

Sexually transmitted disease monitoring, surveillance and impact on breeding farms in the USA

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Introduction

Sexually transmitted (STDs) or venereal disease in horses is of significant concern. Emergence of diseases within breeding operations can restrict or close breeding sheds, leading to significant economic loss. Transmission can occur during various steps in the breeding process, whether from a mare or fomite.

Many STDs are of importance with respect to the international transportation of horses. Outbreaks of STDs here or abroad can have far-reaching implications, leading to restrictions in trade or closure of borders. For these reasons, stallions and broodmares originating from outside North America are required to undergo testing before and/or after importation. These requirements are established and enforced by both the USDA-APHIS and state agricultural officials and will be reviewed below.

Stallions can be asymptomatic carriers of STDs, which can make diagnosis of subfertility difficult and time-consuming. Instead, some clinicians will choose prophylactic antimicrobial treatment (local, systemically or both) in broodmares during reproductive management. However, this practice is controversial and potentially misguided as evidenced by the emergence of multi-drug resistant organisms.¹

It should be noted that the presence of microorganisms on the external genitalia of stallions is not uncommon.² They are usually nonpathogenic and instead represent the natural flora and fauna of the external reproductive tract. There are instances when potentially pathogenic bacteria are isolated, raising concern over shedding of these organisms to mares; for example, certain strains of *Klebsiella pneumoniae* isolated from stallions has been shown to cause metritis and reduced pregnancy rates in mares.³ However, their presence should be interpreted in light of relative fertility.⁴

This paper will provide background information regarding current breeding practices in the USA, as well as commonly encountered STDs in the USA. Specific monitoring and surveillance programs will then be discussed, concluding with a discussion on general consideration to reduce the risk of STDs in breeding sheds.

Overview of equine sexually transmitted diseases

Contagious equine metritis

This particular form of metritis is caused by the bacterium *Taylorella equigenitalis*, a gram-negative coccobacillus that is considered highly contagious.⁵ Transmission via fomites also appears to be likely and was implicated as a cause for an outbreak in the USA in 2008-2010.⁶ Clinical signs vary from mild to copious vulvar discharge originating from the uterus, as well as infertility.⁷ The majority of CEM-affected mares are otherwise clinically normal and will resolve the infection within 2-4 weeks. Unfortunately, by the time clinical signs are observed at least one cycle is lost to endometritis and spread of the disease is rapidly occurring. Inapparent or “silent” carrier stallions are the most common means of transmission because they are much more sexually active. What ensues is a significant drop in productivity—as measured by per cycle pregnancy rates—and increased costs associated with management and veterinary care. Treatment for *T. equigenitalis* is straightforward and consists of careful cleansing of the internal and external genitalia, application of local anti-infective agents and, in some cases, systemic antibiotics.

Equine viral arteritis

Equine viral arteritis (EVA) is another disease that has important regulatory implications. The causative agent is equine arteritisvirus (EAV), which is an enveloped, single-stranded RNA virus.⁸ Clinical signs include: vasculitis, distal limb edema, pyrexia, rhinitis, conjunctivitis and anorexia. The

virus can also cause abortion outbreaks and severe neonatal pneumonia in naïve herds. It is transmitted via respiratory secretions and other body secretions, including semen, fetal fluids/tissues, and fomites.

Equine herpesvirus type 3

Equine herpesvirus type 3 (EHV-3) is the causative agent of equine coital exanthema, characterized by vesicular or pox-like lesions on the genitalia. It is not currently considered a reportable disease in the USA. Transmission occurs most commonly via coitus, but can also occur via fomites. Clinical disease is primarily manifest as vesicular and painful lesions on the external genitalia of both mares and stallions. During the acute phase, these lesions can cause discomfort during reproductive evaluations and during live-cover matings, but infection has no lasting or impressionable effects on fertility.⁹

Dourine

Dourine is a venereal disease of significance due to both its high level of morbidity and its effect on international transport of equids. The etiologic agent is the protozoa *Trypanosoma equiperdum*. Clinical signs include intermittent fever, swelling of and discharge from the genitalia, cutaneous plaques, and neurologic signs.¹⁰ Generally considered a chronic and progressive disease, the onset of recumbancy usually indicates terminal disease. This disease has been eradicated in the USA (and thus considered a foreign animal disease [FAD]), but is still present in Africa, the Middle East, and South and Central America. Sporadic outbreaks have been reported in Asia and Germany.⁵

Breeding management in North America

Live cover

Live cover refers to the process whereby a stallion actively mounts and breeds a mare. Different variations exist, but the two most common methods are: in-hand mating, which is common in commercial Thoroughbred studs; and pasture mating, which is used in large ranch horse herds in the western part of the USA. Past reviews on the venereal disease suggests that both methods pose a higher risk for transmission compared to artificial insemination (AI).¹¹

In-hand mating

This method is commonly used in commercial Thoroughbred studs. Prior to mating, mares are typically exposed to a teaser stallion to determine if the mare is in estrus. They are then prepared for breeding by wrapping the tail and cleansing of the perineum. The breeding process involves restraint of the mare with some or all of the following: lead shank, nose twitch, leather cape, kick boots and/or a leg strap. The stallion is then introduced to the mare. When properly aroused, the stallion will mount and breed the mare. It is common practice to collect the drippings from the stallion as he dismounts for microscopic evaluation of live sperm. Finally, the stallion's external genitalia is cleansed with warm water and practical cotton. Pregnancy examinations are typically performed 14-16 days after the last breeding, and a stallion's performance is judged based upon both early and later (e.g., 45 day) pregnancy and live-foal rates.

Risk factors include nose-to-nose exposure of teaser and mare, expulsion of bodily fluids during teasing and fomite transmission.

Pasture mating

This breeding method is fairly self-explanatory and usually involves running a stallion with a harem of mares during the physiologic breeding season. The stallion is usually turned out for a set period of time and will thus breed mares at his leisure. Pasture breeding is much less controlled and carries inherent risks, such as injury to the stallion or mare. However, management not as intense, requires fewer personnel and resources, and deemed highly effective when managing a large band of broodmares on

open ranges. Pregnancy rates are evaluated by the number of mares pregnant following removal of the stallion.

Risk factors include introduction of a new horse into a herd, significant social and sexual interaction leading to rapid exchange of bodily fluids and an uncontrolled environment.

Artificial insemination

Artificial insemination consists of collecting a stallion's semen with an artificial vagina (AV) followed by extension of the ejaculate into semen extender. The sample can then be prepared for immediate insemination or processed for cooling and shipping. Alternatively, the sample can be frozen and stored for future use. Collection usually occurs off a phantom mount in the presence of a tease mare. When breeding a mare, the sample is deposited manually via a transcervical approach.

Risk factors include fomite transmission, interstate (and potentially international) spread of disease and exposure of tease mare to different horses

Monitoring venereal disease in equine breeding programs

From an epidemiologic standpoint, monitoring systems are used to identify changes in disease status of a particular population. Collection and organization of these data can then be used to evaluate productivity, such as a stallion's breeding efficiency.

Aerobic cultures of the external genitalia of stallions

Swabs of the penis, prepuce, distal urethra (both pre- and post-ejaculation), and urethral fossa and sinus are routinely performed in Thoroughbred breeding sheds. They are commonly done as part of a pre-season stallion reproductive examination and will continue through the breeding season (note: frequency and specific sites will vary based upon individual farms). Isolation of potentially pathogenic bacteria, especially β -streptococci and *Escherichia coli*, in otherwise clinically normal and apparently fertile breeding stallions is not uncommon in live-cover breeding sheds in Central Kentucky. In a recent clinical study involving 15 stallions and 206 mares, the presence of these organisms on the external genitalia of stallions did not appear to transmit to mares as judged by post-breeding uterine cultures taken ~24 hours after mating.⁴ This study also suggested that stallions with positive cultures do not necessarily experience a reduction in fertility. Consequently, treatment of the stallion and mare is not always indicated, which is important due to the potential costs and side effects of therapy.

The environment in which a stallion is housed may influence the type of microorganisms harbored on the external genitalia, and should also be considered. When there is concern about the presence of transmission of potentially pathogenic bacteria, breeding strategies can be implemented to abate the risk. In the case of stallions used for live-cover, semen extender containing an appropriate antibiotic can be infused into the uterus prior to or after mating. Semen from stallions used for AI should be screened for microorganisms and extended in an appropriate semen extender. For mares bred to these stallions, post-breeding management could entail uterine lavages, infusions and ecbolics.

Surveillance programs in equine breeding programs

Surveillance programs differ from monitoring systems in that they entail an ongoing evaluation of data upon which strategies can be established and implemented to mitigate disease.

Aerobic cultures and cytology of the endometrium

Commercial stud farms may require a negative or "clean" uterine culture prior to breeding a mare to one of their stallions. Clinical bacterial endometritis is a common cause of subfertility in broodmares,¹² and identification of these mares prior to mating can prevent a potentially wasted breeding. Contemporary data in Thoroughbreds suggest that a culture should be combined with a cytology to identify the presence and degree of inflammation, since severe inflammation was associated with a reduction in fertility.¹³

Prophylactic treatment with a single infusion of an antibiotic after mating has been used in certain areas of high breeding intensity. A pilot-study conducted in 2015 in 39 maiden Thoroughbred mares in Central Kentucky demonstrated a first cycle pregnancy rate of 83.3% in mares infused with 35 mL of saline and 65.0% in those receiving 5 million units of penicillin G (PPG) qs to 35 mL with saline. Interestingly, early pregnancy loss—as defined by the initial detection of a vesicle 14-15 days after ovulation but subsequent death or absence of a fetus 42-45 days after ovulation—was 9.5% in mares infused with saline, but 0.0% in mares receiving PPG infusions.

Screening programs for contagious equine metritis

Strategies to prevent CEM in a particular locale depend on that country's disease status. For disease-free countries, horses are screened prior to importation and again upon arrival. Infected animals must be treated and test negative prior to entering the breeding population. In CEM-infected countries, control measures include the following:

- Screening all stallions prior to the beginning of the breeding season
- Screening certain mares based upon risk, such as those with copious vulvar discharge and a premature return to estrus
- Proper hygiene of horses and humans
- Sanitation of fomites

In some countries, this disease has been eradicated through surveillance, quarantine and excluding infected animals from the breeding population. To the authors' knowledge, no vaccine exists for this disease. Due to the fairly recent outbreak of CEM, the USA has undergone a reclassification in its status as a CEM-free country. As of this writing (June 2016), international transport of horses to and from the USA was permissible by most agricultural ministries provided proper screening and prophylactic treatments were performed prior to introduction of the animal into the country's breeding population.

With respect to testing, culture swabs of the clitoral fossa, clitoral sinus and endometrium should be placed directly into Amies with charcoal transport medium. Regulations often require serial cultures before declaring an animal CEM-free. In addition, a serum complement fixation (CF) test can be performed on mares two to three weeks after mating to determine if they are producing antibodies against *T. equigenitalis*. These CF tests are commonly done in Central Kentucky breeding sheds for mares bred by a stallion that had also covered a newly imported mare or filly.

Similar screening measures can be employed in other breeding programs, such as those utilizing AI. In fact, it may be as if not more important to utilize a similar protocol in these particular sheds. Doing so may prevent another CEM outbreak like that which occurred in 2008 in the USA.

Mitigation of equine viral arteritis

Current estimates suggest that 30-70% of exposed stallions will become persistently infected when exposed to the virus, and are referred to as "shedder" stallions.¹⁴ Consequently, prevention of the disease is key and the disease has been nearly eradicated in North American Thoroughbreds by screening and vaccination. A modified-live virus vaccine is commercially available in the USA (Arvac[®], Zoetis, Florham park, NJ) and approved for use in breeding stallions. In some breeds that allow AI, breeding with EAV-infected semen is permissible with proper documentation of the mare's vaccination status. Certain breed-registries and state agricultural departments have restrictions regarding vaccination of breeding stock and use of EAV-infected semen. These requirements can be obtained by contacting the breed registry and local regulatory officials.

It appears that the most useful means of mitigating spread of EVA are proper hygiene, evaluating the serologic status of breeding animals and targeted vaccination. An excellent description for control of EVA is provided by the American Association of Equine Practitioners (AAEP),¹⁵ and are summarized below:

- Seronegative stallions
- Seropositive stallions

- Seronegative mares
- Seropositive mares
- Teaser stallions and nurse mares
- Colt foals and weanlings

Abatement of coital exanthema (EHV-3)

Active lesions resulting from EHV-3 infections are readily recognized as a small blistering of the mucocutaneous portion of the genitals. Lesions take four to six weeks to heal and are characterized by focal areas of depigmentation. Gentle cleansing of active lesions may soothe and reduce inflammation. Reports of antiviral treatments are sparse. The author has used docanosol cream (Abreva[®], Glaxo-Smith-Kline, Philadelphia, PA) in four cases with apparently good results in resolving the acute inflammation associated with the lesions and prevention of future lesions. However, no comparison was made regarding the length of the convalescent period between treated and control animals. Transmission to other animals is a concern. Due to the contagious nature of this disease, a period of four to six weeks of sexual rest is recommended to prevent further transmission and allow the lesions to heal. Resumption of breeding activity can occur once the lesions have healed. They often leave rather characteristic areas of hypopigmentation on the external genitalia. Recrudescence of this particular herpes virus is unknown,¹⁶ but experimental administration of dexamethasone has been shown to cause reactivation of the virus.¹⁷ Control is aimed at early detection and withhold from breeding until lesions have subsided. There is no commercially available vaccine against EHV-3 in the USA.

Dourine

Diagnosis is made via compatible clinical signs and laboratory testing. The complement fixation test is prone to false positives from other species of *Trypanosoma*, and newer methods of diagnosis, including ELISA and PCR technologies are currently being explored for both testing and screening purposes. Infected animals should be removed from breeding and culled. Treatment with anti-protozoal drugs has been performed in some endemic areas, but they are unproven and even discouraged owing to concern about inapparent shedding animals. Suspected cases of dourine should be reported immediately to local USDA-APHIS officials.

Impact of STDs on North American breeding operations

In 2006, EVA spread from New Mexico to 18 states via either cooled-shipped semen or horse transport, resulting in numerous cases of respiratory illness, abortion, neonatal pneumonia and death.¹⁸ The consequences were significant, resulting in substantial economic loss in both the breeding and performance sectors for the affected operations.

As described above, surveillance for CEM is fairly rigorous. However, an outbreak was triggered in 2008 from a stallion in Kentucky. Significant resources were expended during the investigation, which included over 1,000 horses screened for CEM in 48 states.⁶ Ripple effects were noteworthy, leading to a change in CEM-status for the USA and altering import and export regulations.

Anecdotal reports exist of coital exanthema emerging on stud farms serving a large number of mares. Infections with EHV-3 can be problematic because they can temporarily halt a stallion's breeding activity due to their highly contagious nature. Assuming lesions take three to four weeks to heal, the stallion could miss several cycles, causing mares to switch off the stallion. The end result is a reduction in stud fee revenues, which, for some stallions commanding large stud fees (e.g., \$30,000+) can translate into a substantial reduction in income.

Because of the various ramifications of STDs, clinicians should be increasingly vigilant and maintain proper protocols for minimizing risk of disease transmission and outbreak.

Strategies to reduce the risk of STDs in equine breeding operations

In addition to the monitoring and surveillance strategies listed above, there are other steps that can be taken to prevent STDs in breeding operations.

Employing experienced and conscientious personnel

These are the first-line of defense in breeding sheds. Handlers should be vigilant for any signs of disease and alert the stallion or breeding manager with any concerns.

Maintaining a veterinary presence in breeding sheds

Among its many advantages, a veterinarian can implement biosecurity strategies and facilitate communication with USDA-APHIS should any concerns arise.

Introduction of a new horse to the herd

Infectious disease testing prior to introduction accompanied by surveillance measures after introduction

Prevent nose-to-nose contact of teaser stallion with mare or vice versa

Instead, position mare so the stallion can sniff the flank.

Expulsion of bodily fluids during teasing

Use ample and absorbent bedding and remove waste as quickly possible help maintain a clean environment.

Washing of the mare

Many STDs have characteristic lesions. Personnel responsible for preparing the mare should be observant of any abnormalities of the external genitalia, including growths, wounds or discharge.

Used materials should be discarded and clean ones used prior to washing another mare.

Suitable methods of washing include the use of a water hose and/or water bucket; practical cotton can be used to cleanse and rinse the mare

Mild detergent soaps, such as Ivory™, are most commonly used.

Handling of the mare and stallion

Proper hand hygiene is instrumental in reducing the risk of disease transmission. All handlers should wear gloves and clean attire.

Handling of semen

Every effort should be made to handle semen cleanly and gently to avoid contamination and thermal stress.

Fomite transmission

The various resources used to restrain the horses should have either protective barriers (e.g., sleeves or cellophane wrap on breeding rolls) or cleaned and disinfected (e.g., rinsing chains and twitches in tap water and soaking in dilute [1% chlorhexidine solution]).

The lower end of the phantom can be wrapped in cellophane and changed in between horses to prevent contamination with bodily fluids.

Prophylactic treatment with antimicrobial agents

This practice is controversial and the pros and cons carefully weighed prior to implementing due to concerns over antimicrobial resistance.

In operations utilizing AI, many semen extenders contain antibiotic agents. However, their use should not preclude proper semen handling and sample preparation. The same is true for keeping the collection area and laboratory clean and organized.

Conclusion

Venereal disease is an important consideration in equine breeding operations. There are several diseases of known importance, and outbreaks can lead to significant economic and emotional loss. Monitoring and surveillance programs should be utilized to screen for diseases, and clinicians should either be familiar with regulations governing their particular locale. Finally, risk of transmission can be mitigated to a large degree by proper hygiene and breeding management practices.

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