EQUINE DISEASE QUARTERLY

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COMMENTARY

IN THIS ISSUE

We no longer listen to music on records or tapes. When we write, we "keyboard," using some form of word processor. Our lives have changed profoundly as a consequence of technology. Therefore, it should be no surprise that changes of comparable or even greater magnitude are occurring in the realm of biology.

Before 2000, the two most significant contributions of veterinary genetics to horses were the discovery of the cause of hemolytic disease of newborn foals (neonatal isoerythrolysis) and the invention of parentage testing. Beyond that, genetic principles simply provided a basis for understanding hereditary diseases. From a practical standpoint, genetics was a black box.

The Human Genome Project changed that.

This $3 billion project led to invention of methods and tools that allowed us to inexpensively sequence genomes of other animals, including horses. The horse genome sequence was completed in 2006 and is routinely used to discover the genetic basis for many hereditary diseases, performance traits, and coat color patterns.

However, molecular genetics is not just about hereditary diseases. Today, the DNA sequence of every economically important animal has been determined. Scientists studying muscle biology, reproduction, infectious diseases, immunology, and pharmacology routinely use DNA sequences to investigate targets for treatment and responses to intervention. Furthermore, the genome sequence is a financial boon to research. Scientists studying horses used to spend months, and tens of thousands of dollars, cloning and sequencing genes. Now an undergraduate student can identify the DNA sequence of any gene in 15 minutes on the computer, for free.

Unfortunately, the horse genome sequence by itself is inadequate to address many important questions. However it is a great foundation for new tools. We can use the horse genome sequence as a basis to investigate gene expression. We can determine what train of events is initiated when we vaccinate a horse, when a horse eats a particular diet, or when a horse experiences an infection. Each of these activities triggers changes in gene expression. If we could identify the set of genes expressed in each tissue under different consequences we would have a powerful way to design therapeutic treatments. This is the kind of information that will help us understand complex metabolic diseases and difficulties in engendering protective immunity to infectious diseases. Scientists are already trying to determine this kind of information, but the magnitude of the problem and the paucity of resources combine to thwart real progress.

A central repository is needed for gene expression information for the horse. The United States Department of Agriculture is encouraging scientists to collaborate in developing these resources for all agriculturally important species. ENCODE is the name of a similar resource developed for human medical research. Consequently, the effort for animals has been called AG-ENCODE. The potential to benefit equine veterinary medicine is huge. We tend to fund research to solve specific problems and poorly support development of research infrastructure. However, developing Equine-ENCODE would not be an answer to a single, specific question but could be key in solving many problems.

CONTACT
Dr. Ernie Bailey
(859) 218-1105
ebailey@email.uky.edu
Maxwell H. Gluck Equine Research Center
University of Kentucky
Lexington, Kentucky

UK UNIVERSITY OF KENTUCKY
College of Agriculture, Food and Environment
Department of Veterinary Science

LLOYD'S

INTERNATIONAL

Third Quarter 2014*

The International Collating Center, Newmarket, United Kingdom, and other sources reported the following disease outbreaks.

Outbreaks of vesicular stomatitis continued to be confirmed in the USA, with the disease diagnosed on 365 premises: 310 in Colorado and 55 in Texas. All cases of infection were due to the New Jersey serotype of the virus. Of the 365 premises, 350 were equine, 13 were bovine, and two involved both equines and bovines. Some 430 virus-infected equines have been reported in Colorado and 78 in Texas.

Outbreaks of strangles were reported by France (12 premises), Germany (four premises), Switzerland (one premises), and the USA (27 premises). In the USA, 59 cases were diagnosed in 14 states; other countries reported isolated cases of the disease.

Ireland, the UK, and the USA confirmed outbreaks of influenza. The disease was diagnosed in non-vaccinated Thoroughbreds in two counties in Ireland. Eight outbreaks were reported by the UK, the vast majority involving isolated cases in non-vaccinated horses and ponies. The USA recorded two outbreaks in Ohio and one in Kentucky.

Equine herpesvirus (EHV)-1 and -4 related diseases were recorded in Argentina, France, Germany, Ireland, the UK, and the USA. Respiratory disease caused by EHV-4 was confirmed in France (three outbreaks), Germany (nine horses), Ireland (nine cases involving two premises), and the UK (isolated cases on nine premises). Abortions due to EHV-1 were diagnosed in Argentina (five outbreaks), and Germany (one case). France reported a single case of EHV-4 abortion in a Thoroughbred mare. Single cases of EHV-1 associated neurologic disease were recorded in France, Switzerland, and the UK.

A few cases of EHV-2 and -5 infections were reported in the USA.

Canada and the USA recorded cases of equine infectious anemia (EIA). A total of 20 cases of the viral infection were confirmed on eight premises in Saskatchewan, Canada, some of which also had cases within the past few years. The USA reported at least 20 cases in California involving Quarter Horse racehorses, the majority illegally smuggled into the country and most engaged in non-sanctioned racing.

Equine piroplasmosis was recorded as endemic in France, Spain, Switzerland, and the United Arab Emirates. The USA reported 11 cases of dual piroplasmosis and EIA infections in California and a number of additional cases in Texas and Florida all in high-risk animals, specifically Quarter Horse racehorses engaged in non-sanctioned racing.

Germany confirmed contagious equine metritis in three stallions and one mare, all non-Thoroughbreds.

Salmonella abortus equi infection was reported in a group of five non-Thoroughbreds on a premises in Singapore. The animals were international performance horses.

The USA recorded an unspecified number of cases of salmonellosis caused by strains belonging to Group B serogroup.

An isolated case of rotavirus infection was reported by France. Switzerland confirmed one case of equine monocytic ehrlichiosis.

During the third quarter of 2014, a total of 114 cases of Eastern equine encephalomyelitis were diagnosed in the USA. Cases were recorded in 16 states with the vast majority (58) confirmed in Florida.

West Nile virus encephalitis was reported by Italy (two cases caused by lineage 2 virus), Turkey (one case), and the USA where 32 states reported 66 cases. California, Missouri, Oklahoma, and Texas had the most positives.

A single case of Hendra virus infection was confirmed in an aged non-vaccinated Thoroughbred in Queensland.

An outbreak of Getah virus infection was recorded at a training center in Japan. Clinical signs were observed in 22 horses of which slightly over 50% had been vaccinated against the disease.

Rhodococcal related disease was reported by the USA. At least 44 outbreaks were diagnosed. The USA also recorded an increase in the frequency and geographic distribution of *Corynebacterium pseudotuberculosis* infection among states.

**Second Quarter Report for Australia*

Equine Disease Quarterly

Editors
Roberta Dwyer
Alan Loynachan
Peter Timoney

Staff
Diane Furry
Tawana Brown
Dennis Duross

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

Responsible Interpretation of Polymerase Chain Reaction Assays

Polymerase chain reaction (PCR) assays are commonly used in veterinary medicine for the detection of infectious agents. These tests have grown in popularity due to their cost effectiveness, rapid turn-around time, and ability to detect unculturable pathogens. The judicious interpretation of PCR results will remain imperative as new assays are developed and implemented. Familiarity with PCR technology and the organism being assessed is essential for the appropriate interpretation of results.

Nucleic acid, such as deoxyribonucleic acid (DNA), is a hereditary material that serves as a template to propagate proteins that perform essential cellular functions. Nucleic acid sequences are unique to each organism and can be used as a genetic fingerprint to identify a particular organism.

PCR is a complex technique used to amplify a small segment of nucleic acid. In a simplified summary, nucleic acid is extracted from a sample; mixed with various reagents; and amplified in a thermocycler. If the nucleic acid of interest is present, then thousands to billions of copies will be made. These copies can be detected by gel-based or real-time platforms. Either platform can be used to determine whether a sample is positive or negative; appropriate positive and negative controls are included in every assay. Using gel-based platforms, results are positive or negative and are not quantitative.

Real-time platforms identify each amplified copy with a fluorescent probe, which can be instantaneously (in real time) detected and displayed by a computer. The detection of the organism's nucleic acid is considered significant once the number of amplified copies meets a statistically determined threshold—CT value. The CT value indicates the number of times that the sample was amplified before it crossed the threshold and allows for quantitation of the original sample; a lower CT value indicates that more target nucleic acid (more organisms) was present in the original sample.

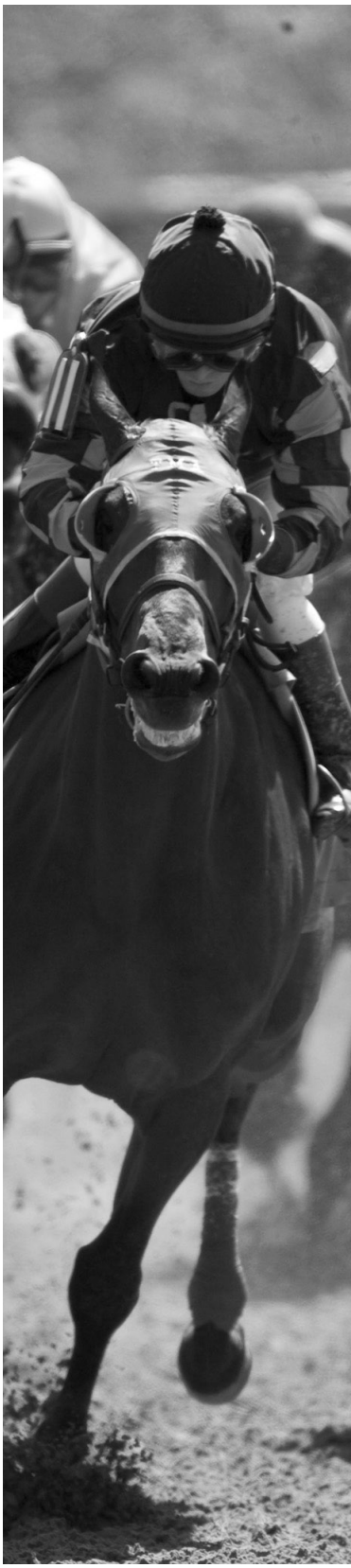
PCR assays are not flawless, and results can be misinterpreted. PCR assays are designed to be highly sensitive (do not incorrectly report positive samples as negative) and specific (do not incorrectly report negative samples as positive). Sensitivity and specificity for a given agent can vary between assays and laboratories, because not all assays use the same reagents or amplify the same segment of nucleic acid.

A negative sample can be incorrectly reported as positive for a number of reasons. Samples can be contaminated, related organisms can have similar fingerprints, and nucleic acid can be detected from non-viable organisms. Similarly, positive samples may be incorrectly identified as negative due to inhibitors in the sample, collection of suboptimal samples (e.g., shallow versus deep nasal swabs for herpesvirus), and collection of samples at suboptimal times (e.g., after the period of peak shedding, or after implementation of antimicrobial therapy). These potential problems mandate that PCR results be interpreted in conjunction with appropriate clinical signs.

PCR assays detect nucleic acid and not a disease. Knowledge of the epidemiology for the agent being assayed, the clinical signs and/or pathology induced by the infectious agent, and vaccination history are important for correct interpretation. Certain infectious agents can be normally found throughout the environment or within nonclinical carriers, thus detection of an agent in an animal without clinical disease should be warily interpreted. Additionally, PCR interpretation following recent vaccination with a modified-live vaccine should be carefully interpreted, because these vaccines may induce positive PCR results.

In summary, PCR assays are extremely useful tools to identify infectious agents. However, results should be cautiously interpreted due to the potential variations in assays, techniques, and samples; detection of pathogens in the absence of disease; and vaccine interference.

CONTACT
Dr. Alan Loynachan
alan.loynachan@uky.edu
(859) 257-8283
Veterinary Diagnostic Laboratory
University of Kentucky
Lexington, Kentucky



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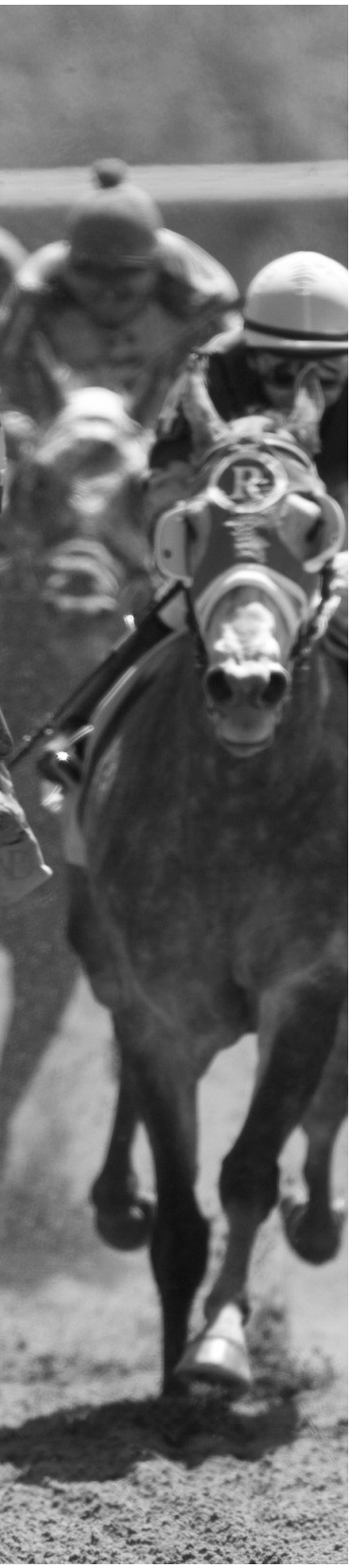

KENTUCKY, AGRICULTURAL COMMUNICATIONS

Heaves, COPD, RAO, or Simply Equine Asthma?

Heaves or broken wind are terms used for decades to describe an allergic respiratory disease of mature to older horses manifested by increased breathing efforts at rest and chronic coughing. Over 40 years ago, the German veterinarian H. Sasse used the term chronic obstructive pulmonary disease (COPD) to describe horses with heaves because of similarities with the human disease. However, our knowledge of respiratory diseases in people and horses has grown considerably since then and it is now clear that heaves in horses is more similar to asthma in people rather than COPD. Veterinarians and scientists prefer using the term "recurrent airway obstruction" (RAO) that implies the reversible nature of the disease once horses are turned out on grass pasture. Equine asthma is used to describe the state of airway hyperresponsiveness following inhalation of dust particles commonly found in barns. Such exposure is usually the result of feeding moldy hay. However, some horses present identical signs while being on pasture during the summer in response to high levels of grass molds and tree pollen.

Feeding round bales at pasture is more likely to trigger equine asthma and is usually associated with more severe disease. Molds are particularly abundant in moldy hay. However, it is important to note that the same types of molds are also present in good quality hay but in lower numbers. A genetic predisposition has also been shown in some breeds, such as Warmblood and Lipizzaner.

The goals of therapy are to avoid exposure to dust and to treat lung irritation. The most effective way to avoid dust is by keeping asthmatic horses outdoors all the time and not feeding hay. Appropriate substitutes to hay and grass are complete pelleted feeds or hay cubes. If horses have to be housed in a barn, it is important to use low-dust feed and bedding. Wetting hay or steaming it will help reduce dust levels, however some very sensitive asthmatic horses may still show signs. Most asthmatic horses improve one to two weeks after being turned outside on pasture with no access to hay but it may take one to two months for horses kept indoors to show the benefits from reduced dust levels. Horses that only improve partially after dust exposure has been reduced should benefit further from drug treatment.

Treatment with corticosteroids and bronchodilators help reduce lung irritation and hasten recovery. Oral or injectable drugs usually cost less than aerosol drugs. However, oral or injectable therapy may result in adverse effects. Albuterol, a commonly used bronchodilator, is not absorbed orally in horses but is effective when given as aerosol although the benefit is very short lived (around one hour). Antihistamines may help some asthmatic horses but most will eventually stop responding to treatment. Recently, we showed that feeding a supplement rich in omega-3 fatty acids helps asthmatic horses breathe better and stop coughing within two to four weeks.

Remember, horses evolved on earth as free roaming grazing animals. Modern use of horses dictated husbandry practices that are not ideal for the horse respiratory health. Asthmatic horses in particular will greatly benefit from returning to their ancestral environment.

CONTACT
Dr. Laurent L. Couetil
(765) 494-8548
couetil@purdue.edu
Director, Equine Research Programs
Purdue University College of Veterinary Medicine
West Lafayette, Indiana

Where Are We Headed with Wobbler Syndrome?

Cervical stenotic myelopathy (CSM), commonly known as wobbler syndrome, is a neurologic disease characterized by malformations of the neck vertebrae. This leads to narrowing of the cervical spinal canal and subsequent compression of the spinal cord. The cord compression manifests clinically as neurologic deficits, typically with the hind limbs being more severely affected than the forelimbs. Depending on the severity of the horse's deficits, euthanasia is often elected for humane and horse and human safety reasons.

Equine CSM is considered to be a multifactorial disease with high planes of nutrition, increased growth rates, alterations in zinc and copper concentrations, and genetic determinants implicated in disease development. Although all these factors are known or suspected to play a role, the exact mechanistic details that lead to clinical disease are still unclear.

Gender, breed, and age factors are well represented in the current knowledge base of this devastating disease. Males are more often affected than females. Breeds such as Thoroughbreds, American

Saddlebreds, Warmbloods, and Tennessee Walking Horses are overrepresented in the identification of the syndrome. Various studies have identified the mean age of CSM horses as less than 2 years leading to the categorization of CSM as a developmental bone disease.

Over the years, assessment methods and analytical approaches for accurate clinical diagnosis of CSM have been developed. All clinical workups begin with a thorough neurologic examination looking primarily for signs of ataxia. The next step is visualization of the neck using radiography. Standard ratios based on skeletal anatomical measurements have been defined at each intervertebral site to identify presumptive areas of spinal canal narrowing. For visualization of actual spinal cord compression, myelograms can be performed.

Once a diagnosis of CSM is made, several management and treatment options are available. More conservative approaches center on dietary modification and anti-inflammatories to slow growth rates, reduce swelling of non-skeletal tissues, and possibly allow vertebral bone remodeling to reduce cord compression. More aggressive approaches involve surgical intervention to alleviate cord compression via cervical vertebral fusion.

Despite all that is known, important questions still remain about wobbler syndrome. Recent research at the Gluck Equine Research Center has focused on CSM. Developments in diagnostic imaging modalities, such as magnetic resonance imaging (MRI) and computerized tomography (CT), enable the characterization of lesions along the entire neck. High-resolution images from multiple angles and the ability to visualize the cervical vertebrae, spinal cord, and associated soft tissues together provide powerful data to study and understand CSM pathology. The combined resources of imaging modalities, clinical resources, and thorough necropsy examinations are providing new insights for CSM research. Although currently used in the research settings, changes in these imaging units to accommodate the size of the horse will allow for the possible use of CT or MRI for clinical diagnosis in the future.

The role of inherited genetic determinants is a long-standing question. Due to rapidly developing genomic technologies, studies are now being conducted to investigate the equine genome to identify specific genes that may contribute to CSM susceptibility. This is an exciting area of research that could have an important impact on breeding decisions and management of potentially susceptible horses.

CONTACT
Dr. Jennifer Janes
Jennifer.janes@uky.edu
University of Kentucky
Lexington, Kentucky

KENTUCKY

Diagnostic Sample Submission Guidelines

A laboratory test is only as good as the sample submitted. That sample should be collected, stored and shipped properly, with appropriate paperwork, for the most reliable and accurate results. Among possible samples that can be submitted to a laboratory for testing are serum, whole blood, feces, urine, swabs, washes, tissue samples, biopsies, feed, hay, water, and entire animals. The following guidelines will help get the most information from diagnostic submissions.

Serum is often required when a titer, or antibody determination, is desired. If serum is needed, allow the blood to clot, and then pour the serum into a different clean tube. Allowing the serum to sit on the clot too long can cause the red blood cells to rupture, or hemolyze. Hemolysis will interfere with many laboratory tests. Gel tubes, or serum separator tubes, are meant to aid the separation of serum from red cells following centrifugation and are not optimal for shipping.

Tests such as complete cell counts, virus isolation, and polymerase chain reaction assays often require submission of plasma or whole blood. For these tests, blood needs to be unclotted, therefore, use tubes containing an appropriate anticoagulant and have been gently inverted five to six times after collection.

Samples should be submitted using proper biosecurity guidelines in capped, clean, leakproof, spill-proof, labeled containers. And, while a palpation sleeve would seem to be the perfect, convenient submission container (especially for fecal specimens), it is not an acceptable vehicle for samples.

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

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Storage on the dashboard or console of a vehicle is not optimal. Most samples need to be kept cool. Tissues in formalin need not be cooled, but must be in leak-proof containers. Serious monetary fines from shipping companies or laboratories may be imposed as formalin is a hazardous substance. Shipping on cold packs for next day delivery is recommended for most biological samples. Samples should be sent in sturdy, insulated packaging that will not allow the specimen or container to be crushed in transit. Padded envelopes are not sufficient for shipping blood tubes.

The paperwork is critical, beginning with the labeling of the specimen. Label the specimen minimally with the name/ID # of the animal, contents, and date. The submission form needs to be filled out as completely as possible. The collection date is important and imperative for regulatory tests. As a general rule, the more history, the better. Be sure to indicate treatments with antibiotics, recent vaccinations, etc., because these can impact test results and interpretation.

If an entire animal is submitted for necropsy, be mindful that decomposition begins quickly in warmer weather. If the animal is submitted for a neurologic examination or is a rabies suspect, do not damage the brain. Gunshot or blunt force trauma to the head is contraindicated for a testable specimen.

Finally, to save valuable time and money, if there are any questions call the laboratory ahead of time.

CONTACT
Dr. Deborah Maples
(859) 257-8283
deborah.maples@uky.edu
Veterinary Diagnostic Laboratory
University of Kentucky
Lexington, Kentucky