

# EQUINE DISEASE QUARTERLY

A PUBLICATION BY THE UNIVERSITY OF KENTUCKY DEPARTMENT OF VETERINARY SCIENCE, MAXWELL H. GLUCK EQUINE RESEARCH CENTER

FUNDED BY: EQUUS / STANDARDBRED STATION, INC.  
M&J INSURANCE

## IN THIS ISSUE

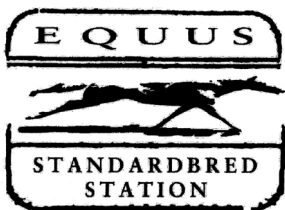
**KENTUCKY, 2-3**  
Application of shotgun metagenomics for diagnosing infectious causes in clinical cases

**NATIONAL, 4**  
A look at poor performance in horses

**INTERNATIONAL, 5-6**  
Third Quarter 2024 International report on equine infectious diseases

Climate change impacts on Central Kentucky horse pastures

## THANK YOU SPONSORS



## RESEARCH SPOTLIGHT

### All hands on deck to conquer herpesviruses – the Goehring Research Laboratory

Equid alphaherpesvirus 1 (EHV-1), formerly known as equine herpesvirus 1, is an important viral infection in horses with global prevalence. EHV-1 causes considerable economic losses for the equine industry while also resulting in a significant emotional hardship to horse owners when their horses become infected.

The virus primarily spreads through the respiratory route, initially replicating in the upper respiratory tract. In some horses, it spreads further into the bloodstream, causing viremia, which can lead to complications such as abortions, neonatal death and Equine Herpesvirus-associated Myeloencephalopathy (EHM). While all of these outcomes are significant, EHM typically garners the most attention when it causes sudden (mild, moderate or severe) spinal cord disease. Transmission of EHV-1 from horse to horse occurs through direct contact, particularly via nose-to-nose contact, and indirectly through airspace or contaminated objects, such as gear and buckets, in addition to the sometimes-forgotten hands that go from horse to horse.

Like most, if not all, herpesviruses, EHV-1 can enter a latent or dormant state after an acute infection. EHV-1 latency can persist for years, and, potentially, for life in the trigeminal ganglion and the lymphatic system. Upon reactivation, this virus returns to the respiratory tract where it may result in new rounds of infection transmission. Understanding EHV-1 latent infection and what circumstances trigger reactivation from latency is one important area of study in the laboratory at the University of Kentucky Department of Veterinary Science’s Gluck Equine Research Center.

Leading the work in this area is Edward Olajide, DVM from Nigeria and PhD student in the lab of Lutz Goehring, DVM, PhD. Early results from Olajide’s research show that, as predicted, EHV-1 DNA most commonly found in the trigeminal ganglion of horses. Interestingly, EHV-1 DNA is also found in the mesenteric lymph node (lymph nodes in the abdomen), which was not anticipated. Further, there was not



Photo Courtesy Dr. Edward Olajide.

consistency with regards to the detection of EHV-1 in the lymph nodes of the upper respiratory tract for the youngest horse with tissue samples positive for EHV-1 DNA at 14 months old. It was also found that there was no linear increase of viral DNA presence with aging despite the fact that it is always assumed there is continuous EHV-1 exposure. These findings add to the complexity of ‘going dormant’ and ‘reawakening’ of herpesviruses and need further attention.

In collaboration with other laboratories, the lab also focuses on evaluating both current and newly developed vaccines and therapeutics, conducting immunogenicity studies and performing clinical trials to ensure the efficacy and safety of these vaccines. With collaborators at Kansas State University and Michigan State University, the lab is evaluating novel RNA vaccines similar to the potent Covid-19 vaccines

introduced during the pandemic. These studies were made possible through a grant by the Grayson Jockey Club Research Foundation and are ongoing.

Vaccination should play an important role in EHV-1 infection control. However, limiting the exposure to EHV-1 is equally important as a regular vaccination schedule. Therefore, the lab is working to advance and standardize techniques and tools to better and more quickly detect EHV-1 in the environment, with the hope to do more studies about dynamics of virus transmission in the future.

While the lab focuses on three major areas, latency and reactivation; transmission and surveillance; and vaccine efficacy studies, with the latency research the lab hopes to get a better understanding through transcriptome studies the “listen” in on virus and host interactions in the near future.

#### CONTACT:

**Edward Olajide, DVM**  
PhD Graduate Student  
Gluck Equine Research Center  
Department of Veterinary Science  
University of Kentucky  
[Edward.olajide@uky.edu](mailto:Edward.olajide@uky.edu)

**Lutz S. Goehring, DVM, MS, PhD**  
Wright – Markey Professor of Equine Infectious Diseases  
Gluck Equine Research Center  
Department of Veterinary Science  
University of Kentucky  
[l.goehring@uky.edu](mailto:l.goehring@uky.edu)

## KENTUCKY

### Application of shotgun metagenomics for diagnosing infectious causes in clinical cases

Next Generation Sequencing (NGS) is a DNA/RNA sequencing technology commercially available since 2005 that overcame the limitation of traditional Sanger sequencing. Traditional Sanger sequencing was a targeted approach that could sequence a few thousand nucleotides at a time, while new sequencing technology allows for massive parallel sequencing of multiple genomes, often referred to as metagenomics. While different distinct approaches such as whole genome sequencing (WGS), targeted next-generation sequencing and shotgun metagenomics NGS exist for infectious disease diagnosis, the shotgun metagenomics approach provides hypothesis-free infectious disease detection and a comprehensive way to identify pathogens directly from clinical samples.

Classical culture-based pathogen detection has limitations as many pathogens grow poorly or not at all in culture. Similarly, newer molecular diagnostic assays (like PCR) rely on some prior knowledge of suspected pathogens, meaning that novel pathogens could remain undetected in such assays. Metagenomics-based sequencing has an advantage over traditional diagnostic assay, as it uses a “search for all” approach. In this approach, genetic material (DNA/RNA) from a clinical sample is subjected to metagenomic sequencing, resulting in millions of copies of genetic material of any microorganisms present in the sample. This data is then analyzed using sophisticated software tools to characterize the genetic material of microorganisms in the sample and in what proportion. This data, along with clinical history and other pathological changes, can help to identify disease-causing organisms, even if they were unexpected or novel to this type of clinical case.

Below are some of the applications of NGS in clinical diagnostics:

- 1. Broad-spectrum pathogen detection:** Shotgun metagenomics is particularly useful in cases where the infectious agent is unknown and/or unculturable. It has been successfully applied in diagnosing infections caused by rare, novel or unexpected pathogens that might be missed by conventional diagnostic methods. For instance, we have recently discovered a novel rotavirus B causing life-threatening diarrhea in neonatal foals, where traditional diagnostic assays, such as bacterial aerobic and anaerobic cultures and PCR tests for *Clostridium perfringens*, *Clostridioides difficile*, *Cryptosporidium* spp, Equine Coronavirus and Equine Rotavirus A, were negative.
- 2. Polymicrobial infections:** Traditional culture-based methods often struggle to identify all the pathogens involved in polymicrobial infections (those infections with multiple microbes of importance). Shotgun metagenomics can detect multiple pathogens in a single sample, providing a comprehensive picture of the disease. This capability is especially important in conditions like disease complexes, such as bovine respiratory disease complex, where timely and accurate identification of all the causative agents is critical for effective treatment.
- 3. Antimicrobial resistance:** Beyond identifying pathogens, metagenomics can also detect any drug resistance present in the pathogen, for instance antimicrobial or antiviral resistance. By analyzing genetic material from the involved pathogens, we can identify resistance mechanisms and tailor antibiotic therapy accordingly. This is particularly valuable in the era of increasing antibiotic resistance, where inappropriate use of antibiotics can lead to treatment failures and further resistance development.
- 4. Outbreak investigation and disease surveillance:** In outbreak settings, shotgun metagenomics can provide rapid and detailed insights into

## EQUINE DISEASE QUARTERLY

### EDITORS

Lynne Cassone  
Lutz Goehring  
Allen Page

### STAFF

Holly Wiemers

[EDQ@uky.edu](mailto:EDQ@uky.edu)

the causative agents and their transmission dynamics. It enables public health officials to identify the source of the outbreak, track its spread and implement control measures more effectively. Metagenomics approaches can also be used for disease surveillance and early detection. This approach was extensively used during the COVID-19 pandemic where wastewater surveillance was used for new variant detection. We can also determine whether mutated pathogens can escape the antibody response induced via vaccination by comparing the genetic makeup of the pathogen to that of vaccine strains. Pathogen typing, for instance *Salmonella* serovar identification or rotavirus genotyping, is an added advantage of this approach and can help clinicians formulate appropriate treatment plans.

Beyond infectious disease, metagenomics sequencing can also be used for non-infectious diseases. Detection of various hereditary diseases, genetic mutations predisposing individuals to certain diseases and early cancer risk prediction are some of the examples successfully used in human medicine.

Despite its advantages, shotgun metagenomics faces several challenges.

- **Complex data analysis (bioinformatics):** The incredible amount of data generated requires sophisticated bioinformatics tools and expertise to interpret. Differentiating between pathogenic organisms and commensal flora can be complex, especially in samples from sites with a high microbial load.
- **Cost and time:** While the cost has decreased significantly since metagenomic sequencing was introduced almost 20 years ago, it remains relatively high compared to traditional diagnostic methods, potentially limiting its widespread adoption. In addition, sequencing and data analysis can take several days, making rapid clinical treatment decisions difficult.
- **Sensitivity and specificity:** The sensitivity of shotgun metagenomics can be affected by the presence of host DNA, which may overshadow microbial sequences. Additionally, a pathogen of interest may not be detected if it is in low abundance in the specimen or the nucleic acid quality has degraded.

Nevertheless, metagenomics approaches for infectious disease diagnosis have been successfully applied to clinical samples like blood, respiratory swabs, bronchoalveolar lavage fluid, transtracheal washes, cerebrospinal fluid, brain tissue and fecal material and fecal swabs, as well as a variety of other body tissues and fluids. Overall, metagenomics-based NGS holds massive potential for infectious disease diagnosis in veterinary medicine and with the continual development of newer sequencing technology, both cost and time for sequencing are expected to decrease further.



AdobeStock.

**CONTACT:**

**Tirth Uprety, DVM, MS, PhD**  
Section Head, NGS Laboratory  
Veterinary Virologist  
[tirth.uprety@uky.edu](mailto:tirth.uprety@uky.edu)

**Erdal Erol, DVM, MS, PhD**  
Professor, Head of Diagnostic Microbiology  
[erdal.erol@uky.edu](mailto:erdal.erol@uky.edu)

Next Generation Sequencing Laboratory  
Veterinary Diagnostic Laboratory  
University of Kentucky  
<https://vdl.uky.edu>

*Material published in the Quarterly is not subject to copyright. Permission is therefore granted to reproduce articles, although acknowledgment of the source and author is requested.*

**Maxwell H. Gluck**  
Equine Research Center  
Lexington, Kentucky USA,  
40546-0099  
Telephone (859) 257-4757  
Fax (859) 257-8542  
[gluck.ca.uky.edu](mailto:gluck.ca.uky.edu)



## A look at poor performance in horses

“They’re not lame, they’re just not...right.”

I commonly hear this as many horses present to our practice for vague signs of poor performance or even behavioral issues. Trainers often will say that the horse is misbehaving and they want to make sure there is not a physical issue before assuming it is a training issue. In my experience, it is very uncommon for a horse to misbehave for no reason and behavioral issues under saddle are usually indicative of an underlying physical problem. Thus, our job as veterinarians (and owners and trainers) is to find the problem and help the owner and/or trainer develop a plan to move forward. While this can sometimes be a challenging and exhaustive process, it is ultimately rewarding if the underlying physical cause can be identified and resolved.

Although there is an almost endless list of things that can cause poor performance and/or behavioral issues under saddle, orthopedic and musculoskeletal disorders are a common culprit. This underscores the importance of a thorough history, physical examination and lameness examination when working up these cases. In more straightforward cases, the horse may present with a visible lameness, therefore the first step is to block and regionalize the lameness. It is important to keep in mind that the site of lameness may or may not be related to the presenting complaint. Re-evaluating the horse under saddle after the lameness has been blocked can clarify if the lameness is the source of the behavioral issue or not.

Conversely, there are multiple orthopedic conditions that can manifest as performance issues rather than lameness, including axial skeletal pain (pain of the neck, thoracolumbar spine and/or pelvis), front foot pain and hind proximal suspensory pain. Therefore, in horses without overt lameness, it can be enlightening to block the front feet and/or hind suspensory ligaments to see if it subsequently changes the behavior under saddle. For evaluation of the axial skeleton, sometimes blocking is also employed. For example, in horses with kissing spine, the horse can be evaluated under saddle before and after blocking the affected sites to assess the extent to which the kissing spine is a contributing factor. Most veterinarians are less comfortable blocking the neck or SI region and, therefore, if involvement of these regions is suspected, many veterinarians rely on imaging and trial treatment of those regions. Response to treatment is then used to help determine if these regions are playing a role in the performance issues.

Looking beyond orthopedic causes, there are multiple muscle diseases that characteristically present with vague signs of poor performance, including stiffness, unwillingness to go forward, inconsistency in the contact, lack of impulsion and loss of quality of gaits. Particularly in Warmbloods, muscle diseases such as myofibrillar myopathy (MFM) and polysaccharide storage myopathy (PSSM) should be on the differential list. In several breeds, including Quarter Horses, Paints, Appaloosas, Belgians and Percherons, many muscle diseases can be diagnosed with genetic and/or blood testing. However, in Warmbloods, a muscle biopsy is almost always necessary to confirm a diagnosis.

Respiratory disease, including disorders of the upper or lower airway, is another common cause of performance issues and often presents as exercise intolerance. Obstructive conditions of the upper airway such as pharyngeal collapse, dorsal displacement of the soft palate and laryngeal hemiplegia can understandably lead to exercise intolerance. The most common lower airway diseases include equine asthma and exercise induced pulmonary hemorrhage (EIPH/ Bleeders). A bronchoalveolar lavage and dynamic endoscopy can help to rule in or out most of these respiratory conditions. The cardiopulmonary system should also be

evaluated in cases that present for exercise intolerance, as certain cardiac arrhythmias and murmurs can, unsurprisingly, lead to performance issues.

While gastric ulcers can cause behavioral issues, they seem to get blamed for behavioral issues at a disproportionate rate. Although gastroscopy (a video camera inserted into the stomach) is the gold standard to diagnose gastric ulcers, riding the horse following “blocking” the stomach with lidocaine or following a dose of sucralfate can potentially help determine if gastric ulcers are contributing to the horse’s clinical signs.

Hormones should also be mentioned when discussing potential sources of performance and behavioral issues, particularly in mares. Oral altrenogest can sometimes be helpful to assess and/or mitigate hormonal influence. Interestingly, there are mares who continue to show behavioral issues while on altrenogest that improve dramatically following ovariectomy, causing some owners to consider this in non-breeding stock.

In summary, there are an exhaustive number of things that can lead to performance issues in horses. A thorough history and examination, in addition to the above investigations, will often lead to an answer in the many of these cases. It is important to keep in mind that most behavioral issues are not behavioral at all but rather a manifestation of an underlying physical problem. Keeping this in mind helps us approach these cases with empathy and an open mind.

### CONTACT:

**Erin K Contino, DVM, MS, DACVSMR**

Associate Professor, Equine Sports Medicine and Rehabilitation  
College of Veterinary Medicine and Biomedical Sciences  
Colorado State University  
Fort Collins, Colorado  
[Erin.contino@colostate.edu](mailto:Erin.contino@colostate.edu)



Photo Courtesy Dr. Allen Page.

# INTERNATIONAL

## Third Quarter 2024

### International report on equine infectious diseases

There is regular reporting of *Strep equi spp equi* outbreaks and single cases throughout North America and Europe.

Several cases of *Rhodococcus* pneumonia in foals have been reported from Europe.

Also, there are isolated notifications of Equine Influenza from North America (Pacific Northwest, USA, Mid Atlantic, USA, and Ontario, Canada), and from Europe (British Isles, Netherlands and Belgium).

An outbreak investigation on Contagious Equine Metritis (CEM) caused by the bacterium *Taylorella equigenitalis* in the United States is ongoing ([see story in our last EDQ edition](#)). A pasture-bred pony mare developed endometritis after mating and uterine fluid was culture-positive for *T. equigenitalis*. This is an ongoing investigation that has currently detected nearly 50 animals on three farms in Florida, and farms in Maine, Maryland and North Carolina. The five farms are connected to the index case. The majority of (culture positive) animals, so far, are geldings. Interestingly, a recent publication in *Equine Veterinary Journal* (Grabatin et al., 2024) showed high prevalence among Icelandic horses of Germany using molecular diagnostics (PCR). These findings are supported by three CEM positive Icelandic horse cases from Germany during the last quarter. More on the ongoing outbreak investigation in the U.S. can be found [here](#).

EHV-3 infection, an alphaherpesvirus and cause of pustular vulvovaginitis, is rarely reported. This quarter, there are reports from Europe (Switzerland and France).

Without further information, Portugal reports a case of Equine Arteritis virus (EAV) infection.

There have been few outbreaks/single cases of respiratory EHV-1

and EHV-4 infection reported from North America and Europe and a few abortions caused by EHV-1. While the third quarter is typically a timespan with the fewest EHM (neurological EHV-1) cases or outbreaks of the year, there has been an unofficial notification of an EHM outbreak in Brazil in a coastal area south of Sao Paulo (Curitiba).

On the other hand, this quarter, as in previous years, has seen a surge in the mosquito-borne (ARBO) diseases Eastern Equine Encephalitis virus (EEE) and West Nile virus (WNV) in North America. A number of WNV cases have also been reported from Europe for all endemic areas. France reports an increase in cases not only from the Mediterranean coastal areas but also from the Atlantic coastal areas. The endemic area around Berlin, Germany, is slowly expanding to the North and West of Germany. In North America, there is increased EEE activity along the East coast including the New England States expanding into Quebec and Ontario, Canada, and to the West with cases reported from Michigan, Wisconsin and Texas, USA.

Kenya reports a case of African Horse Sickness (AHS).

There were isolated Equine Infectious Anemia reports from North America and a single case reported from Europe (Bulgaria).

#### CONTACT:

#### Lutz S. Goehring, DVM, MS, PhD

Warren Wright, Sr. – Lucille Wright Markey Endowed Chair in Equine Infectious Diseases  
Gluck Equine Research Center  
Department of Veterinary Science  
University of Kentucky  
[l.goehring@uky.edu](mailto:l.goehring@uky.edu)



Photo Courtesy Mark Pearson Photography.



## INTERNATIONAL

### Climate change impacts on Central Kentucky horse pastures

Here in Central Kentucky, we are warmer and wetter today than 50 years ago, and these climate trends are predicted to continue.

How do these new climate conditions impact the forage base that supports our equine industry? That is the question our lab posed back in 2008 and received a national grant to examine. We established a manipulative, field experiment elevating temperatures and rainfall on a pasture which we planted in typical Central Kentucky pasture species: Kentucky bluegrass, tall fescue, red and white clover and increasingly common bermudagrass. We used overhead infrared radiant heaters to increase the temperature of the plant canopy by 3oC, day and night, year-round for five consecutive years, and we added +30% of the long-term mean annual rainfall only on wet days, during the growing season, to mimic larger rainfall events on the plots slated for that treatment. We compared the effects of elevated temperature alone, increased rainfall alone and the combination of both treatments to ambient conditions.

We found that warmer conditions significantly increased overall forage production (+19%), in part by stimulating cool-season grass growth earlier in the spring and later into the fall. However, higher temperatures in the summer promoted the growth of warm-season grasses (bermuda and crabgrass) at the expense of traditional cool-season forages (Kentucky bluegrass and tall fescue). Over the five-year period, tall fescue abundance was dramatically reduced (from 40% to 20%) in the warmed treatments compared to ambient controls. Importantly for grazing horses, the tall fescue that remained in the elevated temperature plots was more likely to be endophyte-infected and had significantly higher concentrations of toxic alkaloids such as ergovaline, the putative agent of fescue toxicosis. Endophyte-infected tall fescue plants experiencing warming had 30% higher concentrations of ergovaline than endophyte-infected material grown under ambient conditions, particularly in the fall. The effects of the increased rainfall treatment were less pronounced than that of the temperature response.

Given the significant animal health issues caused by consumption of toxic

tall fescue, the observed increase in alkaloid concentrations with warming is a cause for concern. However, the reduction in tall fescue abundance and the increase in other non-toxic, warm-season forage species may mean that fescue toxicity is diluted for grazing animals. The type of warm-season forage species that responds will be important, as annual species – like crabgrass – will die out in the fall and could potentially leave bare soil exposed over winter, an erosion/mud concern. Some perennial and annual warm-season species in our pasture seedbanks are not preferred horse forages (e.g., nimblewill and goosegrass).

Desirable perennial warm-season species, such as bermudagrass, are not present in most pastures in Kentucky at this time, and thus, would need to be planted and would require different management than our cool-season forages. Bermudagrass also turns brown in the winter (goes dormant) which could affect the aesthetics of the landscape. For horse farms interested in maintaining a cool-season grass base, planting novel endophyte tall fescue may be an option, given the hardiness of tall fescue and the non-toxic nature of these endophytes. While fescue and Kentucky bluegrass abundance decreased over time in our experiment, both species remained after five years, indicating they are able to survive even with increased warm-season species competition.

Although this project did not evaluate the effects of drought and unusually low precipitation, such as Central Kentucky is experiencing this year, these conditions would undoubtedly lower forage production, especially in combination with warming, even for warm season dominated pastures.

#### CONTACT:

#### Rebecca L. McCulley, PhD

Chair and Professor  
Department of Plant & Soil Sciences  
University of Kentucky  
[rebecca.mcculley@uky.edu](mailto:rebecca.mcculley@uky.edu)  
859-257-6388



Photo Courtesy UK Ag Communications.