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# EQUINE DISEASE QUARTERLY

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## GLUCK RESEARCH SPOTLIGHT

### Protective humoral immunity of mares and their foals post equine rotavirus A G3P[12] vaccination

Our research laboratory is interested in understanding, treating and preventing equine infectious diseases. One of our primary interests is to better understand the genetic and antigenic diversity of equine rotavirus groups A and B. In addition, we hope to characterize and identify correlates of protective immunity against rotavirus infections in horses.

Equine rotavirus A (ERVA) globally causes rotaviral diarrhea in foals and was first described in 1975. Two structural proteins, encoded by P and G genotypes, are recognized, of which G3, G14 and P[12] are the most common. Swift and intensive medical treatment typically prevents fatalities but represents a significant economic burden to the equine industry. Due to ERVA's incredibly infectious nature, the high number of virus particles shed in fecal matter, its environmental stability, and the non-enveloped structure, ERVA is very hard to contain once an outbreak occurs, which can lead to a heavy burden on farm staff and veterinarians. Therefore, prevention through biosecurity measures, monitoring programs and vaccination is of the utmost importance. Before the introduction of the monovalent G3 vaccine by Zoetis in 1996, ERVA infection manifested as a disease of neonatal foals. Mares are vaccinated three times during pregnancy at 8, 9, and 10 months of gestation to generate a protective passive immunity to the foal through colostrum. Interestingly, rotaviral diarrhea attributable to ERVA can be observed in foals between three and four months of age born to vaccinated mares and in neonates of unvaccinated dams. Foals at younger ages are much more susceptible to infection and develop more severe clinical signs. With intensive treatment, rotaviral diarrhea has a high survival rate of 94%. However, without treatment, ERVA can be rapidly fatal, especially in neonates.

An ongoing collaborative research project at the University of Kentucky Gluck Equine Research Center is focused on whether vaccination against ERVA G3P[12] provides cross-protection against ERVA G14P[12], with an additional goal of monitoring the duration of maternally-derived protective antibodies. Serum samples were collected from mares pre-vaccination, post-vaccination and for multiple months post-foaling. Foal serum samples were collected prior to nursing and bi-weekly for up to seven months post foaling. Additionally, fecal swabs from both the foals and mares as well as colostrum and milk samples were collected. A total of 50 mare-foal pairs were included in this study, leading to a total of more than 1,500 samples. A virus neutralization assay was used to determine neutralizing antibody titers in serum against ERVA G3P[12] and ERVA G14P[12]. Based on preliminary data, vaccination of mares maintained and increased ERVA G3P[12] virus neutralizing titers. Importantly, serum antibodies were cross-protective against ERVA G14P[12]. Further, ERVA G3P[12] and ERVA G14P[12] neutralizing antibodies are passively transferred through the colostrum as pre-nursing neutralizing antibodies were undetectable and increased greatly in post-nursing samples.

Lastly, we noted that titers of neutralizing antibodies in the foals decreased steadily after birth.

Work on this project is currently ongoing. Once serum virus neutralization assays are completed colostrum and milk samples will be tested using the same assay to determine the level of transfer of neutralizing antibodies from the dam to the colostrum and from the colostrum to the foal. Furthermore, RT-qPCR of fecal swabs for ERVA G3P[12], G14[12] and ERVB will be performed to identify asymptomatic infections due to natural exposure during the project period. In future experiments, we hope to determine the precise antibody titer necessary for protection against ERVA beyond the meonatal period.

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## Second Quarter 2023 International report on equine infectious diseases.

The following report was composed with information provided by the University of Kentucky Veterinary Diagnostic Laboratory and Equine Diagnostic Solutions, Inc. —both in Lexington, Kentucky, United States of America. Furthermore, information from the International Thoroughbred Breeders Federation, International Collating Centre in Newmarket, United Kingdom, and American Association of Equine Practitioners' Equine Disease Communication Center was included. This report is retrospective and does not claim to be complete. However, it provides an indication of heightened activity of relevant contagious or environment-linked diseases among equids. To further improve this data, we encourage entities outside the USA to report laboratory-confirmed (toxico) infectious diseases of Equidae to the ICC.

Reports from Asia, Africa, South America and Australia were not received for this quarter.

The USA reported rapidly spreading vesicular stomatitis virus infections in various cohorts of horses mainly throughout California with fewer outbreaks reported from Texas (first reported in mid-May 2023). VSV is an arbovirus transmitted by a varietyof insects, mainly biting midges and sandflies, but also stable flies. Direct contact and fomites are alternative methods of transmission. VSV outbreaks typically follow a seasonal pattern from late spring and summer in the USA, and reported outbreaks are typically localized to the Western United States. Typical clinical signs consist of painful blisters followed by ulceration of the oral mucosa. Disease is endemic in the USA and South and Central America.

North America and Europe, including the British Isles, consistently report strangles (caused by *Streptococcus equi spp. equi*) cases and outbreaks. It is currently the most consistently reported pathogen. In North America, most cases are reported from several distinct regions. Within the USA, Florida, Michigan and Washington frequently report cases, and most cases from Canada are reported from the province of Ontario.

An outbreak of equine influenza virus was traced to a Standardbred racing facility in the province of Quebec, Canada; the outbreak rapidly spread to other (mainly) Standardbred training facilities. On a smaller scale, another outbreak of EI was reported in the U.S. Pacific Northwest. Scattered EI PCR positive nasal swabs were also reported from various U.S. regions,

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from various U.S. regions including the Midwest, Southwest and East Coast. EI cases and outbreaks were also reported from central Europe.

Equine herpes virus 4 or 1 respiratory disease was reported infrequently throughout the USA. A higher incidence was reported from operations in the United Kingdom, Ireland and from continental Europe. Few EHV abortions were reported from North America. Three abortions caused by EHV-1 were diagnosed in central Kentucky. Of note, there has not been further propagation of disease within the pregnant mares on those farms. This is likely due to high vaccine coverage combined with a fast and effective management intervention. EHV-1 abortions typically occur in the last trimester of pregnancy; therefore it is understandable that the number of abortions significantly increased in continental Europe and the British Isles compared to the spring quarterly report.

Equine herpesvirus myeloencephalopathy also follows a seasonal pattern with the majority of outbreaks occurring in the first, second and fourth quarters in the Northern Hemisphere. Localized outbreaks were reported from across the USA and Canada. Single outbreaks were reported from Scandinavia and the British Isles, and two outbreaks were reported from central Europe. A few scattered equine infectious anemia cases were reported in the USA and Canada. A single report was received from France.

Year-round vector activity occurs in regions bordering the Gulf of Mexico. Four cases of Eastern equine encephalitis were reported in Florida. Although early for the season and quarter, a single case of West Nile virus was identified in the Midwest Region of the USA. During the quarter, there were 35 positive samples for rotavirus A or B and close to 100 *Rhodococcus equi* positive samples reported. These numbers were derived mostly from the Lexington, Kentucky, laboratories. While many samples were submitted from within Kentucky, a significant number were from other states. Nine cases of rotavirus and 11 of *Rhodococcus* were reported from France.

**Miscellaneous**: Contagious equine metritis: Three reports of *Taylorella equigenitalis* (the cause of CEM) were reported from Germany. It is currently unknown whether these cases were epidemiologically connected.

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## The Ridden Horse Checklist

The Ridden Horse Pain Ethogram (RHpE), otherwise known as the ridden horse checklist, was developed as a tool to facilitate recognition of lameness. There is evidence from Denmark, Sweden, Switzerland and the United Kingdom that at least 50% of the sports and leisure horse populations that are considered by their riders to be working comfortably are actually lame, indicating that riders and trainers are poor at recognizing lameness.

Horses are stoical and generally compliant, despite experiencing discomfort. As prey animals, horses have developed adaptations of movement to mask pain. They reduce the range of motion of the thoracolumbosacral region, shorten step length, reduce the height of arc of foot flight and increase duty factor (the proportion of the stride time that a limb bears weight), all to spread load among limbs. These adaptations can lead to muscle atrophy, altered neuromuscular pathways and sometimes the development of other secondary problems, which can make successful treatment and rehabilitation more challenging than if lameness had been recognized earlier.

There are many myths in the equestrian world that have been perpetuated through generations. It has been accepted that there are grumpy, difficult, 'stressy' or lazy horses. Abnormalities of canter (for example, close temporal and spatial placement of the hindlimbs, so-called bunny hopping) have been regarded as training problems.

People say, 'My horse hates dressage but he jumps well, so there cannot be anything wrong," without asking why the horse struggles to work well on the flat. As a result, the horse or the rider get blamed by trainers without anyone questioning why a horse is unwilling or shows tension. Coercive training methods are sometimes adopted ('use a longer whip' or 'use a stronger bit'), with the potential for any underlying problems to worsen.

The RHpE was developed as a tool to facilitate the recognition of pain-related poor performance and pain-induced gait abnormalities. An ethogram is a series of behaviors, each with strict definitions (for example, mouth opening with separation of the teeth for 10 seconds or more). By comparison of extensive video footage of non-lame and lame horses acquired during ridden work, an ethogram comprising 117 behaviors was developed. Through application of this ethogram to non-lame and lame horses,



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24 behaviors were identified (for example, ears pinned back for 5 seconds or more and repeated tail swishing), the majority of which were at least 10 times more likely to be seen in a lame horse compared with a non-lame horse. These 24 behaviors comprise the RHpE and, by comparing non-lame and lame horses, it was shown that the display of eight or more of the 24 behaviors is likely to reflect the presence of musculoskeletal pain. However, some lame horses have a RHpE score less than eight and any of the 24 behaviors could be caused by a variety of factors. Therefore, it is the total score which is important.

The RHpE was applied to ridden horses before and after resolution of lameness using diagnostic anaesthesia (nerve blocks). There was an immediate and substantial reduction in the RHpE scores, demonstrating a causal relationship between the behaviors and pain. This also indicated that the behaviors were not habitual but were a direct response to pain. It has been demonstrated that the RHpE can be used by non-trained individuals from a variety of professional backgrounds to differentiate between lame and non-lame horses, although training improves the accuracy of its application.

The RHpE was applied to 40 horses when ridden by the normal rider and a single professional rider, performing a purpose-designed dressage type test. Overall, the gait quality was improved when the horses were ridden by the professional rider; however, the total RHpE scores were similar, although some of the individual behaviors that were displayed differed. This means that a horse with musculoskeletal pain may appear to move better when ridden by a skilled professional rider than when ridden by the normal rider, but behavioural signs of pain cannot be masked.

The RHpE has the potential to be used in many different circumstances. It can be used as a monitoring tool to facilitate early recognition of musculoskeletal pain, to differentiate a pain-related or training-related problem, to help determine if a saddle fits a horse or causes discomfort, for a trainer to advise a client that their horse probably has a problem and to assess the likely presence of discomfort in a horse which is being tried prior to purchase. Additionally, a veterinarian can use the RHpE as a tool to assess whether nerve blocks have fully resolved pain causing poor performance.

The RHpE has been applied to horses warming up for dressage at five-star three-day events, and during the dressage tests at low-level eventing and various levels of Grand Prix dressage. At all levels, the majority of horses had a low RHpE score (two or three/24 at upper-level competitions; four to six/24 at lower-level competitions), which supports the social licence to ride horses in competitions. However, a minority of horses had higher scores and high scores have been associated with poorer performance at all levels and in all disciplines.

At five-star three-day events, horses that scored seven or more compared with horses which scored less than seven had higher dressage penalties, were more than twice as likely to be eliminated or retire cross-country, and those that completed had lower final places. It is therefore suggested that investigation of horses with higher scores, followed by appropriate treatment and management, has the potential to improve both equine welfare and performance.

The RHpE is a valuable, scientifically-validated tool for assessment of pain in ridden horses.

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## NATIONAL

### Unsanctioned Racing's Role in Equine Infectious Anemia (EIA) and Equine Piroplasmosis (EP) in the United States

Unsanctioned, commonly called "bushtrack", racing has occurred in certain regions of the United States for decades, however, it has only been recently that state and federal animal health officials identified the connection of this activity to widespread transmission of equine blood-borne diseases. Outbreaks of equine piroplasmosis (EP, *Babesia caballi, Theileria equi*) in clusters of Quarter Horse racehorses in Florida in 2008 and Missouri in 2009 were the first instances where involvement in bushtrack racing and iatrogenic transmission were key factors in the disease spread. Increasing numbers of equine infectious anemia (EIA) cases began to be confirmed in Quarter Horse racehorses around 2013 and now are the predominantly affected breed/discipline in annual U.S. EIA cases. There is frequent cross-over of Quarter Horse racehorses between sanctioned and unsanctioned race events,



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so EIA and EP testing requirements to enter sanctioned racetracks provides the primary mechanism by which new disease cases are being identified.

From 2008 through 2022, a total of 541 cases of EP and 409 cases of EIA were confirmed in current or former Quarter Horse racehorses, many of which had ties to unsanctioned racing. Iatrogenic transmission via unhygienic practices was identified as the cause of spread in all cases and some horses were found dually infected with both EIA and EP. The most common methods of transmission included the reuse of needles/syringes/intravenous administration sets between blood contamination of multi-dose drug vials, administration of illegally imported blood and plasma products from other countries and direct blood transfusion between horses for the purpose of increasing athletic performance (blood doping).

The EIA cases in this population have highlighted a significant shift in epidemiology of EIA in the U.S. While EIA cases used to be primarily identified in untested or undertested herds with natural fly-bite transmission as the cause of spread, since 2017, the majority of EIA cases annually are now in Quarter Horse racehorses with iatrogenic transmission identified as the cause of spread. In 2022, 96 cases of EIA were confirmed in 16 states and 84 of the cases were in Quarter Horse racehorses with iatrogenic transmission either suspected or confirmed. Given that iatrogenic transmission is a human-caused event easily prevented by good hygiene and biosecurity, these are cases of disease spread which could have been prevented. Additionally, some of these clusters of cases have an identified source of horses illegally moved into the U.S. from Mexico, where both EIA and EP are endemic and have high prevalence.

Recent investigation of bushtrack racing by state and federal animal health officials has identified at least 121 bushtrack located in 28 states, although many more have yet to be found. Increasing numbers of bushtrack venues and higher participation levels have been fueled in recentyears through social media platforms. These platforms have become the main method by which bushtrack event organizers and participants communicate race dates, advertise match ups and acquire new fans and spectators. While most bushtrack races draw several hundred spectators each race day, some high-volume venues routinely support several thousand spectators. Since no rules are in place for horse or rider safety, many welfare issues are commonly encountered. Participating horses are maintained on performance enhancing drugs and all horses are medicated on race day, with some given narcotics immediately before the race. Horses are raced lame, racing surfaces are poor, whips are used excessively, electronic shock devices are employed and catastrophic breakdowns during the race are common. Most races are recorded on video and posted to social media platforms, so evidence of these welfare issues is readily available. Many other illegal activities have been documented at these venues beyond illegal racing and gambling, so coordination across multiple state and federal authorities would need to be employed to address the situation with any effect.

Bushtrack racing at the current level of occurrence has significant negative impacts on sanctioned horseracing. As previously noted, frequent cross-over of horses between bushtrack and sanctioned races has been documented and exposes sanctioned horses and racetracks to potential EP and EIA transmission. Additionally, rampant drug use in bushtrack horses causes long-term damage to horse health and impacts future breeding performance for stallions and mares recovered from bushtrack circuits. Further, welfare issues observed at bushtrack and seen in internet-posted race videos are perceived by the general public as occurring in all horseracing, resulting in a negative stigma on sanctioned racing.

From an animal health and infectious disease perspective, veterinarians play a critical role in identifying horses that may be participating in bushtrack activities and providing disease, hygiene and biosecurity recommendations to horse owners to prevent EIA or EP infection. Unfortunately, bushtrack participants rarely use a licensed veterinarian, so opportunities to educate or test horses may be sparse. As a result, the best opportunity to test horses leaving this population is during veterinary prepurchase examinations for clients converting these horses to a new athletic focus after racing. Equine practitioners should routinely test current or former Quarter Horse racehorses for both EP and EIA to prevent spread of these diseases into other equine populations.

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## Gastroduodenal ulceration syndrome in foals in central Kentucky from 2020-2022

Gastroduodenal ulceration syndrome (GDUS) in foals is a recognized but poorly understood disease syndrome in which foals may display decreased growth, colic, bruxism or no apparent clinical abnormalities. Ulceration of the stomach can be confirmed via gastroscopy and, in a subset of cases pyloric, duodenal stenosis can be identified. This then leads to decreased gastric emptying and may result in gastric reflux or rupture. Once stenosis occurs, medical management is often unsuccessful and surgical intervention may be required. Surgical intervention carries a significant financial investment and studies report variable prognosis for long term survival. Medical management may include supportive care, anti-ulcer medications and treatment of any inciting disease, such as enteritis.

A portion of foals with GDUS will perforate the stomach or duodenal resulting in septic peritonitis and death. Foals which died or were euthanized due to gastroduodenal ulceration syndrome and submitted to the University of Kentucky Veterinary Diagnostic Laboratory had additional samples collected for testing with specific emphasis on the presence of common infectious agents that cause diarrhea in horses.

From 2020 to 2022, 16 foals with gastric-duodenal ulceration syndrome were submitted for necropsy and further testing. Ages ranged from 3 weeks to 5 months (average of 63 days) with 14 Thoroughbreds, one Warmblood and one unspecific breed. In all cases, aerobic and anerobic culture of the large and small intestine, as well as PCR for equine coronavirus, equine rotavirus A, *Lawsonia intracellularis*, *Salmonella, Clostridium perfringens* and *Clostridioides difficile* was performed. The pH of gastric and duodenal fluid was measured in 15 of 16 cases and a fecal flotation for parasites performed in 14 of16 cases. Equine Rotavirus A was detected in 8 of 16 cases; *Salmonella sp*. in one of 16 cases; and *C. difficile* in two of 16 cases. Gastric pH ranged from 3.22 to 6.87 (average of 5.2) and duodenal pH from 4.49 to 8.1 (average of 6.29). Visually apparent lesions were evident in the stomach in all cases, with ulceration (16 of 16) and perforation (nine of 16) most commonly observed.

Findings within the proximal duodenum and jejunum included enteritis (10 of 16), ulceration (three of 16) and perforation of the duodenum (three of 16). Microscopic findings included severe hyperkeratosis and necro-ulcerative gastritis of the non-glandular stomach, necrosis, ulceration and fibrosis of duodenum and inflammation of the outside of the intestines in cases of perforation. Additional findings included hepatitis (six of 16) and pancreatitis in (five of 16). Interestingly, findings from this group highlight the presence of rotavirus A in 50% of foals with gastroduodenal ulceration syndrome as well as the presence of concurrent hepatitis and pancreatitis in 31% of cases. Inflammation of the intestine, liver and pancreas is similar to the syndrome referred to as triaditis, which is recognized in small companion animals. This extension of infection and inflammation outside of the stomach and intestine may contribute to morbidity and mortality in these cases and may have diagnostic significance if correlations between hepatic values or pancreatic enzyme levels and GDUS can be identified. The significance of the high rate of PCR positivity for equine rotavirus A is not well understood and warrants further investigation.

Importantly, the primary cause of gastric ulceration, hyperkeratosis and gastritis in these cases is still unknown. Possibilities include stress, dietary changes, abnormal gastric pH secondary to motility abnormalities and vascular compromise. While an in-depth epidemiologic evaluation would be required to identify risk factors contributing to this condition, these overall findings provide some interesting insight into a common condition in foals.

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