EQUINE DISEASE QUARTERLY

FUNDED BY UNDERWRITERS AT LLOYD'S, LONDON, BROKERS AND THEIR KENTUCKY AGENTS

OCTOBER 2011
Volume 20, Number 4

COMMENTARY

IN THIS ISSUE

UK
UNIVERSITY OF KENTUCKY
College of Agriculture
Department of Veterinary Science

LLOYD'S

Stem Cells and Regenerative Medicine—Pay Attention

AS A VETERINARIAN and cell biologist trained during the 1980s, I have witnessed firsthand the transformative impact that molecular biology has had on biomedical research, agriculture, and medicine. Characterizing molecular biology as "transformative" is appropriate. Consider how recombinant DNA methods, transgenics, and now genomics have impacted discovery science, farming practices, the pharmaceutical industry, and clinical diagnostics. The process is not winding down—actually, just the opposite. We are entering an era of personalized medicine. Along with medical history, a clinical exam, and diagnostic tests, the evaluation and care of patients will increasingly include consideration of an individual's genome. Factoring in of the genome is already the case for known genetic markers with established disease associations. The full resequencing of a patient's DNA (genome), an overall evaluation of gene expression in clinical samples at the RNA or protein levels, and broad screening of metabolites all appear to be coming to clinical practice in the not-too-distant future. Veterinarians and physicians will use this information to help generate the differential diagnoses list for a patient, customize therapeutic strategies, and improve prognostic assessments.

In contrast to molecular biology, I have more recently recognized that cell biology also will likely have a transformative impact on the practice of medicine in a similar, not-too-distant time frame. Data are now accumulating from several medical disciplines that multipotent "stem cells" have the potential to facilitate tissue repair. I am not a stem cell expert by any stretch, but two broad mechanistic categories appear to be emerging.

The first category includes indirect mechanisms. Stem cells introduced into a patient have the potential to express and deliver the right mix of factors (cytokines, chemokines, growth factors, etc.) at the correct concentrations and with the appropriate molecular structure to positively modulate the patient's immune response and tissue repair capabilities. The patient's own cellular resources for repair are mobilized and activated. Destructive catabolic (tissue breakdown) processes are switched to a self-sustaining anabolic (tissue growth) response. Interestingly, the introduced stem cells may not need to remain viable in the lesion area for very long to affect this switch.

The second category appears to be more challenging. It involves direct mechanisms in which the introduced stem cells themselves are expected to differentiate into the correct cell types, synthesize and organize the appropriate extracellular matrices, and regenerate normal tissue structures to achieve tissue repair. Both mechanisms work toward a restoration of tissue structure and function.

The take-home message that I now embrace and will encourage others to consider is "pay attention." Stem cells and regenerative medicine are poised to yield major clinical advances. As discussed by Dr. Ferraro in this issue, many basic questions remain unanswered, and there is much to learn. Our discovery process needs to be driven by rigorous scientific methods and hypothesis-driven experimental biology. However, through access to our diverse patient population, veterinary medicine has every opportunity to lead development and progress in these important areas. Yes—pay attention.

CONTACT: Dr. James MacLeod, (859) 218-1140, jnmacleod@uky.edu, Maxwell H. Gluck Equine Research Center, University of Kentucky, Lexington, Kentucky.

INTERNATIONAL

Second Quarter 2011*

THE INTERNATIONAL COLLATING Center, Newmarket, United Kingdom, and other sources reported the following disease outbreaks.

Contagious equine metritis (CEM) was reported from Germany, South Africa, and Sweden. The causal agent of CEM was detected in one mare in Germany from embryo flush fluid. The mare was inseminated with semen from a carrier stallion imported previously into South Africa. Sweden reported one case in a Trotter mare inseminated with semen from an imported stallion.

Italy reported outbreaks of dourine *(Trypanosoma equiperdum)* on several premises. The occurrence was linked to the importation of a mare from Holland in September 2010. Mild signs of dourine were described in 10 horses on five premises; two horses were euthanized. Two stallions were confirmed serologically positive. Six more serologically positive cases on another premises near Naples were linked to the index premises.

A single case of equine coital exanthema (equine herpesvirus-3) was reported from the UK.

Equine influenza was diagnosed in three Draft foals at a premises in France on which eight other foals had died, presumably but not stated, from influenza. Cases were also confirmed in adult horses. Ireland, the UK, and the USA recorded isolated cases.

Strangles was reported in Germany (five cases); South Africa experienced ongoing outbreaks. An outbreak involving six horses was confirmed in Sweden.

Numerous outbreaks of equine herpesvirus-1 (EHV-1) were reported. France recorded one case of respiratory disease. Abortions were reported from Argentina (2), France (4), Germany (1), Ireland (5) South Africa (1), Turkey (21), and the UK (6). EHV-1 neurologic disease was confirmed in France (a single case on one premise, several cases on another) and Ireland (three cases involving two premises). EHV-4 was responsible for one mare having abortion and respiratory disease in Germany.

France confirmed isolated equine viral arteritis (EVA) cases on four premises as well as an outbreak later. EVA cases were also confirmed in Germany and Sweden. Ireland reported several seropositive mares and stallions, none with clinical evidence of EVA and some with histories of vaccination.

Equine infectious anemia was reported from Germany (three cases including one clinical case imported from Romania) and Japan (single case).

Reports of equine piroplasmosis were received from France (endemic), Ireland (case of congenitally acquired *Babesia caballi* infection in a neonatal foal out of an imported mare), South Africa (endemic), Switzerland (endemic), and the United Arab Emirates (endemic). In the USA, two *Theileria equi* seropositive Quarter Horses were identified in Texas, both euthanized. An imported horse in Florida was confirmed positive and was exported; two *T. equi* seropositive horses, one in North Carolina and one in Tennessee, are under state quarantine. The majority of positive cases of infection are Quarter Horse racehorses. With the exception of the extensive outbreak of equine piroplasmosis on a large cattle ranch in southeast Texas first detected in November 2009, there has been no evidence of tick transmission in other states.

South Africa reported more than 100 cases of equine encephalosis on numerous premises involving all types of horses. Clinical features of the disease were mild.

One case of Eastern equine encephalomyelitis was diagnosed in a vaccinated Thoroughbred yearling in Florida, USA. Four cases of West Nile encephalitis were reported in the USA and several cases in Greece.

Increased cases of neurologic disease were reported from Australia: New South Wales, Queensland, Victoria, and South Australia. Initial reports suggest association with Kunjin virus or Murray Valley encephalitis virus. Victoria reported evidence of Ross River virus infection associated with a musculoskeletal syndrome.

Four outbreaks of salmonellosis involving 24 horses and five outbreaks (10 cases) of rotaviral enteritis were reported from Ireland. The USA reported foal outbreaks of clostridial enteritis.

Equine grass sickness was confirmed in Switzerland: five cases in non-Thoroughbreds on three premises.

Two separate non-Thoroughbred cases of mild anaplasmosis due to *Anaplasma phagocytophilum* were reported from Switzerland.

**First Quarter Report for Australia*

Equine Disease Quarterly

Editors
Roberta Dwyer
Peter Timoney
Neil Williams

Staff
Diane Furry
Martha Jackson
Linda Millercox

Correspondence should be addressed to the editors, Department of Veterinary Science, Maxwell H. Gluck Equine Research Center, University of Kentucky, Lexington, Kentucky USA, 40546-0099
Telephone (859) 257-4757
Fax (859) 257-8542

Internet address:
http://www.ca.uky.edu/gluck/index.htm

Hendra Virus: An Unprecedented Outbreak in Australia

HENDRA VIRUS IS A POTENTIALLY FATAL zoonotic virus that is transmitted from horses to humans. Together with the closely related Nipah virus, a cause of respiratory disease and encephalitis in pigs and humans, these two viruses form the genus Henipavirus in the family Paramyxyoviridae.

First described following an outbreak of acute, fulminant respiratory disease in horses in 1994 in a north Brisbane suburb, Hendra virus has since been found to naturally infect a variety of Pteropid bat species native to Australia. Since 1994 there have been 26 reported incidents of Hendra infection affecting 63 horses, one dog, and seven people. Of these 26 incidents, 12 have occurred in 2011. No reports of bat-to-human transmission of this virus have been made. All seven cases of Hendra in humans have, to date, been associated with equine infection. The case-fatality rate of human Hendra virus infection is 57%. Horses testing positive for Hendra virus are euthanized, according to national guidelines.

Although the host bat species are native to large regions of coastal Australia, equine and human cases of Hendra virus infection have been reported only in the north of New South Wales and in Queensland (Figure 1). Serological evidence of Hendra infection has been demonstrated in all four species of fructivorous bats found on the Australian mainland; this evidence of seroconversion is not geographically restricted to the areas where equine cases have been reported. Bats with antibodies to Hendra have been identified from Melbourne in the south to the far north of Queensland. Seroprevalence increases with age.

Hendra virus infection of horses is a very problematic disease for equine clinicians. Cases present with a variety of clinical signs, which poses serious human safety concerns. The first large outbreak of Hendra virus infection in 1994 was associated with severe respiratory distress and sudden death that occurred in the majority of affected horses within 36 hours of the onset of clinical signs. Hendra virus has a definite tropism for the vascular endothelium, particularly the arterial endothelium, which explains many of the post-mortem findings, such as foam-filled airways, dilated pulmonary lymphatics, severe pulmonary oedema, and congestion.

However, in 2008 a second large outbreak of Hendra virus infection occurred in a different suburb of Brisbane, involving five horses in residence at a veterinary clinic. The presenting signs in this outbreak were predominantly neurological and included depression, anorexia, and ataxia with rapid deterioration resulting in euthanasia. These clinical signs were not consistent with previous Hendra cases. Both of these outbreaks were associated with human infections and deaths.

Currently no vaccine is commercially available for humans or horses. Recently, vaccine trials at the Australian animal health laboratories in Geelong (Victoria) have demonstrated that vaccination with a soluble Hendra virus G protein protected horses against virus challenge. It is hoped that this vaccine will become available for wider use in 2012.

Quantitative reverse transcriptase polymerase chain reaction (RT-PCR) assays have facilitated the provision of rapid diagnostic results, minimizing potential exposure of the laboratory worker to infectious material. Serological assays to detect Hendra antibodies, including rapid enzyme-linked immunosorbant assay (ELISA)-based serological screening tests, are available, but specificity is not high. Any positive or suspect positive serum samples are then sent to a high security, BSL-4 level laboratory for testing by virus neutralization assay.

Currently, public health authorities in Australia are dealing with an unprecedented number and distribution of Hendra cases. In 2011 there have been 15 equine cases of Hendra on 12 premises. These cases have occurred farther south and west than had been reported previously. Also, the first canine case of Hendra infection has been reported, which is the first report of the infection in a domestic species other than horses. The dog is seropositive, but has not, to date, shown any clinical signs of disease and no virus has been detected. The reasons for the dramatic increase in incidence are not known.

Ongoing virological monitoring of bat populations in eastern Australia has shown an increase in the amount of virus shed by the bats. This outbreak has focused attention on the immensity of what is unknown about Hendra and has stimulated a significant increase in research into this serious disease of horses and humans.

**CONTACT: Dr. J. Gilkerson,
61 3 8344 9969, jrgilk@unimelb.edu.au,
Director, Equine Infectious Disease Laboratory,
The University of Melbourne, Victoria, Australia.**

NATIONAL

Equine Borreliosis (Lyme Disease)

LYME DISEASE IS THE MOST COMMON human disease transmitted by anthropods in North America. *Ixodes* sp. ticks, the vector of Lyme disease, are being described in areas where they have not been reported before. States in the Northeast, mid-Atlantic, and the Great Lakes region have the highest number of reported human Lyme disease cases. Horses in these areas are at high risk of exposure to the Lyme organism, *Borrelia burgdorferi*.

Any outdoor horses are at risk due to increased exposure to ticks and the aggressive feeding of the *Ixodes* ticks. The small ticks are difficult to see in the hair coat. It is thought that ticks must be attached 12 to 24 hours before humans are infected, as is likely to be the case in horses.

Clinical signs associated with equine borreliosis are variable and include shifting leg lameness, myalgia, dermal hypersensitivity, behavior changes, weight loss, uveitis, and neurological signs.

B. burgdorferi diagnostics include culture, direct microscopic visualization, and polymerase chain reaction (PCR), but the most often used diagnostics are serologic tests such as immunofluorescence assay (IFA), Western blot (WB), and enzyme-linked immunosorbent assay (ELISA). The new multiplex bead-based Lyme test combines testing by ELISA and WB proteins in a single, quantitative, bead-based assay.

Antibody-based diagnostics makes diagnosis difficult, since it is thought that over 60 to 70% of horses in endemic areas may be seropositive. Often owners request Lyme testing for "baselines," pre-purchase examinations, assistance in determining causes of poor performance, etc. In the equine borreliosis seropositive horse, it is estimated that a very low percentage actually develop or have signs that may be associated with Lyme disease.

The accepted treatment for equine borreliosis is the use of a member of the tetracycline family of drugs. Response to treatment has been seen by practitioners and owners as a confirmation of a Lyme disease diagnosis, but this response must be cautiously interpreted due to anti-inflammatory effects of tetracycline drugs. It is also difficult to determine response to treatment with serological testing, because *B. burgdorferi* antibody levels are known to persist for years in humans and are also apparently long-lived in horses. Retesting is suggested four to six months after treatment to see if there is a decrease in serological values. This retesting can be questionable in determining response to treatment because horses are likely to be exposed and possibly re-infected post-treatment if living in areas of high tick density. The new veterinary fluorescent bead-based multiplex test, however, may assist in determining response to treatment.

Horse owners should attempt to prevent exposure to ticks by using insect repellants and removing brush and dense undercover, which provide habitats for host mammals infected with ticks. Attention to grooming also may help prevent tick attachment.

Further studies are needed on the effective diagnosis, treatment, immunity, and prevention of equine borreliosis.

**CONTACT: Dr. Linda Mittel,
(607) 253-3633, ldm65@cornell.edu,
Animal Health Diagnostic Center,
Cornell University, Ithaca, New York.**

Updating Equine Influenza

EQUINE INFLUENZA LAST MADE HEADlines in 2007 with the Australia epizootic that affected approximately 50,000 horses. Since eradicated from Australia, equine flu viruses still circulate in much of the world, including the USA. Antigenic drift, which produces new virus strains and gradually undermines the effectiveness of vaccines, necessitates periodic vaccine updating to combat the new virus strains.

The vaccine manufacturers look to scientists to advise them on which vaccine virus strains need to be replaced and with what. In 1995 an ad hoc working group of equine flu scientists was founded for just this purpose. Called the Expert Surveillance Panel, this group includes scientists from the OIE (World Organization for Animal Health) reference laboratories for equine influenza in England, Ireland, Germany, and the USA; other labs specializing in equine flu virus; and the World Health Organization.

Each year the panel assembles and reviews the evidence of equine flu activity worldwide, looking especially for cases of infection in vaccinated horses. It also reviews the data comparing flu strains isolated from the past year's outbreaks with flu strains used in vaccines. The critical piece of evidence is how well the antibodies stimulated by vaccination will react with the circulating flu strain in the exposed horse. A new technique called "antigenic cartography" has been developed by researchers at Cambridge University that makes these analyses easier. Fortunately, in most years the Expert Surveillance Panel reports that the equine flu vaccines are still working effectively. However, constant surveillance is critical.

Scientists now recognize three surviving branches of the equine flu "family tree," one of which currently circulates in the USA: the Florida clade 1 branch typified by strains such as Ohio/03. The Florida clade 2 branch constitutes the majority of recent isolates from Europe and is typified by strains such as Richmond/07. Some older American strains like Kentucky/97 are antigenically similar to Richmond/07. The branch called the "Eurasian lineage" circulated mainly from 1990 to 2005.

Since many horses travel internationally, the latest recommendation is that equine flu vaccines should contain strains of both the clade 1 and clade 2 branches. The panel has stopped recommending the Eurasian branch. The original equine flu branch, the A1 subtype represented by Prague/56, has apparently died out.

It is vitally important to the process of updating vaccines that outbreaks of equine flu are properly diagnosed and virus specimens are collected, which is accomplished by taking nasal swabs from affected horses and submitting them to veterinary diagnostic laboratories. From these swabs, virus strains can be isolated and compared for their antigenic drift and potentially used to make the next vaccine strain. Without virus isolates, the whole process of vaccine updating will break down from lack of information, putting even vaccinated horses at greater risk for equine flu.

Information on collecting and submitting nasal swabs is at www.ca.uky.edu/gluck/ServFlu.asp

The 2011 report of the Expert Surveillance Panel is online at OIE Bulletin, issue 2011 #2: www.oie.int/fileadmin/Home/eng/Publications_%26_Documentation/docs/pdf/Bull_2011-2-ENG.pdf

CONTACT: Dr. Thomas Chambers, (859) 218-1126, tmcham1@uky.edu, Maxwell H. Gluck Equine Research Center, University of Kentucky, Lexington, Kentucky.

Potential Impacts of Regenerative Medicine

THE USE OF THE STEM CELL AS REGENerative medicine therapeutics in the treatment of equine orthopedic injuries has recently received increased attention. Stories of successful "cures" have precipitated a high level of public expectation regarding the efficacy of stem cell therapy in both animals and humans. This expectation has escalated the demand for the use of various regenerative medical products by veterinarians.

While most veterinary scientists and clinicians with experience in regenerative medicine agree that there is ample anecdotal evidence to indicate that stem cell therapy has efficacy in the treatment of equine orthopedic injuries, most would also agree that further basic research and controlled clinical trials are necessary. At the recent North American Veterinary Regenerative Medicine Association conference, scientists and clinicians presented ample evidence for the *in vitro* (in the laboratory) actions and capabilities of stem cells. Demonstrations of their potential capability for tissue regeneration alone and in combination with various growth factors and/or scaffolding materials were quite impressive. These discussions left no doubt in the minds of presenting researchers and attendees of the future potential for biological medicine.

While scientists are able to accomplish rather amazing feats of tissue repair and/or regeneration under laboratory conditions, they have been able to affect or even control those desired actions within the body for relatively few disease conditions. The best dose regimen and route of administration are

(Regenerative Medicine continued from page 5)

questions that have yet to be fully resolved. Controversies exist over how "true stem cells" are identified and counted in animals, which source of cells is best for specific conditions, and which expansion, cryopreservation, and reconstitution techniques are optimal.

Other unanswered questions concern the relative practicality and value added for the clinical application of allogeneic (non-self) over autologous (self-derived) sourced cells. Currently, while autologous cells are most often employed clinically, their use dictates a delay of a few days to a few weeks for cells to be prepared for injection, especially if cell expansion is requested. This extra time often complicates or precludes the treatment of patients with acute injuries.

Since it has been demonstrated that stem cells are not targeted by the body for rejection, the use of pre-prepared and ready-to-use donor cells would seem optimal. In fact, this type of therapy has already been successfully employed in numerous experimental cases. However, several serious scientific and regulatory hurdles need to be overcome before any allogeneic product could be brought to market. These products would be considered drugs by the Food and Drug Administration, so both safety and efficacy trials would first have to be completed. Such trials are expensive and time consuming; therefore, the commercial availability of such products is a long way off.

Regardless of the regulatory and scientific hurdles, the equine industry is beginning to see a myriad of "stem cell" and regenerative medicine products and services brought to market and heavily advertised. While some of these products and services may have legitimate claims to efficacy, others may be questionable. Veterinary practitioners should educate themselves on the basics of stem cell biology and clinical applications so wise therapeutic choices can be made.

CONTACT: Dr. Gregory Ferraro, (530) 752-6433, glferraro@ucdavis.edu, Director, Center for Equine Health, UC Davis, School of Veterinary Medicine, Davis, California.

Equine Disease Quarterly Newsletter

Department of Veterinary Science
Maxwell H. Gluck Equine Research Center
University of Kentucky
Lexington, Kentucky 40546-0099

Address Service Requested

FIGURE 1. **Australia**