Equine Influenza Vaccine Efficacy in Older Horses

W e’ve all heard the statistics about an aging America. The elderly represent the fastest-growing proportion of the U.S. population. In recent years horses have experienced a similar population shift.

A large portion of the equine population (about 15%) is composed of horses older than 20 and, even at this age, many remain actively involved in equestrian sports, reproduction, or as companions. Thus, further understanding of geriatric horses’ immune systems has become increasingly important to preserve good health and quality of life through their golden years.

A goal of the immunology research program at the University of Kentucky Gluck Equine Research Center is to understand how age affects the geriatric horse’s immune response to vaccination, particularly the equine influenza vaccine.

Previous studies have shown the immune response to inactivated equine influenza virus vaccines decreases with age; however, it remains unknown whether aging has an impact on vaccine efficacy. Because alternative vaccination approaches may prove more efficacious in aged horses, a vaccine/challenge study was recently performed at the Gluck Center using a canarypox recombinant virus-vectored vaccine. This live vaccine has limited replication and therefore mimics that of a natural infection, which is expected to induce a more significant and complete stimulation of the immune response compared to killed vaccines.

ARTICLES OF INTEREST

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Upcoming Events
A recent study performed at the Gluck Center by David Horohov, PhD, William Robert Mills chair and professor; Tom Chambers, PhD, professor of veterinary virology; and Amanda Adams, PhD, postdoctoral scholar, addressed whether older horses respond to vaccination and if vaccination provides protection. The study involved 15 horses 20 years of age and older. While the vaccination history of these animals was unknown, they had probably been exposed to the vaccine and/or the equine influenza virus, because all had low-level hemagglutinin inhibition (HI) titers, a measure of antibody production, at the start of the trial. Seven of the horses were vaccinated (one dose) and eight were left unvaccinated. The study also involved 13 young horses (6 months to 1 year old) that had no serological evidence of previous exposure to the virus (naïve animals). Seven of the young horses received the same vaccine as the elderly horses (two doses, as recommended by the manufacturer) and six were left unvaccinated. The vaccine was effective at inducing both humoral (in the blood) and cell-mediated immune responses in naïve horses. By contrast, the antibody response of old horses was not as great as that of the younger animals. All of the older horses had evidence of virus-specific cell-mediated immunity prior to vaccination, and this was enhanced only slightly by the vaccine.

To determine vaccine efficacy, all horses were exposed to aerosolized equine influenza virus for 45 minutes, 14 days after the last vaccination, and were then monitored for clinical signs of infection (nasal discharge, rectal temperature, etc.). In the naïve unvaccinated group of young horses, a temperature peak typically occurred two days after challenge and persisted for five to six days (Fig. 1). This also correlated with increased transcription of pro-inflammatory cytokines in their peripheral blood cells. These animals also shed virus in their upper respiratory tracts for an average of four and a half days.

In the unvaccinated older horses, a febrile peak was also observed post-challenge, although this was not as pronounced as in the young horses (Fig. 1). This also correlated with increased pro-inflammatory cytokine expression. The unvaccinated older horses also shed the virus, but for a shorter time than the naïve young horses.

This study showed that aged horses are susceptible to infection with equine influenza virus. The vaccine was effective at protecting both the young and old horses upon challenge, reducing both clinical signs and virus shedding. Hence, vaccination of aged horses with the recombinant vaccine was effective.

Currently, the immunology group is evaluating the immune response of older horses to inactivated equine influenza vaccines compared to new generation, live-vectored vaccines. The group is also interested in determining how other factors such as nutritional status and underlying conditions (e.g., equine Cushing’s disease) might affect the immune response of geriatric horses to vaccination. The goal of this research is to identify optimal methods for protecting aged horses from infectious disease.

Amanda A. Adams, PhD, is a post-doctoral scholar at the Gluck Equine Research Center.

Figure 1. Mean (±SE) rectal temperatures (°C) of non-vaccinated (closed circle) and vaccinated (open circle) young naïve control horses (A) and old (>20 yrs) horses (B) prior to challenge (Day 50) and for 14 consecutive days post challenge with equine influenza virus.
Equine arteritis virus (EAV) and equine herpesvirus-1 (EHV-1) are the two main topics of research for Udeni Balasuriya, BVSc, MS, PhD, associate professor of virology at the Gluck Equine Research Center.

Balasuriya, who joined the Gluck Center in 2005, focuses on characterizing the molecular epidemiology and molecular basis of pathogenesis of EAV and EHV-1 infections in horses. EAV is the causative agent of equine viral arteritis (EVA), which is characterized by upper respiratory tract disease in adult horses, abortion in mares, and pneumonia in young foals. Stallions can become long-term carriers of the virus and transmit it during breeding. EHV-1 also causes upper respiratory tract disease and abortion. Some EHV-1 strains can cause neurologic disease that could lead to paralysis and even death.

“Both of these viruses pose a significant threat to the global equine industry, as there has been an increase in the number of outbreaks of EVA and the neurologic form of EHV-1 in the U.S. and around the world,” Balasuriya said. “Furthermore, there is increased global dissemination of EAV and rise in the incidence of EVA due to the rapid national and international movement of carrier stallions for breeding and competition. In 2006 and 2007, a multi-state occurrence of EVA was confirmed for the first time in Quarter Horses in the U.S. The recent outbreak of EVA in Quarter Horses increased awareness among horse owners and breeders of a disease that can have significant financial repercussions, especially for the breeding sector of the nation’s equine industry.”

Clinical signs of EVA include respiratory illness, nasal discharge, fever, limb and eye edema, skin rash, swelling of mares’ mammary glands, swelling of stallions’ genital areas, abortion, and pneumonia in young foals. EAV is transmitted by the respiratory and venereal routes. Recently, it has been shown there is a risk of EAV transmission associated with in-vivo (in the live horse) embryo transfer from a donor mare inseminated with EAV-infected semen.

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This laboratory is one of the very few laboratories in the world that is dedicated to EAV research, and our ongoing research projects are focused on the molecular characterization of the virus, viral pathogenesis, virus-host interaction, and molecular epidemiology as well as development of improved diagnostic and vaccine technologies,” Balasuriya said.

While Balasuriya would like to conduct further research on EAV, funding is limited. Balasuriya’s earlier research at the Gluck Center focused more heavily on EAV, and in recent years his work has expanded to also include EHV-1. EHV-1 is highly contagious and responsible for significant economic losses due to respiratory illness, abortion, neurologic (paralytic) disease, and death in horses. The increase in incidence of infection with neuropathogenic EHV-1 strains in recent years has given rise to considerable concern among horse industries and governmental agencies responsible for equine health around the world.

“EHV-1 continues to pose a major threat to equine health and, for the benefit of our relationship with the equine industry, it is vital that we maintain the 60-year-old tradition of conducting pioneering research on these viruses within the Department of Veterinary Science at the University of Kentucky,” Balasuriya said. “This need is even more pressing in the absence of Dr. (George) Allen, who was the driving force behind EHV-1 research at the Gluck Center for many years. There is an urgent need to continue some of Dr. Allen’s research that was in progress at the time of his death.”
"To this end, I have joined with Drs. Frank Cook and Peter Timoney (faculty members at the Gluck Center) to continue some of the equine herpesvirus research," Balasuriya continued.

Ongoing research projects on EHV-1 are focused on the following areas:

- Molecular epidemiology and evolution of EHV-1.
- Identification of virulence determinants of EHV-1 using reverse genetic technology. This involves cloning the full-length genome of a neuropathogenic EHV-1 strain into a bacterial artificial chromosome and reconstitution of infectious virus and genetic manipulation.
- Molecular mechanisms of EHV-1 pathogenesis and host immune response to the virus.
- Development of improved diagnostic assays and vaccines against EHV-1.

"EHV-1 infection is difficult to prevent because the virus can establish latent infection (essentially going into hibernation and causing no clinical signs) and protective immunity is only relatively short-lived following a clinical episode," Balasuriya said. "Therefore, horses may suffer a recrudescence of infection (virus becomes active again after a dormant period) or can become reinfected from another carrier animal once protective immunity wanes. In common with natural infections, the currently available vaccines do not stimulate long-lasting protective immune responses. The ongoing studies in our laboratory will yield important benefits for the equine industry in terms of improved diagnostics and more accurate predictions of the clinical consequences associated with detection of specific neuropathogenic and non-neuropathogenic EHV-1 strains."

In recent months, Balasuriya has received three grants to further his research on EHV-1. Those grants are for the following work:

- Comparison of *in vitro* (in the laboratory) antiviral activity of herpesvirus DNA polymerase inhibitors against neuropathogenic and non-neuropathogenic strains of equine herpesvirus-1, from the Kentucky Horse Racing Commission; and
- Molecular characterization of neurovirulent EHV-1 strains, from the Grayson-Jockey Club Research Foundation.

“I would like to thank the private organizations for funding our research projects on EHV-1. These studies will enable us to better characterize the molecular epidemiology of EHV-1 infection to gain a greater understanding of the genetic basis of emergence of neurologic herpesvirus strains and evolution of EHV-1,” Balasuriya said.

"In addition, these studies will also allow us to develop better guidelines governing the intra- and interstate movement of horses during and/or following EHV-1 disease outbreaks. Findings from these studies could eventually impact control programs designed to prevent EHV-1 infection among Thoroughbreds and other horse breeds in the U.S., as well as development of improved molecular-based diagnostic tests and more effective vaccines. Furthermore, testing of antiviral drugs with the potential to relieve suffering and prevent the spread of EHV-1 will provide the utmost benefit in terms of horse well-being and the financial health of the equine racing industry.”

Studies in his laboratory are also focused on developing improved recombinant vaccines to prevent infection of horses with EAV and EHV-1, as well as developing improved tests to diagnose other equine respiratory viruses.

Balasuriya also spends a considerable amount of time training and supervising postdoctoral fellows and visiting scientists and graduate and undergraduate students at the Gluck Center. His goal is to provide one-on-one instruction of graduate and undergraduate students and afford a multi-disciplinary-based training environment for their benefit.

“My principal goal is to provide leadership, guidance, and support to qualified undergraduate and graduate students and postdoctoral fellows undertaking viral research that utilizes state-of-the-art molecular virology techniques,” he said.

A postdoctoral scholar and three graduate students are currently working on EAV research projects, while another postdoctoral scholar and another graduate student are working on EHV-1 projects. In addition, two undergraduate students and two research staff members work in Balasuriya’s laboratory.

Jenny Blandford is the Gluck Equine Research Foundation assistant at the Gluck Center.
In the early days of education, when a college degree was a privilege and not the social norm, universities seemed unapproachable to most. With the advent of state universities came a new philosophy that knowledge should be accessible not only to students, but to all citizens. Hence, the land grant university was born.

UK was founded in 1865 as one of the nation’s first land grant universities. Land grant institutions were established by the Morrill Acts of 1862 and 1890, which allowed the federal government to appoint a parcel of land to states for sale or development to create state universities. All 50 states in the U.S. have at least one land grant university. The mission of all land grant institutions is a three-fold focus on research, education, and service. In keeping with this mission, Cooperative Extension was established to serve the community and bring knowledge from the lab to the field.

Kentucky’s Cooperative Extension Service is funded at the local, state, and federal levels and places representatives in each of Kentucky’s 120 counties to teach residents about the latest research from UK and Kentucky State University, Kentucky’s other land grant institution. Areas of information and education in each county include agriculture, home and family, youth, environment, and economic development. While there is some variation in the county and state programs, horses are a large part of the life and infrastructure in Kentucky and, therefore, a large part of extension.

Cooperative Extension provides information and programs online and in person to both adults and youth.

An online component of UK’s College of Agriculture Extension Service—eXtension—provides information on a variety of topics to a nationwide audience. UK is also the national host of HorseQuest, the equine corner of eXtension, and provides the latest research and tips to horse owners through articles, webinars, ask-the-expert sections, and a calendar of upcoming educational events related to horse ownership.

**SPOTLIGHT EQUINE**

Kentucky’s Cooperative Extension Service

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**WEED OF THE MONTH**

Common name: Goosegrass
Scientific name: *Eleusine indica* (L.) Gaertn
Life Cycle: Annual
Origin: Eurasia
Poisonous: No

Goosegrass is a warm-season grass that germinates and emerges in spring and grows throughout the summer until the first killing frost. Goosegrass usually produces a prostrate rosette-like growth that is formed from flattened stems almost parallel to the ground. The stems are white to silver in color, which is why this plant is sometimes known as silver crabgrass. Goosegrass produces a fibrous root system and does not root at the nodes. This lack of rooting at the nodes is an easy method to distinguish it from crabgrass. Goosegrass tolerates low mowing heights, heavy grazing, and drought conditions. It is frequently observed in horse paddocks and is rarely grazed by horses if other feed is available. This weed can be difficult to control in horse pastures because it is not controlled by mowing and animals graze it infrequently.

Very few herbicide products are available for goosegrass control in cool-season grass pastures; however, products are available for dormant Bermuda grass pastures. Consult your local Cooperative Extension Service personnel (www.csrees.usda.gov/Extension) for a list of herbicidal controls in your area. **UK**

William W. Witt, MS, PhD, a researcher in the University of Kentucky Plant and Soil Sciences department, provided this information.
In addition to online resources, extension provides hands-on educational activities to adults and youth. The adult education program includes Horse College, a multi-county program that runs from four to five evenings and covers topics such as nutrition, health, basic reproduction, facilities, and behavior. Talks are given in one county and residents of surrounding counties can participate. The program is now using video presentation software to project the classes to extension offices several counties away.

Robert Coleman, PhD, associate director for undergraduate education in equine science and management and extension horse specialist, heads the adult equine extension education. Coleman was one of the early innovators of the Horse College program. He said Horse College has now traveled to 97 of Kentucky’s 120 counties, educating more than 1,500 adults. Horse Colleges are organized largely by each county’s extension agent and taught by university faculty and field experts such as veterinarians and farriers.

“The concept came out of a discussion I had with several agents,” Coleman said. “We realized that if you do this for multiple nights, you’ll develop a relationship with your clientele … it has facilitated not only networking between the agents and the horse owners but between horse owners in a community.”

Coleman said backyard horse owners can also participate in Pastures Please, a grazing school that discusses topics related to local pasture establishment, weed control, and species selection.

Youth participation in extension has always been linked to horses through the 4-H program. The four H’s, which stand for head, hands, heart, and health, reflect the program’s mission to teach and develop well-rounded youth ages 9 to 19. In addition to other agricultural interests, the Kentucky 4-H Youth Program has an equine component that has approximately 6,000 participants each year. The 4-H program teaches the basics of being a horse owner and encourages participants to expand their horsemanship and leadership skills. Participants can partake in educational activities and competitions and might also ride in various disciplines at 4-H horse shows.

Summer is a busy time for the 4-H Horse Program because children are out of school. Upcoming events include the State 4-H Horse

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**Use Caution Bedding Horses on Fescue**

 Tight budgets have caused several Central Kentucky horse farm managers to reduce straw bedding costs by harvesting overmature grass pastures and using the resulting stemmy hay as bedding. On the surface this practice might seem cost-efficient, but horse owners and farm managers should be cautious when using this bedding for pregnant mares during their last trimester, as it can significantly impact the mare’s reproductive cycle if ingested. It is not uncommon for horses to eat some of their bedding, especially if it’s hay (even overmature hay). If the harvested fields contain significant amounts of endophyte-infected tall fescue in the seedhead stage, then the bedding will likely contain toxic levels of ergovaline, a dopaminergic agonist (a drug that stimulates dopamine receptors). Tall fescue at the seedhead stage has the highest levels of toxins, often three to five times higher than fescue at the leafy stage. Additionally, these toxins (i.e., ergot alkaloids) will remain in hay as long as it is stored.

Ray Smith, MS, PhD, forage extension specialist in the University of Kentucky Plant and Soil Sciences department, provided this information.
Judging Contest, State Horse Show, and “Horse Contest,” which showcases photography, crafts, and communications that all revolve around the horse. Another popular event is the horse bowl, a quiz-style competition that tests participants’ knowledge of horse science.

Fernanda Camargo, DVM, PhD, assistant professor in the department of animal and food sciences and equine extension specialist, said boosting public awareness of equine research at UK can help participating youth become better horse owners.

“Research is generally published in scientific journals, which are not available to the general public. It is my role to learn what has been discovered by researchers and pass this knowledge along to horse owners and enthusiasts,” Camargo said.

Kristen Harvey, MS, extension associate, believes the 4-H program in particular has another positive attribute.

“The most valuable element of equine extension in terms of 4-H is providing the leaders with valuable educational resources to help our youth grow and learn,” Harvey said.

Jimmy Henning, PhD, associate dean for Extension and Cooperative Extension Service associate director, believes Cooperative Extension provides guidance to the university, in addition to its benefits to the public.

“The value or importance lies in the nature of Cooperative Extension, to provide a conduit for information and educational programming to move from campus to the communities, and for

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**TOXIN TOPIC**

**RED MAPLE TOXICOSIS**

Red maples (*Acer rubrum*) are beautiful trees that grow extensively throughout eastern North America. However, horse owners should be aware that these trees can pose a serious health risk to horses. Ingestion of wilted or dried maples leaves can cause damage to red blood cells and potentially death in horses (and possibly camelids such as alpacas and llamas).

The red maple tree is a tall hardwood with green leaves that have three large “fingers” or points and five prominent veins in the leaves. The leaves turn a brilliant crimson and sometimes yellow in the fall.

Poisoning typically occurs in late summer or fall or after storms cause tree limbs to fall within reach of horses. Other maple species can be toxic to horses, but most documented cases involve red maple exposure. The toxin responsible has not been identified but is apparently only found in dried or wilted maple leaves, not in fresh growing leaves.

Clinical signs in poisoned horses include depression, poor appetite, yellow or brown color to gums and membranes, dark red or brown urine, colic, and fast heart and respiratory rates. Abortion, sudden death, and kidney failure might also occur. Signs typically present within 12-48 hours of ingestion of leaves, and death can occur within days. If ingestion of dried or wilted maples leaves is witnessed, treatment (i.e., intravenous fluid therapy, blood transfusion, administration of activated charcoal) should be instituted and is effective in many cases. The mortality rate can be high and is dependent upon the dosage ingested, among other factors. As little as 1.5 grams of wilted or dried leaves per kilogram of body weight can be fatal (roughly 1 pound of dried leaves in an adult horse). Post-mortem diagnosis usually is made by finding large amounts of maple leaves in the stomach and intestinal contents.

Prevention is key: Do not plant maples trees near horse pastures or barns, and remove any maples that are already present; do not incorporate maple leaves into bales of hay; and remove fallen maple leaves and downed branches from pastures after storms.

Cynthia Gaskill, DVM, PhD, clinical veterinary toxicologist at the University of Kentucky Livestock Disease Diagnostic Center, provided this information.
UK to Host International Symposium on Equine Reproduction

Equine reproduction specialists from around the world will convene in Lexington July 26-30 for the 10th International Symposium on Equine Reproduction (ISER X). The University of Kentucky will host the prestigious invitation-only meeting, which is held every four years.

“This meeting has a history of providing some of the most cutting-edge research results in equine reproduction. It’s generally at this meeting that the latest information on equine reproduction is presented, which eventually results in new techniques being used in the industry in mares and stallions,” said Ed Squires, international chairman of the International Equine Reproduction Symposium Committee (IERSC), the organizing body of ISER. Squires is also the executive director of the UK Gluck Equine Research Foundation and the director of advancement and industry relations at the Gluck Center.

The first ISER meeting was in Cambridge, United Kingdom, in July 1974. The purpose of the meeting was to provide a forum for biologists and veterinarians interested in equine reproduction to exchange and argue their views, to review the current state of knowledge of the subject, to produce guidelines for future research, and to foster international friendship and collaboration.

Today, the meeting continues to bring together scientists and veterinarians from around the world and it provides a forum for exchanging information on clinical and basic research aspects of equine reproduction. The meeting covers experimental or clinical research in four areas: the non-pregnant mare, stallions, conception and early development, and the pregnant mare and perinatology.

The symposium will feature 164 short communications displayed via poster presentation.
More than 250 farm owners, managers, and Kentucky residents attended the Equine Farm and Facilities Expo hosted by the University of Kentucky Equine Initiative June 1 at Spy Coast Farm in Lexington.

The expo gave attendees the opportunity to view a wide range of farm equipment and supplies appropriate for small- to mid-sized operations. UK specialists provided hands-on instruction for spraying, seeding, fencing, and mowing pastures. Featured demonstrations included weed control basics, mowing and dragging small pastures, seeding and overseeding pastures, and fencing and water systems for rotational grazing. Twenty-two equipment companies also participated.

“The successful Equine Expo showed that horse farm owners in Central Kentucky have a strong interest in improving their pastures,” said Ray Smith, MS, PhD, associate professor and forage extension specialist at the University of Kentucky.

According to Smith, participants were given practical tips on a range of topics including overseeding pastures, using ATV-mounted sprayer systems, setting up temporary fences, and when and how to mow and drag a field to promote new growth.

Farm owners and managers were also able to see and try out equipment that best suited their operations.

“The event was well-attended, and everyone appreciated the vendors and what they brought for people to see and learn about. The support from Spy Coast Farm for allowing the event to be held on their property was also greatly appreciated,” said Robert Coleman, PhD, associate director for undergraduate education in equine science and management and extension horse specialist.


Alexandra Harper is an equine communications intern and a recent graduate in communication.

Jenny Blandford is the Gluck Equine Research Foundation Assistant at the Gluck Center.
Thoroughbred Farm Participation Needed for Lawsonia Intracellularis Study

The University of Kentucky’s Gluck Equine Research Center needs Lexington-area Thoroughbred farms to participate in a study to determine Thoroughbred weanlings’ seroprevalence (presence of positive serum antibodies) to Lawsonia intracellularis, the causative agent of equine proliferative enteropathy (EPE).

Allen Page, DVM, a doctoral student in the Gluck Center laboratory of David Horohov, PhD, William Robert Mills Chair, is conducting the research. Page hopes to include at least 35 farms and 350 weanlings in the study, which is funded by the Equine Drug Research Council of the Kentucky Horse Racing Commission. Thoroughbred farms within 25 miles of Lexington that have had or not had cases of EPE are encouraged to participate.

“The seroprevalence data will provide us with information about the overall exposure of horses to L. intracellularis,” Page said.

Goals of the study are to determine the seroprevalence of antibodies to L. intracellularis in Thoroughbred weanlings; determine the risk factors for exposure to L. intracellularis; determine the effect, if any, of exposure to L. intracellularis on yearling public sales price; and determine the effect, if any, of exposure to L. intracellularis on racing records.

The study will be conducted from August 2010 to January 2011. Study results will be published in a scientific journal. Farms participating will remain anonymous.

Farms interested in participating should contact Page at 859/489-4144 or a.page@uky.edu by July 5.

Jenny Blandford is the Gluck Equine Research Foundation Assistant at the Gluck Center.

UPCOMING EVENTS

July 4-10, State 4-H Horse Show, Kentucky Fair and Expo Center, Louisville.

July 22, 4 p.m., Department of Veterinary Science Equine Diagnostic Research Seminar series at the Kentucky Horse Park. University of Kentucky Veterinary Science Department’s Alan Loynachan, DVM, PhD, Dipl. ACVP, anatomic veterinary diagnostic pathologist and assistant professor at the Livestock Disease Diagnostic Center; Allen Page, DVM, PhD; and Uneeda Bryant, DVM, PhD, assistant professor at the Livestock Disease Diagnostic Center, will speak about the bacterium Lawsonia intracellularis.

July 25-27, National Association of Equine Affiliated Academics (NAEAA) meeting, Cazenovia, N.Y.


July 26-30, 10th International Symposium on Equine Reproduction, UK campus (College of Agriculture and residence halls), Lexington.

July 31, 8 a.m.-noon, International Symposium on Equine Reproduction, Farm Managers Day, UK campus. RSVP is recommended at info@iser2010.com.

Aug. 7, Rood & Riddle Equine Hospital Hats Off Day at the Kentucky Horse Park.
Parasites compete with your horse for nutrition. Left unchecked, they can impair condition, performance and even cause colic. The solution? Deworm daily with STRONGID® C 2X (pyrantel tartrate), which doesn’t allow parasites to get a foothold and can make a visible difference in your horse. To learn more, visit StrongidC2X.com.

Always consult your veterinarian before starting any parasite program.