Scientific studies conducted to help advance equine health and well-being
LETTER FROM THE DEAN

The Louisiana State University School of Veterinary Medicine is pleased to once again present the Equine Health Studies Program's Equine Research Report, which covers scientific activities of the program from 2006 and 2007. The EHSP faculty and staff are dedicated to conducting state-of-the-art research to benefit the horse-owning community in the State of Louisiana and beyond. This multidisciplinary program is a real "Point of Pride" at the SVM and continues to bring recognition and make important scientific advancements in equine research, clinical service, education, and community outreach.

Before embarking on a career in administration at the SVM, I served on the faculty as an equine clinician and surgeon. I am continually impressed by the foresight, dedication, and talent of our EHSP team. Their scientific work in the areas of laminitis, orthopedics and biomechanics, theriogenology, respiratory and gastrointestinal diseases, and surgery will impact equine veterinary care for years to come. With the twin disasters of Hurricanes Katrina and Rita, the SVM was able to step in and help with the rescue and care of thousands of animals in south Louisiana. The SVM is on the forefront of emergency preparedness, and we look to be a model in this area for other veterinary institutions.

The School is extremely pleased that Dr. Frank Andrews will join us as the new Director of the EHSP. Dr. Andrews, a Diplomate American College of Veterinary Internal Medicine, comes to the SVM from the University of Tennessee, where he was professor and section chief of large animal medicine. He brings a strong background in leadership, equine internal medicine, veterinary research, and building collaborations with industry. Dr. Andrews’ experience and skills in building relationships with clients, veterinarians, and industry leaders will serve us well. We are thrilled that he has accepted this position, and we are confident that his talents and abilities will be a tremendous asset. He is preparing for his move to Louisiana and will join us late summer 2008.

The EHSP is committed to improving the health and welfare of the horse through basic and clinical research and through educational, clinical service, and outreach initiatives that will serve our broad-base of external constituents and clients. We continue to improve our infrastructure through facility and equipment enhancements, and we are steadfastly determined to continue on our trajectory to make the EHSP a leader in the field of equine health and well-being.

Sincerely,

Peter F. Haynes, DVM, DACVS
Dean
LETTER FROM THE DIRECTOR

It’s my pleasure to introduce the 2006-07 Equine Research Report from the Equine Health Studies Program (EHSP), Louisiana State University (LSU) School of Veterinary Medicine (SVM)! As the new director of the program, I am honored to take over the reins from interim directors, Dr. Susan Eades and Dr. Daniel Burba. They have been carrying on the important mission and functions of the EHSP over the past 24 months.

Harry Truman once said: “You can accomplish anything in life, provided that you do not mind who gets the credit!” Susan and Dan have devoted countless behind-the-scene hours to the EHSP, not expecting any credit for their efforts. They have kept the EHSP team working toward its primary mission, while continuing to teach, do research and provide clinical service in the SVM. As one can see from this report, the EHSP continues to initiate and complete high-level biomedical research to address the health needs of horses in Louisiana, the region, and the world. Kudos to Susan, Dan, and the EHSP faculty, and staff for their continued commitment and devotion to excellence of the EHSP!

As the new director of the EHSP, I am honored to be a part of a world-class organization and look forward to carrying on the mission of the program. As you read this report, the EHSP biomedical research team at the LSU SVM has diverse research interests with one major goal—to improve the health and welfare of the horse. Investigators represented in this report contributed important information in areas of Emergency Response and Preparedness, Gastrointestinal Disease, Laminitis, Musculoskeletal Injury and Lameness, Reproduction, Respiratory Disease, and Colic Surgery. Each study in this report highlights benefits to and impacts on the equine industry in Louisiana and surrounding regions. Each report also contains a practical “take-home message” to explain the relevance of the results.

The biomedical research outlined in this report and state-of-the-art facilities on campus are a direct result of support from the Louisiana Governor’s Biotechnology Initiative Grants Program, Louisiana Board of Regents Enhancement Grants Program, and recurrent funding through the State Legislature resulting from Louisiana racetrack slot machine revenue. Also, funding for many of these important projects was provided from extramural sources, including The Grayson Jockey Club Research Foundation, The Morris Animal Foundation, Merck-Merial Veterinary Student Summer Research Program, Boehringer-Ingelheim, Pennington Biomedical Research Foundation, Applied Biosystems, Howard Hughes Medical Institute, National Wetlands Research Center, and National Institutes of Health, among others.

Furthermore, we owe our deepest gratitude to the horses that participated in these investigational studies. The research findings presented in this report would not have been possible without the availability and use of horses. All biomedical research on animals at LSU is conducted under Federal Guidelines for the Humane Care and Use of Animals and approved by the Institutional Animal Care and Use Committee (IACUC) within the SVM. Horses were carefully and compassionately used for the advancement of equine health to discover more effective methods to diagnose, treat, and prevent illness and injuries. These horses are valued members of our program and are treated with kindness and dignity.

No client-owned animals were used for research purposes. However, in some cases, data obtained from medical records may have been used to identify specific risk factors and treatment outcomes for important diseases or injuries. All client and animal identity was kept in strict confidence and will not be released under any circumstances.

I am proud to be a part of the EHSP team and look forward to leading and participating in the biomedical research program as it moves forward. As part of the EHSP team, I am committed to furthering the health, well-being and performance of horses to the benefit of the State of Louisiana and the region. Also, with continued support from the horse industry and the State of Louisiana, we can further promote and establish the EHSP as the premier equine biomedical program in the world.

Sincerely,

Frank M. Andrews, DVM, MS, DACVIM
Professor and Director
**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 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.

www.equine.vetmed.lsu.edu

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The Equine Health Studies Program 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 (MS) and Doctor of Philosophy (PhD) 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
Abolghasem Baghian, Assistant Professor, Veterinary Microbiology & Parasitology

Dr. Baghian received his MS in Microbiology from Southeastern Louisiana University in 1981, and he received his PhD 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 BS in 1971, his MS in 1973, and his PhD in 1978, all from the University of Alabama. Dr. Barker is also the director of the Analytical Systems Laboratory.

Ralph E. Beadle, Professor Emeritus, Equine Medicine

Dr. Beadle was born and raised in Montana. He completed his pre-veterinary and veterinary education at Colorado State University, where he was awarded a DVM in 1967. He spent the next five years at the University of Georgia, where he worked in the Equine Clinic and obtained a PhD 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 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.

Daniel J. Burba, Professor, Equine Surgery

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

Ann Chapman, Visiting Assistant Professor, Equine Medicine

Dr. Chapman was born and raised in Harrisburg, Penn. She received her DVM from the LSU School of Veterinary Medicine in 2001. After working in private practice for one year, she began her combination Equine Internal Medicine Residency/Graduate Program at LSU in 2002. Dr. Chapman completed her residency in 2005 and became a Diplomate of the American College of Veterinary Internal Medicine (Large Animal). She performs Mobile Equine Medicine Consultation throughout the state of Louisiana and Equine Ambulatory Medicine to the local Baton Rouge community.
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 Hospital and Clinics. He received his DVM in 1966 and his MVSc in 1970, both from Seoul National University in Korea. In 1973, he received his MS, and in 1978, he received his PhD, both from Kansas State University.

Anderson daCunha, DVM, MS, Assistant Professor of Veterinary Anesthesiology

Dr. daCunha is an assistant professor of veterinary anesthesiology in the Department of Veterinary Clinical Sciences. He is also service chief of Anesthesia Services in the Veterinary Teaching Hospital & Clinics. Dr. daCunha received his DVM from Federal University of Parana in 2000, and he received his MS from Federal University of Santa Maria in 2002. His research interest is the interaction of the Vanilloid receptor (TRPV1) with the pain pathway.

Susan C. Eades, Professor, Equine Medicine

Dr. Eades graduated from the LSU School of Veterinary Medicine in 1982, 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 completed a PhD 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 PhD, 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 a pre-veterinary medicine student. He obtained a BS in veterinary science in 1975 and his DVM in 1977, both from the University of Minnesota. He was in private practice for one year before returning to the University of Minnesota to obtain an MS 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, and his main research interest is intrafollicular insemination in the mare.

Joseph Francis, BVSc, MVSc, PhD, Associate Professor of Comparative Biological Sciences

Dr. Francis is an associate professor in the Department of Comparative Biomedical Sciences. He received his BVSc and his MVSc in 1990 and 1994, respectively, both from Madras Veterinary College (India). In 1999, Dr. Francis received his PhD from Kansas State University. His research focuses on the brain mechanisms regulating cardiovascular function, specifically the understanding of the central nervous system interactions of cytokines renin-angiotensin-aldosterone system, in heart failure.
Dennis D. French, Professor, Food Animal Health and Science
Dr. French, originally from Chatfield, Minn., obtained his BS and DVM degrees from the University of Minnesota in 1976 and 1979, respectively. He is a Diplomate of the American Board of Veterinary Practitioners, certified in equine practice. His clinical interests include equine herd health and sport horse medicine. His research interests include equine parasitology, immunology, and exercise physiology in horses. He is currently a professor of food animal health and 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 equestrian activities throughout the state.

Lorrie Gaschen, Associate Professor, Veterinary Radiology
Dr. Gaschen received her BS and DVM degrees from the University of Florida in 1985 and 1990, respectively. She received her PhD from the University of Utrecht in the Netherlands in 2001. She is a Diplomate of Diagnostic Imaging and a European Specialist in Veterinary Diagnostic Imaging. Dr. Gaschen is a veterinary radiologist and service chief for Radiology in the LSU School of Veterinary Medicine’s Veterinary Teaching Hospital & Clinics. She joined the faculty at LSU in January 2006. Dr. Gaschen’s research interests are in vascular imaging and ultrasound of the gastrointestinal tract and pancreas.

Marjorie S. Gill, Professor, Veterinary Clinical Medicine
Dr. Gill received her DVM and MS degrees from Iowa State University in 1976 and 1984, respectively. She is a Diplomate of the American Board of Veterinary Practitioners (Food Animal Practice). She joined the faculty at LSU in July 1984. Dr. Gill’s clinical interests are in farm animal medicine; small ruminant medicine and surgery; animal behavior; and urogenital, gastrointestinal, and ophthalmological surgery.

Jeff Gimble, MD, PhD, Professor, Pennington Biomedical Research Center
Dr. Gimble is a professor at the Pennington Biomedical Research Center and an adjunct professor in the Department of Veterinary Clinical Sciences. He received his BA from Dartmouth College in 1976. He received his MA, PhD, and MD from Yale University in 1980, 1981, and 1982, respectively.

Giselle Hosgood, BVSc MS, PhD, DACVS, FACVS, Professor, Small Animal Surgery
Dr. Hosgood is a professor of veterinary surgery in the Department of Veterinary Clinical Sciences. She is also a veterinary surgeon and the service chief of Companion Animal Surgery in the Veterinary Teaching Hospital & Clinics. She is a Diplomate of the American College of Veterinary Surgeons and a Fellow of the Australian College of Veterinary Scientists. Dr. Hosgood received her BVSc from Queensland University (Australia) in 1982 and her MS from Purdue University in 1988. Her research interests are in clinical epidemiology, experimental design and analysis; wound reconstruction; skin grafts, gastrointestinal effects of non-steroidal medication.

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. After two years of mixed animal practice in Zimbabwe and the United Kingdom, he completed an equine medicine and surgery internship at LSU.
This was followed by a year in equine practice in the U.K. before embarking on a combined equine surgery residency and MS 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 assistant professor of equine surgery at LSU in October 2001. His clinical interests include upper respiratory tract disease, lameness and orthopedics. His research interests are in 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 her MS 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 immuogenetics and immunology. Current research activities include evaluation of methods of quantifying exercise training using the global positioning system (GPS) and development of tissue culture models to study laminitis and chronic obstructive pulmonary disease using microgravity methods.

Konstantin G. Kousoulas, PhD, Professor of Virology and Biotechnology
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 MS and in 1981, he received his PhD, both from Pennsylvania State University. Dr. Kousoulas’ research interests are in molecular biology and pathogenesis of herpesvirus and coronaviruses; experimental approaches in his laboratory involve advanced molecular biology, molecular genetics and cell biology to study viral attachment, viral penetration into cells, cellular receptors and virus spread. Other interests include the application of viral vectors for gene therapy, the development of DNA based methods for the diagnosis of infectious disease pathogens and genetic diseases, and the utilization of computers for biological research and teaching.

Jim LaCour, DVM, Clinical Instructor of Large Animal Ambulatory Services
Dr. LaCour is a visiting assistant professor of large animal ambulatory services in the Department of Veterinary Clinical Sciences. Dr. LaCour joined the faculty in September 2006. He received his DVM from the LSU School of Veterinary Medicine in 1991.

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 MS and PhD 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 BS in Chemistry at Duke University and her DVM from the University of Florida. She completed a residency in theriogenology in 1989 and her MS 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.

Charles T. “Chuck” McCauley, Assistant Professor, Equine Surgery
Dr. McCauley joined the equine faculty in the Department of Veterinary Clinical Sciences in February 2006. Prior to joining the faculty at LSU, Dr. McCauley was employed in a busy private referral practice in northeast Texas. He completed his BS in Microbiology and his DVM at Texas A&M University. He successfully completed an internship and residency in food animal medicine and surgery at Oklahoma State University. In addition, Dr. McCauley completed a residency in large animal surgery (equine emphasis) at Purdue University. He is double boarded by the American Board of Veterinary Practitioners (Food Animal Practice) and the American College of Veterinary Surgeons (Large Animal Surgery).

Rebecca S. McConnico, Associate Professor, Equine Medicine
Dr. McConnico is originally from north central Ohio, where she lived for 18 years. She obtained her BS in Animal Science from the University of Arkansas, her DVM from Louisiana State University, and her PhD 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.

Colin F. Mitchell, Assistant Professor, Equine Surgery
Dr. Mitchell, originally from Perth, Scotland, received his veterinary medical degree from the University of Edinburgh. He then completed an internship at the University of Prince Edward Island prior to entering a combined three-year equine surgery residency and MS graduate program at the University of Minnesota, which he completed in June 2004. He then remained on the hospital staff at the University of Minnesota, where he worked as the equine emergency clinician/surgeon until July 2005, when he joined the LSU School of Veterinary Medicine. He is board certified by the American College of Veterinary Surgeons. His clinical interests include soft tissue surgery and ultrasound. His research interests include assessment of gastrointestinal motility.

Mustajab Mirza, Visiting Assistant Professor, Equine Medicine
Dr. Mirza received his DVM from the University of Agriculture, Faisalabad, Pakistan in 1992. He received his MS degree from LSU in 1998. Dr. Mirza’s primary interests are repair of long bone fractures and pathogenesis of colics in equids. He primarily provides after-hours emergency services for the LSU Equine Clinic. Dr. Mirza’s research interests are in long bone fractures, performance limitation identification, and gastrointestinal disease in the horse.
Marlene S. Orandle, DVM, PhD, Assistant Professor of Virology
Dr. Orandle was born and raised in Baltimore, Md. She obtained her BA in Biology from Saint Mary’s College of Maryland in 1987. Following four years of research experience at Johns Hopkins University School of Medicine, Dr. Orandle completed her DVM from Iowa State University in 1995 and her PhD in Veterinary Pathology from the University of Florida in 1999. Since receiving her PhD, 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.

Kathy L. O’Reilly, Associate Professor, Veterinary Immunology
Dr. O’Reilly was born in Corona, Calif. She obtained her BS and MS 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 PhD in Veterinary Science (Immunology) at the University of Wisconsin-Madison in 1989. She 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 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.

Dale L. Paccamonti, Head, Department of Veterinary Clinical Sciences, and Professor, Theriogenology
Dr. Paccamonti, originally from Kankakee, Ill., completed his undergraduate and veterinary education at Michigan State University, receiving his DVM in 1981. After four years in a mixed practice in Chestertown, Md., he pursued advanced training at the University of Florida, where he completed a residency in theriogenology and received his MS 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 head of the Department of Veterinary Clinical Sciences and a full professor of theriogenology. 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. 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 BS in 1975, his DVM in 1977, and his MS in 1978, all from Kansas State University. In 1989, he received his PhD from Oklahoma State University. Dr. Paulsen's major research interests 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.

Charles Short, DVM, PhD, Professor Emeritus of Veterinary Pharmacology
Dr. Short is a professor emeritus of veterinary pharmacology in the Department of Comparative Biomedical Sciences. He received his DVM and his MS in 1963 and 1965, respectively, from The Ohio State University. Dr. Short received his PhD from the University of Missouri, Columbia, in 1969. He joined the faculty of the LSU School of Veterinary Medicine in 1974. He is a Diplomate of the American College of Veterinary Clinical Pharmacology.

Gary A. Sod, Assistant Professor, Farm Animal Medicine and Surgery
Dr. Sod received his MA in Mathematics from the University of California at Berkeley in 1975. He earned his PhD 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 DVM in 2001. He has since completed an equine internship and food animal medicine and surgery residency at LSU and is now an assistant professor with the Farm Animal Medicine and Surgery service. Dr. Sod received the American College of Veterinary Surgeons Research Publication Award in 2004 and 2006, and the Mark S. Bloomberg Memorial Resident Research Award from the Veterinary Orthopedic Society in 2004 and 2005. Dr. Sod has completed 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.

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 DVM/PhD program at the LSU School of Veterinary Medicine in the Department of Comparative Biomedical Sciences in 2001 and 2003, respectively. She completed a one-year post-doctorate research fellowship in the summer of 2004 where she continued her doctoral work on the vascular pathophysiology of equine laminitis. As a research assistant professor within the EHSP, Dr. Stokes has continued to focus her efforts in physiology and pharmacology with special emphasis on equine diseases.

Eric Storey, DVM, MVSc, Assistant Professor of Ophthalmology
Dr. Storey is an assistant professor of ophthalmology in the Department of Veterinary Clinical and a veterinary ophthalmologist in the Veterinary Teaching Hospital & Clinics. He received his BS and DVM from Auburn University in 1996 and 1999, respectively. Dr. Storey received his MVSc from University of Saskatchewan (Canada) in 2003. He is a Diplomate of the American College of Veterinary Ophthalmologists. His research interests are in glaucoma, inherited retinal disease, and equine recurrent uveitis.
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 MSc degree in neuropharmacology from Calicut University, India. He received his MS degree in cardiovascular pharmacology and his PhD in pulmonary pharmacology from the Massachusetts College of Pharmacy and Allied Health Sciences in a cooperative program with Harvard University in Boston, Mass. Then he worked as a postdoctoral fellow at Harvard Medical School before joining the faculty at LSU School of Veterinary Medicine in 1981. 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.
Publications


Abstracts


Sod GA, Mitchell CF, Hubert JD, Martin GS, Gill MS. An in vitro biomechanical comparison between AO cortical bone screws with teflon tape wrapped threads and AO cortical bone screws without teflon tape wrapped threads for a limited contact dynamic compression plate fixation of osteotomized equine third metacarpal bones. Veterinary Comparative and Orthopedic Traumatology,


Grants

Eades S, Fugler L: The effects or oral administration of doxycycline on matrix metalloproteinase inhibition and experimentally-induced laminitis in the horse. $25,000. Louisiana State University Equine Health Studies Program, 2007.


Fugler L, Eades S: In vitro effects of inflammatory mediators on laminar explants from normal horses. $6,000. Louisiana State University Equine Health Studies Program, 2007.


Lyle S: Effects of experimental equine placentitis on plasma concentrations of hormones and cytokines. $6,000: Louisiana State University Equine Health Studies Program, 2007.


Moore RM, Peiró JR, Stokes AM, Dietrich MA, Mendes LCN. Evaluation of the modulation of Toll-like receptors and cytokines using ex vivo and in vivo models of endotoxemia in horses. $12,000. Louisiana State University, School of Veterinary Medicine, Equine Health Studies Program. June 2006.


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 100 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, the Laboratory for Equine and Comparative Orthopedic Research, and the Equine Molecular Laboratory. 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 anti-inflammatory 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; and 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, there are 18 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 and a Grass digital interface 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. A kinematic gait analysis system and force plate have been used on studies of orthopedic disease, as well as for evaluation of analgesic clinical efficacy.

Equine Cell and Tissue Culture Laboratory

The Equine Cell and Tissue Culture Laboratory is well equipped to support tissue culture activities. 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. 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, 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 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 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.

Equine Orthopedics and Biomechanics
We have developed a solid research program in the area of equine orthopedics and biomechanics. This has lead to the development of numerous orthopedic implants designed specifically for equine use, which is critical for the advancement of equine orthopedics and fracture repair. These equine specific orthopedic implants have distinct advantages over those intended for human application. The EHSP is unique in that it is the only school of veterinary medicine in the country that is designing and testing equine specific orthopedic implants. An integral part of this research program was the development of a finite element computer model that allows for the biomechanical testing of an orthopedic implant applied to an equine bone or bones. This allows for changes in implant design to be made and tested using the finite element model prior to in vitro biomechanical testing. This computer aided design approach allows for more efficient use of the limited cadaver specimens. This research has direct and often immediate clinical applications. We are presently advising and performing biomechanical tests on prototype large animal orthopedic implants for Synthes Vet, Inc., Innovative Animal Products, and IMEX Veterinary, Inc.

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

Orthopedic-related equipment available in the hospital includes two arthroscopy units, ASIF equipment and implants for fracture repair, surgical lasers (Nd:YAG, diode and CO2), and an extracorporeal shockwave therapy unit. An Equine Lameness and Performance Evaluation (75’ x 125’ covered pavilion) provides a modern facility for evaluation of gait, locomotion, and lameness in
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 is 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 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 to explain the molecular basis of disease with a view to improved clinical approaches; to train scientists, students, and visitors at all levels; and to develop new instruments and methods in equine molecular biology. This laboratory provides refrigerated centrifuge and micro-centrifuges, electrophoresis/transilluminator equipment, refrigerators and ultra-low 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/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, metabolic disease, gastrointestinal disease, summer pasture-associated recurrent obstructive airway disease, bone healing, mechanisms of pain sensation and modulation, and stem cell biology. The capabilities of this laboratory have expanded in light of the increasing importance of the molecular biological approach to the investigation of equine health and disease.
Division of Biotechnology and Molecular Medicine (BIOMMED)

The Division of Biotechnology and Molecular Medicine (BIOMMED), a division within the LSU School of Veterinary Medicine, is a multidisciplinary research, support and development unit that provides centralized access to state-of-the-art equipment and advanced training in molecular and cell biology. In addition, BioMMED oversees three NIH:NCRR funded research cores: The Non-Human Primate Laboratory Core, the Molecular Biology and Immunology Core Laboratories, and a Louisiana undergraduate institution molecular and cell biology training core. The functions of these core laboratories are integrated within BioMMED and Tulane National Primate Research Center (TNPRC) facilities and consist of state-of-the-art equipment and support services that are jointly staffed by BioMMED and TNPRC faculty and personnel.

BioMMED is comprised of five service oriented centralized core laboratories: 1) GeneLab; 2) Cellular and Non-Invasive Whole Animal In Vivo Imaging Laboratory; 3) Bioinformatics, Computational, and Visualization Laboratory; 4) Viral Vector Laboratories (VVL); and 5) Protein and Antibody Production and Purification Laboratory (PAPPL). GeneLab undertakes specific research and training projects, which require expertise in gene cloning, PCR, DNA sequencing, cDNA library construction, gene expression and other molecular methods. In addition, GeneLab performs custom research projects that require other biological experimentation in collaboration with faculty and using facilities of the School of Veterinary Medicine and LSU. Computer analysis of DNA sequences as well as consultation on molecular biological research is provided free of charge. GeneLab provides support letters to LSU-affiliated researchers for federal and state funding agencies on request. The Cellular and Non-Invasive Whole Animal In Vivo Imaging Laboratory focuses on imaging of fluorescent or chemiluminescent probe concentrations within live small rodents (mice, rats). Current equipment include special equipment for the visualization of fluorescent probe concentrations within mice or rats. Specific interests are centered on the visualization of various tumors tagged with fluorescent labels as well as tracking viruses expressing the green fluorescent protein (GFP). Equipment to be purchased includes imaging and computing equipment for the visualization of chemiluminescent probes. The Bioinformatics, Computational, and Visualization Laboratory provides PC server based access to various software used for the analysis of nucleic acid sequences, microarray output data, etc. In the near future this laboratory may provide direct access to the Wisconsin package of software for the analysis of nucleic acids and proteins in collaboration with the LBRN program of the LSU College of Basic Sciences. VVL provides custom baculovirus, adenovirus, vaccinia virus, herpes, and other recombinant virus construction for heterologous gene expression, and vaccine and gene therapy studies. PAPPL 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. 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).
BIOMMED has 2 automated sequencers (ABI3130, Beckman Coulter CEQ 8000), three real-time PCR instruments (two ABI 7900 Sequence Detection Systems and one ABI 7500fast Sequence Detection System), microarray OmniGrid spotter (Gene Machines), microarray reader (AlphaReader 7000, Alpha Innotech), Imager station (Alpha Innotech Fluorochem HD2), Bio-Rad BioPlex, Agilent Bioanalyzer, 5 PCR thermocyclers, a New Brunswick Fermentor, AktaExplorer Chromatography System, Xenogen IVIS 50 Bioluminescent System, KODAK In-Vivo Imaging System FX Pro, LightTools Whole Mouse Imaging Fluorescence equipment, Leica DM IRB Inverted Fluorescent Microscope, Zeiss Axio Observer Z1 Live Imaging Microscope, various computers and software. Additional equipment are available in the departments of Pathobiological Sciences and Comparative Biomedical Sciences that house centralized facilities such as the Organ and Tissue Culture Laboratory, the Glassware and Media Preparation Laboratory, the Electron Microscopy Laboratory, and the Analytical Chemistry Laboratory.

**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, CO_2_ incubators, microscopes, water baths, a pH meter, low speed centrifuges, mixers, stir plates, refrigerators and freezers. One of the laboratories is dedicated specifically for molecular biology procedures and contains all of the equipment and materials for the isolation of and analysis of RNA, DNA, and proteins. Separate refrigerators and freezers for molecular biology samples are found in this laboratory. Spectrophotometers, pH meters, electronic balances, refrigerators and freezer, and other small equipment items are also available in this laboratory.

**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 FACSScan 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 that are performed routinely in this laboratory. There is also a 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 one to four distinct cell populations. Additionally, multicolor immunophenotyping and 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**

VoxBlast, Corel PhotoPaint, QuatroPro, Photoshop 7, IP Toolkit, Fovea Pro, PowerPoint, HazeBuster, and CorelDraw.

The Center features three 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 them to a container for genomic evaluation, 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. The new transmission electron microscope (JEOL JEM 1011) is state-of-the-art with full digital capture of highly detailed sample imaging.

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. This laboratory operates under Federal Good Laboratory Practices regulations and has 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 since 1987. The laboratory screens over 8,000 urine and blood samples per year and has developed sophisticated methodology for detection and confirmation of drugs and their metabolites. The laboratory also serves as a source of information to the racing industry and the public regarding drug pharmacology, metabolism and clearance. The Analytical Systems Laboratory houses advanced mass spectrometry and other analytical equipment that is used to support the research of the School of Veterinary Medicine faculty and graduate students. Equipment in these laboratories includes a Micromass Quattro II LC/MS/MS (+/-) with APCI and ESP interfaces, one HP 1090 II, low-flow HPLC (1 ul/min), two 1090 HPLCs equipped with UV-diode array and fluorescence detectors, five Agilent
1100/1200 HPLC systems, a Thermo LXQ LC/linear ion-trap mass spectrometer, a HP 5973 GC/MS system and beta and gamma counters for radiolabel analyses.

The ASL also has a Waters/Micromass Two-Dimensional-Capillary-Liquid Chromatography/Quadrupole-Time of Flight Mass Spectrometer, which enables separation and comprehensive structural analysis of peptides/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 uses it to identify illegal peptide and protein drug use in racehorses under its contract with the Louisiana State Racing Commission.

The laboratory has developed 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.

Pathology, Histopathology & Immunohistochemistry
The equipment, instrumentation and personnel for gross necropsy, histologic evaluation and immunohistochemical staining are available in this facility. Equipment 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 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 major health sciences library in the greater Baton Rouge area. The SVM Library is a member of the Louisiana Library Network, the South Central Region of the National Network of Libraries of Medicine and the South Central Chapter of the Medical Library Association. The SVM Library is a significant partner in the educational and research programs of the School of Veterinary Medicine. Centrally located on the first floor of the School of Veterinary Medicine, the Library currently holds over 16,000 monographs, provides access to medical journals in print and online, as well as access to several databases dealing with all aspects of veterinary medicine. Patrons have access to not only those resources unique to the SVM Library, but also the resources provided by the main campus libraries. The SVM Library's collection includes topics of human medicine, comparative medicine, public health, animal sciences, and other related areas. Provided each fall is an orientation to incoming students and this orientation is available to anyone upon request throughout the year. Reference and Interlibrary loan services are also available.
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’s 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 31 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 fundraising 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. Newly constructed facilities and new projects soon to be underway offer a wide variety of naming opportunities, including the Equine Intensive Care Unit, Equine Isolation Unit and the Equine Reproduction Unit.

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.equine.vetmed.lsu.edu) or contact Ky Mortensen via telephone (225-578-9590) or e-mail (kmortensen@vetmed.lsu.edu).
LOUISIANA EQUINE RESPONSE IN THE AFTERMATH OF HURRICANES KATRINA AND RITA

Authors/Investigators
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Description of the Problem
The unprecedented devastation caused by Hurricane Katrina, compounded by the effects of Hurricane Rita, presented a unique and challenging situation for animals and their owners in south Louisiana and the Mississippi Gulf Coast regions in the fall of 2005. A report of emergency response activities and lessons learned by faculty and staff of Louisiana State University’s School of Veterinary Medicine (LSU SVM) Equine Health Studies Program (EHSP), members of the Louisiana State Animal Response Team (LSART), state officials, and volunteers has become a useful experiential learning tool for local, state, and national emergency planners.

Study Purpose/Objectives
This account was prepared and provided for the purpose of sharing information and lessons learned that may be useful to those in the veterinary and other animal-care professions in an effort to collectively enhance our abilities nationwide to provide optimum care and resources for animals and their owners when confronted with a natural or man-made disaster.

Approach
Detailed information was obtained from local and state animal officials as well as from individual groups and volunteers. Data collection included pre- and post-storm evacuation records, equine shelter intake records, medical records, LSU Veterinary Teaching Hospital medical records, financial reports, volunteer lists, donation reports, interviews, rescue and response situation reports, after-math reports, USGS surface water information, climatic data, the National Response Plan, and the FEMA Independent Study Program.

Results/Conclusions
While over 30,000 thousand people were ultimately rescued in Louisiana after Hurricane Katrina during the first fifteen days after the storm, there were very minimal state or federal resources available to assist with animal needs. Approximately 500 horses were eventually rescued and cared for by the LSU EHSP/LSART teams during the aftermath of Hurricanes Katrina and Rita. Additionally, the equine rescue groups assisted with the evacuation of over 300 dogs, and other animals including cats, birds, iguanas, goats, potbellied pigs and even people. At the equine response shelter, 382 animals were handled by LSART equine response team (367 equids, 15 goats and three pigs). The most received in a given day was 79 horses on September 9, 2005. The average number of horses cared for per day during this period was 157. Approximately 500 volunteers dedicated time with the Louisiana equine response team.

Lessons learned included: 1) Animal response activities need to be coordinated both locally, regionally, state-wide and nationally using the incident command system. Establishing a network of people and groups with effective communication is vitally important to the overall success. 2) Encouraging the animal-owning public and animal care professionals to have an evacuation plan for their families, including their pets and other animals, and knowledge of local and regional disaster authorities is critical for future disaster response. 3) Every state should have a working state animal response team/plan to support their credentialing program that will work when put to the test. 4) Both permanent and visible forms of animal identification will assist with accurate animal recognition and owner-animal reunions. 5) Educational programs that empower communities to be responsible for caring for their own people and animals are necessary for the future of a successful disaster response. 6) It is important to realize that the goal in disaster
response efforts is for the rescue/shelter team to meet the basic animal medical and husbandry needs. 7) Early establishment and implementation of a shelter exit strategy is vitally important. 8) A clear chain of command, open and effective lines of communication, appropriate pre-disaster planning, maintenance of a degree of flexibility, and identification and empowerment of effective and knowledgeable leaders with sufficient and well-trained back-up personnel are necessary to a successful disaster response.

Benefits to/Impact on the Equine Industry
Improvement of our abilities to provide optimum care and resources for animals and their owners when confronted with a natural or man-made disaster.

Take Home Message
A successful emergency response plan for equine industry stake-holders begins with individual planning in local communities; identification of gaps; networking with local, regional, and state resources; and integration into community and state emergency plans.

Acknowledgements
The LSU Equine Health Studies Program acknowledges those who continue to strive for excellence in emergency response by continuing to work toward integration of personal, local, regional, state, and national emergency response planning in the United States and Worldwide.

Year Completed
2007

Published Manuscripts/Abstracts


Dr. Shannon Gonsoulin (right) and Dr. Jeffrey Artell rescue horses from areas south of New Orleans several days after Hurricane Katrina devastated areas of Southeastern Louisiana and the Mississippi Gulf Coast.
FULL BODY SUPPORT SLING IN HORSES

Authors/Investigators
A. Ishihara, DVM; J.E. Madigan, DVM, MS, DACVIM; Jeremy D. Hubert, BVSc, MS, DACVS; Rebecca McConnico, DVM, PhD, DACVIM

Description of the Problem
Due to their large size and unpredictable demeanor, injured or disabled horses can be difficult and dangerous to manage and treat. Horses do not always tolerate being placed in a sling (particularly during the initial stages), and an unsuitable application may involve the risk of serious injury in both patients and personnel.

Study Purpose/Objectives
To provide instruction for safe and successful handling and management of injured and disabled horses using full body support slinging.

Approach
A review of the literature and referral hospital technical expertise for using equine full body support sling was completed and compiled in the form of two tutorial manuscripts.

Results/Conclusions
A full body support sling is a valuable device as an adjunct to treating horses with neurologic or musculoskeletal disorders. Severely injured or recumbent horses are usually difficult to handle due to their size and behavior. To facilitate the care of such patients using a minimal number of personnel, mechanical suspension devices have been designed and constructed. Horses do not always tolerate being placed in a sling (particularly during the initial stages), and an unsuitable application may involve the risk of serious injury in both patients and personnel. Although horses can subsequently accept a sling, performing an inadequate suspension without appropriate intensive care could lead to a number of complications. For these reason, careful case selection and meticulous patient management are crucial to successful treatment outcomes.

The first report of using a full body support sling for fracture healing and various therapeutic purposes was several centuries ago.

Members of the EHSP Emergency Response Team train with U.S. Coast Guard personnel.
The equine sling has substantially assisted successful helicopter rescues and anesthetic protocols requiring induction and recovery assistance. As equipment and quality of sedation have developed, the equine sling has become a more useful and practical method in a variety of clinical situations.

**Benefits to/Impact on the Equine Industry**
Improved handling of disabled and injured horses with appropriate case selection and safe handling techniques for using large animal slings.

**Take Home Message**
Although the equine full body support sling us fundamentally a supporting tool, it can be very useful when used in appropriate clinical situations.

**Year Completed**
2006

**Published Manuscripts/Abstracts**


Horses with musculoskeletal pain may be successfully managed with the aid of a full body support sling.
RIGHT DORSAL COLONIC PATHOPHYSIOLOGY IN HORSES ADMINISTERED PHENYLButAZONE

Authors/Investigators
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Description of the Problem
Phenylbutazone (PBZ), the most commonly used non-steroidal, anti-inflammatory drug for treating musculoskeletal pain in equine athletes, is often associated with adverse gastrointestinal side effects, including gastric and colonic ulceration, loss of use and even death; but the exact mechanism(s) have yet to be elucidated.

Study Purpose/Objectives
In this study, PBZ was administered to 12 adult horses to determine specific pathophysiologic events that may occur in the right dorsal colon. The overall hypothesis was that administration of PBZ to healthy horses at a standard dosing level and duration causes a decrease in right dorsal colonic blood flow, inhibition of mucosal prostaglandin E2 (PGE2) levels, and a change in production of volatile fatty acids (VFA) leading to mucosal damage and inflammation with subsequent hypoproteinemia and hypoalbuminemia.

Approach
Clinical and serum biochemical parameters, degree of colonic inflammation [mucosal histopathologic grading, myeloperoxidase (MPO) activity, malondialdehyde (MDA) levels, and prostaglandin E2 (PGE2)]; ingesta analyses for volatile fatty acid (VFA) production, and right dorsal colonic arterial blood flow were evaluated every 72 hours for a 21-day period in horses treated with PBZ at standard dosing level (8.8 mg/kg daily).

Results/Conclusions
Data from eight of the 12 horses were used for analysis. Plasma albumin levels decreased during the PBZ-treatment phase.

Plasma albumin levels in horses were significantly decreased as early as four days after PBZ treatment began. Albumin levels remained lower compared to non-treated horses throughout all but one of the time points. Albumin levels were below “normal” lab values after 10 days of treatment and again at day 21 of treatment.

FIG 1
compared with placebo (PLC)-treatment phase from days 10-21 (p< 0.05). PBZ-treatment caused horses to become neutropenic (< 3.0 x 103/ul). No other clinical or hematologic abnormalities were found to show statistical significance between PBZ-treatment phase or PLC-treatment phase, although two horses developed severe colitis while being treated with PBZ. No statistical difference was found between PBZ-phase and PLC-phase right dorsal colonic mucosal tissue analyzed for MPO, MDA, and PGE2 levels for histological evidence of inflammation. Right dorsal colonic blood flow values were increased during the PBZ-phase compared with PLC-phase horses (p < 0.05). Differences were identified in production of VFA ratio in PBZ-treated horses compared with PLC-phase horses with a decrease in acetate levels over time.

Prolonged phenylbutazone administration causes hypoalbuminemia, peripheral neutropenia, changes in right dorsal colonic blood flow and a change in production of VFAs. Equine veterinarians should monitor serum albumin level, peripheral neutrophil count, and be extremely judicious and cautious when making dosing recommendations for PBZ-treatment.

Benefits to/Impact on the Equine Industry
This information results in more judicious use of oral phenylbutazone, increased awareness of adverse effects associated with dosing, and overall improved health and well-being of horses.

Take Home Message
Equine veterinarians should be extremely judicious and cautious when making dosing recommendations for PBZ therapy. There is extreme individual animal variability, and some horses are unable to tolerate even five days of treatment at this dosing level and frequency. Routine blood work beginning as early as three to five days after treatment initiation should be performed, and PBZ dose level decreased or discontinued so that debilitating and life-threatening intestinal adverse effects do not ensue. Alternative NSAIDs with proven clinical efficacy and less adverse side effects (i.e., specific COX1 sparing agents such as firocoxib) are becoming more available for horses and should be considered.

Acknowledgments
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Year Completed
2006

Published Manuscripts/Abstracts
Expression of the Apoptosis-Related Gene Caspase-3 in Equine Laminitis

Authors
Brenna K. Hanly, LSU SVM Class of 2009; Ashley M. Stokes, DVM, PhD; Sharon R. Chirgwin, PhD; Rustin M. Moore, DVM, PhD, DACVS

Description of the Problem
All cells are programmed to self-destruct and cell survival depends on suppression of this cell suicide program. Cell death is crucial for self-organization processes that lead to maturation, immune and nervous system development, control of cell differentiation, matching cell numbers to the environment, and defense against genetic damage and infection.

Cell death involves activation of the family of cysteine proteases, which leads to an orderly process of disintegration, including chromatin margination, cytoplasmic condensation, vacuolization, and fragmentation. Because the cell breaks apart in compact structures, they are phagocytized by neighboring cells, allowing preservation of the surrounding tissues. Active caspase-3 cleaves the DNA repair enzyme and is only expressed in cells going through apoptosis. This project attempts to gain an understanding about programmed cellular death in the equine hoof before and after laminitis development.

Study Purpose/Objectives
We hypothesized that levels of caspase-3 will be upregulated in laminar tissue samples from horses with laminitis. Further, there would be a direct relationship between expression of caspase-3 and the severity of the condition.

Our objectives were: (1) to find, isolate, clone, and sequence equine caspase-3 in laminar tissues; (2) to measure differences between horses with naturally-acquired laminitis and horses with experimentally-induced laminitis; and (3) to determine if there is an effect of laminitis duration on caspase-3 expression.

Clinical Cases of Laminitis

Mean +/- SEM of clinical cases of laminitis, showing the differences between laminitis positive and negative cases for caspase-3 expression.
* denotes significance p < 0.05
Approach
Laminar tissues were collected from 54 horses during various studies of equine laminitis. From these samples, cellular RNA was isolated and purified. Equine caspase-3 sequence was cloned and sequenced from equine laminar tissue using PCR primers designed based on homologous sequences from dogs, cows, and sheep. From this data, primers and probe were designed for use with RT-PCR (TaqMan). Validation of the sequence was completed and RT-PCR (TaqMan) was used to quantify the gene expression of our study samples. Data was standardized to the housekeeping gene \( \alpha \) and analyzed using ANOVA.

Mean +/- SEM of laminitis negative, acute, and chronic cases of laminitis for caspase-3 expression. There was a significant increase in the acute cases and decrease in the chronic cases.

* denotes significance p < 0.05

Mean +/- SEM of caspase-3 copy units associated with P3 movement. Samples with P3 movement noted less caspase-3 than those with no P3 movement. * denotes significance p < 0.05
Results/Conclusions
Higher caspase-3 gene expression was noted in laminitis negative samples as compared to laminitis positive samples. Expression increased as the severity of laminitis increased, but showed significant drops as duration of disease increased beyond three days. Also, significant lower expression of caspase-3 was seen with distal phalanx (P3) rotation and/or sinking in clinical cases.

Benefits to/Impact on the Equine Industry
Equine laminitis is a major concern in the industry. The equine industry in Louisiana is a $2.4 billion industry and laminitis has been identified as the number one disease of horses, requiring focused research. Laminitis is extremely common and in 75% of the cases presented to referral clinics, the horses are euthanized due to the severe pain in their feet. Therefore, there are many reasons to work toward unraveling the pathogenesis of laminitis.

Take Home Message
The changes in expression of the caspase-3 gene suggest a role played by apoptosis in laminitis. The results also mark the differences between the stages, duration, and severity of the condition.

Acknowledgments
This research was supported by the LSU Equine Health Studies Program.

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Published Manuscripts/Abstracts


SERIAL EQUINE LAMINAR TISSUE COLLECTION VIA BIOPSY

Authors/Investigators
Ashley M. Stokes, DVM, PhD; Brenna K. Hanly, LSU SVM Class of 2009; Amy M. Bell; Mike L. Keowen; Daniel J. Paulsen, DVM, MS, PhD, DACVP; Gary A. Sod, DVM, MA, PhD; Jill R. Johnson, DVM, MS, DACVIM, DABVP; Rustin M. Moore, DVM, PhD, DACVS

Description of the Problem
The equine industry in Louisiana is a $2.45 billion industry, and laminitis has been identified as the number one disease of horses requiring focused research. Therefore, there are many reasons to work toward unraveling the pathogenesis of laminitis. Most research studies of laminar alterations unfortunately are impossible without euthanasia of the animal in order to collect laminar tissue samples for histological examination and gene expression studies. With the purpose of gaining a better understanding of equine laminitis, research must be completed regarding the causes, stages, and progression of the disease; however, it is our goal
to develop a new technique for the collection of laminar tissues without the need for euthanasia.

**Study Purpose/Objectives**

We hypothesize that hoof laminar biopsies will provide obtainable and reliable laminar tissues. Our objectives were (1) to determine if laminar biopsies can be performed as an alternative to euthanasia as a way to collect laminar samples; (2) to determine if biopsy samples are of adequate quality and quantity for molecular biological work, especially gene expression studies; (3) to determine if the biopsy samples are of good quality as determined by histological evaluation; and (4) to determine if inflammation occurs as a result of the biopsy procedure thereby, affecting subsequent biopsy samples.

**Approach**

This study was approved by the LSU Animal Care and Use Committee. Six healthy, normal horses (974 lbs. to 1200 lbs. body weight) with no pre-existing lameness were used. The hair around the fetlock was clipped, and the hoof wall was aseptically prepared. Horses were sedated with 125 mg xylazine hydrochloride (1%) IV for each biopsy collection. Medial and lateral abaxial digital nerve blocks were performed using 2 cc of 2% carbocaine (mepivacaine hydrochloride) SQ. An Esmark bandage was applied from the coronary band to just above the fetlock to affect homeostasis. A hole in the hoof wall was drilled until the hoof became thinned and soft. A fine bit was used to drill until 1 mm of stratum medium remained. A disposable 6.0 mm diameter biopsy punch was used to cut the biopsy sample from the laminae to the level of the distal phalanx. Care was taken during biopsy collection to ensure the distal phalanx was not damaged with the biopsy punch. The biopsy core was removed with a Beaver mini-blade. Laminar samples were performed at 3 cm and 5 cm distal to the coronary band and were equally spaced (approximately 3 cm later from the previous site) along the curve parallel and equidistant from the coronary band. Laminar biopsies were performed at 0, 6, 12, and 24 hours to determine the effect of biopsy collection on subsequent biopsy samples. Sterile gauze was applied to the hoof biopsy sites, and the entire foot to the fetlock was bandaged. The bandages were maintained daily for seven to 10 days or until the laminar biopsy sites were dry. Horses were evaluated daily for signs of lameness and oral phenylbutazone was administered as needed after the 24-hour time point. After adequate cornification of the hoof wall and biopsy locations, a hoof repair material (SuperFast Hoof Adhesive) was used to fill the sites. The horse was then returned to pasture.

Laminar biopsies were evaluated histologically with hematoxylin-eosin (H&E) and periodic acid Schiff (PAS) staining (DJP).
Laminar biopsy total RNA was extracted using the standard Tri-reagent technique, quantified using spectrophotometry, and quality-checked using gel electrophoresis. Samples were DNase treated and reverse transcribed to cDNA. TaqMan RT-PCR was conducted for two housekeeping genes, Beta-glucuronidase (β-Gus) and Beta-Actin (β-Actin) to determine if the biopsy samples are appropriate for gene expression studies.

Gene expression data was evaluated for significant effects of any potential variable on housekeeping gene expression.

Results/Conclusions
Adequate laminar biopsies were obtained from all six horses for histological and molecular biological analysis. Five of the six horses showed intermittent grade-1 lameness at subsequent daily evaluations for five days following biopsy. Phenylbutazone (1 gm), administered based on assessment of lameness each day following biopsy, alleviated signs of lameness. One horse showed grade-1 lameness intermittently for three weeks. This horse had poor hoof quality prior to initiation of the experiment. The defects in the hoof wall at the biopsy sites dried and cornified within a week after collection. The hoof wall grew out without long-term ill effects or cracking of the hoof wall adjacent to the biopsy site for over 12 months.

There was no evidence of progressive inflammation or damage in the laminar biopsies as a result of the procedure. In some laminar samples, damage to the tissues occurred due to tissue handling during removal from the biopsy site. Histological examination of the laminar tissues with the H&E and PAS stains found that there was no increase over the 24-hour period in signs for inflammation or basement membrane damage in the laminar tissues when the biopsies were removed easily. Amount of difficulty in the removal of the laminar tissue from the biopsy site determined the degree of inflammation and/or structural damage. RNA extracted from the samples proved to be of high quality and quantity. Laminar housekeeping gene data for both β-Actin and β-Gus were found to be normal. Throughout the course of the 24-hour sampling period, there were not significant differences in expression of the two housekeeping genes β-Actin (p = 0.2996) and β-Gus (p = 0.1504).

Benefits to /Impact on the Equine Industry
The ability to study laminitis has been hampered due to the difficulty in obtaining laminar samples from the equine digit. This technique does not require the euthanasia of the horse for tissue collection and allows for multiple small collections from the same animal. With the use of this new technique, laminitis studies may progress more efficiently by reducing variability and numbers of horses needed to reach significant conclusions.

Take Home Message
Results of this study suggest that laminar biopsy samples can be used in future research and clinical settings. The next step is to take this procedure and use it in conjunction with different laminitis models, studies examining potential treatments, or in clinical settings. The use of this new technique may allow for improved studies of laminitis that will lead to the prevention and treatment of equine laminitis.

Acknowledgments
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SERIAL EQUINE DIGITAL SKIN BIOPSIES FOR THE STUDY OF EQUINE LAMINITIS

Authors/Investigators
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Description of the Problem
Within the equine industry, laminitis is a very costly, painful, and difficult disease to treat, affecting 15% of the horses in the U.S. Because it is such a significant problem to the equine industry, research into this disease is critical. Currently, the collection of laminar tissues for research purposes is technically difficult and limited in the number of samples obtainable. Recent research has demonstrated that laminitis is most often systemic in nature. Alterations frequently found in laminar tissues are also found in other tissues such as the skin. The focus of this study was to validate a technique for the serial collection of skin biopsies located immediately adjacent to the equine hoof, which may serve as an alternative to laminar biopsy collection to evaluate the development of equine laminitis.

Study Purpose/Objectives
Our overall goal is to determine if skin samples collected from skin immediately adjacent to the equine digit are ideal substitutes for laminar tissue collection in studies or clinical evaluation of laminitis. We hypothesized that digital skin biopsies will be obtainable; that serial sampling will not induce inflammation or other alterations from the previous neighboring biopsy site that will preclude use in future laminitis studies; and that 6mm skin biopsies will produce adequate samples for molecular biological studies of gene expression. Our objectives were to devise a procedure for the collection of serial digital skin biopsies; to evaluate them histologically for signs of inflammation and tissue disruption; and to determine the quality and quantity of total RNA found in skin biopsy samples.

Approach
This study was approved by the LSU Animal Care and Use Committee. A thorough physical examination was performed to ensure a healthy status and no pre-existing lameness. After placement in a set of stocks, the horse was sedated with xylazine (150 mg, IV) and acepromazine (25 mg, IV) or detomidine (3 mg, IV); the skin area just proximal to the digit of the forelimb was clipped and prepped; and abaxial nerve blocks were performed using 2 cc of 2% carbocaine. An Esmark tourniquet was placed at the level of the fetlock to decrease hemorrhage. A 6 mm biopsy punch was used to collect skin samples 1 cm proximal to the coronary band. The biopsy punch was used gently to collect the skin sample, taking care not to damage the underlying tissues. Forceps and a curved blade were used carefully to remove the biopsy. We then sutured the skin biopsy cites with a simple interrupted cruciate pattern. The sites were covered with dry gauze, cling, and finally a single layer of elasticon to keep the sites clean.

With every biopsy collected, the size, quality, lameness grade, and attitude of the horse were noted. At every time point, two skin biopsies were collected proximal to the coronary band of the equine digit at time points 0, 6, 12, and 24 hours.
samples of the skin were taken with the second sample taken 1 cm proximal to the first. One sample was placed in a cassette in buffered zinc formalin for paraffin embedding for histological assessment. The other sample was placed in RNase free tubes with Tri-reagent and snap frozen in liquid nitrogen. The time points were 0, 6, 12, and 24 hours and paired skin samples were taken at each of these time points.

Horses were treated with 2 grams of phenylbutazone PO after the 24 hour sample. The bandages were changed daily until the hoof biopsy sites were healed, at which time the sutures were removed.

Skin biopsies were evaluated histologically with hematoxylin-eosin (H&E) staining (DJP).

Total RNA was extracted using the standard Tri-reagent technique, quantified using spectrophotometry, and quality checked using gel electrophoresis. Samples were DNAse treated and reverse transcribed to cDNA. TaqMan RT-PCR was conducted for a housekeeping gene, Beta-glucuronidase (β-Gus) to determine if the biopsy samples are appropriate for gene expression studies.

Gene expression data was evaluated for significant effects of any parameters on housekeeping gene expression.

Results/Conclusions
For each of the time points studied (0, 6, 12, and 24 hours), all of the skin samples were obtained from the six horses. The horses did not have any complications subsequent to biopsy collection, and all sites healed normally.

Histologically, the skin samples did not show significant differences between time points for inflammation and no significant changes from baseline.

There was no evidence of progressive inflammation or damage in the skin biopsies as a result of the procedure over the 24-hour period. Histological examination of the biopsies with the H&E found that there was no increase over the 24-hour period in signs of inflammation or damage. All of the obtained samples were adequate for total RNA extraction and yielded enough for further molecular biological evaluation, such as cDNA production for PCR. The total RNA of the skin was 292.9 ng/ul +/- SEM 29.5. Gel electrophoresis was used to evaluate the quality of the RNA and the samples proved to be of high quality with clean bands present. Laminar housekeeping gene data for β-Gus was found to be normal. Throughout the course of the 24-hour sampling period, there were not significant differences in expression of the housekeeping gene β-Gus.

Benefits to/Impact on the Equine Industry
Finding that serial skin biopsies are a viable technique and that the changes found in laminae during laminitis have been found to occur in the skin, this new technique could be used for further research into laminitis. Taking these serial skin biopsies could be an alternative to using laminar tissue to study laminitis. Serial collection of the skin needs to be studied further to determine if the digital skin and laminae correlate when biopsies are taken serially during the developmental stages of laminitis.

Take Home Message
Overall, we found that the biopsy procedure used in this study was successful in taking serial biopsies of the equine skin near the digit. Results showed that the inflammation did not increase throughout the sample collection period (24 hours). The RNA extraction and gel electrophoresis results showed that this procedure is a viable way of obtaining enough tissue to be able to do further molecular biological analysis.

Since previous research has demonstrated that the development of laminitis involves alterations in the skin that mimic those found in the laminar tissues, we have concluded that this is a viable technique that should be explored further for use in the study of equine laminitis in the pursuit of knowledge into this crippling disease.
Acknowledgments
This research was supported by the LSU Merck-Merial Veterinary Student Summer Scholars Program, the Morris Animal Foundation, the LSU Equine Health Studies Program, and the laboratories of Dr. Ashley Stokes and Dr. Jill Johnson.

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2007

Published Manuscripts/Abstracts

ALTERATIONS IN TOLL-LIKE RECEPTOR REGULATION IN THE LIVER FROM HORSES WITH LAMINITIS

Authors/Investigators
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Description of the Problem
Laminitis is a huge problem in today's world of equine medicine. The disease plagues the backyard pet and Kentucky Derby winners alike, such as Barbaro. Laminitis often occurs as a complication of grain overload, ischemic-reperfusion injury, or other diseases of the gastrointestinal (GI) tract. When the normal population of GI bacteria and/or the GI pH is disrupted due to these conditions, endotoxins and other stimulators of inflammation can be produced and enter into the portal bloodstream. The next point of major organ contact for these endotoxins is the liver. In the presence of these endotoxins, we believe the liver increases its expression of Toll-Like Receptors (TLR). These molecules are known to stimulate many pathways, including the inflammatory cascade resulting in the increased expression of many other inflammatory mediators.

Study Purpose/Objectives
The overall objective is to establish if Toll-like Receptors found in the liver of horses with laminitis are involved in the inflammatory cascade. We hypothesize that TLR-2 will be upregulated in horses with both naturally acquired and black walnut induced laminitis compared with normal horses. The objective of this study was to use RT-PCR of hepatic tissues from normal and laminitic horses to determine if TLR-2 is an early identification of inflammation in the development of laminitis. We hypothesize that TLR-4 will be upregulated in horses with both naturally acquired and black walnut induced laminitis compared with normal horses. The objective of this study was to use RT-PCR of hepatic tissues from normal and laminitic horses to determine if TLR-2 is an early identification of inflammation in the development of laminitis.

Mean (±SEM) Hepatic TLR-4 gene expression expressed in CT units. Acute laminitic horses demonstrated a 5.795 fold increase compared with chronic laminitic horses. A vast majority of the horses with acute laminitis had a primary gastrointestinal disease immediately prior to the development of laminitis (p = 0.013).
**Approach**

Liver samples were collected from a total of 30 adult horses immediately after euthanasia with an overdose of IV sodium pentobarbital. Of these, normal horses (n=18) and horses with naturally-acquired (n=5) and black walnut-induced (n=7) laminitis were collected immediately after euthanasia. TaqMan RT-PCR was used to quantify TLR-2 and -4 presence in both normal and laminitic horses. Beta-Gus was used as the housekeeping gene and standard curves were utilized. Data is presented as mean +/- SEM. Significance was determined using the t-test.

**Results/Conclusions**

Equine TLR-2 and -4 are upregulated in liver collected from laminitic horses compared to liver from normal horses. Specifically, the upregulation of the hepatic TLR-2 gene in laminitic horses is over three-fold higher than in normal horses. The upregulation of TLR-4 was not as substantial and represented only a minor increase in gene expression in horses with laminitis. However, horses with experimentally-induced laminitis (48 hours after black walnut extract administration) had the greatest increase compared with chronic cases having nearly a six-fold increase in TLR-4 gene expression (Figure).

The demonstrated increase in equine TLR-2 and -4 gene expression in the liver of horses with laminitis compared to normal horses is an important finding as we work to discover the pathogenesis of laminitis. The noted increase in expression of TLR-4 is consistent with the use of a laminitis model based on GI disease. These two genes had different patterns of expression depending on disease state, which confirms the need to study these further.

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

The identification of therapeutic targets in the earliest stages of laminitis development may lead to decreased numbers of horse developing this devastating disease.

**Take Home Message**

Inflammation is one of the major pathways associated with the development of laminitis in horses. Identification of mediators of inflammation may aid in the unraveling of the pathogenesis of laminitis in horses. Also, pinpointing an earlier point in this cataclysmic reaction will help in the development of gene specific therapeutics as this technology is developed and utilized in veterinary species.

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**Published Manuscripts/Abstracts**

EVALUATION OF THE MODULATION OF TOLL-LIKE RECEPTORS AND CYTOKINES USING EX VIVO AND IN VIVO MODELS OF ENDOOTOXEMIA IN HORSES

Authors/Investigators
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Description of the Problem
Recently, Toll-Like Receptor (TLR) 4 has been identified as the signal-transducing element of the lipopolysaccharide (LPS) receptor. Regulation of TLR2 and TLR4 expression by LPS is considered to be one of the mechanisms by which the overall responses of immune cells to bacteria are controlled. A greater understanding of how inter-individual variation in innate immune responses is regulated in the general population will facilitate the discovery of new molecular markers of risk for poor outcomes in clinical infections and may help in the discovery of new, innovative treatment strategies.

We performed this study to investigate in an ex vivo model of endotoxemia in adult horses whether low and high responders to LPS can be quantified by measuring cytokine production and expression of TLRs in monocytes, neutrophils and lymphocytes.

Study Purpose/Objectives
The objective of this study was to determine whether horses that produce high concentrations of cytokines after endotoxin stimulation respond to LPS administration with robust expression of TLRs. Cytokine production was measured in an ex vivo model of endotoxemia in adult horses and horses grouped as high and low responders based on cytokine concentrations measured. These high and low responders were then administered endotoxin in vivo, and flow cytometry was used to determine whether their leukocytes demonstrated increased expression of TLRs. The TLR differential expression in neutrophils and monocytes was compared to determine whether expression correlated with cytokine production.

Approach
Ex vivo study whole blood model of endotoxemia – In this model a sample of whole blood is collected in anticoagulant and exposed to endotoxin at a low concentration. Plasma is then collected, and cytokine (TNF-α, IL-1b, IL-6, IL-8) concentrations are measured. Thirty healthy adult horses aged three to 12 years, from various breeds, without a history of GI tract disease or abdominal surgery, were screened to identify a subset of individuals that consistently produce high or low concentrations of cytokines. From these horses, three horses that consistently produce high concentrations of cytokines (high responders) and three horses that consistently produced low concentrations of cytokines (low responders) were selected.

Induction of endotoxemia in vivo – Six adult horses, identified as low or high responders to LPS during the ex vivo model, received 30 ng LPS/kg body weight (IV) as a continuous infusion in 500 mls 0.9% saline through the right jugular catheter over 30 minutes, and were evaluated at 0, 0.5h, 1h, 2h, 4h, 6h, 8h, 12h and 24 hours. Blood was collected for CBC count, cytokines, eicosanoid (TXB2), nitric oxide and flow cytometric analyses to quantify the expression of TLR 1, -2, and -4. After 24 hours, all
animals received flunixin meglumine (1.1 mg/kg bw, IV) to control the clinical signs induced by endotoxin.

Data were analyzed by a two-way ANOVA with repeated measures to determine differences among treatments and over time for all normally distributed variables. When main effects were detected, Student’s t-test was performed to determine differences among individual means. Significance was ascribed to P values < 0.05.

Results/Conclusions
We found that the low responders demonstrated significantly lower levels of TNF-α, IL-6, IL-8, IL-10 than high responders (114.82 vs. 352.14; 0 vs. 14.15; 67.37 vs. 334.84; 0.41 vs. 24.80, respectively [Figure]). There were no differences for TLR1, TLR2 or TLR4 expression in both groups in lymphocytes or monocytes. However, TLR1, TLR2 or TLR4 expression was significantly decreased in lower responders than in high responders over the surface of neutrophils (1.09 vs. 2.24; 3.12 vs. 5.80; 6.37 vs. 8.10, respectively [Figure]). WBC and neutrophil counts were significantly higher in low responders than in high responders (8.12 vs. 6.79; 6.42 vs. 5.25, respectively).

In this study we were able to identify two subsets of horses that differentially responded to LPS. We also demonstrated that neutrophils of the low responders were less activated than in high responders. Our findings may indicate that the milder inflammatory response to LPS in low responders was related to the slight stimulation of TLRs in neutrophils.

Benefits to/Impact on the Equine Industry
Endotoxemia is a major cause of mortality in horses. A greater understanding of how inter-individual variation in innate immune responses is regulated in the general population will facilitate the discovery of new molecular markers of risk for poor outcomes in clinical cases and to develop new innovative therapeutic strategies. Therefore, there are many reasons to work toward unraveling the pathogenesis of endotoxemia and the role played by TLR.

Take Home Message
The importance of these three TLR has been shown to occur in horses as occurs in humans. This study demonstrates their importance and regulation during endotoxemia in horses, leading to studies of their potential involvement in many diseases of the gastrointestinal tract. This study also demonstrates evidence for continued investigation into the role of TLRs during endotoxemia and possible treatment and prevention of this devastating disease.

Acknowledgements
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Year Completed
2006

Published Manuscripts/Abstracts

LOAD AND THERMAL EFFECTS ON EQUINE DIGITAL HEMODYNAMICS MEASURED USING MICROSPHERES

Authors/Investigators
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Description of the Problem
The role of weight-bearing alterations in the development of equine laminitis is an important consideration and concern for orthopedic surgeons. Following orthopedic injury, increased weight-bearing by the contralateral limb is often associated with the development of laminitis. Additionally, controversy exists regarding cryotherapy and subsequent effects on laminar blood flow in the prevention and/or treatment of acute laminitis. The goals of the studies presented here were to determine laminar perfusion in the normal equine digit under various weight-bearing and thermal conditions. Direct measurement of laminar capillary perfusion is limited due to the anatomic location between the hoof wall and bone. The use of colored microspheres (15 um in diameter) allows for differentiation between flow through the laminar capillaries (nutrient vasculature approximately 8 um in diameter) and flow bypassing the laminae via arteriovenous shunts (AVAs approximately 40 um diameter).

Study Purpose/Objectives
Our first hypothesis is that laminar perfusion will be negatively correlated with limb-load distribution. The objective was to determine the effects of limb load on laminar perfusion and flow through AVAs as assessed by measuring digital hemodynamics and administering colored isotopic-labeled microspheres into the metacarpal arterial circulation in normal standing adult horses during zero loading (leg held off of ground), intermediate loading (normal; both forelimbs bearing equal weight) and full loading (contralateral limb held off of ground). Since the size of the microspheres is such that they should not traverse the capillary bed, their presence in digital venous blood collected at designated times would indicate flow through open AVAs. Our second hypothesis is that laminar capillary perfusion will be positively correlated with digital thermic conditions. The objective was to determine the effects of normothermia (25°C), local cold (ice) therapy (0°C) and local warm therapy (60°C) on laminar perfusion and flow through AVAs as assessed by administering colored isotopic-labeled microspheres into the metacarpal arterial circulation in normal standing adult horses.

Approach
Palmar digital arterial blood flow was measured by use of an ultrasonic flow probe. Hoof wall surface temperature was measured with a thermistor probe. Colored isotopic-labeled microspheres were injected into the digital artery with subsequent withdrawal of digital venous blood for measurement of microspheres bypassing the digital capillary bed (passage through AVAs). Microspheres were injected under three different weight-bearing states: normal load with equal load between forelimbs (intermediate load);
maximal load with contralateral limb held off of the ground (full load); and minimal load with the instrumented limb held off of the
ground (zero load). The amount of load on the digit was quantified using a digital scale in order to correlate weight-bearing forces
with measured variables. Microspheres were injected under three thermal conditions: room temperature (25°C), local cold (ice,
0°C) and local warm (60°C) therapy. Upon completion, laminar tissues were collected for quantification of colored isotopic-labeled
microspheres within laminar capillaries. Data were analyzed using a mixed-effects general linear model (with repeated measures
and the horse considered as a random variable where applicable) and pre-determined post-hoc comparisons were made using the
least squares mean. Bonferonni’s correction was used for multiple comparisons.

Results/Conclusions
The most significant finding of our data is that full loading of a forelimb (weight on instrumented limb) results in decreased laminar
perfusion compared to normal conditions (p < 0.05; Figure). Zero weight bearing (weight off of instrumented limb) does not appear
to have a significant effect on laminar perfusion and does not seem to alter flow through AVAs compared to normal weight bearing.
The thermal data suggests that ice therapy (cold) decreases laminar perfusion and increases flow through AVAs compared to
normothermic conditions (Figure); however, this was not statistically significant. Warm therapy (hot) increases laminar perfusion,
but does not seem to decrease flow through AVAs.

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 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
Much information has been gained in recent years regarding laminitis pathogenesis; however, laminitis researchers agree that
more studies should focus on gaining information regarding the normal physiology of the equine digit. Our study is providing very
useful data regarding the physiological changes that take place under various weight-bearing and local digital thermal conditions.
This study has given us a much better understanding of the potential physiological reasons altered weight-bearing leads to the
development of support limb laminitis. Further work will take place to explore the relationship between weight-bearing and laminar
perfusion alterations.

Acknowledgments
This study was supported by the Morris Animal Foundation.

Year Completed
2007

Published Manuscripts/Abstracts
Stokes AM, Keowen ML, Eades SC, Moore RM. Load and thermal effects on equine digital hemodynamics measured using

Effects of clenbuterol on skeletal muscle composition, cardiac function and biochemical markers of muscle injury in exercising horses

Authors/Investigators
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Description of the Problem
Considerable potential for abuse of clenbuterol exists in the racehorse and performance horse industry due to the widespread perception that the drug increases performance and lean muscle mass.

Study Purpose/Objectives
Clenbuterol is a beta-adrenergic agonist licensed for veterinary use as a bronchodilator in horses with obstructive airway disease. Considerable potential for abuse exists in the racehorse and performance horse industry, due to the widespread perception that the drug increases performance and lean muscle mass. Due to the confirmed and tragic deaths of two valuable racing Quarter Horses and the unconfirmed reported deaths of several other race horses in South Louisiana following ingestion of an overdose of an illegally prepared and marketed form of clenbuterol in fall of 2006, a study was designed to determine if clenbuterol administered at recommended dosing level may have detrimental effects on skeletal muscle composition, cardiac function and biochemical markers of muscle injury in exercising horses.

Approach
The study examines the effects of oral clenbuterol, administered at doses up to 3.2ug/kg for 14 days, on skeletal and cardiac muscle injury in clinically healthy Thoroughbred horses undergoing treadmill exercise, as compared to a control group (saline placebo). Muscle biopsies are collected before and after treatment for determination of percent myocyte necrosis and apoptosis. Echocardiographic measurements and activities of creatinine kinase, aspartate aminotransferase, and cardiac troponin I are measured before, during and after treatment. Serum clenbuterol levels are measured before, during and after treatment, and horses are monitored for any adverse effects of treatment.

Results/Conclusions
No significant effect of clenbuterol or exercise on serum biochemical or echocardiographic variables was found between treatment and control groups at any time point, nor within groups over time. Muscle biopsy results are pending.

Benefits to/Impact on the Equine Industry
The study may support the fact that recommended dose levels of clenbuterol syrup are safe to use in race horses being treated for reactive airway disease.

Acknowledgements
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Year Completed
2007

Published Manuscripts/Abstracts
Thompson J. First do no harm? Preliminary investigation of clenbuterol-induced muscle injury in exercising horses. LSU School of Veterinary Medicine, House Officer Seminar Series, Baton Rouge, Louisiana, Feb. 8, 2008.
CHARACTERIZATION OF EQUINE ADIPOSE TISSUE-DERIVED STROMAL CELLS: ADIPOGENIC AND OSTEOPGENIC CAPACITY AND COMPARISON WITH BONE MARROW-DERIVED MESENCHYMAL STROMAL CELLS.

Authors/Investigators
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Description of the Problem
The stromal composition of postnatal adipose tissue contains mesenchymal cells, which are now commonly referred to as adipose-derived stem cells (ASC). These cells were defined based on their multipotent characteristics and capability of in vitro differentiation along a number of lineage pathways. The majority of the published work documenting mesenchymal stromal cell differentiation potential, however, has been performed in species other than the horse, with the exception of the ability of equine mesenchymal cells to differentiate into chondrocytes. More recently cell-based approaches to tendon and ligament injury repair have employed high numbers of ASCs. However, the growth characteristics of equine ASCs as a basis for further tissue engineering approaches has not yet been established.

Study Purpose/Objectives
The objectives of this study were to document and quantify the growth and differentiation characteristics of equine adipose-derived ASCs using limit dilution assays for fibroblasts, adipocytes and osteoblasts. This data will assist future efforts to standardize the isolation, expansion, and transplantation of equine ASCs in clinical practice.

Approach
Adipose tissue was collected from the supragluteal subcutaneous fat depots from five horses (aged nine months to five years) for the isolation and primary culture of adipose-derived stem cells (ASCs). Cell doubling times and numbers for each passage were calculated. Limit dilution assays were used for ASCs to quantify colony forming units for fibroblasts (CFU-F), and cells capable of differentiation into adipocytes (CFU-Ad) and osteoblasts (CFU-Ob), and alkaline phosphatase-expressing cells (CFU-ALP). Cells were cultured for 12 days during which daily examinations were performed to assess lineage-specific differentiation. For CFU-F, cell doubling times and number of primary and passaged ACSs were calculated.

Figure 1 – Cell Doubling Time and Number of Primary and Passaged ACSs

Cell Doubling Times (DT) were compiled from duplicate cell cultures from young horses (n=5). All values reflect the arithmetic mean ± standard deviation. Data from passage 0 – 7 represent n = 5 whereas data from passage 8 – 10 represent n = 3. The over all data distribution was best described with a quadratic model (y = 1.73 + 0.369*X - 0.046*X²). The overall DT of primary and subcultured cells was 2.1 ± 0.9 days/cell doubling. No significant differences in DT were observed between individual passages. By passage 10 the equine ASCs had undergone 28 ± 2 cell doublings. The original CD data behaved in a linear fashion (y = 2.24 + 2.4*X, R² = 0.95).
cells were cultured for nine days then fixed (10% formalin) and stained with toluidine blue. CFU-Ad cells were exposed to an adipogenic induction medium for three days and maintained in adipocyte maintenance medium until day six when the cells were fixed with 10% formalin and stained for neutral lipid accumulation with Oil-Red-O. For CFU-Ob assays, cells were induced and maintained in osteogenic medium then fixed with 70% ethanol and stained with Alizarin Red. For CFU-ALP assays, cells were fixed with 100% ethanol then incubated with BCIP/NBT solution.

Results/Conclusions
ASC isolates exhibited an average cell-doubling time of 2.1 +/- 0.9 days during the first 10 cell doublings. Approximately 1 in 2.3 +/- 0.4 of the total stromal vascular fraction nucleated cells were ASCs, based on the CFU-F assays, and 1 in 3.6 +/- expressed alkaline phosphatase, an osteogenic marker. Primary ASCs differentiated in response to adipogenic (1 in 4.9 +/- 5.4, CFU-Ad) and osteogenic (1 in < 2.44, CFU-Ob) inductive conditions and maintained their differentiation potential during subsequent passages (P2 and P4). The frequency, in vitro growth rate, and adipogenic and osteogenic differentiation potential of equine ASCs show some differences to those documented for ASCs in other mammalian species.

Benefits to/Impact on the Equine Industry
The isolation, expansion in culture and transformation into desired cell types is imperative for the application of stem cells to medical problems. This study demonstrated that cell numbers can be increased and the cells can be induced to change into different cell types based on the culture conditions.

Take Home Message
Our data imply that for a 10mL adipose tissue sample with a yield of 300,000 nucleated cells/ml of adipose tissue, the immediate available ASC number at a frequency of 43% would be 1.3 x 10/6 mL. After 21 days in culture, the total ASC number would be 1.8x 10/9 cells, which is significantly greater than comparable calculations for bone-marrow derived cells. The frequency of ACSs in horses is approximately 10-fold higher than that reported for humans.

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Year Completed
2006
Published Manuscripts/Abstracts


COMPARATIVE CHARACTERIZATION OF CHONDROGENIC DIFFERENTIATION IN EQUINE BONE MARROW AND ADIPOSE TISSUE-DERIVED MESENCHYMAL STROMAL CELLS: COMPOSITIONAL ANALYSIS AND GENE EXPRESSION

Authors/Investigators
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Description of the Problem
The two most common types of adult equine stem cells currently used and studied for regenerative tissue repair are those derived from bone marrow (MSC) and adipose tissue (ASC). While in vitro chondrogenic potential has been described for equine MSCs, little attention has thus far been directed toward equine ASCs. Our recent work has shown that there are differences between MSCs and ASCs with respect to cell frequency in their respective tissues, in vitro growth characteristics and their osteogenic and adipogenic potential. Human ASCs appear to have reduced chondrogenic potential relative to MSCs and show reduced expression of the bone morphogenetic proteins (BMP-2, -4, and -6) and TGFβ receptor-1. Based on human and rodent models, it is important
to evaluate potential differences between equine MSCs and ASCs and to establish which cells may be optimal for specific regenerative tissue applications.

**Study Purpose/Objectives**
The aim of this study was to compare the chondrogenic potential of equine ASCs and MSCs pellet cultures in the presence or absence of a robust growth factor stimulus and to establish potential differences in their extracellular matrix composition.

**Approach**
Sternal bone marrow and supragluteal subcutaneous adipose tissue were collected to isolate MSCs and ASCs, enrich the populations by plastic adherence and expand them in stromal cell culture. Cells were cultured with or without transforming growth factor (TGF-B3) and bone morphogenic protein (BMP-6) pellet cultures as a model for chondrogenesis. Differences in the pellet cultures between the cell types were compared by quantitative compositional analysis of glycosaminoglycans (GAGs), total DNA, and pellet size as well as expression of collagen Type 2 determined by immunohistochemistry and electron microscopy.

**Results/Conclusions**
Collagen type II synthesis was predominantly observed in MSC pellets from Day 7 onward. Unlike ASC cultures, MSC pellets showed hyaline-like matrix by Day 14. Proteoglycan deposition occurred earlier in MSC cultures compared to ASC cultures and growth factors enhanced both GAG concentrations (P<.0077) and pellet size (P<.0033) after two weeks in culture. This study illustrates the superior chondrogenic potential of equine MSCs compared to ASCs and points toward possible differences in ASC growth factor response compared to other species. Elucidation of equine ASC and MSC receptor profiles will enhance the use of these cells in regenerative cartilage repair.

![Figure 1: Collagen Type II Immunohistochemistry of ASC and MSC Pellets](image)

The figure shows convincing Collagen Type II expression in MSC pellets when cultured with BMP6 and TGFβ-3. ASC pellets did not show any evidence of collagen staining under the same conditions and are therefore not shown. Pellet cultures under chondrogenic conditions without growth factors showed mild staining by Day 14 whereas ASC pellets again did not express antigen for the collagen Type II antibody. All pellets were exposed to conditioned medium serving as negative control for unspecific staining and mature cartilage from the proximal surface of the third tarsus (T3) was used as positive control (data not shown).
Benefits to/Impact on the Equine Industry
This study showed that MSCs may be the more efficacious stem cell type for the application toward regenerative cartilage repair. These data have direct implication in the advancement of tissue engineering of equine cartilage.

Take Home Message
Under current culture conditions equine MSCs are superior in their chondrogenic potential compared with equine ASCs. Elucidation of ASC and MSC receptor profiles is necessary to use these cells in regenerative cartilage repair.

Acknowledgements
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Year Completed
2007

Published Manuscripts/Abstracts

Effects of Athletic Conditioning on Equine Degenerative Suspensory Ligament Desmitis

Authors/Investigators
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Description of the Problem
Degenerative suspensory ligament desmitis (DSLD) is characterized by progressive enlargement of the suspensory ligament and lameness. Peruvian Paso and Paso Fino horses are frequently affected, though the condition has been reported in the majority of horse breeds. An enlarged, hyperextended fetlock is a cardinal sign of DSLD (Figure 1). Microscopically, DSLD is consistently characterized by degeneration and swelling of collagen bundles forming the fascicular system of the suspensory ligament. Degenerative changes result in the failure of collagen fiber maintenance and the loss of tensile strength. DSLD is considered a chronically progressive disease with no known cure. Empirical and supportive treatment is not effective in halting disease progression.

Study Purpose/Objectives
This study was designed to test the hypothesis that signs of minor to moderate DSLD are reduced with athletic conditioning.

Figure 1. A radiographic image showing a hyperextended fetlock of a horse affected with DSLD.

Figure 2. Horse exercising on treadmill with active motion analysis markers in place to record dynamic fetlock angle.
Approach

Six horses (n=2/normal; n=4/DSLD) performed 30 minutes of treadmill exercise (Figure 2) every other day for eight weeks. Weight was recorded weekly. Following the exercise trial, horses were pasture rested for four months. Gait analysis (Figure 3), distal limb radiographs, and suspensory ligament ultrasound (Figure 4) were performed prior to the exercise trial, after four and eight weeks of exercise, and after four months of pasture rest following the exercise trial. Static and dynamic fetlock flexion was measured at each time point (Figure 2). All outcome measures except ligament fiber disruption scores were evaluated for significance at p<0.05.

Results/Conclusions

Mean ± SD weight loss was 9.3±6.8, 11.7±11.2 and 12.2±22.1 kg after four and eight weeks of exercise and after four months of pasture rest, respectively. There was a significant improvement in soundness after eight weeks of exercise and four months of pasture rest and also improvements in ligament surface area and fiber disruption in affected horses after four and eight weeks of exercise and after four months of pasture rest. Static and dynamic fetlock angles did not change significantly during the study. This study demonstrates that signs of DSLD are not exacerbated by and may be reduced with athletic conditioning.

Benefits to/Impact on the Equine Industry

The results of this study indicate that exercise does not exacerbate and may improve the signs of DSLD in mildly to moderately affected horses. There was improved soundness with exercise in affected horses. Additionally, there was significant improvement in ligament size and structure with exercise, and the improvements were retained through four months of pasture rest. Ultrasound proved to be the best way to monitor DSLD in this investigation. Further studies are necessary to investigate the metabolic mechanism of exercise induced improvements in signs of DSLD.
Take Home Message
This study demonstrates the signs of DSLD, a disease previously thought to be incurable, appear to be reversible to some degree with athletic conditioning, and that the observed changes are maintained for a period of time after consistent exercise has ceased.

Acknowledgements
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Year Completed
2007-2008

EVALUATION OF THE EFFECTS OF MONOPOLAR RADIOFREQUENCY ENERGY AND DIODE LASER ENERGY ON EQUINE DISTAL INTERTARSAL AND TARSOMETATARSAL ARTICULAR CARTILAGE

Authors/Investigators
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Description of the Problem
Osteoarthritis or bone spavin is a frequent cause of hindlimb lameness in the athletic horse affecting as many as 30% of all performance and sports horse. The disease results from repeated shearing forces between the central and third tarsal bones and between the third tarsal bone and third metatarsal bone. These forces are produced by the complex maneuvers associated with athletic and competitive activities such as show jumping, racing, reining, and dressage. Bone spavin is a degenerative and progressive disease that can ultimately result in fusion of the affected joint(s). Bony fusion causes joint immobility resulting in alleviation of pain and subsequent soundness. Unfortunately, the natural ankylosing process is slow and unreliable and often results in only partial fusion of the joints.

![Figure 1. Images of distal third tarsal (A) and proximal third metatarsal (B) articular surfaces illustrating the location of the drill track.](image-url)
Management of bone spavin includes anti-inflammatory medications, chemical and surgical fusion and cunean tenectomy (CT). As many as 50% of the medically managed horses with bone spavin fail to respond and remain lame. The prognosis after chemical fusion of the distal intertarsal (DIT) and tarsometatarsal (TMT) with sodium monooiodoacetate (MIA) has been previously performed, however, complications of MIA treatment include intense pain and continued lameness. Sixty-seven to 85% of horses treated with surgical fusion return to soundness over three to 12 months. Surgical fusion is a major procedure that requires general anesthesia, is relatively expensive and has a convalescence period of up to 12 months. Another surgical approach for the treatment of bone spavin is cunean tenectomy (CT), which can be performed with sedation and local anesthesia. The efficacy of CT is debated, and there are no current studies or experimental evidence in the literature to support the validity of its success.

Study Purpose/Objectives
The purpose of this study was to determine if monopolar radiofrequency energy (MRFE) or diode laser energy (DLE) applied to the articular cartilage of the hock joints would produce sufficient chondrocyte death to promote joint fusion.

The objectives were:

1. To develop and validate minimally invasive methods for application of MRFE and DLE to equine hock joints.
2. To quantify cartilage and joint tissue changes following treatment with MRFE and DLE.
Approach

Fresh limbs were collected from horses euthanized for reasons unrelated to this study. The DIT and TMT joints were approached through the soft tissues on the medial aspect of each joint. Each joint was assigned to one of two groups: 1) MRFE or 2) DLE.

**MRFE Treatment:** Following joint identification, a bone tunnel was created using a 3.2mm drill bit for a MRFE probe to pass through (Figure 1). Energy was applied as the probe was passed from lateral to medial.

**Laser Treatment:** A bone tunnel was created using a 3.2 mm drill bit for the laser probe to pass through as described above. Energy was applied as the probe was passed from lateral to medial.

Immediately after treatment, the hock was disarticulated. Each of the treated surfaces from the central and third tarsal bones and the third metatarsal bones was divided into three equal, corresponding sagittal sections. Thin slice sections (1.5mm and 2.5 mm) from the center of each quadrant were then collected and prepared for confocal laser and light microscopy.

**Microscopy:** Light microscopy: Samples were fixed in 10% neutral buffered formalin, decalcified, and embedded in paraffin. Sections (5.0 um) were stained with H&E to demonstrate chondrocyte morphology, and with safranin O to stain proteoglycan within the extracellular matrix.

**Confocal Light Microscopy:** Sections (1.5 mm) were stained by incubation in 1.0ml of PBS containing 4ul calcein-Am per 13 ul EthD-1 (Live/ Dead Viability/ Cytotoxicity Kit). Sections were objectively evaluated and scored using a previously established system. Confocal light microscopic images from each section were digitalized and the total area of viable versus non-viable tissue determined using graphics software.

The mean + SEM affected area ([viable tissue/total area] x 100) were determined for each representative section from each treated articular surface. Statistical comparisons between affected area and histologic scores for each treatment modality and articular surface were accomplished with two-way ANOVAs. One way ANOVA with Tukey’s post-hoc test was used to further evaluate significant differences. Significance was set at P<.05.

**Results/Conclusions**

The preliminary results of this study indicate that treatment of articular cartilage in the equine distal tarsal joints with MRFE and DLE significantly enhanced chondrocyte destruction over drilling alone (Figure 2). There was no difference in chondrocyte or osteocyte death among articular surfaces for either treatment (Figure 3). Given that cartilage ablation is required for joint fusion,
our results support the concept that MRFE and DLE may prove to be an effective and minimally invasive way to facilitate joint ankylosis as a treatment for bone spavin. Further investigation is necessary to determine the clinical applicability of MRFE and DLE. Planned and ongoing studies will include further investigation of treatment effects on subchondral bone, light microscopic evaluation of tissue changes, and analysis of the effects of age, gender, breed, and concurrent orthopedic disease on treatment.

Benefits to/Impact on the Equine Industry
The results of this study support the use of MRFE to facilitate hock joint fusion by effectively removing articular cartilage. This study has the potential to contribute significantly to the advancement of equine health by providing an effective, minimally invasive treatment for bone spavin.

Take Home Message
Treatment of the hock joints with MRFE and DLE may be minimally invasive alternatives for bone spavin.

Acknowledgements
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Year Completed
2007

Molecular identification of Phialophora oxyspora as the cause of mycetoma in a horse

Authors/Investigators
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Description of the Problem
Evaluation of an 18-year-old mare who developed an unknown tissue mass in the oral cavity where a broken incisor had been previously extracted (Figure 1).

Study Purpose/Objectives
Positive identification of the infectious agent that caused the oral mass needed to be obtained in order to effectively treat the infection. Due to a lack of fresh tissue, standard culture methods were not applicable. Molecular biology techniques were used to isolate and identify the fungal agent.

Figure 1. Mass in the oral cavity.
**Approach**

The DNA from four paraffin embedded sections of the mycetoma mass was isolated and purified using a commercially available DNA isolation kit. Polymerase chain reaction was carried out, and the amplified PCR products were purified and sequenced using an automated sequencer. The acquired sequences were then compared to known sequences in the Genbank database and were identified as having 96% homology with the DNA of *Phialophora oxyspora* (Figure 2).

**Results/Conclusions**

Molecular biology techniques were employed to definitively identify the causative organism because standard culture methods were inconclusive. DNA was isolated from paraffin embedded sections. The subsequent PCR product was sequenced and made identification of the fungus possible.

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

Mycetoma should be a differential diagnosis for equine oral masses. Identification of the fungal agent can be critical for selection of optimal treatments. Molecular methods may permit definitive identification when standard identification criteria are inconclusive.

**Take Home Message**

Proper treatment of fungal infections can only be accomplished after appropriate identification of the causative agent. Accurate and timely identification of the causative agent can have a positive impact on the treatment and outcomes.

**Acknowledgements**

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**Year Completed**

2006

**Published Manuscripts/Abstracts**


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**ADIPOGENIC DIFFERENTIATION AND CHARACTERIZATION OF EQUINE BONE MARROW-DERIVED MESENCHYMAL STROMAL CELLS**

**Authors/Investigators**

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Description of the Problem

During the past 20 years, enormous advances have been made in the area of adult stem cell biology. Since the 1980s, many studies have set out to characterize and investigate the origins and differentiation potential of adult stem cells, also known as mesenchymal stromal cells (MSC). MSCs have the ability to form clones and therefore have a self-renewal capacity. These cells can be cultured through many passages without differentiating until an appropriate environment is provided to induce the cells to differentiate and commit to a specific cell lineage.

Bone marrow represents one of many different tissue types from which adult mesenchymal stem cells can be harvested. MSCs have been isolated from bone marrow of a variety of species and have been shown to form adherent cell cultures. MSCs have a fibroblastic phenotype and have the ability to differentiate into cell lines of various stromal tissues such as muscle, bone, fat and nervous tissue. Recently, adult equine MSCs have been shown to differentiate into adipocytes using histochemical staining techniques.

Study Purpose/Objectives

Adult equine bone marrow-derived MSCs are capable of adipogenic differentiation and subsequent mRNA expression of vascular endothelial growth factor (VEGF) and the adipose tissue markers insulin receptor substrate 1 (Irs1), adipose differentiation-related protein (ADFP), and leptin (Lep). The objectives are the collection of equine bone marrow and isolation of MSCs in culture; adipogenic differentiation of equine bone marrow-derived MSCs; demonstration of adipogenic differentiation by histochemical staining with Oil-Red-O; and demonstration of adipogenic differentiation using adipose tissue markers through mRNA expression of adipocyte specific genes.

Approach

MSC collection, isolation and culture – Sternal bone marrow was collected from a three-year-old gelding and nucleated cells (EB060624.P0) were isolated by Ficoll centrifugation. Primary cells were cultured in stromal medium (DMEM/Ham's F-12, 1% antibiotic/antimycotic solution and 10% characterized fetal bovine serum) to approximately 80% confluence and then trypsinized before freezing.

MSC Freezing – Primary cells were frozen (-80°C) at 2.0 x 106 cells/ml in 80% FBS, 10% DMSO and 10% stromal medium and stored in liquid nitrogen.

Adipogenesis – Thawed equine MSCs from two, three-year-old geldings (EB060624 and E09) were used for this experiment. Cells were seeded in 6-well culture plates at ~ 5,000 cells/cm2. Cells were cultured in stromal medium at 37°C (5% CO2) for ~ three days to approximately 80% confluence. Treatment plates were incubated for 48 hours with adipogenesis induction medium. Induced MSCs were maintained in adipocyte maintenance medium while control cells were maintained in stromal medium. Media

Fibroblastic equine bone marrow-derived MSCs at 40% (Panel A) and approximately 80 to 90% (Panel B) confluence, at which stage, cells were induced with adipogenic induction medium.
were changed every two days, and on Day 6 cells from two wells of each plate were fixed and those from the remaining four wells were harvested with 1 ml of Tri-reagent and frozen at -20°C for later RNA isolation.

**Histochemical Staining** – Two wells per plate were rinsed with PBS, fixed in 10% formalin and stained with 60% Oil-Red-O (ORO) to highlight intracellular fat droplets.

**Primer Design and Reconstitution** – Primer sequences for four fat genes (ADFP [human], LEP [equine], Irs1 [human, mouse], HSPB3 [human]) and VEGF [equine] were designed using available sequences on the NCBI database and Primer Express® software. Primers were synthesized by Integrated DNA Technologies, Inc, and verified by dissociation curve analysis with SYBR Green qRT-PCR and then optimized with semi-quantitative PCR.

**RNA Isolation** – RNA was isolated from equine bone and adipose tissue to serve as a control for gene expression in cultured adipocyte differentiated MSCs. Equine tibial bone chaff and minced peri-tibial adipose tissues were collected from two horses (15 and 21 years old) immediately post-mortem and stored in 1 ml RNALater® (Ambion, Inc.) for RNA isolation. Trizol Reagent, chloroform extraction and isopropanol precipitation was then used to isolate RNA from the equine tissues as well as the cultured cells. RNA was purified in DNAse (TURBO DNA-Free Kit, Ambion, Inc) and quantified at 260/280 wavelength.

**cDNA Synthesis** – Reverse transcriptase PCR was used to make complimentary DNA (cDNA). Up to 1 ug of RNA was added to Sprint™ PowerScript™ Pre-primed Random Hexamer tubes (BD Biosciences Clontech) in a total volume of 20 ul in DEPC treated water. A thermocycler (MJ Research PTC-200) was used to synthesize cDNA and stored at -20°C.

**Gene Expression via PCR and Gel Electrophoresis** – Semi-quantitative PCR reactions were set up at optimized primer conditions (ADFP [10 uM, 54°C], LEP [10 uM, 57.5°C], Irs1 [2.5 uM, 62°C], VEGF [10 uM, 60°C]). PCR products were loaded onto an Ethidium Bromide-stained 2% agarose gel in TAE buffer. A DNA ladder molecular weight marker (Bio-Rad, EZ Load 100bp) was run on every gel to confirm the expected molecular weight of the amplification product. Molecular mass rulers (Bio-Rad, EZ Load Precision) were then used for gel electrophoresis, and their images were captured with a digital camera (UVP Bioimaging System) to analyze mRNA concentrations with the Labworks™ imaging software (version 4.6).

**Results/Conclusions**

Bone marrow stromal cells are easily separated from the non-adherent hemopoietic cell fractions by culture and adherence to plastic dishes as early studies on human MSCs have shown. The low frequency of the primary MSC populations was greatly enriched with subsequent passages, which was evident by the increasing morphological cell homogeneity. Further work is needed to demonstrate whether equine MSCs show increasing percentages of stromal cell surface markers such as CD13, CD29, CD44, CD73 and CD90, as it has been shown in human adipose tissue–derived mesenchymal stromal cells (ASCs). Similar to other reports on human, rat and mouse MSCs, this study on equine MSCs showed significant adipogenesis enhancement by the addition of rabbit serum.

The markers ADFP, Irs1 and leptin were chosen for their association with adipocyte or related protein and carbohydrate synthesis and metabolism. It was, therefore, expected that these markers would be expressed by differentiated adipocytes. The markers were not expected to be expressed in equine bone. Further work establishing mRNA concentrations in repeated samples is required before significant conclusions can be drawn regarding mRNA expression differences between MSCs and adipocytes.

Contrary to our hypothesis, non-differentiated MSCs also expressed ADFP, Irs1 and leptin. However, it is not unreasonable to assume that MSCs may include the genomic codes for these genes. The role of ADFP is not yet understood beyond its association on the surface of lipid droplets and transcriptional regulation by peroxisome proliferator-activated receptor (PPAR). Leptin has recently been shown to have a concentration-dependent proliferation and inhibition effect on preadipocyte and primary ASCs in
stromal vascular fractions (SVF). The expression of the vascular endothelial growth factor (VEGF) by non-differentiated MSCs was expected, as these cells are recruited to sites of ischemia, where they promote tissue vascularization; hence, their migratory response is dependent upon VEGF and its receptor (VEGFR1). Finally, the insulin receptor substrate, Irs1, is involved in insulin signaling and response associated with somatic growth and carbohydrate metabolism, and, therefore, it is likely that both MSCs, as well as differentiated adipocytes, will express Irs1. Further work is required to demonstrate protein expression in equine MSCs and differentiated adipocytes associated with these genes.

**Take Home Message**

Equine MSCs can be differentiated into adipocytes under in vitro conditions supplemented with rabbit serum. Equine MSCs and differentiated adipocytes both express VEGF and the adipose tissue markers ADFP, Irs1 and Lep.

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**Year Completed**

2006

**PULLOUT STRENGTH OF AO CORTICAL SCREWS WITH TEFLOM TAPE WRAPPED THREADS IN FOAL THIRD METACARPAL BONES**

**Authors/Investigators**

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**Description of the Problem**

The ability of a screw to generate compression between the plate and the bone or between bone fragments to produce 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. Histological studies have shown that immediately after its insertion, a limited area of contact between screw threads and bone exists. Only at the level of the horizontal thread surface of an AO cortical screw, which is oriented towards the head of the screw, do the threads firmly oppose the bone. The contact between the horizontal thread surface and bone is partly caused by tightening the screw to the plate or the cis-cortex, in the case of lag screw fixation. In tightening the screw, the horizontal surface compresses the bone while the oblique under-surface of the thread, which is oriented towards the tip of the screw, is lifted away from the bone (Figure 1). For traditional AO screw implantation, the thread hole (3.2 mm for the 4.5 mm and 4.0 for the 5.5 mm cortical screws) is larger than the inner core diameter of the screws (3.0 mm for the 4.5 mm and 3.8 mm for the 5.5 mm cortical screws), which results in decreased bone thread depth and less contact. The tap often
cuts threads in the bone whose diameter is larger than the outer thread diameter of the screw, which results in an even smaller area of contact between the screw thread and bone, with the crest of the screw threads losing contact with the bone. These spaces between the screw threads and bone can be up to 0.150 mm thick. The limited area of contact and the presence of spaces between screw and bone can permit micromovement of the screw in its bed. To improve the fit between the screw thread and bone the use of Teflon tape (Figure 2) wrapped around the screw threads was considered (Figure 3).

Study Purpose/Objectives
The objective of this in vitro study was to determine the pullout force and strength of 4.5 mm and 5.5 mm AO cortical screws with and without Teflon wrapped threads inserted in the diaphysis of foal third metacarpal (MC3) bones. It is hypothesized that the mean cortical screw pullout force and mean cortical screw pullout strength per mm of bone will be significantly greater for foal MC3 bones with cortical screws with Teflon wrapped threads than in foal MC3 bones with cortical screws without Teflon wrapped threads.

Approach
Eight pairs of MC3 bones were collected from foals that had been euthanized for reasons unrelated to the bones used in this study. For each matched pair of MC3, one was selected at random to have screws with Teflon wrapped threads inserted. For each pair, the mid-diaphysis of each bone was marked and then the bones were transected with an oscillating saw, perpendicular to the longitudinal axis of the bone and 4.0 cm either side of the midpoint of the diaphysis. Each 8.0 cm diaphyseal segment was transected at the mid-diaphysis, perpendicular to the longitudinal axis of the bone (Figure 4). One half (proximal or distal) of each MC3 bone received a 4.5 mm cortical screw while the other half received a 5.5 mm cortical screw, each with Teflon wrapped threads. The particular screw assignment to either the proximal or distal half was randomized, and then screw allocation was reversed for the contralateral MC3 bone. Thread holes were drilled and tapped transversely from the lateral through the medial aspect of the bone at the midpoint of each diaphyseal section. Screws were then inserted into the tapped holes until the screw protruded 2.0 mm through the trans cortex. The bone-screw constructs were subjected to a tensile loading at 19.0 mm/sec using servo-hydraulic biaxial material testing system. 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 < 0.05.

Results/Conclusions
Wrapping the threads of the 4.5 mm AO cortical screws (and 5.5 mm AO cortical screws) with Teflon tape increased (P < 0.001) the mean pullout force and mean pullout strength in foal MC3 bone compared to AO cortical screws without Teflon wrapped threads. A 31% increase in mean cortical screw pullout force for 4.5 mm cortical screws and a 29% increase in mean cortical screw pullout force for 5.5 mm cortical screws were achieved when the threads were wrapped with Teflon tape. This demonstrates that increasing the area of contact and reducing the spaces between the screw and bone increases the screw pullout force in foal MC3 bone, which contributes to the overall stability of the fixation.
Benefits to/Impact on the Equine Industry

The possibility of return to athletic use is enhanced by fracture stability, which keeps the limb functional during healing. Applying a torque to a cortical bone screw results in the screw driving the plate against the underlying bone surface. The frictional contact between the plate and the bone is governed by the force of the screw acting against the plate as well as the coefficient of friction between the plate and bone. A screw with greater pullout strength can generate greater force against the plate, increasing the friction between the plate and bone, hence increasing the stability of the fixation.

Take Home Message

Using AO cortical screws with Teflon tape wrapped threads results in increased pullout strength, which can result in increased fixation stability of equine long bone fractures.

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AN IN VITRO BIOMECHANICAL COMPARISON BETWEEN AO CORTICAL BONE SCREWS WITH TEFLOM TAPE WRAPPED THREADS AND AO CORTICAL BONE SCREWS WITHOUT TEFLOM TAPE WRAPPED THREADS FOR A LIMITED CONTACT DYNAMIC COMPRESSION PLATE FIXATION OF OSTEOTOMIZED EQUINE THIRD METACARPAL BONES

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Description of the Problem
Cortical bone screws are widely used for internal fixation of fractures, either alone (applied in lag fashion) or to secure plates. Histological studies have shown that immediately after its insertion, a limited area of contact between screw threads and bone exists. Only at the level of the horizontal thread surface of an AO cortical screw, which is oriented towards the head of the screw, do the threads firmly oppose the bone. The contact between the horizontal thread surface and bone is partly caused by tightening the screw to the plate or the cis cortex, in the case of lag screw fixation. In tightening the screw, the horizontal surface compresses the bone while the oblique under-surface of the thread, which is oriented towards the tip of the screw, is lifted away from the bone. For traditional AO screw implantation, the thread hole (3.2 mm for the 4.5 mm and 4.0 for the 5.5 mm cortical screws) is larger than the inner core diameter of the screws (3.0 mm for the 4.5 mm and 3.8 mm for the 5.5 mm cortical screws), which results in decreased bone thread depth and less contact. The tap often cuts threads in the bone whose diameter is larger than the outer thread diameter of the screw, which results in an even smaller area of contact between the screw thread and bone, with the crest of the screw threads losing contact with the bone. These spaces between the screw threads and bone can be up to 0.150 mm thick. The limited area of contact and the presence of spaces between screw and bone can permit micomovement of the screw in its bed. To improve the fit between the screw thread and bone the use of Teflon tape wrapped around the screw threads was considered.

Study Purpose/Objectives
The objective of this in vitro study was to compare the monotonic biomechanical properties of a limited contact-dynamic compression plate (LC-DCP) fixation with the same properties between the AO cortical screw with Teflon tape wrapped threads and AO cortical screw for an LC-DCP fixation of osteotomized equine MC3 bones. It was hypothesized that osteotomized equine LC-DCP-MC3 constructs secured with AO cortical screws with Teflon tape wrapped threads (TLC-DCP-MC3) would provide significantly better stability under static loading (palmarodorsal 4-point bending and torsion) and cyclic fatigue testing (palmarodorsal 4-point bending) than the LC-DCP-MC3 construct secured with AO cortical screws (LC-DCP-MC3).
Approach

Twelve pairs of equine MC3 bones were collected from adult Thoroughbred horses. A broad LC-DCP (8 hole, 4.5 mm) was applied to the dorsal surface of a pair of MC3 bones. The LC-DCP was secured in place, on one MC3 bone chosen at random from each pair, with four 5.5 mm AO cortical screws with Teflon wrapped threads (proximal and distal holes in the plate and the holes immediately adjacent to the osteotomies), and four 4.5 mm AO cortical screws with Teflon wrapped threads in the remaining holes using standard AO/ASIF techniques. The LC-DCP was secured in place on the contralateral MC3 bone from each pair, with four 5.5 mm AO (proximal and distal holes in the plate and the holes immediately adjacent to the osteotomies) and four 4.5 mm AO cortical screws in the remaining holes using standard AO/ASIF techniques. In all constructs, the screws were loosened, mid-diaphyseal osteotomies performed, and the screws tightened uniformly to a final torque of 4.2 N-m. Four matching pairs of constructs were tested in palmarodorsal four-point bending in single cycle to failure. A load was applied at a constant displacement rate of 15 mm/sec to failure using servo-hydraulic material testing system. Four pairs of constructs were tested for cyclic fatigue under palmarodorsal four-point bending. A cyclic load of 0 to 7.5 kN was applied at a rate of 2.0 Hz. Four pairs of constructs were tested under torsional loading. Load was applied at a constant displacement rate of 0.17 rad/sec until a rotation of 0.87 rad was attained. Mean test variable values for each method were compared using a paired t-test within each group with a statistical significance of P < 0.05.

Results/Conclusions

In single cycle to failure, four-point bending, the mean values for the yield bending moment, composite rigidity, and the failure bending moment were 1.9 times, 1.8 times and 1.8 times greater, respectively for the TLC-DCP-MC3 composite compared to the LC-DCP-MC3 composite. In cyclic fatigue, four-point bending, the mean number of cycles to failure was 2.9 times greater for the TLC-DCP-MC3 composite compared to the LC-DCP-MC3 composite. In torsion, the mean stiffness was 1.8 times greater for the TLC-DCP-MC3 composite compared to the LC-DCP-MC3 composite.

The TLC-DCP offers increased stability in cyclic fatigue testing, resulting in a significant increase in the number of cycles to failure. This demonstrates that the micromovement between the screw threads and bone, due to the limited area of contact and the presence of spaces between screw and bone, contributes to the overall stability of the fixation. Using AO cortical screws with Teflon tape wrapped threads reduces the screw wobble (similar to a locking screw in a locking compression plate) and reduces micromovements between the LC-DCP and bone during cycling. Increased micromovements between the internal fixation and the bone leads to increased loads on the plate or screws resulting in high-strain cyclic fatigue of the implant.

Benefits to/ Impact on the Equine Industry

The possibility of return to athletic use is enhanced by fracture stability, which keeps the limb functional during healing. Screw wobble is a major component in the destabilization of equine long bone fracture repair during repeated loading of the limb during the recovery period. Coating the screw threads with Teflon dramatically reduced screw wobble and significantly improved stability. It is hypothesized that the use of plasma sprayed hydroxyapatite-coated cortical screws to secure an orthopedic plate to an equine long bone fracture would not only increase the fracture stability during the early stages of fracture healing, but also result in improved fracture stability during later stages of fracture healing due to osteointegration, that is, bone into the hydroxyapatite coating of the screw.

Take Home Message

Using AO cortical screws with Teflon tape wrapped threads reduces screw wobble and micromovements between the LC-DCP and MC3 bone during cycling, resulting in superior stability for MC3 diaphyseal fracture fixation.

Year Completed

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**AN IN VITRO BIOMECHANICAL COMPARISON OF A 5.5 mm LIMITED-CONTACT COMPRESSION PLATE FIXATION WITH A 4.5 mm LIMITED-CONTACT DYNAMIC COMPRESSION PLATE FIXATION OF OSTEOTOMIZED EQUINE THIRD METACARPAL BONES**

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**Description of the Problem**
Most equine long bone fractures are repaired with dynamics compression plates (DCP), introduced in 1969 and designed for use in human patients. The adaptation of human implants to equine fractures has been difficult because of the long-bone size, the greater loads imposed on the implant, and the need for horses to be ambulatory and fully weight-bearing in the immediate post-operative period. Post-operative complications, such as operative site infection and osteomyelitis, implant loosening and implant failure, bone failure and laminitis, contribute to failure of the fracture fixation. The current trend in AO internal fixation is biological

![Figure 1](image1.png) *(Top)* 5.5 mm broad limited-contact dynamic compression plate viewed from above. *(Bottom)* 5.5 mm broad limited-contact dynamic compression plate viewed from below.
plating, which has led to the design of the limited-contact dynamic compression plate (LC-DCP). The evenly distributed undercuts of the LC-DCP reduce contact between the plate and the bone.

A new limited contact-dynamic compression plate (LC-DCP), the 5.5 mm broad LC-DCP was recently introduced for the large animal, particularly, the equine patient (Figure 1). The 5.5 mm broad LC-DCP (5.5-LC-DCP) is thicker and narrower than the 4.5 mm broad LC-DCP (4.5-LC-DCP).

Study Purpose/Objectives
The objective of this in vitro study was to compare the monotonic biomechanical properties of a 5.5-LC-DCP fixation with a 4.5-LC-DCP fixation to repair osteotomized equine third metacarpal (MC3) bones. We hypothesized that the 5.5-LC-DCP would provide significantly better stability under palmarodorsal four-point bending, single cycle to failure, than 4.5-LC-DCP fixation. It was further hypothesized that the 4.5-LC-DCP fixation would provide significantly better stability in cyclic fatigue testing under palmarodorsal four-point bending than the 5.5-LC-DCP fixation. And finally, it was hypothesized that there would be no significant difference in stability under torsional testing, single cycle to failure, between the 5.5-LC-DCP fixation and the 4.5-LC-DCP fixation.

Approach
Paired MC3 bones from 12 adult Thoroughbred horses, each having mid-diaphyseal osteotomies, were randomly chosen to receive the 5.5-LC-DCP (8-hole, 4.5 mm broad) or the 4.5-LC-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 osteotomy, arrows) and four 4.5 mm cortical screws in the remaining holes (Figure 2). Four matching pairs of constructs were tested in palmarodorsal four-point bending in a single cycle to failure, four pairs of constructs were tested for cyclic fatigue under palmarodorsal 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.

Results/Conclusions
The mean values for the yield bending moment, composite rigidity and the failure bending moment were 1.2 times, 1.6 times and 1.3 times greater, respectively for the 5.5-LC-DCP-MC3 composite compared to the 4.5-LC-DCP-MC3 composite. In cyclic fatigue, the mean number of cycles to failure was 1.3 times greater for the 4.5-LCP-MC3 composite compared to the 5.5-LC-DCP-MC3 composite. In torsion, there was no significant difference in mean yield and failure bending moments and mean composite rigidity for the 5.5-LC-DCP-MC3 composite and the 4.5-LC-DCP-MC3 composite.

The number of cycles to failure under palmarodorsal four-point bending for the 5.5-LC-DCP fixation was significantly less (a 20% reduction) compared with the 4.5-LC-DCP fixation. This can be explained by comparing the area of surface contact between the 5.5-LC-DCP and the bone with that of the 4.5-LC-DCP and the bone. The evenly distributed undercuts on both the 5.5-LC-DCP and the 4.5-LC-DCP have the same dimensions, yet the 5.5-LC-DCP is 1.5 mm narrower than the 4.5-LC-DCP, which results in a
22% reduction in area of surface contact between the 5.5-LC-DCP and the bone compared to that of the 4.5-LC-DCP. This results in increased micromovements between the 5.5-LC-DCP and the bone, with resulting decreased stability, compared to the 4.5-LC-DCP fixation and a significantly lower number of cycles to failure with the 5.5-LC-DCP-MC3. This suggests that in cyclic fatigue testing, under palmarodoral four-point bending, between two plate-MC3 constructs where the same type and size of screw, as well as the same pattern of screw placement, is utilized, a major factor affecting the number of cycles to failure is the relative plate-bone contact of the different constructs.

Benefits to/Impact on the Equine Industry

While the 5.5-LC-DCP was specifically designed for the equine patient, it was significantly less stable than the 4.5-LC-DCP (designed for human patients). 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

In cyclic fatigue testing, under palmarodoral four-point bending, between two plate-MC3 constructs where the same type and size of screw, as well as the same pattern of screw placement, is utilized, a major factor affecting the number of cycles to failure is the relative plate-bone contact of the different constructs.

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AN IN VITRO BIOMECHANICAL COMPARISON OF A LOCKING COMPRESSION PLATE FIXATION WITH A LIMITED-CONTACT DYNAMIC COMPRESSION PLATE FIXATION OF OSTEOTOMIZED EQUINE THIRD METACARPAL BONES

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Description of the Problem

The goal of internal fixation is to have an athletically sound horse. Potential for return to athletic use is enhanced by fracture stability, to maintain a functional limb during healing. A recent advance has been the development of the locking compression...
plate (LCP) (Figure 1). The LCP is an internally positioned external skeletal fixation device. The LCP combines locking screw technology with the conventional AO dynamic compression plate techniques (Figure 2). The LCP can be used in compression with conventional AO cortical screws, in a bridging fashion with AO locking screws, or in a combination of compression and bridging. Locking screws provide the ability to create a fixed-angle construct, which does not rely on plate-to-bone compression to maintain stability and limits the need for exact plate contouring (Figure 3).

**Study Purpose/Objectives**

The objective of this in vitro study was to compare the monotonic biomechanical properties of a 4.5 mm broad LCP (4.5-LCP) fixation with a 4.5 mm broad limited-contact dynamics compression plate (4.5-LC-DCP) fixation to repair osteotomized equine third metacarpal (MC3) bones. We hypothesized that the 4.5-LCP-MC3 construct would provide significantly better stability under both static loading (palmarodorsal four-point bending and torsional) and cyclic fatigue testing (palmarodorsal four-point bending) than the 4.5-LC-DCP-MC3 construct.

**Approach**

Paired MC3 bones from 12 adult Thoroughbred horses, each having mid-diaphyseal osteotomies, were randomly chosen to receive the 4.5-LCP (8-hole, 4.5 mm broad) or the 4.5-LC-DCP (8-hole, 4.5 mm broad) applied to the dorsal surface. The 4.5-LCP was secured on the dorsal surface of each randomly selected MC3 with four 5.0 mm locking screws in the 1st, 3rd, 6th and 8th holes (all holes were numbered from the proximal end of the plate), two 5.5 mm cortical screws in the 4th and 5th holes (immediately adjacent to the osteotomies), and two 4.5 mm cortical screws in the remaining holes (Figure 4). The 4.5-LC-DCP 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 5). Four matching pairs of constructs were tested in palmarodorsal four-point bending in a single cycle to failure, four pairs of constructs were tested for cyclic fatigue under palmarodorsal 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.
Results/Conclusions
The mean values for the yield bending moment, composite rigidity and the failure bending moment were 1.9 times, 2.5 times and 3.3 times greater, respectively, for the 4.5-LCP-MC3 composite compared to the 4.5-LC-DCP-MC3 composite. In cyclic fatigue, the mean number of cycles to failure was 2.8 times greater for the 4.5-LCP-MC3 composite compared to the 4.5-LC-DCP-MC3 composite. In torsion, the mean composite rigidity was 2.0 times greater for the 4.5-LCP-MC3 composite compared to the 4.5-LC-DCP-MC3 composite.

The results of previous studies have demonstrated that an important factor affecting the number of cycles to failure is the relative plate-bone contact of the different constructs. However, there are other factors that contribute to the difference between the number of cycles to failure under palmarodorsal four-point bending, for the two fixation methods. Such factors include the screw size, screw type and the pattern of screw placement utilized in securing the plate. This point has been demonstrated in a previous study and is demonstrated by the results of this study. The 4.5-LCP and 4.5-LC-DCP have equal area moments of inertia and equal area of surface contact with bone. Yet, the number of cycles to failure under palmarodorsal four-point bending for the 4.5-LCP fixation was significantly greater (a 2.8-fold increase) compared with the 4.5-LC-DCP fixation. The locking mechanism between the locking screws and the 4.5-LCP created a rigid system, a single beam construct between the plate, screws and bone, where there is reduced motion between its components. Thus, there should be less micromovements between the 4.5-LCP fixation and the bone, with a resulting greater stability, compared to the 4.5-LC-DCP fixation.

Benefits to/Impact on the Equine Industry
The results of this in vitro study provide information to aid in the selection of a biological plate (developed for human patients) for the repair of a long bone fracture in the equine patient. 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
In the case of cyclic fatigue testing, under palmarodoral four-point bending, while the relative plate-bone contact of the different constructs is an important factor, there are other factors that contribute to the difference between the number of cycles to failure for two fixation methods. One such factor is the screw size, screw type and the pattern of screw placement utilized in securing the plate.

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AN IN VITRO BIOMECHANICAL COMPARISON OF ROUGH PLATE FIXATION VERSUS SMOOTH PLATE FIXATIONS OF OSTEOTOMIZED EQUINE THIRD METACARPAL BONES

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Description of the Problem
Most equine long bone fractures are repaired with dynamics compression plates (DCP), introduced in 1969 and designed for use in human patients. The adaptation of human implants to equine fractures has been difficult because of the long-bone size, the greater loads imposed on the implant, and the need for horse to be ambulatory and fully weight-bearing in the immediate post-operative period. The current trend in AO internal fixation is biological plating, which has lead to the design of the limited-contact dynamic
compression plate (LC-DCP). The evenly distributed undercuts of the LC-DCP reduce contact between the plate and the bone.

In previous studies it has been shown that a major factor affecting the number of cycles to failure is the relative plate-bone contact of the different constructs. Rather than a smooth plate, a textured plate having a rough surface to increase its surface area was developed. The term “contact surface” shall be used to refer to the implant surface intended to be brought into contact with the bone to form an interface. The rough plate had a contact surface with micro-roughness having fine pitting superimposed. The texture of the surface is defined by the maximum peak-to-valley height of the microroughness, Rt, and roughness spacing, Rs (Figure 1). Roughness spacing has been found to be more important than common roughness itself. The small value of roughness spacing indicates a heavily pitted surface having a very large surface area.

Study Purpose/Objectives
The objective of this in vitro study was to compare the cyclic fatigue life under palmarosorsal four-point bending of rough plate fixation with smooth plate fixation to repair osteotomized equine third metacarpal (MC3) bones. Rough versions of three of the most commonly used plates in equine orthopedics—the 4.5 mm broad DCP (rDCP), the 4.5 mm broad LC-DCP (4.5-rLC-DCP) and the 5.5 mm LC-DCP (5.5-rLC-DCP)—were compared with their corresponding smooth counterpart, denoted by DCP, 4.5-LC-DCP and 5.5-LC-DCP, respectively. We hypothesized that for each pair of plates—rDCP vs. DCP, 4.5-rLC-DCP vs. 4.5-LC-DCP and 5.5-rLC-DCP vs. 5.5-LC-DCP—the rough plate fixation would provide significantly better stability in cyclic fatigue testing under palmarodorsal four-point bending than the smooth plate fixation.

Approach
To create the rough version of a plate, each plate was initially sandblasted. The particles sandblasted onto the contact surface was subsequently dissolved by a reducing acid—hydrochloric acid (HCl)—made to exert its action on the implant in its boiling state. This helped avoid the contamination by the blasted particles and produce a contact surface with a fine pitting, having mean value of Rs of 2 µm, superimposed upon a micro-roughness having an Rt of 25 µm as impressed by the sandblasted grains. In this case process the required contact surface structure is integral with the metal mass rather than merely a coating as in a plasma-coated plate.

Paired MC3 bones from 12 adult Thoroughbred horses, each having mid-diaphyseal osteotomies, were randomly chosen to receive the rough (8-hole) or the smooth (8-hole) 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. Four matching pairs of constructs were tested for cyclic fatigue under palmarodorsal four-point bending, for each of the three pairs of plates, rDCP vs. DCP, 4.5-rLC-DCP vs. 4.5-LC-DCP and 5.5-rLC-DCP vs. 5.5-LC-DCP—the rough plate fixation would provide significantly better stability in cyclic fatigue testing under palmarodorsal four-point bending than the smooth plate fixation.

Results/Conclusions
The mean number of cycles to failure for the rDCP, 4.5-rLC-DCP, and 5.5-rLC-DCP fixations were 2.7 times, 2.1 times and 1.9 times greater than the DCP, 4.5-LC-DCP and 5.5-LC-DCP fixations, respectively.
The number of cycles to failure under palmarodorsal four-point bending for each rough plate fixation was significantly greater compared with the corresponding smooth plate fixation. This can be explained by considering the texture of the rough plate having micro-roughness with superimposed fine pitting that resulted in a significantly greater contact surface area compared to that of the corresponding smooth plate. The increased contact surface of each rough plate fixation resulted in decreased micromovements between the rough plate and the bone, with resulting increased stability, compared to smooth plate fixation. This further substantiates that in cyclic fatigue testing, under palmarodoral four-point bending, between two plate-MC3 constructs where the same type and size of screw, as well as the same pattern of screw placement is utilized, a major factor affecting the number of cycles to failure is the relative plate-bone contact of the different constructs.

Benefits to/Impact on the Equine Industry
The possibility of return to athletic use is enhanced by fracture stability, which keeps the limb functional during healing. An orthopedic plate with a rough texture may result in a more stable fixation of equine long bone fractures.

Take Home Message
A rough texture on the contact surface of an orthopedic plate was created, which resulted in an implant that had a significantly increased cyclic fatigue life compared to the smooth counterpart.

Year Completed
2007

Published Manuscripts/Abstracts
Effect of centrifugation on equine spermatozoa motility, plasma membrane integrity and acrosomal integrity immediately and after cooling for 24 hours

Authors/Investigators
Jose A. Len, DVM; Jill A Jenkins, PhD; Bruce E. Eilts, DVM, MS, DACT; Dale L. Paccamonti, DVM, MS, DACT; Sara K. Lyle, DVM, MS, DACT; Giselle Hosgood, BVSc, MS, DACVS, FACVS

Description of the Problem
Centrifugation of stallion semen to remove seminal plasma is commonly performed when processing semen for cooling or freezing; however, the effects of centrifugation on equine spermatozoa have not been fully elucidated. Centrifugal forces of 400 to 500 x g are most commonly used for processing equine semen. Some investigators have suggested that forces above 500 x g are deleterious to equine spermatozoa, although controlled studies to compare the effect of different forces on in vitro parameters are lacking.
Study Purpose/Objectives

The objectives of this study were to determine the effects of centrifugal forces between 400 and 4500 x g on equine sperm motility, plasma membrane integrity and acrosomal integrity immediately and after 24 hours of cooling.

Approach

Ejaculates from six stallions were collected, extended (INRA96, IMV Technologies, USA) to a concentration of 25 x 106 cells/mL, and subjected to one of four centrifugation treatments for 10 minutes; 1) non-centrifuged (NC), 2) 400 x g (400), 3) 900 x g (900) and 4) 4500 x g (4500). Before and after centrifugation (D0) and after 24 hours of cooling (D1), spermatozoa motility was assessed (Sperm Vision®, Minitube, USA) and samples were stained with SYBR-14/propidium iodide (PI) and PI/Arachis Hypogaea (Molecular Probes®, Eugene, OR, USA) to assess plasma membrane and acrosomal integrity respectively. The mean (± SD) percent progressive motility, percent intact plasma membrane, percent intact acrosome and spermatozoa recovery rate were analyzed using Shapiro-Wilk’s statistics (SAS/STAT®, USA).

Results/Conclusions

The 4500 treatment group had lower (p<0.05) spermatozoa motility than the other groups (D0: NC = 82.6 ± 6.7%, 400 = 85.6 ± 8.7%, 900 = 84.2 ± 7.4% and 4500 = 74.2 ± 6.2%; D1: NC = 76.4 ± 7.1%, 400 = 74.9 ± 12.2%, 900 = 73.4 ± 10.8% and 4500 = 66.5 ± 11.5%). The 4500 treatment group had fewer (p<0.05) intact plasma membranes than the other groups (D0: NC = 81.1 ± 6.2%, 400 = 85.5 ± 5.6%, 900 = 81.7 ± 5.2% and 4500 = 72.8 ± 6.6%; D1: NC = 76.4 ± 5.2%, 400 = 81.1 ± 2.8%, 900 = 79.0 ± 4.6% and 4500 = 70.7 ± 7.9%). The 4500 treatment group also had fewer (p<0.05) intact acrosomes (D0: NC = 96.0 ± 0.3%, 400 = 92.4 ± 0.2%, 900 = 89.0 ± 0.1% and 4500 = 78.2 ± 0.1%; D1: NC = 91.9 ± 0.4%, 400 = 96.5 ± 0.3%, 900 = 94.7 ± 0.3% and 4500 = 88.2 ± 0.4%). The 400 and 900 treatments had lower (p<0.05) recovery rates than the 4500 treatment (NC= 100.0 ± 0.0%, 400 = 54.4 ± 8.6%, 900 = 75.0 ± 7.1% and 4500 = 97.9 ± 2.8%).

Benefits to/Impact on the Equine Industry

This study demonstrates that centrifugation of equine sperm cells at forces up to 900 x g does not cause significant damage to the acrosomal or plasma membrane and does not significantly reduce spermatozoa motility.

Take Home Message

Of the measured parameters, centrifugation at 400 or 900 x g does not cause damage to equine spermatozoa. However, the number of undamaged spermatozoa recovered was greater with the 4500 treatment. Further investigations of forces between 900 and 4500 x g are warranted to identify the optimum force that maximizes recovery rate with minimal spermatozoal damage.

Acknowledgements

Funding was provided by the Equine Health Studies Program at the Louisiana State University School of Veterinary Medicine and the National Wetlands Research Center, Lafayette, Louisiana.

Year Completed

2007

Published Manuscripts/Abstracts

EQUINE ENDOMETRITIS: EVALUATION OF LOW VOLUME UTERINE LAVAGE FOR DIAGNOSTIC PURPOSES AND ASSOCIATION OF MUCUS WITH CHRONIC ENDOMETRITIS

Authors/Investigators
Tina Miletello, LSU SVM Class of 2010; Brenna Hanly, LSU SVM Class of 2009; Sara K. Lyle, DVM, MS, DACT; Bruce E. Eilts, DVM, MS, DACT; Robert A. Godke, PhD; M.L. LeBlanc; R.C. Causey; Dale L. Paccamonti, DVM, MS, DACT

Description of the Problem
Endometritis is a major cause of infertility in mares. Accurate diagnosis requires the identification of false positives (diagnosis of infection when infection is not present) and false negatives (no infection identified when bacterial infection is present). It is currently recommended to obtain uterine culture concurrently with cytology samples. A negative cytology indicates isolated bacteria are contaminants from the sampling procedure, while a positive cytology indicates pathogenic bacteria. Recent reports, however, indicate that certain bacteria may reside in the uterus without stimulating an inflammatory response.

Study Purpose/Objectives
We hypothesize that using a small volume lavage will improve the efficiency of bacterial recovery from apparently reproductively normal mares and produce fewer false negatives than the swab technique. We also hypothesize that chronically infertile mares produce more mucus. Mucus may contribute to false negative culture results. The objectives of this study are 1) to determine which of two techniques for obtaining cytology and culture samples have fewer false negatives or positives; and 2) to examine the presence of mucus produced by the endometrium and correlate mucus with the ability of bacteria to reside in the uterus without stimulating an inflammatory response.

Approach
Reproductively normal mares were selected based on standard breeding soundness examinations, including a negative uterine culture and cytology obtained by swab, and no significant histological inflammation. Samples from Group 1 mares (n=5) were obtained first during estrus, then diestrus while Group 2 mares (n=5) were sampled first during diestrus then estrus. The following samples were collected in an aseptic manner: endometrial cytology and culture using a brush and swab, low volume lavage, and endometrial biopsy. For the lavage, sterile saline (60 ml) was infused into the uterus via a catheter, the uterus manipulated 30 sec per rectum, and the efflux collected into a sterile 50 ml tube (Figure 1). Efflux clarity, volume and pH were recorded. The efflux was centrifuged, the supernatant removed and the pellet cultured on MacConkey’s and blood agar. A sample of the pellet was also stained for cytological examination. Cytology samples were stained with a modified Wright’s stain and evaluated for the presence of endometrial cells and neutrophils (positive= >2 neutrophils per field at 1000x [Figure 2]). Following lavage, a uterine biopsy was obtained, fixed in Bouin’s solution, paraffin embedded, and stained with H&E and with PAS & Alcian Blue to evaluate mucus production by epithelial cells. Culture results were compared with cytological & histological findings, efflux clarity and pH.

Results/Conclusions
Nine of 10 reproductively normal mares had negative culture results from both the swab technique and the lavage technique. These results were supported with concurrently negative cytologies and biopsies. One mare during estrus had a negative culture and cytology based on the swab, but the lavage had a hypocellular cytology and a positive culture.
During diestrus, the same mare again had a negative culture and cytology based on the swab, but on the lavage had a positive culture. Subsequently, the clitoral fossa, vestibule and vagina of this mare had bacterial cultures taken and were found to be positive for the same organism.

There were no false positives on culture and cytology in nine of 10 mares. No mares had histologic inflammation, therefore there was no ability to detect false negatives.

Benefits to/Impact on the Equine Industry
In this study, examination of low volume uterine lavage was determined to be a feasible technique for obtaining uterine samples for culture and cytology. Bacterial infection without concurrent inflammation was identified in the uterus, vestibule, and vagina of one mare.

Take Home Message
These results suggest that low volume uterine lavage may be a superior technique for identification of uterine infection in mares by reducing the number of false negative samples. Mares examined in this study will serve as controls for a group of infertile mares examined as part of a collaborative study.

INFLUENCE OF THE TIME OF DAY OF HUMAN CHORIONIC GONADOTROPIN ADMINISTRATION ON CIRCADIAN PATTERN OF OVULATION IN THE MARE

Authors/Investigators
Brenna K. Hanly, LSU SVM Class of 2009; Tina Miletello, LSU SVM Class of 2010; Sara K. Lyle, DVM, MS, DACT; Jose Len, DVM; A. Snyder; Bruce E. Elits, DVM, MS, DACT; Robert A. Godke, PhD; Dale L. Paccamonti, DVM, MS, DACT

Description of the Problem
Mares have a variable period of estrus lasting five to seven days that usually ends one to two days following ovulation. A previous study by this group found that ovulation time in mares is circadian, and is most likely to occur between 16:00 to 24:00 hours. Breeding close to ovulation improves pregnancy rates. The ability to time insemination to coincide with ovulation is beneficial when breeding to stallions that are in high demand or have reduced fertility; is needed when using fresh, chilled, shipped semen; and is essential when using frozen semen. To optimize breeding management, human chorionic gonadotropin (hCG) is commonly administered to estrual mares with a follicle > 35 mm, causing approximately 80% of mares to ovulate within 36 to 48 hours. Because of the shortened longevity of frozen semen, knowledge of the expected time of ovulation is beneficial to improving the per cycle conception rate of frozen-thawed semen. If hCG is not effective in overriding the circadian ovulation pattern in the mare, late night breeding may still be required to attain optimum pregnancy rates. We hypothesized that the normal circadian pattern of ovulation could be altered depending upon the time of day of hCG administration.
Study Purpose/Objectives
The goal of this project was to determine if mares ovulated at a consistent time after hCG administration or if mares still tended to follow a circadian rhythm and ovulate between 16:00 and 24:00.

Approach
Transrectal ultrasonography and semi-quantitative progesterone analysis were performed to detect estrus. Mares in estrus with a follicle 35 to 38 mm were assigned to one of two treatment groups. Group A (n=4) received 2,000 IU hCG, i.v., at 08:00 and Group B (n=4) received 2,000 IU hCG, i.v., at 20:00. Beginning 24 hours after hCG administration, transrectal ultrasonography was performed every six hours until ovulation occurred. A post-ovulation rise in progesterone was confirmed by RIA of a plasma sample obtained three to five days after ovulation. A Fisher’s Exact test and the Wilcoxon Two-Sample test were used to compare the distribution frequency between ovulation time of treatment Groups.

Results/Conclusions
There was no difference in time from treatment to ovulation. The time to ovulation after hCG treatment was 38.5 hours ± 3.0 and 38.5 hours ± 7.6 for Group A and B mares, respectively (Mean ± SD). The modal time period of ovulation for Group A mares was 18:00 to 24:00 and for Group B mares 06:00 to 12:00.

Benefits to/Impact on the Equine Industry
These results support the hypothesis that the normal circadian pattern of ovulation could be altered depending upon the time of day of hCG administration.

Take Home Message
In summary, because the interval to ovulation was unaffected by the time of hCG administration, scheduling hCG administration can be used to influence the anticipated time of ovulation.

Acknowledgements
Funding was provided by the Equine Health Studies Program at the Louisiana State University School of Veterinary Medicine and the National Wetlands Research Center, Lafayette, Louisiana.

Year Completed
2007

Published Manuscripts/Abstracts

A typical ultrasound of a mare’s ovary (a) and uterus (b) during estrus.
membrane integrity and acrosomal integrity immediately and after cooling for 24 hours. Society for Theriogenology 2008 Annual Conference, St. Louis, Missouri.

Hours until ovulation in the AM vs PM treated mares.
ROLE OF VANILLOID RECEPTORS IN SUMMER PASTURE-ASSOCIATED OBSTRUCTIVE PULMONARY DISEASE (SPAOPD) IN HORSES

Authors/Investigators
Changaram S. Venugopal, BVSc, MS, PhD; Ralph E. Beadle, DVM, PhD; Rustin M. Moore DVM, PhD, DACVS

Description of the Problem
SPAOPD is one of the two forms of recurrent airway obstruction (RAO), which is characterized by severe bronchoconstriction, airway spasm, hypersecretion of mucus, chronic coughing, exercise intolerance and dyspnea. SPAOPD is seen usually in the temperate regions world wide, such as the southern regions of the United States. Several inflammatory mediators are released during disease episodes. These mediators are responsible for bronchoconstriction, increased vascular permeability, increased mucus secretion, damage to the airway epithelium, oxidative stress and airway inflammation. A positive correlation exists between the intensity of airway hyperreactivity and the quantity of chemical mediators released.

Recently, it has been suggested that a wide range of etiological factors such as charged particles, various forms of dust, fumes, and irritating agents induce release of mediators through stimulation of a unique type of receptors known as vanilloid receptors. These receptors are capsaicin-sensitive sensory receptors that initiate the sensory neuron impulses leading to release of inflammatory mediators. These receptors belong to a superfamily of receptors and the specific class of receptors causing this effect is termed transient receptor potential vanilloid-1 (TRPV1). These capsaicin-sensitive receptors are sensors of environmental changes located in skin, alimentary tract and airways.

The existence of a capsaicin-sensitive tachykinergic excitatory receptor has been demonstrated in several species including guinea pigs and man. A large body of evidence has now established that capsaicin-sensitive sensory nerves play a dual role of 1) sensory transmission (afferent) from the capsaicin-sensitive receptor to the CNS and 2) an efferent function in the peripheral organs via release of mediators at the peripheral nerve endings.

Study Purpose/Objectives
The intent of this study was 1) to characterize the responses of bronchial rings caused by stimulation of vanilloid receptors by capsaicin, and 2) to compare the responses of bronchial rings of RAO-affected and unaffected horses. We also hypothesized that bronchial rings from RAO-affected horses will show a greater contractile response than those of the unaffected clinically healthy horses.

Approach
Two groups of horses–RAO-affected and unaffected–were used. Grouping was performed on the basis of history, physical examination, clinical scoring and transpleural pressure determination.

Bronchial tissues were obtained immediately after euthanasia, which was performed by administration of an overdose of pentobarbital sodium (90 mg/kg of body weight, iv). Bronchial rings were prepared according to previously described methods for preparation of equine bronchial rings. Bronchial rings (4 mm wide) obtained from airways (4th to 7th generation bronchi) of the right diaphragmatic lung lobe were prepared from all horses. Bronchial rings were placed in 10 ml organ baths; one side of the ring was fixed to the floor of the bath, and the other end was attached to a force transducer interfaced with a polygraph. The bath was filled with Tyrode's solution maintained at 37°C by a circulating water bath and was continuously oxygenated with a gas mixture of 95% O₂ and 5% CO₂. An initial tension of 2 grams was applied to the rings to mimic the airway tone observed under conditions in vivo. The rings were allowed to equilibrate for 45 minutes, and the solution was gently replaced with fresh warm Tyrode’s solution.
at 15 minute intervals. After each solution change, tension was reapplied to maintain 2 g of tension except after the last solution change.

After a 45-minute equilibration period, all rings were precontracted with carbachol (10-7M) and allowed to reach a plateau. One ring was used as a carbachol control, one as a DMSO control and the other rings received Capsaicin in DMSO. Capsaicin(10-4M) was added to the bath and the response was monitored for 30 minutes. Two rings were used for each condition.

**Results/Conclusions**

Capsaicin induced a contractile response on the bronchial rings from both unaffected, clinically healthy horse (Figure 1) and RAO-affected (Figure 2). The solvent DMSO caused relaxation of the rings, but a contraction due to Capsaicin was seen over the 30 minutes. Comparison of responses of bronchial rings from RAO-affected and unaffected horses demonstrated dramatically more contraction in the RAO-affected horses.

Capsaicin, a vanilloid receptor agonist, produced contractile responses on bronchial rings of clinically healthy as well as RAO-affected horses suggesting its role in the mediation of bronchial tone in normal as well as RAO-affected horses. The contractile responses of the rings from RAO-affected horses were greater than those of unaffected horses suggesting a important role for vanilloid receptors in RAO-associated bronchoconstriction. TRPV1 receptors are distributed in bronchi of both clinically healthy, as well as RAO-affected horses. From the enhanced responses of rings of RAO-affected animals, it seems that vanilloid receptors are altered, perhaps upregulated in RAO. This indicates that capsaicin-sensitive receptors play an important role in equine airway hyperreactivity.

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

Since vanilloid receptors play a role in equine health and disease conditions, blockade of these receptors could be beneficial in controlling and management of RAO.
Take Home Message
Antagonists of vanilloid receptors could be effective in the management of bronchoconstriction in RAO.

Acknowledgements
This study was supported by the Equine Health Studies Program of the LSU School of Veterinary Medicine.

Year Completed
2007

Published Manuscripts/Abstracts

**TRANSCRIPTIONAL CHANGES ASSOCIATED WITH EQUINE RECURRENT AIRWAY OBSTRUCTION**

Authors/Investigators
Changaram S. Venugopal, BVSc, MS, PhD; Louis Mendes; Juliana Peiró, DVM, PhD; Ashley M. Stokes, DVM, PhD; Rustin M. Moore, DVM, PhD; DACVS

Description of the Problem
Recurrent airway obstruction (RAO), a common respiratory disease of horses, is a severely debilitating, devastating and often career-ending or life-threatening disease. It is a disease of airways with a subsequent economic burden. The etiology and pathogenesis of the disease are not clearly understood. Heaves, or equine chronic obstructive pulmonary disease (COPD), and summer pasture-associated obstructive pulmonary disease (SPAOPD) are two forms of RAO. The two forms of disease show similar signs and pathology that are characterized by airway hyperreactivity and mucus hypersecretion. The current therapeutic measures for RAO are avoidance of potential causative agents if identified, immunotherapy if an allergic component is identified, and control of the disease by symptomatic treatments. There is a paucity of knowledge of the gene changes in this disease.

Many inflammatory mediators have been implicated in RAO over the years and currently some are being investigated. However, while most RAO research has tended to focus on physiological and pharmacological studies in the past, more recently an interest has developed in identifying transcriptional changes occurring in lung tissues during disease progression. Thus far, no such work has been done in equine RAO.

Study Purpose/Objectives
1) To determine whether or not any genes are regulated (either up or down) in the lung tissues of RAO-affected horses compared with unaffected horses using a differential display (DD) methodology.  2) To identify the genes already determined in objective 1 using DD.

Approach
Lung tissues collected from RAO-affected and unaffected horses and stored at -80°C were used for this study. Total RNA was extracted from each sample per lobe and equal amounts of RNA from each lobe sample were pooled to represent pooled sample of a horse. Samples from each horse were again pooled to make two groups.
<table>
<thead>
<tr>
<th>Clone #</th>
<th>Regulation Direction</th>
<th>Identify</th>
<th>GeneBank acc.#</th>
<th>Base pairs sequenced</th>
<th>Homology</th>
<th>Protein function</th>
</tr>
</thead>
<tbody>
<tr>
<td>25A</td>
<td>Up</td>
<td>Uridine phosphorylase 1</td>
<td>XM_592968</td>
<td>407</td>
<td>108/123 (87%)</td>
<td>Catalyze the reversible phosphorolysis of uridine and thymidine or deoxyuridine, respectively, to free bases and ribose-1-phosphate or deoxyribose-1-phosphate</td>
</tr>
<tr>
<td>28A3</td>
<td>Up</td>
<td>Cystatin C</td>
<td>DO661047</td>
<td>716</td>
<td>165/196 (84%)</td>
<td>Inhibits lysosomal proteinases</td>
</tr>
<tr>
<td>36A</td>
<td>Up</td>
<td>Translation elongation factor 1</td>
<td>XM_84805</td>
<td>541</td>
<td>345/371 (92%)</td>
<td>Enzymatic delivery of aminoacyl tRNAs to the ribosome</td>
</tr>
<tr>
<td>37A1</td>
<td>Up</td>
<td>Plecstrin homology domain-containing protein</td>
<td>XM_847491</td>
<td>547</td>
<td>153/162 (94%) 77/85 (90%)</td>
<td>Interacts with PDZ domain-containing proteins</td>
</tr>
<tr>
<td>41A2</td>
<td>Up</td>
<td>Solute carrier</td>
<td>XM_532844</td>
<td>521</td>
<td>348/374 (93%)</td>
<td>Forms a gated pore through which ADP is moved from the matrix into the cytoplasm</td>
</tr>
<tr>
<td>45C</td>
<td>Down</td>
<td>Secretoglobin</td>
<td>AY885562</td>
<td>476</td>
<td>227/232 (97%) 75/76 (98%)</td>
<td>Candidate tumor suppressor gene and a putative growth inhibitory cytokine</td>
</tr>
<tr>
<td>46A</td>
<td>Up</td>
<td>Ferritin light chain</td>
<td>AB175617</td>
<td>712</td>
<td>279/280 (99%)</td>
<td>Intracellular iron storage protein</td>
</tr>
<tr>
<td>53A</td>
<td>Up</td>
<td>Insulin-like growth factor 2</td>
<td>ECILGF22</td>
<td>287</td>
<td>48/48 (100%)</td>
<td>Mediation of growth hormone action, stimulation of growth of cultured cells, stimulation of the action of insulin, involvement in development and growth, and autocrine regulators of cell proliferation</td>
</tr>
<tr>
<td>61A</td>
<td>Up</td>
<td>Annexin A2</td>
<td>BC013843</td>
<td>665</td>
<td>177/184 (96%) 226/258 (87%)</td>
<td>Enhances osteoclast formation and bone resorption</td>
</tr>
<tr>
<td>63A</td>
<td>Up</td>
<td>Eukaryotic translation initiation factor 1A</td>
<td>BC008710</td>
<td>640</td>
<td>323/367 (88%)</td>
<td>Translation initiation pathway enhancing ribosome dissociation into subunits and stabilizes the finding of the initiator Met-tRNA to 40S ribosomal subunits</td>
</tr>
<tr>
<td>69B</td>
<td>Up</td>
<td>Major histocompatibility complex</td>
<td>NM_002124</td>
<td>549</td>
<td>159-181 (87%) 39/44 (88%)</td>
<td>Present processed foreign antigens to T cells</td>
</tr>
<tr>
<td>72C</td>
<td>Up</td>
<td>Fuse-binding protein-interacting repressor</td>
<td>XM_851947</td>
<td>442</td>
<td>216/232 (93%)</td>
<td>Binds the single-stranded FUSE of activity MYC genes, possesses potent transcription activation and repression domains, and is necessary for MYC expression</td>
</tr>
<tr>
<td>75A</td>
<td>Up</td>
<td>Beta-2-microglobulin</td>
<td>X69083</td>
<td>967</td>
<td>526-532 (98%)</td>
<td>Found in association with the major histocompatibility complex</td>
</tr>
<tr>
<td>76B</td>
<td>Up</td>
<td>Cold-shock domain-containing E1</td>
<td>XM_851743</td>
<td>355</td>
<td>219/224 (97%)</td>
<td>Critical factor in mCRD-mediated mRNA turnover</td>
</tr>
<tr>
<td>77B</td>
<td>Up</td>
<td>Thmosin beta-4</td>
<td>NM_001002885</td>
<td>532</td>
<td>303/323 (93%) 32/32 (100%)</td>
<td>Induces the expression of terminal deoxynucleotidyl transferase activity</td>
</tr>
<tr>
<td>78A</td>
<td>Up</td>
<td>Kelch-like ECH-associated protein 1</td>
<td>XM_592838</td>
<td>259</td>
<td>120-121 (99%)</td>
<td>Expression of detoxifying enzymes and oxidative stress-inducible genes to protect against DNA damage</td>
</tr>
<tr>
<td>83F</td>
<td>Up</td>
<td>Glucose-6-phosphate isomerase</td>
<td>XM_848765</td>
<td>990</td>
<td>821/914 (89%)</td>
<td>Catalyzes the interconversion of glucose-6-P-glucosephosphate isomerase and phosphoglucone isomerase</td>
</tr>
<tr>
<td>92F</td>
<td>Up</td>
<td>Lectin, galactoside-binding</td>
<td>NM_002305</td>
<td>227</td>
<td>78/83 (93%)</td>
<td>Autocrine regulator of cell proliferation with a role in the maintenance of G0 and in the control of G2 traverse</td>
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</table>
The RNA concentration was determined by spectrophotometry and was analyzed by agarose gel electrophoresis. The differential display PCR was performed with the GeneFishing DEGs 103 to 110 systems following the manufacturer's instructions. Briefly, first strand cDNA was synthesized using reagents provided in the GeneFishing DEGs Premix Kit. Differential display PCR was set up using 50ng cDNA with the annealing control primer paired with one of the 80 arbitrary primers. The PCR reaction was run using the following program: an initial three minutes at 94°C was followed by 50°C for three minutes and one minute at 72°C, followed by 40 cycles at 94°C for 40s, 54°C for 40s, 72°C for 40s, and a final extension at 72°C for five minutes. PCR amplicons were analyzed on 2% agarose gel and visualized using ethidium bromide. The differentially expressed bands were excised from the gel and purified using an UltraClean GelSpin DNA Purification kit (MoBio, Inc) following the manufacturer's instructions. The purified PCR products were cloned using the pTOPO TA Cloning kit (Invitrogen, Inc.). At least three colonies resulting from the transformation were analyzed for recombinant plasmids by PCR with universal primers. PCR amplicons were analyzed on 1.2% agarose gel and visualized using ethidium bromide. The recombinant colonies had the plasmid extracted from culture using QiaPrep Spin Miniprep kit (Qiagen, Inc.). Purified DNA was sequenced. The results were validated by use of real time PCR, using the primers and probes (FAM-BHQ), in a 7900HT Fast Real-Time PCR System (Applied Biosystems) with Beta-glucoronidase (beta-gus) as endogenous control.

Results/Conclusions
There were 88 differentially expressed genes identified after blasting against the NCBI database, of which 18 were identified. Of these, 17 were viewed as upregulated and one was down regulated (Table 1). Real-time PCR was performed on four selected upregulated genes: uridine phosphorylase 1 (catalyzes phosphorolysis of uridine and thymidine), annexin A2 (enhances osteoclast formation), beta 2 microglobulin (associated with major histocompatibility complex) and thymosin beta 4 (induces expression of terminal deoxynucleotidyl transferase activity). These results demonstrate the complexity of RAO and contribute to a better understanding of molecular mechanisms responsible for the progression of this devastating disease.

Benefits to/Impact on the Equine Industry
The study showed that four genes were upregulated in RAO, of which three are involved in the expression of RAO symptoms.

Take Home Message
Differential display PCR technique is useful in detecting differentially displayed genes. Although this procedure is easy to perform, it has only limited usefulness in diseases that are well established with known mediators.

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Published Manuscripts/Abstracts
Role of Neurokinin-A (NKA) in Equine Obstructive Pulmonary Disease

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

Description of the Problem
Summer pasture-associated obstructive pulmonary disease, a common respiratory disease of horses in the southern region of the United States, is characterized by airway inflammation and obstruction with increased secretion of mucus. Several potent airway inflammatory mediators are released into airway secretions and around the bronchial smooth muscle subsequent to degranulation of mast cells caused by allergic reactions. These mediators are responsible for contraction of smooth muscles in airways, increased vascular permeability, increased mucus secretion, damage to airway epithelium, and coughing and exercise intolerance with dyspnea. Commonly accepted airway inflammatory mediators are histamine, bradykinin, prostaglandins, leukotrienes and platelet aggregating factor. More recently identified mediators are endothelin and neurokinins.

Neurokinin-A (NKA), a member of the tachykinin family of neuropeptides with patho-physiological roles in airway function, mediates bronchoconstriction and neurogenic inflammation in asthmatics. Recently, it gained attention warranting investigation of selective NKA receptor antagonists for therapeutic usefulness by pharmaceutical companies. Neurokinin-A is also reported to mediate nonadrenergic, noncholinergic (NANC) excitatory neurotransmission in human and guinea pig airways under healthy conditions. There is ample evidence for interaction between the neurogenic and immune systems possibly through release of cytokines that are mediated by neurokinins. However, there is a paucity of information in horses regarding involvement of NKA in RAO.

There are three major members in the tachykinin family, namely NKA, NKB and substance P. These agents cause their effect by binding to neurokinin receptors. The preferred receptors for these agents are termed NK-2, NK-3 and NK-1, respectively. Our previous studies and other published studies have shown that substance P did not yield a consistent concentration-dependent contraction of equine airway rings. The next important agent in this family, NKA, produces its action by binding to NK-2 receptors.
Study Purpose/Objectives

Since tachykinins play an important role in the airway hyper-reactivity in humans and guinea pigs, we hypothesized that NKA will induce contraction in equine bronchial rings in a dose-dependent manner. A greater contractile response should be observed in the bronchial rings from RAO-affected horses than those from unaffected horses due to upregulation of NK-2 receptors in the lungs of RAO-affected horses.

Specific Objectives are: 1) to determine and compare the in vitro contractile responses of equine bronchial rings to graded concentrations of NKA (10⁻⁷M to 10⁻⁴M) in RAO-affected and unaffected horses; 2) to determine the NK-2 receptor distribution in the pulmonary tissues using immunohistochemical staining methods; and 3) to determine the NK-2 receptor protein expression in the pulmonary tissues of RAO-affected and unaffected horses using Western blot analysis.

Approach

Twelve horses, aged 10-16 years were grouped into SPAOPD-affected and unaffected based on history, physical examination,
RESPIRATORY clinical scoring, and pulmonary function testing. None of the horses received medications within seven days prior to assessment.

Immediately after euthanasia, a large segment of the right diaphragmatic lobe was removed for pharmacological studies. Pulmonary tissue samples from all five lobes of the lung were also collected, immediately frozen in liquid nitrogen and stored in a -80°C freezer until analyzed for receptor protein expression. A section from each lobe was also fixed in zinc formalin for 12 hours, embedded in paraffin, and processed for routine histological evaluation and immunohistochemical staining studies.

Bronchial rings (4 mm wide) were prepared from the right diaphragmatic lobe around the region of 4th to 7th generation bronchi and mounted in organ baths containing 95% oxygenated Tyrode’s solution at 37°C. An initial tension of 2 grams was applied to each ring to mimic in vivo airway tone. Rings were allowed to equilibrate for 45 minutes, and the bath fluid was gently replaced with fresh, warm Tyrode’s solution at 15-minute intervals. After each solution change except the last, tension was reapplied to maintain 2 grams of tension. After the completion of the equilibration period, cumulative concentration-response (CR) relationships were determined by adding graded concentrations of NKA. The responses were normalized to mg tension/mg dry tissue weight.

Pulmonary tissues collected and placed in zinc-formalin fixative solution were dehydrated using graded concentrations of ethyl alcohol (70%, 90% and 100%) and subsequently cleared using xylene. The specimens were embedded in paraffin for immunohistochemical and pathological analyses. Hypothalamic specimens obtained from rats and horses were used as positive control samples, which were collected and processed in a similar manner. The tissues in paraffin blocks were sectioned (4µM in thick) and stained by immunohistochemical staining methods. One slide of the two prepared was used for detecting the NK-2 receptors (NKA receptors) and the other slide was used as a negative control (without primary antibody). A commercially available polyclonal primary antibody, Anti-Neurokinin-A (Abcam, Inc., Cambridge, Mass.), raised in rabbit was used. Positive and negative
control samples were included in all immunohistochemical staining procedures. Immunohistochemical staining was performed using an automated autostainer (Dako autostainer). Two slides from each lobe were independently evaluated by three authors for staining intensity. The staining intensities for NK-2 receptors were scored after comparing them to that of negative control, the score of which was considered zero. The results were interpreted as absence of staining (0), weak staining (1+), moderate staining (2+) and strong staining (3+).

Five slides/horse (one from each lung lobe) in each group were evaluated for the presence of pathological changes in the bronchioles, blood vessels and alveoli. Each slide was evaluated independently by three authors and scores were assigned based upon the intensity of the pathological change (4). The changes evaluated included alveolar infiltration, epithelial hyperplasia, mucus plugs in the bronchioles, neutrophils in the bronchiolar lumen and peribronchiolar inflammation. The results were interpreted as 0 (absence of the pathological change), 1+ (weak presence), 2+ (moderate presence) and 3+ (strong presence).

Since the pharmacological and immunohistochemistry studies supported the contention of increased NKA receptors in the lungs, a protocol was designed to determine NKA receptor protein expression from the total protein extracts of the pulmonary tissues. The five lobes from each horses were pooled. Total proteins were extracted and receptor expression was determined by Western blotting. The blots were analyzed using Bio-Rad’s Gel DocXR gel documentation system and average intensity of the bands was determined by using Bio Rad’s Quantity One Quantitation software.

Results/Conclusions
The results of the study indicated that NKA induced a concentration-dependent contractile response on the bronchial rings of RAO-affected and unaffected horses. The response was significantly greater in RAO-affected horses (Figure 1).

Representative pictures of immunohistochemical staining of NK-2 receptors from RAO-affected and unaffected horses are shown in Figure 2 A-D. The overall immunohistochemical staining (intensity score) of pulmonary tissues was significantly greater in RAO-affected horses than those of unaffected horses. The scores of the NK-2 staining intensity in the bronchial smooth muscle, bronchial epithelium, vascular smooth muscle and vascular endothelium in RAO-affected and unaffected are shown in Figure 3. The significance is denoted by asterisks. The densitometric analysis of neurokinin-2 receptor protein expression in pooled lung
lobe samples of RAO-affected and unaffected horses are shown in Figure 4.

Benefits to/Impact on the Equine Industry

NK-2 antagonists could be a useful therapeutic agent for recurrent airway obstruction in horses.

Absobserved in RAO-affected horses. The findings also suggest that there is an upregulation of NK-2 receptors in RAO. Antagonists of these receptors could have a therapeutic potential.

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EQUINE AIRWAY SMOOTH MUSCLE CULTURE: A MODEL FOR THE STUDY OF PATHOGENESIS OF RECURRENT AIRWAY OBSTRUCTION (RAO)

Authors/Investigators

Crystal Steib, LSU SVM Class of 2009; Truax R., Larry G. Lomax, DVM, PhD; Changaram S. Venugopal, BVSc, MS, PhD

Description of the Problem

Recurrent Airway Obstruction (RAO), most commonly known as heaves, is a disease that affects horses throughout the world. RAO is actually a broad term that is used to group two similar diseases that affect horses in different climates. Summer pasture...
associated obstructive pulmonary disease (SPAOPD) strikes horses in the subtropical regions, while chronic obstructive pulmonary disease (COPD) is known to affect horses in colder climates. RAO has been characterized as an allergic respiratory disease whose most common symptoms are chronic cough, nasal discharge, and respiratory difficulty. This disease results as a reaction to environmental factors that trigger mucus production, inflammation of small airways, and constriction of the smooth muscle that surrounds them. Airway wall thickening due to increased airway smooth muscle content is thought to be a major contributor to the pathogenesis of RAO, though there is a lack of information available about the mechanisms of airway remodeling in horses. Because RAO is a disease that is triggered by environmental factors, it is most often a seasonal affliction, causing a shortage of suitable research subjects at some point during the year. This could be one reason why more effective treatments have not been studied and compared. The widely believed notion that the pathogenesis of RAO is linked to an increase in smooth muscle cell content indicates the need for further research on this point. Therefore, the overall objective of this project is to generate a protocol for the growth and maintenance of equine bronchial smooth muscle cells in culture to facilitate the study of airway diseases throughout the year. Currently there is no established cell line of equine airway smooth muscle cells (E-ASM) available. This, in turn, means that individual horses must be sacrificed and used for the study of RAO.

Study Purpose/Objectives
The objective of this study is to grow airway smooth muscle cells in a cell culture. Eventually, this will lead to create an established cell line of E-ASM cells to allow researchers to broaden their research avenues. The immediate goal of this project was to confirm the E-ASM in culture using H&E staining for morphology and testing for actin and desmin using immunohistochemical (IHC) staining for confirmation.

Approach
Equine airway smooth muscle was collected aseptically from the region between the 4th and 7th bronchial generation of clinically normal horses. The tissue was digested and allowed to grow in human airway medium (SmGM-2). The cells were frozen in liquid nitrogen at their 6th passage. A portion of the frozen cells were later thawed and approximately 10 million cells were transferred to SmGM-2 medium in a T75 flask. The cells were incubated at 37°C with 5% CO₂. After three to four days of incubation, the cells
were passaged and placed in fresh medium. On the 10th, 12th, and 15th passages approximately 100,000 cells per chamber were transferred to a four chambered slide. These slides were incubated as before for up to five days or until confluent. Once the cells reached confluency, the medium and chambers were removed leaving a monolayer of E-ASM cells firmly attached to the slide. The cells were fixed to the slide using 10% neutral buffered formalin. The slide containing cells from the 10th passage were run through the LEICA Autostainer XL to produce the H & E stain. The slides containing cells from the 12th and 15th passages were stained immunohistochemically for actin and desmin using the DAKO Autostainer. After being fixed and stained, digital images of the results were captured.

**Results/Conclusions**

The H & E stained slides, when examined under light microscopy, showed spindle-shaped cells with central oval nuclei, the most common characteristics of smooth muscle cells. Confluent airway smooth muscle cells commonly align themselves in parallel with the wide nuclear region of one cell lying adjacent to the thinner cytoplasmic region of the neighboring cells, which is what we observed (Figures 1 and 2). The slides stained immunohistochemically for actin and desmin were also examined with light microscopy. Positive staining for actin and desmin both yield a brown color where the protein is found (Figures 3 and 4). On the slides containing cells from the 12th passage, approximately 20% of the cells stained positive for actin (Figure 5); however, no cells stained positive for desmin (Figure 6). The slides containing cells from the 15th passage had more cells stained positive for actin (Figure 7) and desmin (Figure 8).

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

The results of this study will help equine research on airway smooth muscle. Various parameters can be investigated using cell lines.

**Take Home Message**

Equine airway smooth muscle can be cultured, and a cell line can be produced.

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EVALUATION OF INTESTINAL ANASTOMOSIS STRENGTH: MUCOSAL-SUB-MUCOSAL LAYER INDEPENDENT OF SEROMUSCULAR LAYER

Authors/Investigators
Mustajab Mirza, DVM, MS

Description of the Problem
Numerous post operative complications are seen in horses after surgery for small intestinal disease including trauma and paralysis in recovery, postoperative ileus, incisional dehiscence, intestinal stricture, and abdominal adhesions. Although the mechanism(s) in the pathogenesis of these complications is (are) not well understood, it is generally accepted that reduction of surgical times, reduced bowel manipulations, optimal anastomotic closure, and management of abdominal contamination are important. Controversy has been expressed over the optimal anastomotic closure; one question being the number of layers of closure necessary for adequate incisional strength. Reduction of the number of layers closed reduces surgical time but may also reduce the final strength of the incision.

Study Purpose/Objectives
The study was conducted to answer a clinical question whether closure of the mucosa and sub mucosa as a separate layer contribute significantly to the strength of an anastomosis in the equine jejunum. The second objective was to establish a model of an incomplete ring end-to-end anastomosis facilitator (I-ring EEA facilitator).

Approach
Small intestinal segments (30 cm long) were harvested immediately from horses euthanized for reasons other than intestinal disease. The segment was divided into two segments in the middle. The ring of an I-Ring EEA facilitator was passed into both segments and secured with 2 stay sutures in each segment that did not penetrate into the lumen of the bowel. This applied constant equal tension to oppose the edges of the two segments. A single layer simple continuous closure of the mucosa and submucosa using monofilament and braided suture was performed. The I-Ring EEA facilitator was not incorporated into the final closure.

A Foley catheter was placed into the end of one segment and attached by use of umbilical tape to tighten the seal and prevent leakage. The Foley catheter was connected to an air gauge manometer. A bulb air inflator was attached to the lumen of the intestinal segment via polyethylene tubing and an 18 gauge needle.

The intestinal segment was slowly filled with air and the pressure in the segment monitored continuously via visual inspection. The failure point was noted as the point at which the pressure recorded on the air gauge decreased abruptly and/or the sound of air leaking was noted. The pressure at the point when the intestinal incision failed was recorded.

Results/Conclusions
The average pressure necessary to cause failure of the mucosa-submucosa constructs was exceeds the pressures in intestinal segments postoperatively; therefore, it is expected that a two layer closure (mucosa-submucosa and seromuscular) provides more than adequate strength to an intestinal anastomosis. Therefore, a third layer of closure is not necessary. It was also observed that short bites promoted that integrity of an airtight seal. Leakage did not occur after two layer closures. Intestinal samples maintained adequate integrity for testing for 4 hours. The I-Ring EEA facilitator aided with opposition at constant equal tensions while avoiding assistant fatigue and contamination.
Benefits to/Impact on the Equine Industry
The observations provide objective information to assist surgeons with choices of surgical techniques for intestinal anastomosis during colic surgery.

Take Home Message
A two layer closure provides adequate strength and could be incorporated into future studies.

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