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Disease transmission in horses

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Abstract

Bacterial, viral and protozoal infections may cause severe reproductive losses. The present paper reviews the risk factors, clinical signs and preventive measures for the most important venereal or potential sexually transmitted diseases in horses. The stallion and use of semen for artificial insemination represent major risk factors for the transmission of bacterial contaminants of the penis, including *Streptococcus equi* subspecies *zooepidemicus*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*, known to cause endometritis and infertility in the mare. The role of the stallion in disease transmission is also due to the non-clinical manifestation of diseases such as contagious equine metritis and equine viral arteritis. Dourine has been eradicated from many countries, but continues to be a problem in other areas of the globe. Strategies for the prevention of introduction and transmission of diseases in breeding operation are discussed.
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1. Introduction

Managerial procedures have enabled stallion owners, managers and veterinarians to increase the number of mares bred to desirable stallions in a given year. Since geographical localization is no longer a barrier for disease transmission, in recent years the stallion has become an important epidemiological risk factor in spreading bacterial, viral or protozoal organisms. In the sport horse industry or in standardbreds, semen can be shipped in its liquid state or frozen [1]. In the thoroughbred industry, stallions are moved easily either from one continent to another, e.g. from the northern to the southern hemisphere or vice versa, on a regular basis.

The high risk that the stallion poses to the mare population to which he, or his semen, is exposed, becomes a more serious threat when we realize that the stallion is an asymptomatic carrier of most reproductive diseases [2]. Although natural mating poses the highest risk for venereal transmission of disease, there are other risk factors that will increase the threat of contaminating a population of mares or contaminating a stallion. Managerial and hygienic procedures in the housing and bedding of a stallion are important factors that must not be overlooked due to the possibility of the colonization of the penis by certain bacteria. In breeds that permit artificial insemination, it is not uncommon for stallion to alternate between natural cover and artificial insemination (AI) without veterinary supervision. This inconsistency of breeding method can increase the risk of a stallion getting contaminated or of spreading microorganisms to several mares. Artificial insemination has been used as a management technique to increase reproductive efficiency and reduce the risk of spreading

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disease. Stallions breeding artificially could breed >300 mares during a year; these horses have usually been carefully tested for the presence of venereal diseases. In addition, they are typically housed with other animals of similar health status. However, other factors, e.g. cleanliness of semen collection equipment and lubricants, can be important sources of disease.

The objective of this paper is to review the main epidemiological and clinical features as well as preventive measures for sexually transmitted infections in the equine.

2. Bacterial infections

2.1. Contaminants of the surface of the penis or urethra

Many commensal bacteria including *Escherichia coli*, *Streptococcus zooepidemicus*, *Streptococcus equisimilis*, *Staphylococcus aureus*, *Bacillus* spp., *Klebsiella* spp. and *Pseudomonas* spp. are part of the exterior of the stallion penis and are not regarded as pathogenic and may be cultured from an ejaculate. However, alterations of the normal bacterial flora on the exterior genitalia may cause the growth of opportunistic bacteria such as *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *S. zooepidemicus*, which, if inseminated, may cause infertility in susceptible mares.

Streptococcus equi subspecies *zooepidemicus*, *E. coli*, *P. aeruginosa* and *K. pneumoniae* are the most common isolates in cases of endometritis in the mare [3,4]. Infections due to *P. aeruginosa* or *K. pneumoniae* are often considered venereal due to the mode of transmission of these organisms (coitus, insemination with infected semen and genital manipulations) [5–14].

Stallions whose bacterial flora is disrupted and can shed pathogenic bacteria are asymptomatic carriers; however, at breeding they can cause persistent mating-induced endometritis or infectious endometritis with reduced fertility in susceptible mares. Factors that contribute to the colonization of the penis by these organisms are not clearly determined. The intact penile skin and the normal desquamation of superficial skin cells help combat the proliferation of pathogenic bacteria. However, frequent washing of the penis with soaps and detergents that change the texture of the skin will increase the susceptibility of the penis and prepuce to colonization by pathogenic organisms [15–17]. The environment in which a stallion is housed may influence the type of organisms harboured on the external genitalia. These organisms can also be acquired at the time of coitus with a mare that is genitally infected.

Careful evaluation of breeding records with a sudden and unexplained drop in early pregnancy rates or an increase in early embryonic death, should warn the stallion manager about a possible problem. Definitive diagnosis is achieved by obtaining a pure culture of the suspected microorganism from the stallion's reproductive tract. In addition, isolation of the same microorganism with a similar sensitivity pattern from the non-pregnant mares will help confirm the diagnosis.

Treatment of penile colonization depends on the type of bacteria and method of breeding. For stallions breeding by AI, a thorough penile wash prior to semen collection is recommended. The filtered semen is then diluted with extender containing antibiotic for which the bacterial is sensitive. Semen incubation should be done for at least 30 min prior to insemination. Stallions breeding by natural cover should be washed and scrubbed thoroughly. After washing, the penis is dried. After the mare is "covered", 4–6 h later, a uterine lavage is performed, followed by uterine infusion with an appropriate antibiotic. Stallions with penile colonization by *Klebsiella* or *Pseudomonas* can have their penis washed washed with a weak solution of HCl (0.2%) or sodium hypochlorite (bleach 5.25%), respectively [10,18–20]. Systemic treatment with antibiotics should be avoided since it has proved unrewarding in most cases. Field observations from practitioners suggest that inoculation of the penile surface with bacteria (smegma) from a normal stallion may be helpful in re-establishing the normal flora of the penis.

2.2. Contagious equine metritis

The only true venereal sexually transmissible disease in horses is known as contagious equine metritis (CEM). The causative agent is a fastidious growing cocco bacillus known as *Taylorella equigenitalis*, which is not present in North America but is endemic in Europe. Efforts to eradicate the disease have been successful in some places, but CEM still occurs sporadically in some countries [21,22].

Stallions that are imported from other continents where CEM is present are all tested during mandatory quarantine. Stallions infected with CEM are asymptomatic carriers and harbour the organism in the urethral fossa, the urethra or the sheath. Diagnosis of CEM is achieved by culturing the organism from these sites as well as from semen [23–25]. The recommended transport medium is Aimes supplemented with charcoal. Swabs are plated on Columbia blood–chocolate agar at 37 °C and 7% carbon dioxide. Because of the slow growth of *T. equigenitalis*, the possibility of false

negative results is relatively high. Another test that is commonly used is the polymerase chain reaction (PCR) assay, which appears to be very sensitive. However, it is possible that due to its sensitivity an increase in the number of false positives could be recorded [26–29].

Mares bred to infected stallions will develop a severe purulent vaginitis, cervicitis and endometritis. These mares will appear to ''clean up'' but will remain infected and the organism can be cultured from the clitoral fossa [23].

Positive stallions must be removed from breeding. Treatment consists of daily washing of the penis and urethral fossa with 2% chlorhexidine gluconate, followed by packing of the risk areas with nitrofurazone (0.2%) ointment daily for 7 consecutive days [23,30,31]. Association of the local antiseptic treatment with systemic treatment by sulfamethazole trimethroprim® (30 mg/kg) every 12 h for 10 days is recommended [25]. Treated stallions should be tested several times within 6 weeks after treatment before they are used for breeding. Regular washing with chlorhexidine is not 100% efficacious for the prevention of CEM in stallions [32].

2.2.1. Bacterial infection from the accessory sex glands

Colonization by bacteria of the accessory genital glands is restricted to the seminal vesicles [33–37]. Although uncommon in stallions, these infections can be clinically important because of their persistent nature, possibility for venereal transmission and detrimental effect on fertility. The most common isolates from cases of infectious seminal vesiculitis in the stallion are *P. aeroginosa*, *K. pneumoniae*, *Streptococcus* spp., *Staphyloccocus* spp., *Proteus vulgaris* and *Brucella abortus* [6,33,35–42]. A case of seminal vesiculitis due to *Acinobacter calcoaceticus* was recently described [43]. Infected stallions are usually identified initially by the presence of excessive neutrophils, erythrocytes, or both, in their ejaculates, along with a history of infertility or subfertility [33,37,43,44]. Confirmation is achieved by culturing as well as ultrasonographic and endoscopical evaluation of the seminal vesicles [45–47]. Direct sampling from the seminal vesicles can be obtained by video-endoscopy and catheterization of the ejaculatory duct [47–49]. Treatment of seminal vesiculitis is very difficult because the majority of antimicrobials cannot reach the gland in sufficient concentrations to be effective [33,43,44]. Broad-spectrum antimicrobials, e.g. trimethoprim sulfa, may be used systemically [44]. Enrofloxacin has been reported to reach sufficient concentration in the seminal vesicles following parenteral administration [43]. Direct flushing and local infusion of affected glands with an appropriate antimicrobial is the preferred method of treatment [35,47]. Local lavage with amikacin and oral treatment with trimethoprim sulfa for 8 days has been successful for the treatment in a case of seminal vesiculitis due to *P. vulgaris* [35]. Infusion of an extender containing a specific, non-spermatoxic, antimicrobial agent in the uterus of a mare before breeding, combined with post-breeding lavage, helps prolong the survival of semen and control bacterial growth [6,33,43,50–54].

3 Viral infections

Although many viruses have the potential to be found in semen during the viremic phase of the disease, only equine arteritis virus (EAV), responsible for equine viral arteritis (EVA) and equine herpes virus III (EHV III), the etiologic agent for equine coital exanthema, are considered to be sexually transmissible [55].

2.3. Equine arteritis virus

Equine arteritis virus is a small Togaviridae virus present in most countries except Iceland and Japan [56–58]. The virus is non-arthropod-borne and primarily transmitted from stallion to mare [59,60]. Horizontal transmission from stallion to another via fomites or contaminated bedding is possible [61]. At present, EAV is responsible for major restrictions in the international movement of horses and semen. The prevalence of seropositive stallions in some countries reaches 60–80% [59,62–64].

The acute phase of the disease is characterized by fever and panvasculitis resulting in limb and ventral edema, depression, rhinitis and conjunctivitis [56,57,65–67]. The virus may cause abortion and has caused mortality in neonates. Stallions will have scrotal edema and hyperthermia resulting in an increased number of sperm abnormalities [68,69]. Experimentally infected stallion experience a necrotizing vasculitis in the testes, epididymis and accessory sex glands. Natural EAV exposure results in long-term immunity to disease. Mares and geldings eliminate virus within 60 days, but 30–60% of acutely infected stallions will become persistently infected, temporarily or permanently shedding virus in the semen [56,57]. Mares infected venereally may not show any clinical signs, but they can shed large amounts of virus in nasopharyngeal secretions and in urine, which may result in the lateral spread of infection by the aerosol route.

Venereally acquired infection with EAV does not affect fertility, although mares infected at later stages of gestation may abort. Identification of carrier stallions is crucial in controlling the dissemination of EAV. These animals can be identified by serological screening using a virus neutralization (VN) test. If positive at a titer of 1:4, the stallion should be tested for persistent infection by virus isolation from the sperm-rich fraction of the ejaculate or by test mating. Shedding stallions should not be used for breeding or should be bred only to mares which are seropositive via either natural infection or vaccination [56,65,70–74]. Total spontaneous elimination of viral shedding has been reported in some stallions [75].

Abortion is one of the greatest risks of EAV infection. In cases of natural exposure, the abortion rate has varied from <10 to $>60\%$ and can occur from 3 to 10 months of gestation [63,76,77]. Abortion is due to a severe edema and necrosis of the endometrium leading to placental detachment [56,57]. Abortions appear to result from the direct impairment of maternal–fetal support and not from fetal infection.

Although mares and geldings are able to eliminate virus from all body tissues by 60 days post-infection, 30–60% of stallions become persistently infected. In these animals, virus is maintained in the accessory organs of the reproductive tract, principally the ampullae or the vasa deferentia and shed constantly in the semen. The development and maintenance of virus persistence is dependent on the presence of testosterone [78]. Persistently infected stallions that were castrated but given exogenous testosterone continued to shed virus, whereas those administered a placebo ceased virus shedding. Furthermore, stallions treated with an anti-GnRH product stopped shedding the virus during the treatment period, but resumed within a few weeks after treatment ceased [75]. There are three carrier states known in the stallion: short-term during convalescence, medium-term (lasting for 3–9 months) and chronic shedder (which may persist for years after the initial infection). Virus shedding in mares and geldings is limited to the convalescent state.

After clinical recovery from initial infection, there was no significant decrease in the fertility of shedding stallions. Mares infected after service by a carrier stallion do not appear to have any related fertility problems during the same or subsequent years and there are no reports of mares becoming EAV carriers or chronic shedders, nor of virus passage by the venereal route from a seropositive mare causing clinical disease or seroconversion in a stallion.

The EAV is transmitted mostly through aerosols generated from respiratory or urinary secretions from acutely infected animals or from secretions from recent abortions. The other route of transmission is venereally via semen from a shedding stallion. Close contact between animals is generally required for efficient virus spread in aerosol transmission. Personnel and fomites may play a minor role in virus dissemination. The virus remains viable in fresh, chilled and frozen semen, and venereal transmission is efficient, with 85–100% of seronegative mares seroconverting after being bred to a stallion shedding virus. In several cases, outbreaks of clinical disease have been traced to a persistently infected stallion. Diagnosis requires laboratory confirmation with acute infections having a four-fold or greater increase in neutralizing antibodies between acute and convalescent serum samples. Definitive diagnosis is reached by the cytopathic effect of the virus on monolayers of rabbit kidney cells. In the case of abortion, virus isolation can be attempted from fetal and placental tissues. Persistent infection in stallions can be diagnosed by first screening for antibody with a serum neutralization (SN) test. If seropositive at a titer of 1:4, virus isolation should be performed on the untreated, sperm-rich fraction of the ejaculate or the stallion should be test mated to seronegative mares that are subsequently monitored for seroconversion. Some countries require semen testing of all stallions for viral shedding by culture and isolation or by PCR technique [57,62,63,79].

For suspect animals, several samples can be submitted for diagnosis of EVA. In abortions, both fetus and placenta contain large amounts of virus. Samples of fresh placenta, spleen, lung and kidney along with fetal and placental fluids should be collected and submitted for virus isolation. Blood should be obtained from the mare at the time of the abortion and 3 weeks later for testing by SN.

Sample submissions from stallions include serum and semen. Semen should be collected using an artificial vagina or a condom. Although less satisfactory, a dismount sample can be collected at the time of breeding. The sample should be from the sperm-rich fraction of the full ejaculate and should be chilled immediately and shipped at 4 °C to arrive at the diagnostic facility within 24 h. If this is not possible, the sample should be frozen in dry ice and shipped to the diagnostic facility under these conditions. Washing of the penis with antiseptics or disinfectants prior to collection of the samples should be avoided. Samples of commercial frozen semen could also be tested but it is necessary to have at least 2 billion sperm cells for the

sample to be representative. False negatives have been reported due to the lack of seminal plasma in cryopreserved semen.

Prevention and control are implemented by rigorous vaccination schemes using a modified live vaccine [57,69,80–82]. The vaccine does not appear to produce any side effects in vaccinated stallions apart from a possible short-term abnormality of sperm morphology and a mild fever with no overt clinical signs [67,83–85]. However, live virus can be isolated sporadically from the nasopharynx and blood after MLV vaccination [86,87]. Vaccinated horses should not be mixed with naïve pregnant mares for at least 1 month. Increased SN antibody titers are induced within 5–8 days and persist for at least 2 years.

2.4. Coital exanthema

Another virus which, although less important than EAV, can be more alarming to the inexperienced breeder, is equine herpes virus type 3, the etiologic agent of equine coital exanthema. The disease can be transmitted by the stallion to the mare or from the mare to the stallion at the time of coitus. The disease may also be transmitted by infected artificial insemination equipment or gynecological examination instruments [88–92]. It is characterized by the formation of small (0.5–1.5 cm) blister-like lesions on the penis and prepuce or on the perineal area of the mare [93–95]. These lesions will eventually break to form skin ulcers that usually resolve completely in 3–4 weeks leaving some round white scars in the area that was affected. Sometimes mild fever and slight depression can be observed. Diagnosis can be obtained from the typical clinical signs or definitive diagnosis can be done by observing the inclusion bodies on histopathology.

The effect on fertility is thought to be minimal, but stallions and mares during the acute phase of the disease should be sexually rested to avoid further spread. Prevention includes good hygiene and sexual rest of affected animals or the use of artificial insemination in breeds that allow the procedure.

3. Protozoal infections

Dourine, caused by *Trypanosoma equiperidum*, is a venereal disease found in Africa, South and Central America and the Middle East. Dourine achieved a global distribution during World War I and has been eradicated from North America and most of Europe. It is still reported in Africa (Bostwana, Etiopia, Namibia and South Africa), Asia (Kyrgyzstan, Mongolia, Pakistan, Russia, Turkmanistan and Uzbekistan). Suspected cases have been reported in other areas (Germany and Middle East) [96–99]. It is perhaps the only protozoal organism that can be transmitted venereally in horses. Tentative diagnosis is made via the clinical signs, which include intermittent fever, depression, progressive loss of body condition and severe purulent discharge from the urethra. Characteristic cutaneous lesions, from which the disease derives its name ‘‘dourine’’, have been described as circular elevated plaques of thickened skin ranging in size from 1 to 10 cm in diameter resembling money or ‘‘douros’’. These plaques are observed mostly on the neck, hip and ventral abdomen. Terminal stages of the disease are characterized by severe anemia and nervous signs, progressive ataxia, hind limb paralysis or paraplegia followed by death. Diagnosis is performed via a complement fixation test. However, recent studies have shown that this test cannot distinguish between *T. equiperdum*, *T. evansi* and *T. b. brucei* [96,98,100,101].

Piroplasmosis has been recently gained attention due to the increase movement of horses from infected areas. The disease is caused by the hemoparasite *Babesia equi* or by a less severe strain, *Babesia caballi*. It is considered to be enzootic in many areas of the southern US, as well as being found throughout the world. The protozoal agent is most often spread by ticks, but mechanical transmission has also been documented; therefore, there is concern of possible venereal transmission if blood from an infected horse contaminates the semen.

4. Other microorganisms

Other microorganisms that can be potentially be transmitted venereally include *Chlamydia* spp. and *Micoplasma* spp. *Mycoplasma* spp. have been isolated from the external genital and semen of clinical normal and infertile stallions but their exact role in uterine infection is not well established [102–104]. *Mycoplasma equigenitalium*, *M. subdolum* and *Acholeplasma* spp. have been associated with infertility, endometritis, vulvitis and abortions in mares and with reduced fertility and balanoposthitis in stallions [102,105–107]. *M. equigenitalium* and *M. subdolum* were isolated from the genital tract of mares (5–34%) and aborted equine fetuses (7%). However, the detection of *Mycoplasma* spp. was not always associated with reduced fertility [102,105–108].

Genital chlamydiosis of horses has been reported to result in mild chronic salpingitis [109] reduced reproductive rates [110,111], low ejaculate quality

[112] and occasional abortion [113–116]. Detection of chlamydial organisms from aborted equine fetuses ranges from 20 to 55% [116–118]. However, these reported rates may be too high because other infections were usually present and it is difficult to culture this organism. Chlamydial organisms that have been found in the horse include *Chlamydophila pneumonia*, equine biovar associated with respiratory diseases and *Chlamydophila abortus* and *Chlamydophila psittaci* which were both detected in equine abortion cases [115].

It is important to be aware of the possibility of these and other agents causing infertility both in mares and stallions. For example, *Candida* spp. and *Aspergillus* spp., although not commonly present in semen or the genital tract of the stallion, can be potential pathogens particularly in artificial insemination programs where the hygiene of the collection and processing equipment is not well monitored.

5. Conclusion

To prevent the spread of any disease to susceptible populations through breeding, correct identification of infected animals as well as the implementation of appropriate managerial procedures is critical. A stallion carrier of a bacterial disease should not be used for breeding through natural service and his semen should treated with appropriate antibiotics prior to insemination. Stallions shedding arteritis (EVA) virus can still be used, provided that the mare owners are first informed of the fact, and agreeable. The option to use a particular stallion in a breeding facility may depend on the value of the stallion as a breeding animal as well as individual regional regulations. Whatever the case may be, all stallions should have a diagnosed disease status prior to each breeding season.

Stallions should undergo complete semen evaluation as well as microbiological examination from semen as well as pre- and post-ejaculation urethral swabs. Vaccination status as well as previous exposure to specific disease agents should be determined.

Quarantine of recently introduced animals should be considered, particularly in regions with high risk of contagious diseases. Animals returning from events where commingling has occurred (breeding farms, shows, etc.) should be placed in quarantine for a minimum of 3 weeks. Animals in quarantine should be monitored on a daily basis and promptly isolated with proper veterinary care if abnormal demeanor or clinical signs of illness are seen. Personnel attending quarantined animals should always don protective clothing (coveralls, etc.) and boots or shoe covers that are devoted solely to the quarantine facility. Clothing and boots should be washable and boots or shoe covers should be made of rubber or other impervious materials. All other equipment and supplies used in a quarantine facility (halters, ropes, blankets, feeders, buckets, etc.) must be solely devoted to the facility.

Breeding hygiene should be strictly observed to avoid transmission of contaminants to mares. The surface of the penis may harbour several organisms that may be potentially pathogenic [119–121]. If artificial insemination is used, particular attention should be paid to the origin of semen and health certificates of stallions at the time of collection [7,122,123]. Antibiotic containing extenders do not eliminate risk of transmission of organisms. Quality controls of semen processing, particularly, shipped cooled semen, are often lacking. Health importation requirements for frozen semen should be verified for each country of origin and strictly followed [79]. The stallion status with regard to EVA and CEM are of particular importance. Guidelines are available for use of stallions that are EVA-virus shedders [124].

Prevention of introduction of diseases into the herd should also take into account other vectors animals (insects, birds and rodents) as well as proximity with other species (donkeys and others). Pest control may be difficult but should not be overlooked. Regular cleaning and disinfection of the barns and common areas is critical to breaking transmission cycles of disease agents that contaminate housing, feeding and treatment equipment or other vectors or fomites.

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