

EQUINE DISEASE QUARTERLY

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COMMENTARY

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PIROPLASMOSIS, GLANDERS, AND DOURINE ARE reportable equine diseases, designated List B diseases by the International Office for Epizootic Diseases (OIE). Although differing in global distributions, each disease is endemic in equine populations in various parts of the world. Many countries, including the United States, require that Equidae presented for importation be serologically negative for these infections.

The complement fixation test (CFT) has been the most commonly used OIE-prescribed serodiagnostic test for piroplasmosis, glanders, and dourine. Alternative serologic procedures approved by OIE include the indirect fluorescent antibody test (IFAT) for piroplasmosis, the intradermal palpebral mallein (IPM) test for glanders, and the IPAT, enzyme-linked immunosorbent assay (ELISA) or agar gel immunodiffusion (AGID) test for dourine. Though of proven value in detecting serological reactors, the CFT is not without certain shortcomings. These shortcomings have been associated with certain sera having anticomplementary activity, anti-erythrocyte activity, or high levels of immunoglobulin G (I) activity that will not fix complement.

The need to develop more sensitive and more specific serodiagnostic assays for piroplasmosis, glanders, and dourine that are less prone to subjective interpretation has been recognized for some years. Greatest promise is offered by competitive ELISAs (cELISAs) based on inhibition of specific monoclonal antibodies to immunodominant and highly conserved antigenic epitopes that are unique to the etiological agents of these diseases: *Babesia caballi* and *B. equi* (piroplasmosis), *Burkholderia mallei* (glanders) and *Trypano-*

soma equiperdum (dourine). Such assays would negate the shortcomings mentioned earlier.

In the case of piroplasmosis, cELISAs have been developed for both *B. caballi* and *B. equi* that offer at least equivalent specificity and significantly greater sensitivity than the CFT. It has been recognized for many years that CF titers to either of these parasites can be suppressed by treating seropositive horses with certain antibabesial drugs. Treatment of such animals 30 to 60 days before import can result in temporarily reducing antibody titers to below-positive levels. Since treatment is not always curative, especially in the case of *B. equi* infections, antibody titers can revert to their former levels, starting perhaps 30 days after the conclusion of treatment. Treatment of horses seropositive for *B. caballi* or *B. equi* prior to export as a means of avoiding compliance with the regulatory requirements of the importing country is a long-standing subterfuge. Replacement of the CFT with an assay of superior sensitivity such as the cELISA should help address this problem.

The CFT is also the OIE-prescribed test for international trade for both glanders and dourine. Aside from any consideration of test sensitivity, problems occur from time to time with horses from countries historically known to be free from either disease reacting in the CFT with either *B. mallei* or *T. equiperdum* antigens. Significant economic hardship can result from such spurious test results. More specific, sensitive serodiagnostic tests for both diseases are long overdue.

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LLOYD'S



INTERNATIONAL Second Quarter 2003

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

A significant increase in the number of equine cases of Eastern Equine Encephalitis (EEE) was reported in the USA during June compared to numbers in recent years. States reporting cases since are shown in Figure 1. The number of equine cases of West Nile Virus (WNV) reported by the USDA as of September 9 is 1,205, with Colorado, New Mexico, and Wyoming each reporting more than 100 cases. Only two states, Oregon and Nevada, are currently recognized as completely free of WNV.

Equine-confirmed or probable cases of WNV have been reported in five provinces of Canada—Alberta, Manitoba, Ontario, Québec, and Saskatchewan.

Twelve abortions attributable to equine herpes virus (EHV-1) based on serological diagnosis were reported on six farms in Japan. Respiratory disease caused by EHV-4 was diagnosed among several different breeds of horses in France. Coital exanthema (EHV-3) was confirmed in a Thoroughbred stallion, and three mares showed clinical signs in Wales.

Equine influenza was reported from Denmark, France, and on three racetracks in Italy—Milan, Rome, and Turin. In the United Kingdom many racing stables in the Newmarket area were diagnosed with influenza as well as numerous other equine premises in England and Scotland. Among vaccinated horses, particularly those in Newmarket, the clinical signs were mild, including a cough at exercise and some horses with nasal discharge but little evidence of fever. Among non-vaccinated horses in the rest of England, the signs were more severe, including fever, a mucopurulent discharge, and frequent coughing at rest. Laboratory findings indicated the influenza virus causing the outbreak virus was closely related to an American lineage H3N8 virus isolated in Kentucky in 2002.

In the 2003 breeding season there was a dramatic reduction in the number of cases of Mare Reproductive Loss Syndrome (MRLS) in Central Kentucky compared to 2001 and 2002, with less than 10 reproductive and no eye or heart cases. Outbreaks of strangles were reported in Ireland, Switzerland, and the United Kingdom.



Equine Disease Quarterly

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Glanders

GLANDERS CAUSED BY *BURKHOLDERIA MALLEI* IS one of the oldest documented plagues of solipedes. In the times of war from Middle Ages onward and as late as World War I, losses of horses through glanders in the armed forces of several nations had a considerable devastating impact. Although the disease has been completely eradicated from North America, England, and Australia through destruction of affected and suspected animals, the disease still occurs in Pakistan, India, China, Mongolia, Brazil, Middle East, Eastern Europe, and North Africa. Veterinary laws in these countries call for destruction of affected animals. In Pakistan a low indemnity (less than a U.S. dollar) paid to the owners and lack of a rigorous implementation of the Glanders and Farcy Act, 1899 does not encourage affected animals to be destroyed. The report describes an effective glanders cure with a combination of enrofloxacin and potentiated sulphonamide (trimethoprim-sulfadiazine). All animals received both drugs intravenously for 21 days (double doses for the

first week and standard doses subsequently).

An outbreak of glanders was recorded in 13 of 18 horses maintained by Faisalabad Metropolitan Mounted Police in February 1999. In August, five equines (three draught horses, one mule, and a donkey) owned by private clients were also diagnosed with glanders. Four of 13 police horses suffering from glanders died before initiation of treatment. The disease was diagnosed based on clinical signs, a positive mallein reaction, and isolation of the causative agent. Intraperitoneal inoculations of guinea pigs were attended with severe orchitis, culminating in deaths after 72 hours. The glanders isolates were found sensitive to enrofloxacin and potentiated sulphonamide. The clinical signs registered in different combinations were fever, decreased appetite, dyspnoea, occasional cough, enlarged and tortuous submandibular lymph nodes, purulent nasal discharge, epistaxis, nasal septum ulcers, nodules and crater-like ulcers on different parts of the body, cording of lymphatics of hind limbs

and ventral abdomen, and orchitis. Hematologic findings prior to treatment confirmed a macrocytic hypochromic anaemia.

After one week of treatment, there was a noticeable improvement in clinical condition. After completion of 14 days treatment, skin ulcers changed to scabs, and development of nodules totally ceased. Enlarged, tortuous submandibular lymph nodes and cough were still present. After completion of the third week of treatment, all animals resumed normal feed intake. Treatment was not associated with significant untoward effects except in two cases, which suffered from slight alopecia which resolved within two days after completion of therapy and a slight delirium with head shaking, quivering of breast muscle, and incoordination of gait lasting for about ten minutes. Subsequently, all animals re-checked at six and 12 months post-treatment were pronounced healthy. Two of the treated mounted police

horses died after the 14th month post-treatment due to unrelated causes (i.e., severe stomach bots infestation and hindquarter oedema). Autopsies revealed a milliary calcified area in lungs devoid of any hyperaemic zone, and culturing of lung specimens was negative. Animals examined two and three years later remained healthy. Treatment of this zoonotic disease with enrofloxacin and sulphadiazine-trimethoprim appears to be a viable alternative in countries where a test and destruction policy cannot be implemented for socio-economic reasons. Treatment of glanders-affected equines is also warranted on animal-welfare grounds.

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NATIONAL Equine Babesiosis (Piroplasmosis)—A Barrier in the International Movement of Horses

EQUINE BABESIOSIS (PIROPLASMOSIS), A DISEASE of equids in many regions of the world, is caused by the tick-borne hemoprotozoans *Babesia equi* and *Babesia caballi*. The central concern is the risk and consequences of entry of these parasites through international movement of horses into the United States, where equine piroplasmosis is limited to Florida and apparently not established. This concern continues to have dramatic impacts, such as those occurring during the 1996 Olympics. The horse population within the United States is presumed to be entirely susceptible to infection; therefore, management safeguards against the entry and/or dissemination of piroplasmosis continues. For owners whose horses become infected while residing in countries with endemic piroplasmosis, the main consequence of infection is that their horses are restricted from re-entry into the United States. Regardless of on which side of the issue you stand, the central question remains: is the regulatory impact on the international movement of horses justified? Below are discussions of the key factors, which must be understood to adequately address this question. The first and foremost factor concerns the tick population within the United

States and its ability to transmit these parasites. Since ticks are biological vectors of *B. equi* and *B. caballi*, it is assumed that without ticks capable of transmission, infection cannot occur.

In the United States, *B. caballi* is known to be experimentally transmissible by three native tick species: *Anocentor nitens*, *Dermacentor albipictus*, and *D. variabilis*. In a recently completed study, five North American tick species—*Amblyomma americanum*, *Boophilus microplus*, *Dermacentor andersoni*, *D. occidentalis* and *D. variabilis*—were tested for their ability to transmit *B. equi*. Intrastadial transmission was demonstrated by *D. variabilis* males and transstadial transmission by *B. microplus* adults. These collective data indicate that the possibility exists for natural transmission of equine piroplasmosis within the United States. All of these ticks are native to the United States, although *B. microplus* has been prevented, through acaricide use, from becoming re-established north of Mexico. Obviously, vector control by use of acaricides is a viable part of a piroplasmosis control program; however, there are concerns of the development of acaricide resistance. While the

Figure 1.

Equine Cases of BEE, S

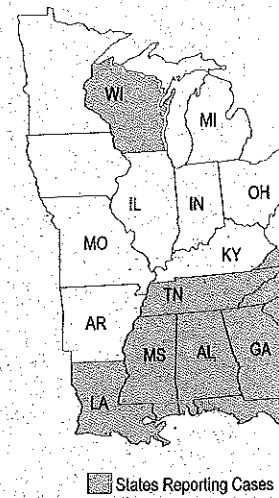
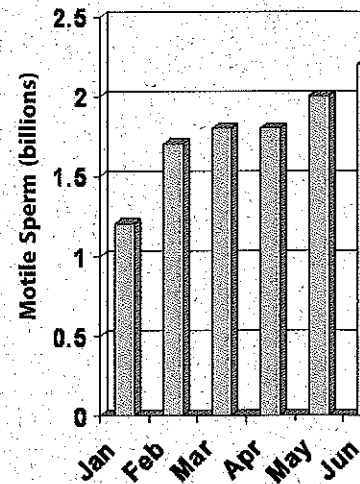
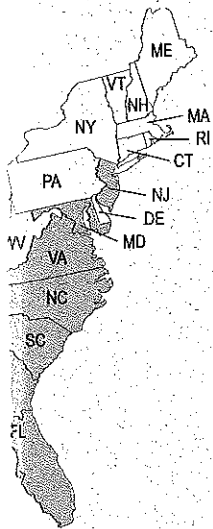


Figure 2.

Sperm Number Corre



Summer 2003



control of potential tick vectors is one method to prevent transmission of equine piroplasmiasis, the United States has adopted the strategy of preventing infected horses from entering the country based on finding anti-*B. caballi* and/or anti-*B. equi* antibodies in horses presented for importation. One of the re-occurring problems of this strategy is that the majority of horses that are found to be serologically positive for one or both of these parasites are in other measurements clinically normal. Stated more clearly, the majority of infected horses do not have indications of decreased performance.

This collective information indicates that the potential for tick transmission of equine piroplasmiasis exists within the United States. Is the regulatory impact of *B. equi* and *B. caballi* infection on the international movement of horses justified? There are also many other factors that influence whether infection and clinical disease caused by these parasites could become endemic in the United States. We have minimal understanding concerning influences of a number of factors that determine whether or not an infected horse shows clinical disease.

Immunogenetics of the horse, the virulence of the infecting strain, the tick burden, the tick infection rate and the challenge dose are factors impacting disease expression and possibly transmission.

Therefore, since there are tick vectors within the United States that have been shown experimentally to transmit *B. caballi* and *B. equi*, and considering our lack of knowledge concerning these other factors that influence the establishment of endemicity and disease expression, it must be concluded that the restricted movement into the United States of horses infected with one or both of these parasites must continue. An exciting current area of research is examining the development of these parasites within their tick vectors with the goal of developing vaccines that would block transmission. This endeavor may one day lead to a vaccine that would aid in alleviating the burden of equine piroplasmiasis on the international movement of horses.

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Breeding Soundness Examination of the Stallion

THE BREEDING SOUNDNESS EXAMINATION (BSE) of the stallion is an attempt to provide an estimate of a stallion's future fertility and is composed of the following:

- History—past breeding records, illness, medications, pedigree.
- General physical condition—body condition, inheritable conditions, external genitalia, testicular evaluations, and measurements.
- Semen collection and evaluation.
- Evaluation of libido and mating behavior.
- Examination of the internal and external genitalia.
- Ancillary procedures—bacterial cultures, cytology, special stains, hormone analysis.

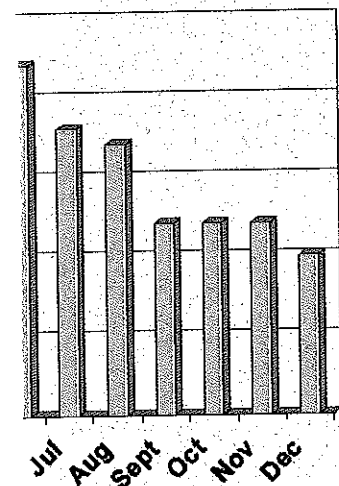
Other factors, such as management of the stallion and his mares, may have a large impact on the overall fertility of the stallion. Therefore, the BSE is an attempt to provide an estimate of a stallion's potential fertility and should not be interpreted as an absolute measure of the stallion's fertility. Annual assessments for actively breeding stallions prior to the breeding season can help with management decisions and provide a baseline for comparison if a problem should arise.

At the time of semen collection, mating behavior and libido are evaluated, as is inspection of the penis. Bacterial cultures of the external genitalia are taken at this time. Aerobic bacterial cultures are obtained from the penis, fossa glandis, and urethra prior to ejaculation and the urethra after ejaculation. Potential pathogens are *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*, but isolation does not always indicate a venereal problem.

Tylorella equigenitalis, causing Contagious Equine Metritis (CEM), is the only known bacterium that constantly produces venereal disease in the horse.

Two semen samples are collected from the stallion one hour apart. The two ejaculates are considered representative if volume of the ejaculates are similar and the second contains approximately one-half the number of spermatozoa as the first with comparable or increased sperm motility. A semen sample is generally obtained by use of a jump mare or phantom and an artificial vagina. If it is impossible to obtain a sample with an artificial vagina, a condom can be used, but it provides an inferior sample.

ed for Season



Immediately after collection, the sample is evaluated for color, clarity, and volume. The gel portion of the ejaculate should be removed by filtration. Next, the motility of the sperm should be assessed and sperm concentration determined using a densometer, hemocytometer, or spectrophotometer. Sperm concentration multiplied by gel-free semen volume will give the total number of sperm. Sperm morphology is also addressed as well as longevity of spermatozoa.

The internal genitalia (ampullae, seminal vesicles, prostate, bulbourethral glands) are not routinely evaluated unless there is a suspicion of problems. These are generally evaluated per-rectum utilizing ultrasound.

The testes should be evaluated and measured as well as total scrotal width. Sperm production is directly related to testicular size. The volume of each testes is calculated so that theoretical sperm production can be compared to actual production. Stallions with a total scrotal width of less than 8 centimeters should be suspected of testicular hypoplasia or degeneration.

The Society for Theriogenology has developed *Guidelines for Classification of Breeding Stallions* based on breeding 40 mares by natural service or 120 mares by artificial insemination. Stallions are classified as satisfactory, questionable, or unsatisfactory breeding prospects.

To be classified as satisfactory, the following criteria must be met:

- Stallion demonstrates good libido and mating ability.
- Penis normal size without inflammatory lesions.
- Free of venereal or transmissible diseases or potentially heritable defects.
- Negative EIA (Coggins) test.
- Two scrotal testes and epididymides of normal size, consistency, and shape. Total scrotal width greater than 8.0 centimeters.
- Stallion produces a minimum of one billion morphologically normal, progressively motile spermatozoa in the second of two ejaculates (corrected for season, see Figure 2).

If a stallion fails these criteria, he is classified as a questionable or unsatisfactory. Many stallions are not used for 40/120 mares and may be able to settle a smaller book with adequate reproductive management.

Reassessment after 60 days may be in order. Many stallions breed larger books of mares (100+) and some breed in both the northern and southern hemisphere seasons. Ancillary testing to assess DNA integrity of spermatozoa, fertilizing capacity of spermatozoa, etc. are available for selected problem cases.

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Manure Concerns

THINK HORSE OWNERS DON'T HAVE TO BE CONCERNED with the U.S. Environmental Protection Agency (EPA)? Think again!

The first judicial civil action that the EPA took against a concentrated animal feeding operation (CAFO) was against a Thoroughbred race-track in Oregon in 2000. This action occurred after repeated, continuous discharges of manures and other wastes to a river in violation of the 1972 Federal Clean Water Act, which allows a maximum penalty of \$27,500 a day until the discharger complies with the law. The objective of the Clean Water Act is to restore "fishable and swimmable" quality to lakes, streams, and estuaries. To achieve this, a permit system was established to regulate pollutants at point discharges to the "waters of the United States." The law defined animal feedlots as point sources if they had a large concentration of animals or had the potential to pollute. States were allowed by the Clean Water Act to have stricter

standards than the law commanded.

In February 2003, new concentrated animal feeding operation rules were published by the EPA as a result of significant changes in animal production and housing during the last 20 years. The major causes of water pollution from food production are confinement facilities for swine, dairy, beef, and poultry. However, facilities for boarding, training, breeding, and racing of horses can have similar impact on water quality.

Because of intensive production methods, animals are concentrated in high numbers, resulting in manure and animal waste production in smaller areas. The runoff of nutrients from manure in unroofed confined feeding areas, storage of wastes outside roofed facilities, and runoff from fields on which these wastes are spread contributes to pollution of waterways.

To understand if the new regulations apply to an existing or proposed horse facility, the terms AFO and CAFO need to be defined. An AFO is

an animal feeding operation and is defined as a place where animals are confined, fed, and/or maintained for 45 days or more for any 12-month period in an area that manure is generated and/or concentrated and there is no grass or other vegetation in the confinement area during the normal growing season. These concentrated sites can be for "temporary confinement." An AFO does not include those animals that are exercising, grazing, or feeding on the forage in pastures, fields, or rangeland in which they may be restricted. The 45 days do not have to be consecutive, and a day can be any portion of a day to count as a day. The year does not have to be a calendar year but any consecutive 365 days.

A CAFO has been redefined in the new regulations and is identified by a specific number of animals in an AFO and by site conditions. A horse AFO is a large CAFO if there are more than 500 or more horses. A horse AFO is a medium CAFO if a constructed ditch or a pipe carries manure or wastewater from a confinement area to the waters of the United States or the horses are in contact with running surface water through a confinement area, and there are 150 or more horses. An AFO can be designated as a CAFO no matter what the number of horses if the state regulatory agency has

determined that the AFO is contributing significant pollutants to surface water. Situations where this may apply include a site that is too near surface water, a steep sloping site, or surface water is designated as a drinking water supply or an outstanding water resource.

If an AFO is considered a CAFO, the operator/owner is required to apply for a permit from the state water regulatory authority. The permit will require the operator/owner to develop a waste management plan that includes proper waste storage, diverting clean water from concentrated wastes, testing the nutrient content of the waste, and spreading the waste on fields at nutrient levels that soil testing results recommend. Records of waste disposal must be kept for five years. If animal wastes are transferred to another person or entity, records of date, recipient, amount, and nutrient content must be maintained.

If you have questions about the classification of an equine facility as a CAFO, contact the water regulatory agency in your state.

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