

EQUINE DISEASE QUARTERLY

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COMMENTARY

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MARE REPRODUCTIVE LOSS SYNDROME (MRLS) has acutely sensitized us to the financial and emotional losses associated with abortion or the premature delivery of a highly anticipated foal. A search of the literature indicates that approximately 40% of equine abortions go undiagnosed.

The pathologist is part of a team needed to identify the cause of an abortion, which also includes the farm manager or owner and attending veterinarian. The pathologist is responsible for interpreting the gross and histological findings; others are responsible for submitting the fetus and placenta to the laboratory in a timely manner and providing a thorough history. Most foals are aborted either alive or soon after they die, so tissues are fresh but will quickly autolyze if they remain above 5° C for more than six to eight hours. Autolysis decreases the likelihood of making a diagnosis. A good reproductive history provides clues to possible causes. This history should include the age of the fetus or last breeding date, vaccination history of the mare, illnesses experienced by the mare or other members of the group, and past reproductive performance.

The causes of abortion include infectious, non-infectious, developmental, and toxic conditions. Placentitis is currently the most common cause of abortion. Most fetal infections result from bacteria that normally reside in the posterior genital tract, ascending through the vagina into the uterus through a relaxed cervix. The placenta detaches from the endometrium in the area of the cervical star, decreasing nutritional support to the fetus. The fetus is aborted secondarily to placental insufficiency or as a result of death from septicemia. Some bacterial infections are blood borne, but it is not clear how others, such as *Nocardia*, gain entry.

Other less common causes of abortion include fetal growth retardation secondary to uterine degeneration, umbilical cord torsion, and congenital cardiac abnormalities. Fetal growth retardation is associated with fibrosis of endometrial glands in older, pluriparous mares; placentitis; and metabolic disturbances of the placenta. The cause of fetal growth retardation is often undiagnosed. Umbilical cords that are long (more than 85 cm) are associated with fetal death from excessive twisting and strangulation of the blood supply. Those that are short (less than 45 cm) are associated with tearing at the body wall. A fetus may die following maternal illness or after surgery, especially if surgery is performed in the last 30 days of gestation and the mare is anesthetized for more than 60 minutes. Toxic conditions associated with abortion include ingestion of endophyte-infected fescue during late gestation or ingestion of eastern tent caterpillars.

What is the incidence of abortion in a well-managed broodmare band? Veterinary clinicians that specialize in reproduction would answer 4 to 5%, but there are few published reports on the subject. One source states a rate of 9.3% in Thoroughbred mares after 60 days of gestation. After the losses experienced in 2001 and 2002 from MRLS, a monitoring system is needed to identify increases in abortion rates. An equine reproductive health monitoring program is being developed in Central Kentucky in cooperation with the local equine industry, equine practitioners, and the UK Livestock Disease Diagnostic Center. By working together, the equine community can develop improved methods to identify health problems.

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INTERNATIONAL

Expert Surveillance Panel on Equine Influenza Vaccines

THESE INTERIM RECOMMENDATIONS WERE MADE following a meeting of the OIE Expert Surveillance Panel on Jan. 13, 2003, and relate to the composition of vaccines for the forthcoming year (2003).

Influenza activity, January - December 2002

Outbreaks of equine influenza in the Benelux, Canada, France, Germany, Israel, Italy, Sweden, the United Kingdom, and the United States were reported during 2002. Activity was mainly sporadic, with no epidemics and no reports of international transmission of equine influenza into countries previously free of the disease. All influenza activity was associated with H3N8 viruses. There were no reports of serological or virological evidence of H7N7 (equine-1) subtype viruses circulating in the equine population. Nevertheless, diagnostic laboratories should continue serological and virological monitoring and, when using PCR for rapid diagnosis, should ensure that primers specific for H7N7 virus as well as H3N8 virus are used.

Characteristics of recent isolates

In haemagglutination inhibition (HI) tests with post-infection ferret sera, the European isolates from 2002 were antigenically related to the European lineage prototype strains A/eq/Newmarket/2/93 and A/eq/Suffolk/89, although there was some heterogeneity among the isolates. Isolates from the United States and Canada were all antigenically similar to the American lineage prototype strains A/eq/Newmarket/1/93 and A/eq/Kentucky/94.

The haemagglutinin (HA) sequences of most European viruses from 2002 (including direct sequencing from clinical specimens) were similar to those viruses circulating in the previous year, although a few were genetically more related to viruses circulating in 1991 to 1995. The United States 2002 isolates were genetically similar to other recent American isolates.

European viruses belonging to the American lineage continue to co-circulate with European viruses.

The panel urges the need for increased effort to isolate more viruses from a greater geographic area to increase the likelihood of early detection of emerging antigenic variants.

Recommendations for the composition of equine influenza vaccines

It is recommended that vaccines to be used in 2003 contain the following:

- An A/eq/Newmarket/1/93 (H3N8) or A/eq/Kentucky/94 (H3N8)-like virus (American lineage).
- An A/eq/Newmarket/2/93 (H3N8)-like virus* (European lineage).

**A/eq/Suffolk/89 and A/eq/Borlänge/91, currently used in vaccine strains, continue to be acceptable.*

A Web site devoted to equine influenza is available at <www.equiflunet.org.uk>.

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Equine Disease Quarterly

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Fourth Quarter 2002

THE INTERNATIONAL COLLATING CENTRE, Newmarket, reported the following disease outbreaks:

- Reports from France and Switzerland confirmed the death of horses during November and December in many different locations. Mortality occurred within 12 to 72 hours of illness among young horses on pasture. Clinical signs included ataxia, significant muscle stiffness, depression, dark-colored urine, and sweating. The cause of

the illness has not been identified.

- Three cases of Contagious Equine Metritis (CEM) were diagnosed among Thoroughbreds on separate premises in Japan. A Warmblood stallion imported to the United Kingdom from Germany in 2002 was also positive.
- An increased incidence of the isolation of *Corynebacterium pseudotuberculosis* was reported at the end of the year from horses in Colorado and Kentucky. The disease causes

illness in several large animal species and is often referred to in the horse as *Pigeon Fever* due to the formation of abscesses in the pectoral muscles. The bacteria survive in the soil. Infection develops via skin wounds and may be transmitted by flies.

- Cases of equine herpes virus abortion (EHV-1) were reported from several states in Australia, particularly New South Wales and Victoria, with sporadic cases diagnosed in France and the United Kingdom. A national survey for equine viral arteritis (EVA) has been undertaken in Argentina by the National Services of Animal Health (SENASA) on 381 registered stallions of 21 different breeds. Seven serologically positive Thoroughbred stallions were identified, all imported from the United States with a history of EVA vaccination. Mi-

nor outbreaks of influenza were diagnosed in France, Sweden, and the United Kingdom. Two cases of *Leptospira* abortion were reported during November among mares in Central Kentucky. Multiple cases of strangles were reported on the east coast of Australia and in Ireland, Sweden, and Switzerland.

- In 2002 West Nile Virus (WNV) spread across the United States, reaching the West Coast states of California and Washington. Presently only Alaska, Arizona, Hawaii, Nevada, Oregon, and Utah are considered free of the disease, which is now officially recognized as endemic in the United States. As of Dec. 31, 2002, the USDA reported 14,717 equine cases in 40 states during the year. Four states—Illinois, Iowa, Nebraska and Texas—each reported over 1,000 cases.



NATIONAL Pigeon Fever

HORSES AND OTHER LARGE ANIMALS WORLDWIDE are infected by *Corynebacterium pseudotuberculosis*. There are also reports of infection in humans resulting from exposure to infected sheep and horses. Pigeon Fever, the most common form of the disease in horses, is characterized by external abscesses, primarily in the pectoral area and ventral part of the abdomen. It is commonly diagnosed in California and other arid regions of the western United States and appears to be increasing in other, wetter areas of the country such as Kentucky and Colorado.

Treatment of this form of disease involves establishing drainage of the abscess with lavage and disposal of the abscess contents to prevent further contamination of the environment. Internal abscesses, which can be fatal if not treated appropriately with antimicrobials, are less common than external abscesses. A third form of disease, ulcerative lymphangitis, can result in lameness and lymphatic damage (*big leg*) unless treated aggressively with antimicrobials.

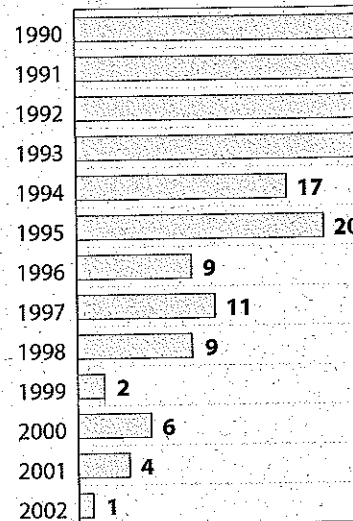
The portal of entry for *Corynebacterium pseudotuberculosis*, a potentially soil-borne organism, is thought to be through abrasions or wounds in the skin and mucous membranes. Insects such as the horn fly, house fly, stable fly, and biting midge have been suggested as potential vectors in horses but have not been confirmed experimentally. Using molecular

techniques, a team of researchers at University of California, Davis is determining the importance of these flies in the transmission of the bacterium to horses.

Corynebacterium pseudotuberculosis is considered to survive for long periods in the soil, and it appears endemic on most California farms and ranches. The prevalence of disease is estimated at 10%, making the syndrome one of the most common infectious bacterial diseases of horses in the state. Persistence of the pathogen in the soil indicates that management techniques rather than eradication efforts will be the best first step toward reducing disease incidence. Further research is needed to better understand the epidemiology and pathogenesis of this disease.

Controlling Pigeon Fever could be accomplished with a coordinated strategy involving two complementary tactics: reducing the probability of transmission and reducing the probability of infection should transmission occur. The disease is seasonal, with peak incidence in the fall months, and the incidence fluctuates from year to year within a site. It is not known whether these patterns result from population growth of the pathogen during the warm months, from population fluctuations of the potential vectors, or both. While vaccines are commercially available for sheep and goats,

Figure 1.
Surveillance Testing in Kentucky
(samples testing positive follow ea



none are available for horses. Administration of bacterins or toxoids offers excellent protection in sheep (more than 90% against experimental challenge). There are strain differences between bacteria infecting horses and small ruminants, so that research is needed to develop a product for use in horses. Dr. Spier has been working with autogenous bacterins/toxoids with the in-

tention of developing a vaccine for horses. Dr. Janet Foley, a UC Davis researcher, is also investigating the strain homogeneity of *C. pseudotuberculosis* collected from horses in California, Kentucky, and Colorado.

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KENTUCKY

EIA Surveillance 2002

DURING THE 2002 CALENDAR YEAR, A TOTAL OF 110,811 samples were tested for Equine Infectious Anemia (EIA) in Kentucky. Private testing accounted for 91,541 samples in order to comply with state regulations regarding the sale and exhibition of equine in Kentucky and meet interstate transportation requirements.

In addition to the private sampling, another 19,270 samples were collected through our state market surveillance program or through epidemiological testing. The surveillance testing resulted in one animal being identified as EIA positive.

As can be seen in the accompanying **Figure 1**, testing of samples has been steadily increasing each year. The evidence suggests that the prevalence of this virus is diminishing in Kentucky's equine population. Late in 2002, the Kentucky Department of Agriculture amended administrative regulations from requiring a negative test within six months to a negative test within 12 months for animals changing ownership or being offered for sale.

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Diagnostic Approaches to Equine Abortion

THE ABORTION OF A FOAL CAN BE A VERY COSTLY occurrence. A potentially valuable individual is lost; a year's production by the mare is lost, even though the fixed costs of maintaining the mare are unchanged; and psychological effects occur from the loss of an anticipated birth after considerable work and planning. Finding the cause of abortion is important. Some diseases causing abortion may require treatment of the mare or can pose a risk to other pregnant mares. Other conditions causing abortion may necessitate changes in management practices, and clusters or outbreaks of abortions can indicate unusual or new diseases or conditions. The University of Kentucky (UK) Livestock Disease Diagnostic Center is uniquely positioned to address this situation.

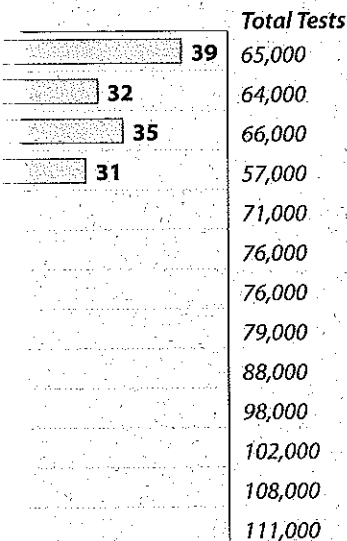
Each foaling season the UK Livestock Disease Diagnostic Center receives over 600 fetuses, placentas, and term foals. These cases represent approximately one-third of all equine submissions. Most involve fetuses from five

months of gestation to term. The UK Livestock Disease Diagnostic Center performs a complete work-up using a standardized approach to the examination of these fetuses and placentas.

Following an abortion, the fetus and fetal membranes should be collected in an undisturbed state, kept cool, and submitted to the laboratory as soon as possible. A detailed history of the mare's current and past reproductive performance is important in the evaluation of an abortion, and a completely filled-out accession sheet is a necessity.

As a part of the case work-up, the fetus is measured and weighed, and the weight of the membranes and length of the umbilical cord are obtained. Examination includes a complete necropsy of the fetus and membranes with fetal tissues and fluids (stomach content, heart blood, and pericardial fluid) obtained. Also, a representative set of tissue is placed in fixative to be processed for microscopic examination. The allantochorion is spread with the chorionic sur-

for Equine Infectious Anemia (bar).



5 face outward, and changes in texture, color, and thickness are noted. The amnion and umbilical cord are also examined for lesions. The laboratory does testing on every submitted fetus for bacterial infection by culturing lung, liver, stomach content, and placental membranes and by dark-field examination of stomach fluid. The fluorescent antibody (FA) test is performed on kidney, liver, and placenta for the presence of leptospire. Tissues are tested for equine herpesvirus by the FA test, and virus isolation is performed to test for the presence of arteritis virus and other equine viruses. In addition, body fluids are tested for the presence of antibodies to leptospira species.

A comprehensive study by pathologists at the UK Livestock Disease Diagnostic Center (Giles, RC, et. al., JAVMA, 203: 1170-1175) of causes of abortion, stillbirth, and perinatal death over a six-year period revealed that infection of the fetus and/or placenta caused by bacteria, equine herpesvirus, fungi, or placentitis of unknown cause was the most common cause of abortion and accounted for 34% of the diagnoses. The second most common cause involved birth complications, including asphyxia, dystocia, and

trauma (19% of cases). Other common diagnoses were placental edema or premature separation of the placenta (7%), twins (6%), contracted foal syndrome (5%), other congenital abnormalities (5%), and umbilical cord abnormalities including torsion of the cord (3%). Less common conditions included placental villous atrophy, body pregnancy, fetal diarrhea, and other miscellaneous conditions. A diagnosis was not made in 16% of the cases.

Such a large caseload of aborted fetuses and placentas provides a rich resource of materials by which to study and monitor equine reproductive conditions. Testing of fetuses at the UK Livestock Disease Diagnostic Center has led to the recognition of leptospirosis as an important cause of abortion in horses and emergence of nocardioform placentitis as a cause of chronic placentitis in mares. It has also allowed for the rapid recognition and characterization of changes in fetuses associated with the Mare Reproductive Loss Syndrome (MRLS) outbreak in 2001.

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Summary of the 2002 MRLS Pasture Monitoring Program

DURING THE SPRING AND SUMMER OF 2002, DATA were collected and analyzed from 13 Central Kentucky farms (12 horse farms and one hay production farm) on a biweekly basis from February 21 to June 28, 2002, to help determine the causal agent or agents responsible for Mare Reproductive Loss Syndrome (MRLS).

Sentinel Farm Activities

- An average of nine visits per farm, 13 farm visits every two weeks.
- An average of 175 samples per farm, which did not include blood and urine samples.
- Six of the monitored farms collected blood (378) and urine (205) samples for future analysis.
- Over 3,000 samples were collected, including blood and urine samples and samples from farm referrals. A total of 83 fields were sampled from the 13 sentinel farms, averaging 6.4 fields/farm.
- Six of the 12 sentinel horse farms experienced losses: 29 early fetal losses (EFL) and 9 late fetal losses (LFL) between April 25, 2002, and June 13, 2002.

- University of Kentucky (UK) personnel spent over 1,700 person-hours of actual time on monitoring activities.

Other farms (nine) were visited when veterinarians or farm managers found MRLS type symptoms and referred them to the University of Kentucky. Losses among these farms totaled 27 EFL and three LFL.

Correlations of Pasture Data to MRLS

Statistical analysis of monitoring data found a significant correlation between cases of MRLS and the presence of black cherry trees in proximity to pasture. There was also an indication that tall fescue alkaloids could account for some late-term abortions on farms without clear exposure to eastern tent caterpillar or black cherry trees. The 2002 monitoring data found that other pasture and environmental parameters were not correlated to MRLS. These included presence of poisonous plants, cyanide content of white clover, mineral content of forages, fungal mycotoxins, soil microbiology, and weather.

Pasture Parameters

Ergovaline and ergovalinine are produced in tall fescue that is infected with the endophyte and can lead to agalactia, foaling difficulty, and prolonged gestation in mares. Some tall fescue had reached or exceeded the toxicity threshold (0.600 ppm) in early May and remained high in ergot alkaloids even into late June. However, no typical fescue toxicity symptoms were observed, possibly because mares were not in late gestation at this time or because tall fescue formed a minor part of the diet.

Total cyanide potential for white clover averaged less than 350 ppm across all farms and was as high as 816 ppm on one farm. All values were below reported values for some agronomically important varieties and therefore would be considered within the normal range.

Nitrate-nitrogen (NO₃-N) levels ranged from zero to 2,112 ppm and averaged less than 600 ppm across all pastures. Horses are much less sensitive to nitrate than cattle, and the levels reported in 2002 were generally safe, even for cattle. Elevated levels of nitrate were not associated with any losses.

One early theory for the cause of MRLS was that pastures in 2001 had excessively high ra-

tios of potassium to calcium. Values for K/Ca greater than 5:1 were suggested to lead to mineral imbalances in the pregnant mare. K/Ca values for most sampling dates in 2002 were less than 5 for all pastures and never exceeded 7. For perspective, the average K/Ca ratio for May 2001 across several farms was 6.76 to 1. However, the K/Ca for 1996 for the same farms in May was 7.58 to 1.

Most fields did not contain detectable levels of any of several fungal mycotoxins. The three principal compounds that did appear in a few pastures were DON (deoxynivalenol), T-2, and zearalenone.

Yeast and mold counts were determined in the soil and were between 200,000 and 2,000,000 CFU/g. High levels of yeasts and molds in soil (especially molds) were theorized to be a good predictor of a field's potential to produce fungal mycotoxins if environmental conditions were suitable. However, no relationship between soil microbial counts and mycotoxins was observed.

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