EQUINE DISEASE QUARTERLY

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IN THIS ISSUE

COMMENTARY

I have not failed. I have just found 10,000 ways that don't work.—THOMAS A. EDISON (1847-1931)

WITH SICK ANIMALS, PEOPLE OFTEN THINK negative diagnostic results are "bad" results, since the cause of disease is still unknown. However, we would do well to remember Thomas Edison's mindset, shown in his famous quote above. If blood test results indicate that a horse has normal bloodwork (including kidney and liver function tests) and no evidence of anemia or infectious disease, much information can be gained from these "negative" results. If basic test results are all normal, it tells the veterinarian which organ systems are functioning within normal limits. Far from being unnecessary expenses, these so-called "negative" test results can point the way to the next steps in diagnosing the real problem. As Dr. Neil Williams points out in his article in this issue, despite a complete postmortem and multiple diagnostic tests for infectious diseases, the cause of equine abortions sometimes cannot be determined.

In 1977, Thoroughbred mares in Newmarket, England, developed an unusual grayish vaginal discharge after being bred. Their failure to conceive caused significant concern. Standard aerobic culturing failed to detect conventional bacterial causes for endometritis; examination of cells showed a white blood cell response. Since all routine diagnostic methods had shown negative results, other diagnostic laboratories were consulted for further assistance. Eventually a vaginal swab from an affected mare was taken to Dr. Eddie Taylor's Public Health Laboratories in Cambridge for testing using methods to detect human gonorrhea (reduced oxygen culturing on hemolyzed blood agar). This testing showed the growth of a new, slow-growing Gram-negative coccobacillus. The equine disease is now known as contagious equine metritis (fortunately not horse gonorrhea!); the bacterial cause is *Taylorella equigenitalis*.

Who would have thought to take a mare vaginal swab to a public health laboratory? By ruling out other causes of equine vaginal discharge and still running into negative results, veterinarians and scientists were forced to think outside the box.

In another example, in 1966 a mathematician wrote an article speculating that a protein could replicate on its own. "Self-replication and Scrapie" was written by J.S. Griffith of Bedford College, London. He proposed a self-replicating protein in association with scrapie, now known to be caused by a prion protein. Scrapie is a transmissible spongiform encephalopathy of sheep, just as bovine spongiform encephalopathy (BSE) is of cattle. Griffith never wrote again on scrapie, yet his paper was read (out of a textbook in a library) by researchers some 15 years later, shortly before the devastating outbreak of BSE began in England.

While no transmissible spongiform encephalopathy of horses yet exists, let us hope that libraries, textbooks, and "think-outside-the-box" scientists never go out of vogue.

Just because something doesn't do what you planned it to do doesn't mean it's useless.—THOMAS A. EDISON

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LLOYD'S

INTERNATIONAL

Third Quarter 2010*

THE INTERNATIONAL COLLATING CENTER, Newmarket, United Kingdom (UK), and other sources reported the following disease outbreaks.

One case of contagious equine metritis (CEM) was reported from France and limited numbers of cases were reported from Italy. The USA reported no spread of infection from the Arabian stallion in California that was cultured positive for *T. equigenitalis* earlier this year. Also, 226 stallions in 24 states were tested as part of a voluntary national stallion screening program for CEM, all with negative results.

Outbreaks of equine herpesvirus-1 (EHV-1) were reported from Germany (one case), Ireland (one case of abortion), Japan (two cases of abortion), and South Africa (10 abortions). EHV-1 myeloencephalopathy was recorded in Italy (three cases in Standardbreds at a racetrack), Japan (one case), and the UK. The UK outbreak involved seven recently imported donkeys, most of which developed respiratory signs, with two also exhibiting signs of mild ataxia and bladder paralysis. Ireland reported one case of respiratory disease due to EHV-4.

Equine influenza was reported from the UK and the USA. Six outbreaks were diagnosed in the UK, most involving isolated cases in non-vaccinated equids. In some instances, affected animals had a recent history of importation or having come from a dealer's yard. The causal strain of virus in all instances belonged to the Florida sublineage clade 2 of the American lineage H3N8 equine influenza virus. The USA reported four cases at a racetrack in Kentucky.

Outbreaks of strangles were recorded in France (10 premises), Germany, Ireland, Singapore, South Africa (five premises), Sweden, Switzerland, the UK, and the USA. Sweden, the UK, and the USA consider the disease endemic in their respective equine populations.

France, Spain, and the United Arab Emirates (UAE) consider equine piroplasmosis (EP) endemic in their respective equine populations. The USA reported infection with *Theileria equi* in 410 of 2,362 horses tested, the vast majority on the index premises in southern Texas reported previously. Positive horses in six states were epidemiologically linked to that premises. Testing for EP is required for entry to sanctioned racetracks in New Mexico, Texas, Oklahoma, Colorado, and Louisiana. While nearly all of the seropositive horses identified so far have been positive for *T. equi*, a very small number of horses with *Babesia caballi* have also been detected. Epidemiologic investigations and testing are continuing.

The USA reported 188 cases of Eastern equine encephalomyelitis (EEE) during the third quarter of 2010. Florida (91) and Michigan (56) had the majority of cases, with fewer cases in eight additional states.

West Nile encephalitis (WNE) was reported from Italy, Spain, the UAE, and the USA. There were outbreaks in three locations in Italy. Spain and the UAE each confirmed mild infections in two horses. In the USA, 101 cases were diagnosed: California (38 cases), Florida (17 cases), and the remainder in 22 other states. The vast majority of affected horses were not vaccinated against WNE or the vaccination history was not up to date.

Equine infectious anemia (EIA) was reported in France, Germany, Italy, and the UK. Cases were diagnosed on three premises in France, some representing horses imported from Romania. Germany reported 12 EIA cases. Movement restrictions have been imposed, and epidemiologic investigations are ongoing. Italy reported one outbreak of EIA and provided findings from a national survey of all equids in the country. Inapparent EIA infections were diagnosed in 133 of 150,733 horses (<0.1%), 12 of 7,433 donkeys (0.16%) and 92 of 861 mules (10.7%). The UK reported isolated cases on separate premises, including one equid that had recently been imported.

Reports of equine viral arteritis were received from Germany (three clinical cases) and Italy (7.2% prevalence, with the highest prevalence in Standardbreds). Four carrier stallions (mostly Standardbreds) were allowed to breed under restrictions.

Potomac horse fever (five cases in Kentucky), Hendra virus infection (one case in Queensland), and grass sickness (two cases in Switzerland) were also reported.

**Second Quarter Report for Australia*

Equine Disease Quarterly

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NATIONAL

Microbial Colonization of the Foal's Gastrointestinal Tract

THE GASTROINTESTINAL (GI) TRACT OF THE normal adult horse contains a complex community of microorganisms. Most of these microbes are located in the large intestine, where they assist in the digestion of food (especially fiber) and synthesize compounds (such as some vitamins) that are important to the horse's health. Most of the helpful microbes in the gut of the normal horse are non-pathogenic, and they are typically called the normal flora. It is generally believed that some pathogenic organisms also reside in the GI tract, but these organisms are kept in check because they have to compete with the normal flora.

In foals, the colonization of the GI tract by pathogenic organisms can lead to diarrhea. Considerable research has focused on identifying the organisms responsible for neonatal diarrhea, but less effort has been made to identify the factors that allow the pathogenic organisms to become established in the foal's GI tract. Additionally, very little is known about how or when the normal GI flora becomes established in the foal.

Researchers at the University of Kentucky have been studying the development of digestive capacity in the foal for several years. An initial project indicated that the very young foals have a low capacity for fiber digestion but older foals have a capacity similar to their dams. Because fiber digestion is performed by specific bacteria in the large intestine, these observations suggested that foals develop a normal microbial population in their GI tract by at least 1 or 2 months of age.

In the past, study of the GI flora has been limited by the availability of accurate, economical, and relatively time-efficient laboratory methods. New molecular techniques are now being developed that will facilitate the study of the equine flora and hopefully enhance our knowledge of this important part of equine health. We have recently used an analysis called denaturing-gradient-gel-electrophoresis (DGGE) to compare the similarity between the fecal flora of neonatal foals and their dams from birth through about 12 weeks of age.

Because most of the microbial population of the horse's GI tract resides in the large intestine, feces are an accepted and non-invasive substitute for actual large intestinal contents. For DGGE, microbial metagenomic DNA is isolated from the feces and amplified by polymerase chain reaction (PCR). The resulting amplicons are then separated on a gradient gel to produce a banding pattern that represents the diversity of the microbial community. The number and placement of the bands are distinctive to the community that was sampled.

On the first day after foaling, the similarity between the microbial DNA in mare feces and foal feces was low. However, the similarity rapidly increased, and by 2 weeks of age mares and foals appeared to have similar microbial populations in their feces. None of the foals in this study suffered from pathogenic diarrhea, so these observations suggest that the foal's gastrointestinal tract is colonized by normal microbes within a few days of birth.

Several questions remain in our study of the colonization of the foal's GI tract. It is unknown whether a failure of the normal flora to colonize the gastrointestinal tract increases the susceptibility of the foal to pathogenic diarrhea. If so, strategies to enhance the development of the normal flora might be beneficial to the foal. Our research group examined the effect of a prebiotic on the incidence of diarrhea in foals in a two-year study. In the first year, there was a trend for the prebiotic to decrease the number of days foals were treated for diarrhea. However, in the second year there was a low incidence of diarrhea in both treatment groups (control and prebiotic-treated), and therefore no significant effect of the prebiotic was noted.

In the future we hope to study the effects of both prebiotics and probiotics on the development of the GI flora of the foal. In addition we would like to identify the normal progression of microbes that colonize the foal's gastrointestinal tract in the first few days of life.

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What are Prebiotics and Probiotics?

Prebiotics and probiotics are used for a similar purpose: to optimize the microbial community of the gastrointestinal tract. However, they have different mechanisms of action.

- **A prebiotic is a substance that provides nutrients to specific and desirable microbes in the digestive tract. Common prebiotics are various types of fermentable compounds (usually carbohydrates). The goal of a prebiotic is to stimulate the growth of beneficial organisms that already inhabit the gastrointestinal tract, particularly the large intestine.**
- **A probiotic contains live organisms, such as *Lactobacillus* or other microbes. The goal of the probiotic is to introduce beneficial organisms to the gastrointestinal tract. To be effective, the probiotic organisms must be alive at the time of ingestion and must be able to live in the gastrointestinal tract.**

The Changing Face of Mosquito-Borne Diseases: 2010

MOSQUITO-BORNE VIRUS INFECTIONS OF the horse pose a continuous and expanding threat to equine health in the United States and internationally. The majority of equine mosquito-borne diseases in the United States today are preventable by immunization; however, the delivery of preventive health care to the equine industry can be improved.

In the last 40 years, two arthropod-borne equine pathogens were introduced into the United States. The virulent 1AB epidemic strain of Venezuelan Equine Encephalomyelitis (VEE) virus made its entry in 1971 with much warning and sufficient time to prepare. It was eradicated by strict local quarantines, large-scale aerial spraying for vectors, and extensive mandatory immunization of equids. The effective response was accomplished at great expense because VEE was judged an important human as well as equine disease.

West Nile virus (WNV), first seen in the United States in 1999, was unanticipated and challenged the U.S. diagnostic infrastructure. Once the virus spread from the initial epicenter in New York State, it quickly established itself in multiple mosquito species and a diversity of susceptible vertebrates. This establishment caused unprecedented viremia levels in some bird species and high mortality rates in numerous bird species. Its transcontinental spread was unparalleled. A safe and effective vaccine to protect horses against the now-endemic WNV was rapidly developed and conditionally licensed in August 2001. Several additional WNE vaccines have since been produced.

The distribution and host range of Eastern Equine Encephalomyelitis (EEE) virus has not significantly changed over the last 20 years. EEE virus is the arbovirus of great concern to horses and humans because of high case-fatality rates (approximately 40% in humans and 90% in horses). Safe and effective vaccines for EEE in horses are widely available. Regretfully, the vast majority of cases in horses each year continue to be in animals that have had no history or an incomplete history of EEE immunization. Although the capability of stimulating durable protective immunity against EEE with a single immunization would be welcome, available vaccines provide an excellent level of protection if administered appropriately. The American Association of Equine Practitioners considers both WNV encephalitis and EEE of such significance that it recommends the vaccines against those diseases be considered "core vaccines."

Even though the United States has not had an equine case of VEE since 1971, the risk of its re-introduction remains. The virus exists as multiple subtypes in nature (I-VI), and while subtypes 1AB and 1C are the epidemic strains of the virus, the endemic 1E subtype caused clinical disease and deaths in horses in southwestern Mexico in 1993 and 1996. Recent outbreaks of a horse-virulent strain in Central/South America serve as a reminder that active surveillance must continue if we are to be prepared to protect our equine population.

It has been nearly 20 years since the last reported equine case of Western Equine Encephalomyelitis (WEE) occurred in the United States. The fact that the virus is endemic in some of the western states should not be overlooked; mutations could yield strains with greater potential for epidemic disease.

Active surveillance and reporting must remain at a heightened level to prevent or limit the spread of "exotic" vector-borne diseases that could affect horses in the future. Furthermore, increased education and awareness of the value of vaccination in preventing the major diseases of horses are critical.

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Expect changes in mosquito-borne disease epidemiology in the future, because a single mutation can:

- Change the insect host range of a virus
- Alter the vertebrate host range of a virus
- Affect the pathogenicity of a virus

AND:

- Mutation is a constant finding in insect-transmitted RNA viruses.

KENTUCKY

Equine Abortion of Unknown Cause

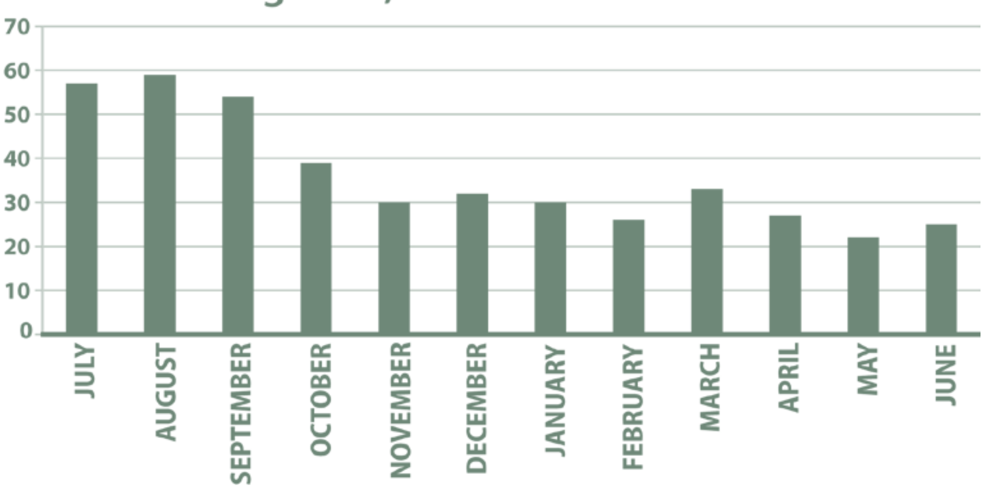
UNFORTUNATELY, LOSS OF A PREGNANCY is a relatively common occurrence in horses. It is generally accepted that only 80% of mares bred will give birth to a live foal at term. The loss of the developing foal (fetus) during gestation is somewhat arbitrarily divided into two groups. The loss of a fetus in the early stages of pregnancy is referred to as an early embryonic loss (generally less than 40 days of gestation); a loss later in gestation is referred to as an abortion. Determining the cause of early fetal losses is very difficult, and in many instances, the aborted fetus is reabsorbed or lost. The success of determining the cause of an abortion later in gestation is greater, but unfortunately the actual cause in many of these cases goes undiagnosed as well. Studies in cattle have shown that in up to 50% of bovine abortions the cause is not determined.

In humans, it is estimated that 15-20% of all known pregnancies in North America end in miscarriage (miscarriage is a lay term for a spontaneous abortion in women). The majority of human miscarriages occur during the first three months of pregnancy, and more than 60% of these are believed to result from chromosomal defects in the fetus due to maternal or paternal factors.

A review of equine fetuses submitted to the University of Kentucky Veterinary Diagnostic Laboratory over a two-year period encompassing the 2008 and 2009 foaling seasons revealed that 1,308 fetuses/fetuses and placentas were submitted. Full-term fetuses that died from birth-related trauma, dystocia, or asphyxia were excluded, leaving a total of 921 aborted fetuses. The most common diagnosis category was abortion due to an infectious cause, with 301 cases, or 33%. Of these, the more common diagnoses were placentitis, with 174 cases (19% of overall total), bacterial abortion/septicemia (48 cases, 5%), and viral abortion (25 cases, 3%). The most common non-infectious cause by far was torsion of the umbilical cord (126 cases, 14%). However, there were 289 cases (31%) in which no cause for the abortion was found. The highest percent of non-diagnosed abortions occurred in the months of July to October, when the fetuses were typically of younger gestational age (Figure 1). As the fetuses aged and approached their due dates, the likelihood of a diagnosis increased.

Cases of abortion in which the cause is not determined are frustrating to the owner/manager, veterinarian, and the pathologist. These cases can be referred to as an idiopathic abortion, no diagnosis, or an abortion of undetermined etiology. While these diagnoses are not welcome on a report, all is not lost. Such a diagnosis simply means that there is no explanation for the abortion by examination and testing of the fetus. A positive outcome is that an infectious disease was not found in the fetus or membranes. Diagnostic laboratories are very adept at diagnosing infections of the fetus/membranes, and if pathogens are not found, the likelihood of an infectious abortion is low. Since infectious agents are often those that can result in multiple abortions or "abortion storms" in a

Percentage of Equine Abortions with No Diagnosis, 2008 - 2009



herd, this determination allows the farm owner and staff to rest easier.

Likewise, a number of other common causes of abortion can be excluded through routine testing and pathology. Therefore, even if the etiology of the abortion is still not found, many diseases and conditions can be documented as not existing. Often the pathologist also will note additional information about the case that can be helpful. When no fetal reason for the abortion is found, other possible explanations are considered. In addition to undetected genetic components or as yet unknown fetal factors, maternal problems should be considered, including those of genetic, metabolic, anatomic, endocrinologic, immunologic, and microbiologic origin. Horse breeders generally recognize the importance of diagnostic testing on all abortions but should also realize that in a significant number of cases, a precise cause of abortion may not be found by examination of the fetus and placental membranes.

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