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IN THIS ISSUE

University of Kentucky

College of Agriculture

Department of Veterinary Science

LLOYD'S

COMMENTARY

Equine protozoal myelitis (EPM) is a significant equine neurologic disorder which can result in loss of use, debilitating disease or fatality. The causative organism, *Sarcocystis neurona*, which was first identified in the United States during the 1970s, affects the central nervous system (CNS) including the spinal cord, brainstem or brain. These regions of the CNS can be affected individual (focally) or in combination (multifocally). Therefore, clinical signs can be quite variable.

The disease should be included in the differential diagnosis of a wide variety of equine neurologic conditions. However, recently EPM has been blamed for everything short of high taxes and El Niño. Antemortem diagnosis of EPM is difficult. Isolation of the causative agent and the subsequent development of immunoblot testing that is specific for *S. neurona* have provided clinicians with an antibody test which can aid in the diagnosis of EPM. However, reliance on the western blot (WB) has resulted in over-diagnosis of the disease.

Serum positive WB indicates exposure to *S. neurona*. At postmortem, the cerebrospinal fluid (CSF) WB performs well, with a sensitivity and specificity of 89%. However, the test performs differently when applied in the antemortem situation. Surveys of neurologically normal yearlings at individual farms have identified as high as 100% CSF WB antibody positive. A review of more than 1000 cerebrospinal fluid taps performed over the last five years found 31% of horses "screened for EPM" (neurologically normal) to be WB positive while 29% of horses with a neurologic condition, gait abnormality or other client complaint were CSF WB positive.

There are numerous explanations for the presence of *S. neurona* antibody in CSF of clinically normal horses. Blood contamination of CSF at the time of collection is one of the more obvious potential causes of CSF *S. neurona* antibody presence. How much blood from a serum positive horse is adequate to result in a positive CSF WB reaction? Is simply counting red cells adequate? These questions are yet to be answered.

A second plausible explanation for CSF positive normal individuals is the fact that in health the intrathecal immunoglobulin profile is a representation (microfiltrate) of peripheral immunoglobulin. Therefore it is not possible that the WB identifies normal CSF antibody presence in individuals that are chronically antigenically stimulated.

Other explanations for CSF *S. neurona* positive are blood brain barrier compromise, the presence of the organism with the absence of clinical disease, and persistence of antibody response subsequent to antigen removal.

In conclusion, the WB for *S. neurona* antibody has improved the antemortem evaluation of the neurologic patient. As with other diseases, antibody tests are an adjunct to diagnosis and rarely can be considered as definitive. The *S. neurona* WB should be considered a useful addition in the diagnosis of equine neurologic disease. The use of the WB alone or the use of the WB in clinically normal individuals will lead to false positive diagnosis and inappropriate therapy.

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INTERNATIONAL

Fourth Quarter 1997

The International Collating Center, Newmarket and other sources provided the following information.

The period was relatively quiet with no major epizootics. Cases of influenza were reported from France, Sweden, United Kingdom and the United States, where horses of many different breeds throughout the state of California showed respiratory signs. EHV-1 abortion cases were reported from Australia and New Zealand, and strangles from Australia, Sweden and the United Kingdom.

A few cases of African horse sickness were reported from South Africa and an increasing incidence of equine piroplasmosis was reported from Switzerland. Cases of respiratory disease attributable to EHV-4 were common among weanlings in Kentucky.

The USDA reported that the last case of vesicular stomatitis was confirmed in the western states during mid-November. Consequently, as of the beginning of February, Kentucky no longer required horses entering Colorado, New Mexico, Utah and Arizona to be tested for vesicular stomatitis.

NATIONAL

CEM in the USA

During December 1997 and January and February 1998, the bacteria causing contagious equine metritis (CEM), *Taylorella equigenitalis*, was identified from three Warmblood stallions recently imported from Germany. The organism was isolated from the test mares each stallion covered or the reproductive tract of the stallions during the mandatory testing procedure prior to release from Federal Quarantine. The stallions were tested in New Jersey, West Virginia and Kentucky with the streptomycin sensitive strain of *T. equigenitalis* isolated on each occasion.

A CEM-like organism was isolated from a male donkey in California and two male donkeys in Kentucky during the same time period. Although the bacteria is similar in many respects to *T. equigenitalis* there are differences observed in the laboratory and further studies are underway to characterize the bacteria. The donkey in California has had semen collected over the last three years to artificially inseminate mares and female donkeys but to date no further isolations have been made based on extensive tracing and testing of mares both within and outside the state of California.

In Kentucky three mares recently covered by another male donkey tested positive to a CEM-like bacteria. From additional investigations undertaken by federal and state officials, a second male donkey, Paint horse stallion and three mares on the premises from which the male donkey originated were also identified as positive. Of 13 nurse mares examined which the initial donkey covered in 1997, the majority of which are pregnant, one has tested positive. All swabs from these animals have been examined at the Livestock Disease Diagnostic Center in Lexington by microbiologist Dr. J. Michael Donahue.

Prior to the onset of the breeding season it was recommended by the Kentucky Department of Agriculture that teaser stallions and nurse mares in the state should be tested for the presence of this CEM-like organism.

Genetic Marker Analysis of Feral Horses

Since the passage of the Wild and Free-Roaming Horse and Burro Act in 1971, the Bureau of Land Management (BLM) has been charged with managing wild horse populations on public lands. The management policy must often obtain a balance between preserving the horse herds and maintaining the often delicate ecosystems in which the horses live. To maintain this balance, horse population sizes must be kept at levels low enough to prevent the herds from damaging the public lands.

Since horses have few natural predators this means periodic removal of horses. However, population sizes small enough to prevent ecological damage may pose problems to the long-term health of the horse herds. Small population size is directly related to the loss of genetic variability and an increase in inbreed-

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ing, and both processes can have deleterious consequences. Modern techniques of genetic marker analysis can be used to develop management plans that can help maintain genetic variation in small populations. The Equine Blood Typing Research Laboratory (EBTRL) of the Veterinary Science Department of the University of Kentucky has been involved in such studies of feral horse populations for a number of years.

Genetic marker analysis has several uses that relate to the development of management strategies. The primary use is to give an estimate of the level of genetic variation present in the current population. The type of management decisions that can be made will be different for populations that have relatively high variation compared to those that already have low genetic variability.

Another use is to determine if there is population substructure. It is easier to preserve genetic variation in a naturally subdivided population than in one that is not subdivided. This is because each different subdivision will maintain different sets of the total genic diversity of the total population. Genetic markers also can identify populations that would be most suitable as a source of immigrants if a population has low variation and needs an infusion of new genes to restore variation and reverse inbreeding.

There are still a large number of feral horse populations on public lands managed by either the BLM or the National Park Service (NPS). Most of the wild horse populations are in the Western states but there are still a few on East coast barrier islands.

At the EBTRL we have now genetically examined one herd from Cumberland Island, Georgia, five from the outer banks of North Carolina and the Chincoteague Virginia herd. From the western United States we have tested herds in the Theodore Roosevelt National Park, North Dakota, the Pryor Mountain, Montana, and several populations from BLM management areas in Colorado, Wyoming, New Mexico, Utah, Arizona and Oregon. The Theodore Roosevelt and Pryor Mountain herds both have been tested three times since 1991, although the 1997 data have not been fully analyzed. This repeated testing allows for a detailed genetic analysis of these populations and the opportunity to examine trends in genetic variation through time.

There is a great range in genetic variation within the feral horse populations. Most populations were extremely healthy; however, two of the populations with the lowest genetic variation showed problems that could be related to inbreeding. One herd had very low reproductive rates (possibly due to inbreeding but also possibly due to mountain lion predation on foals) while the other had a significant number of blind horses and a few dwarf horses in the herd.

Management strategies for each herd must be developed on a case by case basis as the circumstances related to the management of individual herds are unique. Genetic marker analysis is a powerful tool that increases the probability that the management plans that are put into operation will be successful.

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Foal Vaccination for Equine Influenza

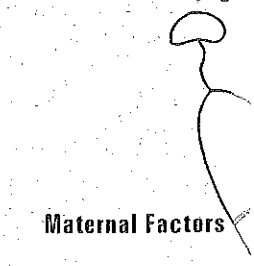
Influenza is among the most common upper respiratory diseases of horses. Foals are not usually clinically infected with influenza because the dam's colostrum provides the newborn with protective maternal antibodies (assuming the colostrum has antibodies to influenza). As foals get older their maternal antibodies decay and they become susceptible to influenza, especially as yearlings and two-year-olds.

The current veterinary practice is to begin an immunization schedule for equine influenza between two and six months of age. However, researchers in Europe have questioned the effectiveness of this strategy due to possible interference from maternal antibodies. Recent work in our laboratory, presented by Dr. H.S. Conboy at the 1997 American Association of Equine Practitioners Convention, gauged the effectiveness of influenza vaccination starting at different ages of the foal to determine any maternal antibody interference. This information is needed by practitioners to determine an effective influenza program in foals.

Our study sample included 187 foals whose dams were vaccinated with inactivated influenza vaccine approximately 30 days before parturition. Foals received maternal antibodies in the first 24 hours of life via colostrum, as verified by serology. The hemagglutination inhibition (HI) serological assay was used to test foals' sera for antibodies to the A1 and A2 subtypes of equine influenza.

The 187 foals, divided into 6 groups, were vaccinated with the standard 2 doses of vaccine 1 month

Figure 1
Maternal Recogni



apart, starting at either 2, 3, 4, 5, 6 or 7 months age. The 2-dose course of vaccination did not induce detectable antibody production in *any* foal against A2 virus, the subtype presently found in nature. Even three doses of vaccine given at 7, 8 and 9 months of age induced seroconversion to A2 virus in only 25% (3/12) of foals.

Vaccination against A1 virus was slightly more efficient although three doses (at 7, 8 and 9 months) were still required to produce a seroconversion rate greater than 50%. Therefore, during approximately the first seven months of life, conventional equine influenza vaccines fail to produce a protective antibody response in foals from vaccinated dams.

In another study, foals which lacked maternal antibodies to either A1 or A2 influenza would seroconvert specifically to that strain after vaccination at just 3 to 4 months of age. This is the first direct evidence that maternal antibodies are responsible for the failure of foal vaccinations. Since virtually every dam in the US has prior exposure to influenza, thereby having some level of maternal antibody to it, we expect that most foals will fail to seroconvert to influenza vaccination.

At present the only known solution is to wait until foals are old enough, i.e., weanlings of at least 8 months age, before beginning a vaccination program for influenza. This information should not be extrapolated to other vaccinations until specific studies are completed to determine the influence of maternal antibody on specific vaccine antigens.

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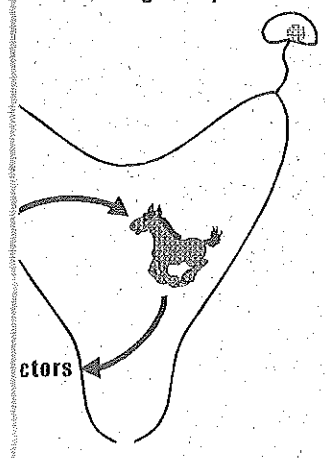
tion of Pregnancy



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Conceptus-Maternal Communication in Horses

Early conceptus loss causes a significant financial burden to horse breeders. In the January 1998 issue of the Lloyd's *Equine Disease Quarterly*, maternal recognition of pregnancy and the relationship of progesterone, prostaglandin $F_2\alpha$ (PGF) and oxytocin were discussed. This article will examine some aspects of the communication that occur between the conceptus and the maternal environment during the first few critical weeks of gestation. (See **Figure 1.**)

One of our approaches in studying conceptus-maternal interactions is to look at changes in protein production. We have examined proteins synthesized and released by equine conceptuses and from uterine endometrium and oviducts of cycling and pregnant mares. Major proteins synthesized and secreted by the uterine endometrium include retinol binding protein and oxytocin.

Retinol binding protein carries metabolites of vitamin A to the developing embryo and placenta. Studies in laboratory animals have shown that these metabolites are responsible for proper formation of the developing embryo. Either too much or too little of the vitamin A metabolites are lethal to the embryo. Uterine oxytocin may be involved in regulating PGF production and maternal recognition of pregnancy, as discussed previously.

Major protein products of the equine conceptus include transferrin and alpha-fetoprotein. Transferrin serves to carry iron to the developing embryo and placenta. Iron is important in a variety of functions, including formation of hemoglobin, and is necessary during early development to establish the circulatory system for the embryo and the placenta. Transferrin also functions in cell growth, immunoregulation and organ differentiation.

Alpha-fetoprotein serves a variety of functions, including acting as a transport protein for estrogens and prostaglandins. Alpha-fetoprotein is the main protein of the fetal circulation; it serves many of the same functions that serum albumin serves in the adult animal.

The signals between the conceptus and the maternal reproductive system are controlled primarily by changes in, and regulation of, gene expression. Conceptus-maternal communication can be successful only if the appropriate genes are expressed in the appropriate tissues at the appropriate times during gestation. For example, "turning on" or "turning off" expression of certain genes will allow the conceptus or uterus to start or stop making particular products.

What are some of the genes which the horse conceptus expresses around the time of maternal recognition of pregnancy? In the past 2 years, we have identified 50 gene sequences which are expressed during this time (Simpson, Adams, Behrendt, Baker & McDowell: Identification of genes expressed in day 12 and 15 horse conceptuses by suppression subtractive hybridization. *Biol Reprod 1997*, Suppl 1:88). In addition to alpha-fetoprotein and transferrin, these latest studies identified conceptus production of phospholipase A2 (PLA2), calcyclin, and equine pregnancy associated glycoprotein (ePAG).

PLA2 is involved in PGF synthesis. We know that the conceptus produces a variety of prostaglandins, including PGF, but at this time we do not know how conceptus prostaglandins interact with uterine prostaglandins. Calcyclin is a protein that binds calcium. Studies in laboratory animals have indicated that it may help regulate secretion of other products that the conceptuses produce. It may also be important for the differentiation of the nervous system in the developing embryo.

The function of ePAG is unclear at this time, but it may be involved in mediating maternal immune responses during pregnancy. In addition, studies in ruminants have shown that the PAG's of sheep and cattle conceptuses play a role in maternal recognition of pregnancy in those species.

Understanding more about gene regulation is essential in deciphering how the conceptus and mother communicate with each other. Appropriate conceptus-maternal communication is critical for a successful outcome to pregnancy of all species.

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EPM Workshop

Diagnosis and treatment of equine protozoal myelitis (EPM) have been topics of intensive research and discussion since the disease was first recognized. To address these topics, 40 invited participants attended a workshop in November 1997 sponsored by the Maxwell H. Gluck Equine Research Center, Bayer Animal Health, Neogen Corp., and Equine Biodiagnostics, Inc.

Dr. Noah Cohen of Texas A&M University College of Veterinary Medicine summarized the discussion of the immunoblot testing of equine cerebrospinal fluid (CSF) from neurologically normal and abnormal horses for antibodies to *Sarcocystis neurona*.

Testing of normal horses should be discouraged because the positive predictive value of a positive CSF test result (i.e., the probability that a positive result is from a horse with EPM) is very low in a clinically normal horse. A positive CSF test in a clinically normal horse is much more likely to be a false positive than a true positive. In contrast, the negative predicative value of a negative test in a clinically normal horse is very high, indicating a high probability that a negative test result indicates the horse does not have EPM.

The diagnostic significance of a CSF positive test result is higher (but still undefined) in a horse with neurologic disease. A positive test result in a horse with neurological disease is more likely to be a true positive in certain situations (e.g., a horse with classical clinical signs of EPM) than in others (e.g., a horse without classic EPM signs).

More information regarding the biology and natural history of infection of *S. neurona* is needed to more fully understand the meaning of detecting antibody to this organism in CSF. Because it is improbable that a test will be developed that perfectly differentiates between horses with active infection of the central nervous system with *S. neurona* and those without such infection, some amount of uncertainty and inaccuracy will be inherent in the diagnostic process.

Dr. Tom Divers of Cornell University College of Veterinary Medicine compiled the following information relating to the treatment of EPM: Our ability to treat EPM may be more advanced than our ability to accurately diagnose the disease. Currently the most common treatment is a combination of pyrimethamine (1 mg/kg PO daily) and a sulfonamide (20-30 mg/kg PO daily), given as distant as possible from feedings. No work has established which treatment protocol (pyrimethamine-sulfadiazine vs. pyrimethamine-trimethoprim/sulfa) is more efficacious. About 70% of EPM cases show improvement in their clinical signs with either treatment protocol.

There is a justifiable concern about enhanced toxicity with both pyrimethamine and trimethoprim, although the incidence appears low. The risk of toxicity is greater in pregnant mares, and confidence in accuracy of the diagnosis and/or severity and/or progression of the disease should all be considered prior to treating pregnant mares. Historical experience suggests that mares can be treated without toxicity to the mare or foal. Folic acid supplementation cannot be recommended at this time because it enhances toxicity in conjunction with pyrimethamine.

In some severe peracute EPM cases pyrimethamine has been given at a "double" loading dose (2 mg/kg) for the initial 1 to 2 weeks of treatment. However, enhanced efficacy has not been proven; similarly, the incidence of toxicity may be increased by this approach. The recommended duration of treatment with pyrimethamine-sulfonamides is unknown. Most horses are treated for a minimum of 3 months. If the CSF becomes negative for *S. neurona*

antibody, treatment can be safely discontinued.

It would be ideal if all horses had a negative CSF test prior to discontinuing treatment; this is difficult to recommend for all horses because of the suspicion that some of the antibody in CSF samples submitted to laboratories may be serum derived. A substantial number of observations indicate that the great majority of treated cases are still positive in CSF after 90 days of treatment.

A new development has been the experimental use of diclazuril in horses with EPM. The clinical response is reported to be at least equal to the current pyrimethamine/sulfonamide treatment. There are no reports of serious side effects, and the duration of treatment (21-28 days) and cost of diclazuril are less than for pyrimethamine/sulfonamide treatment. More long-term studies on diclazuril are necessary.

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K E N T U C K Y

1997 EIA Testing Summary

During 1997 a total of 79,291 samples were tested for equine infectious anemia in Kentucky. Private tests accounted for 66,363 samples that were submitted to comply with state regulations, five of which were positive.

In addition, 12,928 samples were collected through our Market Surveillance Program or through epidemiological testing. Of these samples six were reported as positive; four through market testing and the remaining two through epidemiological testing. This compares with 9 EIA positives in 1996; 20 positives in 1995; and 17 positives in 1994.

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