lesions.

Acknowledgments:
This study was funded by the LSU Equine Health Studies Program. The authors thank Catherine Koch and Frank Garza for their technical assistance. The LigaSure Atlas™ was generously supplied by Valleylab, Boulder, Co.

Year Completed: 2002

Published Manuscripts/Abstracts:
Electrolyte supplementation in wild horses gathered and transported long-distance

Authors/Investigators:
Ann Davidson, DVM; Rebecca S. McConnico DVM, PhD, DACVIM; Mark A. Mitchell DVM, MS, PhD; Jeremy D. Hubert BVSc, MRCVS, MS, ACVS; Linda Coates-Markle BSc, MSc.

Description of the Problem:
The health and well-being of wild horses and burros under their management is identified as a priority of the Bureau of Land Management (BLM). Electrolyte supplementation prior to horse transport is debated as to its efficacy yet no scientific information was available to assess its value in wild horses gathered and transported long-distance.

Study Purpose/Objectives:
The objectives of these studies were to (1) determine if electrolyte supplementation prior to long distance transport would reduce dehydration and stress in BLM-managed wild horses transported cross-country and (2) determine if stress levels were affected by electrolyte supplementation and if electrolyte flavor affected water consumption during gathering and holding activities.

Approach:
Hematological data were collected from horses at the beginning and end of the trials (experiment 1 = long-distance transport by tractor-trailer; experiment two = 7-day holding period) for assessment of dehydration and stress. Blood values evaluated included total WBC, PCV, total plasma protein, plasma sodium, chloride, blood urea nitrogen (BUN) cortisol and glucose levels. Daily water consumption was recorded for each group.

Accomplishments/Results/Conclusions:
Experiment 1 - Elevated plasma cortisol was observed in all groups before and after transport with no significant difference noted between or within groups. Sodium, chloride, total protein, and BUN increased significantly between pre- and post samples in all groups, but remained within normal reference ranges for domestic horses. No difference in water consumption was found between groups supplemented with electrolytes and those not supplemented.

Experiment 2 - Elevated plasma cortisol levels were detected before and after the 7-day holding period. Sodium, chloride, BUN, and glucose values were within normal reference ranges for domestic horses. There were no significant differences between groups for total protein, BUN, chloride, PCV, glucose, cortisol, and WBC. No significant difference was found for water consumption when evaluated for treatment or gender effects.

Benefits to/Impact on the Equine Industry:
These studies demonstrate that gathering, holding and transportation are stressful in wild horses; however, the level of stress does not appear to be excessive. Appropriate steps should probably be taken to minimize the stress associated with these procedures, but supplementation with electrolytes does not appear beneficial and thus is not warranted.

Take Home Message
Findings in these two studies support the contention that transportation and gathering/holding are stressful for wild horses. However, these results suggest that the stress level is not severe and neither long distance transport nor gathering and holding activities cause significant dehydration or stress irrespective of electrolyte supplementation.
Acknowledgments:
The views and conclusions contained in this document are those of the authors and should not be interpreted as representing the opinions or policies of the U.S. Government. Mention of trade names or commercial products does not constitute their endorsement by the U.S. Government. This study was conducted with the collaboration and support of the U.S. Department of Interior, Bureau of Land Management, Wild Horse and Burro Program in partnership with the U.S. Department of Agriculture, Animal and Plant Health Inspection Service, Veterinary Services.

Year Completed: 2002

Published Manuscripts/Abstracts:


Practical aspects of airlifting horses by helicopter

Authors/Investigators:
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Description of the Problem:
Flooding that occurs in the state of Louisiana gives us the opportunity to be involved in the airlift rescue of horses. Safe helicopter lifting of horses requires trained personnel and precise preparation of the horse in order to be successful.

Study Purpose/Objectives:
The purpose was to train faculty and associated personnel of the Equine Health Studies Program in the safe handling of horses for helicopter rescue.

Approach:
Four horses were air-lifted during a mock rescue which was coordinated with the US Coast Guard from New Orleans. Each horse was prepared for a helicopter airlift in a similar manner. Horses had a jugular catheter placed at least 1 hour before the exercise began. Horses were sedated with xylazine (0.5 mg/kg IV) approximately 2-5 minutes before cuing the helicopter for pick-up. This decreased stress and anxiety, and minimized movement. Each horse was vertically airlifted less than 30 feet into the air and then gently placed back into the same location, whereby the sling was removed immediately. The Coast Guard coordinated the airlift with 2 different pilots and 4 crew members, while veterinarians, veterinary technicians, or veterinary students prepared the animals.

Accomplishments/Results/Conclusions:
This training exercise was successful in training 16 veterinarians, veterinary technicians and veterinary students in the safe preparation, handling, and delivery of horses during helicopter rescue activities.

Benefits to/Impact on the Equine Industry:
The LSU-SVM-Equine Health Studies Program is committed to helping provide horse-owners with the latest technology and methods for the safe lifting and transportation of horses.
in Louisiana and surrounding states a better chance of their livestock being successfully rescued from the perils of disasters. The LSU EHSP will collaborate with the Louisiana Office of Emergency Preparedness and the US Coast Guard in rescue efforts to provide veterinary assistance in certain declared disasters in the Southeaster United States.

**Take Home Message:**
This practice session provided essential training to prepare LSU-EHSP personnel for future airlift rescues of horses.

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**Year Completed:** 2001

**Published Manuscripts/Abstracts:**
Pettifer GR, Hubert JD, McConnico RS. Practical aspects of airlifting horses by helicopter. 26th Annual Meeting of the American College of Veterinary Anesthesiologists, New Orleans, LA, 2001.

Pettifer GR, Hubert JD, McConnico RS. Practical aspects of airlifting horses by helicopter. 2nd International Conference on the Transportation of Horses by Road, Sea and Air, Hartbury College, Gloucestershire, UK, 2003.
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