Equine Research Hall of Fame Inductees Honored

The University of Kentucky Gluck Equine Research Foundation inducted three scientists into the UK Equine Research Hall of Fame Oct. 9 at the UK Hilary J. Boone Center.

Michelle LeBlanc, a posthumous inductee formerly of Rood and Riddle Equine Hospital, Ernie Bailey, professor at the UK Gluck Equine Research Center, and Elwyn Firth, a professor at the University of Auckland in New Zealand, were honored for their contributions to equine science and research. Nominated by their peers and colleagues, LeBlanc, Bailey and Firth were selected by past Hall of Fame inductees.

“On behalf of the Gluck Equine Research Foundation board, I would like to congratulate this year’s inductees,” said Case Clay, chairman of the foundation’s board of directors. “The inductees were selected from a strong group of nominees who have dedicated their lives to equine research. We looked forward to celebrating the accomplishments of Drs. LeBlanc, Bailey and Firth at the induction ceremony.”

LeBlanc’s career extended over 35 years and included teaching, administration and mentoring. She was a theriogenologist, reproductive specialist, with interests in mare infertility, embryo transfer, placental infections in mares and acupuncture in infertile mares. LeBlanc was awarded the Lifetime Achievement Award from the World Equine Veterinary Association in 2011 and was named 2000 Theriogenologist of the Year by the American College of Theriogenologists. She died in April 2013 after a battle with ovarian cancer.

According to the nomination letter from Wayne McIlwraith, university distinguished professor at Colorado State University, and Ed Squires, professor at the UK Gluck Equine Research Center, LeBlanc “led research in the development of innovative equipment and the development of novel research and treatment techniques in mares and foals. Her contributions as a teacher, administrator and mentor defied quantification. Her passion for the horse and for equine research up to her untimely passing makes her a very worthy recipient.”

Bailey joined the Department of Veterinary Science at UK in 1979 and established a research program for horse genetics. Beginning in the 1990s, Bailey, with his students and colleagues, conducted gene-mapping research and provided leadership for the international horse genome project. This work led to sequencing of the horse genome at the National Human Genome Research Institute in 2006. The tools resulting from these initiatives empowered all areas of equine research. With his students and co-workers, Bailey used molecular genetic tools to uncover mutations responsible for coat color patterns, developmental defects, cytogenetic abnormalities and genes influencing viral susceptibility in horses.

“I have had the pleasure of working with wonderful colleagues at a time when genetics technologies have just exploded. It is gratifying that colleagues and peers nominated and elected me to the Equine Research Hall of Fame,” Bailey said. “I am especially pleased with the recognition accorded the field of genetics. During the last 20 years our research field consisted of about 150 scientists and students, worldwide, who shared time, knowledge and resources to create gene maps and other genomic tools for the horse. These new tools are empowering veterinarians and breeders to solve problems that plagued horses and horse owners for centuries. It’s been a pleasure to work with these amazing scientists; the accomplishments actually belong to this extended community.”

Bailey was nominated by James MacLeod, professor at the UK Gluck Equine Research Center.
The mission of the Gluck Equine Research Center is scientific discovery, education and dissemination of knowledge for the benefit of the health and well-being of horses.

The UK Gluck Equine Research & Service Report is produced by UKGERF and the Department of Veterinary Science. It is published twice a year on behalf of all equine researchers and veterinarians and others in the horse industry who are committed to the continued improvements in equine research and technology.

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Hall of Fame, continued

“Over the past 35 years, Dr. Bailey’s direct research contributions, combined with his vision, cultivation, facilitation and leadership of the international community of scientists working on horse genetics and genomics, has had a profound impact on equine science. Just as our understanding of the human genome is changing all aspects of human health, equine genomics is proving to be transformative for equine biomedical disciplines,” MacLeod said.

Firth has worked in North America, The Netherlands and New Zealand in university research, teaching and surgical referral clinics. He classified bone infection in foals and studied other bone and joint diseases of the young horse. This led to the quantification of bone, joint and tendon changes in young foals exposed to exercise or confinement early in life, and in 2-year-olds trained for racing. A later multi-national collaborative study showed that appropriate early exercise in pastured foals was not harmful and had positive effects on tissues, on adult musculoskeletal health and on the welfare of the horse.

“To have been selected for induction into the University of Kentucky Equine Research Hall of Fame is a great honor. Receiving this prestigious award is a source of great pleasure to me, and I feel very proud to have been so recognized. It is full circle in some ways, since when I first left New Zealand it was to Lexington that I traveled, and I have been back many times since. It will be very nice to return,” Firth said.

Firth’s nomination letter by McIlwraith and I.G. Joe Mayhew, professor at Massey University, commended him for his 35 years of contributions to research in equine musculoskeletal disease: “Dr. Firth has made outstanding contributions to research in equine musculoskeletal disease and comparative knowledge emanating from that.”

Equine Research Hall of Fame nominees can be living or deceased, active or retired in the field of equine research. Established in 1990, the Equine Research Hall of Fame honors international scientific community members biennially who have made equine research a key part of their careers, recognizing their work, dedication and achievements in equine research.


--Jenny Evans

Testing Available for Specific Dwarfism Gene in Miniature Horses

The University of Kentucky Animal Genetic Testing and Research Laboratory (AGTRL) is now offering DNA-based tests for four mutations in the aggrecan gene (ACAN) associated with dwarfism in the Miniature Horse. The mutations were discovered by John Eberth, MS, a PhD student of Ernie Bailey, PhD, professor in genetics and genomics at the UK Gluck Equine Research Center.

It is important to note that these mutations are not associated with another type of dwarfism known as skeletal atavism seen in Miniature Horses and Shetland Ponies. The ACAN mutations are also not associated with the osteochondrodysplasia dwarfism found in some breeds of horse such as the Friesian.

A horse that is a carrier for any one of the four ACAN mutations appears to be normal and does not exhibit any dwarf traits. Because carriers have the normal phenotype, it is important to test breeding stock for these mutations to avoid matings that might produce a dwarf or aborted/absorbed fetus. One of these mutations (D1) is lethal in combination with any of the other mutations and will cause early pregnancy loss. Care must also be taken in breeding two horses together that are carriers for the other ACAN mutations, as the presence of two mutations in any combination will produce a dwarf foal with a range of physical ailments. Some of these defects seriously affect the health of the horse and include breathing problems, malformed mouths which cause eating difficulties, and abnormal bone growth leading to chronic soundness issues.

The four identified mutations are designated D1, D2, D3, and D4. The normal copy of the gene is designated as N.

Information on test price and instructions for sample submission is available at http://www2.ca.uky.edu/gluck/AGTRL.asp#Dwarfism.

For questions regarding the dwarfism test, please contact Kathryn Graves, PhD, assistant clinical professor and director of the AGTRL, at ktgraves@uky.edu or 859-218-1193.

--Kathryn Graves
Update: Balasuriya’s EVA Research

Udeni Balasuriya, BVSc, MS, PhD, professor at the University of Kentucky Gluck Equine Research Center, recently spoke at a UK Equine Forum meeting where he discussed “Equine Infectious Diseases in the Genomic Era: Identification of Putative Host Factors Associated with EAV (Equine Arteritis Virus) Carrier State in the Stallion.”

EAV is a virus that causes equine viral Arteritis (EVA) an upper respiratory tract and reproductive disease in horses. EAV can be transmitted by respiratory or venereal routes. Symptoms of EVA include skin rash, watery eyes, swelling of the eyes, edema on the legs, sheath and scrotum, and establishment of persistent infection in stallion. Most of the EAV infections are subclinical in nature but animals develop moderate to severe clinical signs. The severity of the clinical signs is determined by many factors, including the strain of EAV, route of infection, age, sex, breed, immunity, climate, and management practices. Standardbreds and European Warmbloods have the highest seroprevalence, or disease occurrence within a population, followed by the American Saddlebred. Thoroughbreds and American Quarter Horses have low seroprevalence of EAV.

Balasuriya’s research focus is to identify the viral and host factors involved in the establishment of EAV carrier state in the stallion. The identification of the host factors was made possible by sequence analysis of equine genome from EAV carrier and non-carrier stallions. His work has identified two genes that may be associates with the establishment of EAV carrier state in stallions. These two genes encode for two proteins (CXCL16 and CXCR6) that are present in equine cells that are associated with the immunity.

The research is important to the equine industry because the EAV is a threat to the breeding industry. The persistently infected stallions can transmit the virus to susceptible mares during breeding and precipitate outbreaks of EVA. Furthermore, virus can be transmitted to a naïve recipient mare via embryo transfer from a donor mares inseminated with EAV-infective semen.

In early 2013, Balasuriya was awarded a five-year $2.9 million grant from the U.S. Department of Agriculture to identify genetic factors responsible for the establishment of EAV. The grant is titled “Identification of genetic factors responsible for establishment of equine arteritis virus carrier state in stallions.”

A portion of the grant will fund the Controlling EAV and Other Infectious Agents in Stallions, Semen and Embryos Symposium organized by the UK Gluck Equine Research Center on Nov. 22, 2014, at the Embassy Suites in Lexington. For more information about this event and to register, visit www.eavsymposium.eventbrite.com

Graduate Student Research: Ashish Tiwari

Influenza virus is one of the important pathogens of animals and humans. Viruses have always intrigued Ashish Tiwari, and it inspired him to pursue a career researching influenza virus.

The laboratory of Thomas Chambers, PhD, professor of veterinary virology at the University of Kentucky Gluck Equine Research Center is the only OIE (World Organisation for Animal Health) reference laboratory for equine influenza in the Western hemisphere and is one of the best places to work on influenza virus, Tiwari said.

One of the major complications and causes of mortality during influenza infection is bacterial pneumonia. Secondary bacterial pneumonia during equine influenza could potentially be fatal or at least significantly reduce the rate of recovery, resulting in significant economic losses to the equine industry. Tiwari is exploring how influenza virus modulates the host innate immune system for secondary bacterial infection.

“In my PhD project, I am investigating how influenza virus inhibits a cytokine (IL-23/IL-17) response, which is important in the antibacterial defense in the lungs of the host,” he said.

Tiwari is also investigating whether external supplementation of IL-23 could help in preventing secondary pneumonia and possibly speed up recovery as well as reduce the associated mortality. The project is funded by Kentucky Equine Drug Research Council.

“In addition to my project, I have worked on development of a real-time PCR based diagnostic assay for influenza viruses, and I have also studied the molecular epidemiology of equine influenza virus,” he said.

With emerging drug resistance among bacterial populations, it is important to develop alternative treatment strategies. Results from this study will identify the targets that could help prevent and/or treat secondary bacterial infections even in case of a drug resistant infection, Tiwari said.

After finishing his PhD, Tiwari said he would like to pursue his research interests in virology with emphasis on antiviral immunity.
Jennifer Janes came to the University of Kentucky Gluck Equine Research Foundation for a doctoral degree because it allowed her the unique educational opportunity to pursue her interests in anatomic pathology and equine musculoskeletal disease. This was accomplished through a dual-degree program comprised of an anatomical pathology residency at the UK Veterinary Diagnostic Laboratory and a doctoral degree under the mentorship of James MacLeod, VMD, PhD, John S. and Elizabeth A. Knight chair and professor of veterinary science at the Gluck Center.

“The opportunity to learn and train with respected equine scientists was very exciting and has been a wonderful experience,” Janes said.

Janes’ main research focus has been investigating the roles of orthopaedic pathology and genetic determinants in equine cervical stenotic myelopathy, commonly known as Wobbler Syndrome. Wobbler Syndrome is an important neurologic and musculoskeletal disease that often has a significant impact on the health and athletic future of a horse.

Gaining a better understanding of how this disease develops, will enhance identification of susceptible horses, treatment, and management decisions.

“Wobbler Syndrome is a multifactorial disease, but a major knowledge gap in our understanding is the interactions of these variables -- for instance nutrition, growth rates, possibly genetics, that lead to disease development. So the focus of our project was twofold,” Janes said. “First, we used current imaging techniques, specifically MRI and micro-CT in conjunction with classic anatomic pathology, to re-evaluate and identify lesions associated with the neck vertebrae in this disease.”

Understanding the types of lesions present in the bone can provide clues about possible disease mechanisms, Janes said.

“Secondly, given recent advances in equine genomics, we were also able to re-examine the long-standing question of the role of genetic determinants in Wobbler Syndrome,” she said.

Together these two aims hope to address current knowledge gaps in the cause and development of Wobbler Syndrome.

“My future plans include pursuing my interests in diagnostic anatomic pathology and collaborative research in an academic setting. I hope to continue to make contributions to the further understanding of musculoskeletal diseases in the horse,” Janes said.

--Shaila Sigsgaard

Antibiotics in Semen Extenders can Prevent CEM Bacteria Transmission

Researchers from the University of Kentucky Gluck Equine Research Center recently investigated if antibiotics in a semen extender can inhibit the growth of the bacteria *Tayorella equigenitalis*, the cause of contagious equine metritis (CEM), and escape detection of the bacteria in horses bred by artificial insemination.

CEM has a devastating effect on fertility; it is a very costly and serious disease in horse operations that practice natural cover. The United States has largely been considered free from CEM since 1978, when an outbreak occurred in Kentucky with significant costs to the Thoroughbred industry. Importation regulations currently apply to horses imported from countries considered endemic for the disease.

“Although there is no current threat to the U.S. horse population, it always raises concerns that imported horses may bring the disease into the U.S.,” said Mats Troedsson, DVM, PhD, Dipl. ACT, professor at the Gluck Center.

In 2006, three imported stallions in Wisconsin tested positive for the bacterium that causes CEM, *T. equigenitalis*. All stallions had tested negative prior to exportation to the U.S. and again upon arrival into the U.S. They had bred several mares resulting in normal fertility and no clinical signs of disease, Troedsson said.

In 2008, a Quarter Horse stallion tested positive for *T. equigenitalis* upon routine testing required for frozen semen to be exported to Brazil. However, no clinical observations of disease or infertility had been reported in mares bred to this stallion.

Following a subsequent national disease investigation, 22 stallions tested positive for *T. equigenitalis* resulting in 715 mares having been exposed, Troedsson said, while only five of these mares (0.7%) tested positive for *T. equigenitalis*. This is in sharp contrast to previous outbreaks in Thoroughbred populations in the U.S., France, Great Britain, etc., where natural breeding is practiced exclusively.

According to Troedsson, a possible explanation for the low CEM transmission during these outbreaks in 2006 and 2008 was that all mares were bred by artificial insemination with the semen extended in media containing antibiotics. Semen extenders with antibiotics are routinely used for artificial insemination in order to minimize a negative effect of contaminating bacteria on semen quality. However, CEM can potentially go undetected when semen is extended in media with antibiotics. If CEM becomes endemic in breeds that practice artificial insemination, it could potentially spread with devastating consequences and even enter Thoroughbreds through cross breeding using natural cover.

Therefore, the rationale for conducting the present study was the low CEM transmission in mares bred via artificial insemination in an extender containing antibiotics during these outbreaks.

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CEM, continued

“We hypothesized that the inclusion of antibiotics in semen extender prevents growth of \textit{T. equigenitalis} in extended semen and therefore reduces the risk of CEM transmission. It also makes it difficult to diagnose CEM in horses that are bred exclusively with artificial insemination,” Troedsson said. “However, the purpose of the present study was not to show that it is possible to treat semen from infected carrier stallions. That could potentially make the disease endemic in the U.S., largely because of the difficulties to diagnose CEM in mares bred to a stallion.”

The study

The study compared the growth of \textit{T. equigenitalis} in raw semen from a CEM positive stallion and the same semen extended in media containing antibiotics. The outcome in mares inseminated with raw, extended, or frozen semen from the same stallion was also assessed.

Twenty-one adult mares were randomly chosen and assigned into three different groups to be inseminated with either raw, extended, or frozen/thawed and extended CEM positive semen or insemination with CEM negative semen (control group). Additionally, the researchers cultured semen from an experimentally infected stallion to test the effect of antibiotics in extenders on the growth of \textit{T. equigenitalis}.

Upon analyzing the data, the researchers found the following:

- Commercial semen extender (EquiPro) with amikacin and penicillin contained enough antibiotics to prevent transmission of \textit{T. equigenitalis} to any of the mares in this study through artificial insemination with fresh, cooled, and frozen semen.
- Six mares inseminated with raw CEM-positive semen had clinical signs of CEM (e.g. vaginal discharge) at each sampling point after artificial insemination.
- In contrast, none of the mares inseminated with extended or frozen semen from the CEM-positive stallion or mares in the control group inseminated with raw CEM-negative semen developed clinical signs of vaginitis.

---Shaila Sigsgaard

Graduate Student Research: Anthony Claes

One of the reasons Anthony Claes, DVM, said he came to the University of Kentucky Gluck Equine Research Center for his doctoral degree in veterinary science is because the Gluck Center has an excellent reputation for conducting high-quality research. In addition to a large research herd, it has state-of-the-art facilities, equipment, and technology.

“The ability to work for distinguished Gluck Equine Research Center faculty such as Drs. (Barry) Ball (DVM, PhD, Dipl. ACT, Albert G Clay Endowed Chair in Equine Reproduction), (Mats) Troedsson (DVM, PhD, Dipl. ACT, professor at the Gluck Center and chair of the department of veterinary science), (Ed) Squires (PhD, Dipl. ACT (hon.), professor at the UK Gluck Equine Research Center) and (Karen) McDowell (PhD, EMB, associate professor) allowed me to expand my research skills in equine reproduction considerably,” Claes said.

Claes’ research is primarily focused on anti-Müllerian hormone (AMH), a hormone that is exclusively produced by the testes or ovaries and secreted in the circulation of mares and stallions. AMH plays an important role in male and female reproduction.

“Over the last three years, I examined variations in circulating AMH concentrations in intact stallions and mares of different ages and studied molecular and endocrine changes in the equine follicle during follicular development,” Claes said. “In addition to the more advanced research techniques in the laboratory, we investigate how all our experimental findings can be translated into a clinical setting. AMH has different clinical applications in stallions and mares.”

Claes said the study initially showed that AMH is a valuable diagnostic marker for cryptorchidism, a condition in which one or both testes fail to descend in the abdomen. Furthermore, concentrations of AMH in intact mature stallions are influenced by season, with higher concentrations during the physiological breeding season when the production of spermatozoa are increased.

Determination of AMH concentrations also has some clinical utility in mares. As AMH is strongly correlated with the number of follicles in the ovary, measurement of AMH can be useful to predict ovarian reserve in older mares.

Claes graduated this summer. He was subsequently hired as a visiting researcher at Utrecht University in the Netherlands.

---Sheila Sigsgaard

The Facts: Contagious Equine Metritis (CEM)

CEM is a highly contagious and sexually transmitted disease caused by the bacterium \textit{Taylorella equigenitalis}. The disease is spread through breeding or contaminated instruments and causes temporary infertility in mares, and a non-symptomatic carrier state in stallions. In addition to costs associated with infertility of the mares, infected stallions need to be treated and rigorously tested free from the disease before they can breed again. The disease can be catastrophic to the horse industry if it goes undetected. Stallions show no clinical signs, but can carry the CEM bacteria on their genitalia for months or even years. If tested positive, they need to be taken out of breeding for several weeks or even months.

---Sheila Sigsgaard
Several researchers and students from the University of Kentucky College of Agriculture, Food and Environment presented at the 66th Annual Midwestern Conference of Parasitologists (AMCOP) June 5-7. The conference was hosted by UK’s Gluck Equine Research Center.

Daniel Howe, PhD, professor and molecular parasitologist at the Gluck Center, was the program officer for the meeting. The conference topic was “Parasite adaptation and anthelmintic resistance.” Symposium keynote speakers were Craig Reinemeyer, DVM, PhD, Dipl. ACVM, president of East Tennessee Clinical Research, and Martin Nielsen, DVM, PhD, EVPC, Dipl. ACVM, assistant professor at the Gluck Center.

The conference featured oral and poster presentations, as well as a graduate student competition. Allison Young, an agriculture biotechnology major at UK and undergraduate student in Howe’s laboratory at the Gluck Center won the R.M. Cable Award for best presentation by an undergraduate student with her presentation titled, “Identification of surface antigens in the llama and alpaca parasite Sarcocystis aucheniae.”

Below is a recap of other abstract titles and corresponding UK presenters:

- “Evaluation of growth rate responses to anthelmintic regimens in young Thoroughbreds,” Jennifer Bellaw, MS candidate in Nielsen’s laboratory
- “Characterization and localization of Sarcocystis neurona Rhoptry Protein SroP9,” Maggie Schlich, undergraduate student in Howe’s laboratory
- “Vaccine-induced responses in ponies – are they modulated by anthelmintic treatment?” Emily Rubinson, MS candidate in Nielsen’s laboratory
- “Measurement of antibodies to Strongylus vulgaris in equine colic cases,” Holl Gravatte, research analyst at the Gluck Center

--Jenny Evans

**UK to host Racetrack Injury Prevention Symposium and EAV Symposium**

The University of Kentucky Gluck Equine Research Center and UK Veterinary Diagnostic Laboratory (UKVDL)—two UK Ag Equine Programs—will host a free Racetrack Injury Prevention Symposium on Monday, Oct. 20 from 1:30-5 p.m. at the UKVDL.

The Racetrack Injury Prevention Symposium will offer an in-depth look at current efforts to reduce injuries in race horses. The event is targeted toward veterinarians and anyone else with an interest in learning more. Registration is not required.

Speakers and topics include:

- The Kentucky Horse Racing Necropsy Program: "The safe international movement of stallions, trade in semen and embryos is essential to the economic well-being of the equine industry. Outbreaks of venereal diseases can have a disastrous impact on the horse industry. The symposium focuses on strategies for the control of Equine Arteritis Virus (EVA) and other infectious agents in stallions, semen, and embryos. International experts will discuss topics such as the importance of the carrier stallion and methods to eliminate EVA from the stallion; consequences of an EVA outbreak from a national and international perspective; and safety and efficacy of vaccines against EVA and the veterinarian’s role in adapting a code of practice for minimizing the spread of venereal diseases. The symposium is targeted toward veterinarians, regulatory officials, farm managers, and breed registry representatives. The symposium is partially funded by a USDA-NIFA-AFRI grant titled “Identification of genetic factors responsible for establishment of equine arteritis virus carrier state in stallions.” However, registration is required and the event costs $25. To register, visit https://eavsymposium.eventbrite.com.

--Jenny Evans
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