American Association of Equine Practitioners (AAEP)

Biosecurity Guidelines for Control of Venereally Transmitted Diseases

Introduction

These guidelines are intended to serve as recommendations. These guidelines are neither regulations nor directives for standard of care and should not be interpreted as such. It is the responsibility of attending veterinarians, through an appropriate veterinarian-client-patient relationship, to utilize relevant information to determine optimal management of the horses in their practice. It is incumbent on each individual practitioner to reach a decision on actions based on the circumstances of each unique situation and his or her professional experience. (See last page of guidelines for full disclaimer.)

Guidelines

Although these guidelines are primarily intended for the prevention and control of venereally transmitted diseases, they are also useful for control of other infectious diseases caused by viruses, bacteria and parasites. Other AAEP guidelines such as Infectious Disease Control Guidelines and Vaccination Guidelines are recommended reading for members. For this document, the term horse will be used to refer to all equids (horse, donkey, mule, and pony).

The emphasis of these guidelines is to control the transmission of the following disease agents:

Taylorella equigenitalis/asinigenitalis; Contagious Equine Metritis Organism (CEMO)
Equine arteritis virus (EAV)
Equine herpesvirus-3 (EHV-3) or equine coital exanthema virus

Although not included in the foregoing list, mention should be made of *Trypanosoma equiperdum,* the cause of dourine that the United States has been free from for many years. Dourine has long been known to be a venereally transmitted disease of horses. Transmission of dourine takes place almost exclusively by coitus, especially from an infected stallion to a mare. While spread of the causal agent has not been confirmed by AI, this could potentially occur since *T. equiperdum* is present in seminal fluid.

Other infectious organisms are known to be associated with venereally transmitted disease; however, at this time, there is lack of scientific information as to the pathogenicity of different strains to cause disease. These types of organisms include:

Klebsiella pneumoniae
Pseudomonas aeruginosa
Streptococcus zooepidemicus

Venereally transmitted diseases are of great concern to horse breeders and veterinarians involved in breeding management of mares and stallions. Whether horses are part of a natural breeding program or an artificial insemination program, some of these diseases are highly contagious and have been shown to be transmittable between animals by direct horse-to-horse contact, contaminated semen, and also by indirect venereal contact through the use of contaminated semen collection equipment (including, but not limited to artificial vaginas and breeding phantoms) and personnel (hands and clothing) participating in the semen-collection process. These guidelines are specifically written and endorsed by the American Association of Equine Practitioners to help protect horses and breeding facilities from the economically damaging effects of such diseases.

General biosecurity considerations for equine breeding facilities, concentrating on sexually transmitted diseases

Introduction: The goal of a biosecurity program at a breeding facility is to reduce the risk of introduction of an infectious disease organism onto the site and/or to reduce the risk of spread of an infectious disease organism at a facility.

Techniques for breeding, including natural service (live cover) and artificial insemination (AI), are associated with inherent infectious disease risks. Advanced reproductive procedures, such as embryo transfer, oocyte transfer and intracytoplasmic sperm injection, may also be mechanisms for transmission of infectious disease agents. Although horses used for breeding are the focus of these guidelines, non-breeding equids must also be considered when evaluating biosecurity. Foals are particularly susceptible to infectious diseases. A biosecurity plan for an equine breeding farm must include provisions for management of newborn foals, weanlings and yearlings.

Live cover or natural service refers to mating in which a stallion mounts a mare, intromits the penis into the vagina and ejaculates. Pasture breeding and breeding-in-hand are two types of natural service. Live cover breeding is generally associated with

a greater risk of venereal disease transmission than artificial insemination (AI) for certain disease agents. Artificial insemination can be performed using fresh, cool-transported or frozen semen.

Infectious diseases that are important in the equine breeding industry are not limited strictly to agents that may be transmitted during coitus or insemination. Stallions, mares and foals are at risk of disease from a wide variety of infectious organisms. The mode(s) of transmission and propensity to spread to other horses on a farm vary with each organism, and the susceptibility or resistance of the at-risk population. Viral, bacterial and parasitic agents impact equine health. Internal parasites continue to be a problem at horse facilities, including breeding farms. Resistance to certain anthelmintics has been documented as a problem of increasing importance in some geographical areas. Appropriate identification of parasitized horses and development of a strategic deworming program should be a high priority for an overall herd health preventive medicine program.

General guideline considerations: Biosecurity guidelines for equine breeding programs should be tailored for each individual operation. Specific biosecurity endpoints will depend on risk, geographic area, equine density, horse-traffic, ages of resident horses, breeding management procedures (e.g. live cover versus AI) and other factors, such as importation of preserved semen or embryos. Biosecurity protocols should be understood by all facility personnel and followed on a routine basis.

Managers of breeding operation, and their attending veterinarians, must understand and comply with all federal and state/provincial regulations regarding health status testing. Examples include testing for Equine Infectious Anemia (EIA; Coggins/AGID and/or ELISA testing) and procurement of a health certificate from an accredited and licensed veterinarian prior to transport, as well as testing of stallions for Equine Viral Arteritis virus and *T. equigenitalis* in certain states (such as Kentucky). To verify testing requirements in your area, please check with your state/provincial animal health office.

While this communication is specifically directed at venerally transmitted diseases, the potential modes of transmission of any disease agent on a breeding farm include:

1. Direct horse-to-horses, especially nose-to-nose, contact.
2. Respiratory transmission by aerosol or droplet of an agent from one horse to another.
3. Oral (ingestion)--especially contaminated hay, grain or water or contact with contaminated surfaces (stall floor mats, walls, drains, stocks).
4. Venereal transmission by live cover or artificial insemination.

5. Fomite--exposure to contaminated AI collection equipment, phantom, breeding rolls, stocks, trailers, stalls, farm equipment, vehicles, grooming equipment, halters, lead ropes, twitch, water buckets, water hoses, shared needles, clothing, footwear and hands of personnel, etc.
6. Transmission of infectious agents through advanced reproductive techniques, such as embryo transfer, oocyte transfer, or intracytoplasmic sperm injection (ICSI).
7. Vector-borne transmission— insects, ticks.

How to determine a known biosecurity risk (existing or historical) on the premises:

1. Evaluate the method of breeding, whether natural cover or AI. If performed properly, AI reduces the risk of bacterial contamination of the uterus by eliminating physical contact between the mare and stallion and allowing the incorporation of antimicrobial drugs (AMD) in the semen. Artificial insemination with shipped semen allows the mare (and foal) to stay at home and thus not be exposed to potential pathogens at another location; however, viral and, to a lesser extent, bacterial pathogens, may gain access onto a breeding facility via infective semen. Some stallions may shed potentially pathogenic organisms in the semen. Procedures used to collect semen may be less than optimal at some facilities. Use of AMDs does not necessarily eliminate all bacteria in a semen sample. Viruses are not killed by AMDs nor are they eliminated by cooling or freezing.

2. Consider whether the stallion is at risk for carrying an infectious disease agent. Evaluate whether the horse has been tested for Equine Infectious Anemia, *T. equigenitalis*, and Equine Arteritis virus (serology, agent detection). Assess whether there are potential pathogens on the external genitalia or in the semen.

3. If a mare has to travel to an outside breeding facility, assess whether she and/or the foal by her side are at risk of contracting a disease. Quarantine of the mare (and foal) is highly recommended on their return to the home farm.

Recommendations for a biosecurity program for horses on a breeding facility:

1. Allow only healthy horses to enter the facility. Entrance should require a Certificate of Veterinary Inspection (CVI) from an accredited veterinarian dated within the past 14 days for all new arrivals. All horses should be

required to be vaccinated (core and risk-based vaccines as listed in the AAEP Vaccination Guidelines, as appropriate). The horse owner/agent should provide a statement of the disease status of origin herd and premises.

2. Examine all new horses upon arrival for signs of contagious disease and to verify that the CVI, vaccination history, other tests required by the facility and the owner/agent statement match the horses being delivered and are in compliance with the requirements. Special attention should be paid to leased teaser stallions and nurse mares which can be responsible for the introduction of certain diseases, e.g. CEM and EVA, onto a premises.

3. Isolate new arrivals to prevent contact with resident horses (especially pregnant mares). The period of isolation should be 7 to 14 days for horses arriving from a facility with minimal perceived risk and 21 to 28 days for horses coming from a facility of unknown risk. Do not allow horses with overt signs of disease or a high risk of infection onto the property. An alternative would be to unload such horse(s) and accommodate them at a separate isolation facility.

4. Immediately isolate any horse on the property suspected of having a contagious disease, such as respiratory infection, diarrhea or fever of unknown origin. There should be a situation evaluation by a veterinarian to determine etiology, biosecurity risk and containment plan. Any treatment and follow-up procedures depend on the diagnosis. Appropriate cleaning and disinfection of the stall the horse resided in is essential.

5. Vaccinate all resident horses. Use AAEP Guidelines for Vaccination (adult horses and foals) to include core and risk-based vaccines.

6. All horses on the property should be observed frequently for signs of infectious disease. All farm personnel should be familiar with signs of infectious diseases and report any signs of disease promptly to a supervisor.

7. Separate pregnant mares from all other horses on the property, especially horses that travel frequently to other equine venues (e.g., shows, racetracks). Also consider separating mares into small groups (8 to10 mares per group) and keep groups physically separated to reduce cross contact. This will limit the on-facility spread of a disease if it occurs in an individual horse (such as EHV-1 abortion).

8. Limit access of visitors on the breeding facility to areas where they would have minimal contact with horses. For key personnel that need to have access to horses, have protocols in place to minimize the risk they pose. Vehicles and people are potential carriers of infectious organisms. Strategies of minimizing risk of transmission by humans include the required use of clean coveralls and shoe covers dedicated to a given facility (or disposable barrier protection) within each separated group of mares and foals. Personnel should wash their hands prior to contacting resident horses and prior to departure from a group of animals or the facility. In addition, foot baths should occur. This may include thorough hand cleansing with soap and water or the use of an alcohol based-hand sanitizer.

9. Use separate/dedicated equipment such as halters, lead ropes and blankets for each horse. Clean shared equipment and disinfect prior to use between horses (remove loose material, then appropriately clean, rinse, dry and disinfect).

Pre-breeding care of stallions (breeding and teaser): Determine the status of the stallion for selected infections prior to use. Stallions should be tested for venereally transmitted diseases, as well as EIA. Follow current recommendations (see Guidelines for Breeding a Mare to an Equine Arteritis Virus Shedding Stallion in the AAEP Resource Guide and Membership Directory) for breeding a mare to a known carrier/shedder stallion.

Use hygienic procedures during breeding. The breeding facility should be clean and well maintained. Wrap the mare's tail with clean disposable or washable material to prevent contamination from the tail hairs at breeding. The stallion’s penis, including the urethral diverticulum, should be rinsed with only clean water and then patted dry with a clean dry disposable towel. The person washing the genitalia should wear disposable gloves and change these between horses. The routine use of soap or disinfectants should be discouraged as it may increase the risk of removal of the normal flora and repopulation of the penile integument with *Klebsiella and/or Pseudomonas.*

To minimize the risk of cross-contamination between stallions, the external genitalia should be cleaned using a bucket of water. The bucket should be cleaned between stallions. It is recommended that a disposable liner be used in the bucket and changed between stallions. Ensure that the water source is not contaminated with potentially pathogenic bacteria, such as *Klebseilla* and/or *Pseudomonas*. Use an appropriate cleansing technique to minimize contamination of the clean water source by incorporating a standard “clean hand, contaminated hand” technique.

Pre-breeding care of the mare: Mares being bred by either natural service or artificial insemination (AI) should be examined to ensure that they are in the correct stage of the estrous cycle and free of potentially pathogenic microorganisms. In addition, the pre-breeding reproductive examination should be thorough enough to detect abnormalities that may interfere with the ability of a mare to become pregnant or carry a foal to term.

The facility for breeding mares should be safe for horses and facility personnel, and must be able to be cleaned and disinfected appropriately. Prior to either live cover or AI, the tail of the mare should be wrapped with a clean disposable or washable material to prevent contamination from the tail hairs. Thorough cleansing of the perineum is critical prior to breeding to limit infection of the reproductive tract. The person washing the mare should wear disposable gloves and change gloves between mares. The wash procedure may be accomplished with the gloved hand alone or with use of roll cotton, disposable paper towels or similar supplies. A ready supply of clean water should be available, either via a water hose or a water bucket with a disposable liner. It should be standard procedure to use a new bucket liner and fresh cotton or paper towel for each mare. In addition, appropriate washing procedures should be used to minimize contamination of the clean water source (e.g., a bucket with a disposable liner) by incorporating a standard 'clean hand, contaminated hand' technique. A non-residual liquid soap or a povidone-iodine scrub may be used.

A liberal amount of clean water is applied to the perineal area to remove gross debris. A small amount of soap or scrub is applied to either the gloved hand, cotton/paper towels or directly onto the perineal area. The perineal area is gently, but thoroughly scrubbed. Cleaning should begin in the central region around the vulvar lips and then moved to the anus and beneath the tail head, followed by the regions lateral to the vulva. After the entire area has been scrubbed, the region should be rinsed with clean water. The procedure should be repeated as needed until the area is clean. The entire washed area should be dried with clean disposable paper towels after the final rinse. The drying procedure should be initiated directly on the vulvar lips, then moved to the anus, and then to each side. Special consideration should be given to the area at the base of the tail where water tends to accumulate within the tail wrap material. Failure to completely rinse away soap or failure to dry water from the perineum can adversely affect spermatozoal motility. In addition, it is important to gently part the vulvar lips and pass a moist paper towel or cotton pledge between the vulvar lips to insure that any particulate material, soap or water are removed from the vestibule. It is also advisable to remove any accumulations of smegma from the clitoral fossa and clitoral sinuses, and to also clean the clitoral fossa when washing the vulvar area. The clitoral fossa should be rinsed thoroughly with water to avoid the irritating effects of residual soap on this area.

Protocol for breeding via live cover: Before a mare is allowed to enter a shed for breeding, a veterinary certificate may be required by a stallion station to confirm that a mare is not a carrier of a venereally transmitted disease and is ready to be bred. Definitive identification of the mare should be required. All regulatory requirements must be followed. To verify requirements in your area, please check with your state/provincial animal health office. A current negative test result for Equine Infectious Anemia virus (i.e. either a Coggins test or EIA ELISA) and a Certificate of Veterinary Inspection (CVI) may be required.

Mares may be required to have been vaccinated against EHV-1 from 7 to 90 days prior to visiting the breeding shed. A negative aerobic bacterial uterine culture collected at least 48 hours previously may be required. The stallion station may require an additional culture for each heat period in which the mare is bred (cultures greater than 30 days old may not be accepted). Cultures and complement fixation (CF) tests for *Taylorella equigenitalis/asinigenitali* may be required for imported mares.

A vaginal speculum examination is often an integral part of the pre-breeding evaluation in a live cover program, but may not be required as part of the veterinary health certificate. Breeding shed requirements may vary between maiden, foaling, barren and imported mares.

Disposable gloves should be worn by the person directing the penis of a stallion into a mare. These gloves should be discarded into a covered trash receptacle prior to contacting other surfaces such as areas in the breeding shed or equipment used to handle the mare or other stallions. If a breeding roll is used during a live cover, the roll should be covered with a disposable plastic sleeve, which is changed between horses. If a twitch is used to restrain a mare during a live cover, the twitch should be disinfected with Nolvasan (chlorhexadine) solution prior to use on another horse.

Protocol for maintenance of artificial vaginas: Appropriate cleaning and storage of artificial vaginas will aid in controlling horizontal transmission of venereally transmitted diseases. Personnel cleaning the artificial vagina should wear disposable gloves/sleeves and change these between each cleaning procedure. To clean an artificial vagina, one accepted method is provided below:

1. Rinse the artificial vagina liner (inside and outside) thoroughly in hot (greater than 50-degrees Celsius/122-degrees Fahrenheit) running tap water and use a clean brush (brush should be cleaned with hot water and 70% isopropyl

alcohol between uses) to aid in removal of any particulate matter that is adhered to the latex rubber.
2. Rinse the artificial vagina with deionized or distilled water to remove any impurities in the tap water that could negatively impact sperm function.
3. Rinse the artificial vagina with 70% isopropyl or ethyl alcohol. This can be accomplished with a squirt or spray bottle that is designated for this purpose.
4. Hang the artificial vagina in a clean covered cabinet for drying and storage (protected from UV rays or natural sunlight to avoid drying and cracking of the liner over time).
5. Thoroughly rinse the sink used for cleaning artificial vaginas with tap water after each use, then wipe or rinse sink with 70% alcohol.

Soaps or disinfectants (other than alcohol) can permeate the latex rubber and later leach into semen, resulting in decreased semen quality; as such it is critical to thoroughly rinse the artificial vaginas if these products are used.

It would be ideal to have a latex artificial vagina liner dedicated and labeled for each individual stallion at facilities with multiple stallions. It would also be ideal to clean used artificial vagina liners in a sink separate from the one used to fill clean ones. Alternatively, disposable artificial vagina liners could be used for each stallion. Plastic disposable liners can be used to collect semen from stallions to reduce transmission of venereally transmitted diseases, but the texture of these liners is not tolerated well by some stallions.

Latex artificial vaginas that show material breakdown (cracks, thin spots, sticky spots or black areas) should be discarded and replaced.

Between uses, the case/cover of the artificial vagina should be thoroughly cleaned with hot water, then sprayed with 70% isopropyl alcohol and allowed to air dry. Other disinfectants should not be used if these will come in contact the stallion's penis as they can negatively impact semen quality.

Protocol for maintenance of breeding phantoms: Contaminated breeding phantoms have a high potential for horizontal transmission of venereally transmitted pathogens; therefore, precautions should be taken to eliminate such pathogens from this equipment.

It is advisable to thoroughly cover the back end of the breeding phantom (the portion that comes in contact with the stallion's genitalia) with a disposable plastic wrap prior to use. The wrapping material should be fitted properly to the breeding phantom such that

it will not easily come off during the semen-collection process. The wrapping material should be removed after each use and discarded. The cover of the breeding phantom should then be cleaned after each use. Personnel should wear disposable gloves/sleeves when cleaning a breeding phantom and the gloves should be discarded as soon as the cleaning process is completed. The phantom can be washed with soap and water, if visible debris is present, and then disinfected by applying 70% alcohol or 2% chlorhexadine solution. To avoid damage to semen during collection, chlorhexadine solution should only be used on the breeding phantom if the residual chlorhexidine will not contaminate the semen during collection and impair its quality. Reusable and washable covers can be made for individual stallion use.

Protocol for breeding via artificial insemination (AI): Routine evaluation of the semen should be performed. If an infectious agent is suspected to be contained in the semen, bacterial culture, virus isolation or polymerase chain reaction (PCR) testing should be considered, as appropriate. An aliquot of semen may be appropriately frozen and stored for testing or swabs placed in appropriate media for microbial testing at a later date.

Dilute semen in an extender containing appropriate antimicrobial drugs (AMDs), which may limit bacterial growth and transmission of pathogenic bacteria by AI. Most commercial extenders for cooled semen contain AMDs, or an AMD can be added to the base ingredients. Occasionally, a bacterial pathogen may survive in extended semen and may infect a mare following insemination.

Consider the contents of a cooled-semen shipping container as potentially contaminated. Wear disposable gloves when opening container and handling packages of extended semen. Clean all surfaces of the laboratory area in contact with the semen container and semen packages. Discard all disposable equipment promptly and properly. Wash hands thoroughly with soap and water after removing disposable gloves. Use sterile disposable equipment for insemination, including syringes, insemination pipette, obstetrical lubricant and obstetrical sleeve.

Clean and disinfect entire area, including examination equipment and flooring, after breeding/insemination procedure. Discard all disposable equipment promptly and properly. Wash hands with soap and water once all procedures are completed.

Disease-specific Information

Contagious Equine Metritis; *Taylorella equigenitalis/ asinigenitalis*

Questions and Answers: Contagious Equine Metritis Fact Sheet (USDA/APHIS/VS)

Definition: *T. equigenitalis* is the causal agent of contagious equine metritis (CEM). It is a non-systemic venereally transmissible disease of equids that can cause short-term infertility in mares and very rarely, abortion. It was first reported in England and Ireland in 1977 and named *Taylorella equigenitalis*. This bacterium is a fastidious, microaerophilic, Gram-negative coccobacillus. This organism was first recognized in the United States when it was found in Kentucky Thoroughbreds in 1978.

Carrier stallions have been found in the U.S. among horses imported in 2004 from Slovakia, and most recently in 2008-2010, in a variety of breeds of stallions (11 breeds) in seven states. Contagious equine metritis is considered a foreign animal disease and is therefore a reportable disease in the U.S.; positive samples must be reported by laboratories to the state veterinarian and USDA: APHIS: VS. In Canada, positive samples are reported to the Canadian Food Inspection Agency (CFIA). There is no evidence that *T. equigenitalis* is transmissible to humans.

Clinical Signs: The stallion is a carrier of the organism and does not develop any clinical signs. Infection in the mare is confined to the reproductive tract. Mares bred to a carrier stallion by natural cover or with contaminated semen by AI may develop a purulent vaginal discharge and a variable degree of inflammation of the uterus, cervix and vagina for up to 2 weeks post breeding. A mare bred with contaminated semen may not conceive on the first or second estrus after exposure. However, after being exposed and infected, subsequent breeding(s) will usually result in pregnancy.

Although stated in literature that abortion may occur, *T. equigenitalis* is very rarely a cause of abortion. Mares that become infected may also display shorter heat cycles than normal, but most mares eventually clear the infection, and most do not become long-term carriers. However, some mares remain persistently infected and can be a source of the organism for stallions either through natural cover or through contact with the stallion during teasing.

Testing Recommendations: It is recommended that all stallions being bred or collected at a semen-collection facility have a minimum of 1 set (4 swabs) that are culture negative for *T. equigenitalis* prior to the start of the breeding season. These test results with identification of the stallion and date of sampling should be provided by the veterinarian who collected and submitted the swabs for testing. This means the

stallions that are permanent residents or are sent for breeding management only to the primary facility (not bred at home) are tested once a year for *T. equigenitalis* prior to the beginning of each breeding season.

- Wait for negative results prior to breeding with the horse (approximately 7 to 10 days), unless the stallion is being bred or collected where it is resident, with the stallion owner's equipment.
- Educate stallion owners prior to each breeding season about newly suggested testing procedures and explain the reason for such changes as these may add to the cost of stallion management.
- Stallions that culture positive for *T. equigenitalis* cannot be used for breeding until treated, retested and cleared by the USDA: APHIS: VS and/or the state veterinarian (or following appropriate testing and treatment for each country).

Diagnostic Testing: Based on the recent occurrence (2008-2010) of CEM in the U.S., veterinarians need to be alert to this foreign animal disease (FAD). These guidelines recommend prophylactic, annual testing of stallions prior to the breeding season. In addition, mares with clinical signs of vaginal discharge or short cycling (i.e., early return to estrus as a result of prostaglandin release from acute endometritis), particularly after breeding to untested or imported stallions, should be evaluated for *T. equigenitalis*.

It is acknowledged that a single set of swabs lacks the sensitivity of more thorough testing as required for post-entry testing of male and female equines above 731 days of age being imported into the U.S. from CEM-affected countries. However, one single annual testing (point-in-time sampling) will aid in screening the stallion (and female) resident population in the U.S. and Canada. Such testing will provide greater reassurance of the breeding population's freedom from *T. equigenitalis* and could help in persuading international trading partners to reduce the level of testing required of horses imported from the U.S. and Canada.

*Taylorella equi*genitalis can be detected by collecting swabs from specific sites in the mare and the stallion and placing each swab in a separate tube of Amies transport medium with charcoal and sent to a laboratory approved for testing for CEM. The organism is difficult to culture, requires a CO_2 incubator, and samples must be shipped on ice packs and arrive cool at the laboratory and be plated out within 48 hours of collection. The collection of these samples should be performed by a veterinarian who has been trained in how to collect, handle and transport the samples by a state or USDA: APHIS: VS veterinarian. These are similar to requirements of the Canadian Food Inspection Agency (CFIA).

It is recommended that swabs are obtained, wearing disposable gloves, from four sites on the unwashed external genitalia of the stallion. The sites that are to be swabbed are the shaft of the penis and prepuce, diverticulum of the glans penis (urethral sinus), the fossa glandis and the distal urethra. Multiple sets of swabs can increase the chances of detecting the presence of *T. equigenitalis* in a high-risk stallion, such as a recently imported stallion or a stallion that may have been treated with antimicrobials. Serial sets of swabs must be collected at least 72 hours apart.

Where there are strong grounds for suspecting the carrier status of a particular stallion, test mating (live cover) may be required to detect *T. equi*genitalis in a stallion negative for the bacterium on culture and/or PCR assay. This entails collecting of swabs from each of two "approved" mares (in which *T. equi*genitalis has not been isolated by appropriate culturing and a negative complement fixation [CF] test), following live cover by the suspect carrier stallion (test mating). Blood should also be collected from the test mares 21 to 28 days after live cover by the suspect carrier stallion to determine if seroconversion to *T. equigenitalis* had occurred. Swabs from test mares should be obtained from the clitoral fossa using a standard sized swab and the clitoral sinuses should be cultured with mini-tipped swabs that are small enough to enter the clitoral sinuses. Many mares have multiple sinuses and each one should be cultured if possible prior to washing the vulva. One mini-tipped swab can be used to sample multiple clitoral sinuses. After the vulva is washed, a deep cervical or an endometrial swab should be obtained. Test mares are to be swabbed on three occasions within a 12-day period, with swabbing starting no earlier than the third day and no later than the fourteenth day with a minimum of 72 hours between sampling. One of the three sets of swabs must include an endometrial or deep cervical swab. Swabs in Amies transport medium with charcoal must be kept chilled in transit and should either be hand delivered or sent by overnight courier Monday through Thursday to a laboratory approved by the USDA to culture for *T. equigenitalis*.

Any regulatory work should be reviewed in the Code of Federal Regulations (CFR) prior to initiation of any sample procurement to insure the most up to date requirements.

A combination of test types (ie, culture and serology) is indicated when evaluating the status of test mares, as no one test may detect infection in one or both test mares. A polymerase chain reaction (PCR) test is currently being validated for the detection of *T. equi*genitalis.

Specific Control Measures – Semen Collection: These are recommended for stallions used for semen collection (semen evaluation, fresh semen, cooled-transported semen, semen freezing):

- Swab samples prior to the onset of a stallion's breeding season
  - A minimum of one set of swabs (consisting of swabs from the four designated sites of the stallion as described above) should be negative for *Taylorella equigenitalis* on bacteriological culture prior to semen collection (results can take up to 7-10 days). Samples should be sent by overnight courier Monday through Thursday to a laboratory approved by the USDA to culture for CEMO.
  - Stallions shall not be subjected to semen collection or natural cover while awaiting results of CEM testing.
  - Optimally, testing should be repeated if the stallion is bred by natural cover or his semen is collected at a facility other than the primary collection facility before subsequen semen collections are again performed at the primary facility.

- Teaser stallions
  - Teaser stallions should be tested for CEM prior to the beginning of each breeding season. Teaser stallions pose a potential risk of disease transmission due to their frequent to exposure to multiple mares on the premises thus their health status should be monitored regularly and they should receive preventative care such as vaccinations similar to the other horses at the facility.

- Teaser mares—suggested recommendations are indicated due to the risk of inadvertent infection and transmission of disease
  - Use of a live mare for semen collection (jump mare) should be avoided when possible. Use of a jump mare should only be for stallions deemed low risk for CEM (novice stallions or those recently cultured negative). Jump mares and teaser mares should be tested for CEM prior to the beginning of each breeding season (culture of swabs of clitoral fossa, clitoral sinus and cervix/uterus).
  - If semen is collected using a jump mare rather than a phantom, attempts should be made to divert the penis into the AV so as to avoid penile contact with the hindquarters of the mare.

Specific Control Measures - Natural Cover:

- Annual set of CEM swabs prior to that stallion's breeding season
  - One set of swabs (from each of the four designated sites as described above) should be negative for *T. equigenitalis* on

bacteriological culture prior to breeding the stallion (results can take up to 7-10 days). Samples should be sent by overnight courier Monday-Thursday to a laboratory approved by the USDA for the culture for *T. equigenitalis*. Stallions should not be collected for semen evaluation or bred natural cover while awaiting results of CEM testing.

- Teaser stallions. The contact surface of any shields used to prevent the stallion from breeding the mare should be laundered or cleaned of all visual debris and sprayed with 2% chlorhexadine solution prior to reuse. Teaser stallions should be tested for CEM annually.

- Sampling of dismount semen. Personnel obtaining dismount samples should wear disposable gloves and use a semen receptacle that is appropriately disposed of after use.

- Cleaning perineal area of mares. Personnel should use a disposable tail wrap and gloves, which are changed between mares. The vulvar area is to be cleaned with water from a handheld spray nozzle, or with cotton and water from a bucket with a disposable liner. A new liner is used for each mare. The hose nozzle should not touch the hand that is used to wash the mare, and the nozzle should be disinfected daily.

- Breeding rolls. These should be covered with a clean shoulder-length disposable sleeve that is changed between uses and the breeding roll surface cleaned and disinfected.

Equine Viral Arteritis (EVA)

Definition: Equine viral arteritis (EVA) is a contagious disease of equids caused by equine arteritis virus (EAV), an RNA virus that is found in horse populations in many countries. While typically not life-threatening to otherwise healthy adult horses, EAV can cause abortion in pregnant mares (and uncommonly cause death in young foals) and establish a long-term carrier state in breeding stallions. While various horse breeds appear equally susceptible to EAV, the prevalence of infection can vary widely with higher seropositivity rates occurring in Standardbred and Warmblood horses.

Historically, outbreaks of EVA have been relatively infrequent. However, the number of confirmed occurrences appears to be increasing, likely attributable to increases in the:

- Global movement of horses.
- Accessibility of carrier stallions.
- Use of shipped cooled or frozen virus-infective semen.

Transmission most frequently occurs through direct contact with virus-infective respiratory secretions, leading to widespread dissemination of the virus among susceptible horses that are close to each other. Venereal transmission by infected stallions has a significant role in virus spread on or between breeding farms.

Equine arteritis virus can be efficiently spread through artificial insemination (AI) and the use of raw, cooled-transported or frozen semen. The virus has been shown to remain viable for considerable periods of time in raw (fresh), extended or frozen semen held at temperatures equal to or less than 4^0C. Indirect transmission, though less significant, can occur through contact with virus-contaminated fomites.

The majority of primary EAV infections are subclinical or asymptomatic. EVA can vary in clinical severity both between and within outbreaks. EVA cannot be diagnosed on clinical signs alone, as case presentation is similar to various other infectious and non-infectious equine diseases. Laboratory confirmation is required for diagnosis.

Clinical signs: Clinical signs, if they occur, typically develop 3-13 days post exposure (6-8 days where transmission has occurred by the venereal route). They may include all or any combination of the following:

- Fever
- Depression (Lethargy)
- Anorexia
- Dependent edema ventral (ventral thorax/abdomen, lower limbs, scrotum and prepuce or mammary glands)
- Localized or generalized urticaria
- Supra or periorbital edema
- Conjunctivitis
- Serous to mucoid nasal discharge

Abortion is a frequent sequel to infection and can occur from 2 months to term in the unprotected pregnant mare. Young foals exposed to EAV can develop a life-threatening pneumonia or pneumoenteritis.

A carrier state can develop following EAV infection in the post-pubertal colt or stallion. The virus can persist in certain of the accessary sex glands of the reproductive tract of stallions for many years and may result in lifelong infection. The carrier stallion is widely accepted as the natural reservoir of EAV and the source of diversity among naturally occurring strains of the virus.

Diagnostic Testing: EVA cannot be diagnosed based on clinical signs alone, as case presentation is frequently similar to various other infectious and non-infectious equine diseases. Laboratory confirmation is required for diagnosis.

This is based on virus isolation, RT-PCR testing, and/or serological examination of paired sera.

Virus detection is accomplished by virus isolation in cell culture and/or RT-PCR testing of whole blood (EDTA or citrate but not heparin), nasal, nasopharyngeal swabs/washings, fetal and placental tissues/fluids.

Sampling for virus detection should be initiated as early as possible after onset of clinical signs. EAV is stable at refrigeration or lower temperatures. With the exception of unclotted blood samples, specimens should be refrigerated or frozen and shipped on frozen freezer packs. Unclotted bloods should be kept cold but not frozen in transit to the laboratory.

Paired (acute and convalescent) sera should be collected over an interval of 2-4 weeks. Previous vaccination history against EVA should be considered when interpreting positive titers. Vaccinated individuals may develop a serologic response or rapid rise in titer in response to natural exposure to infection.

Vaccine: The current licensed vaccine in North America is a highly attenuated, modified live virus (MLV) product. It has been shown to be safe and effective in stallions and non-pregnant mares. Mild post-vaccinal febrile reactions with transient lymphopenia have been observed in a small percentage of first-time vaccinated horses. Primary vaccination provides clinical protection against EVA but does not prevent re-infection and limited replication of challenge virus. However, in first-time vaccinates, the frequency, duration and amount of vaccine virus that is shed via the respiratory tract is significantly less than that observed with natural infection. The occasional stallion may shed very low concentrations of vaccine virus in its semen within the first week after vaccination.

Vaccination in the face of an EVA outbreak has been successful in controlling further spread of the virus within 7 to 10 days. Immunization with the MLV vaccine stimulates a rapid protective response, which in turn restricts development of the cell-associated viremia and viral shedding via the respiratory tract or in semen in horses naturally exposed to infection. As a consequence, the amount of EAV in circulation is greatly decreased, limiting further spread of the virus.

Vaccination Schedules: In planning a vaccination program against EVA, it is important to consult with state and/or federal animal health officials to ensure that any such program is in compliance with the state's control program for EVA, if one exists.

The indications for vaccination against EVA have been to:

- Protect stallions against infection and subsequent development of the carrier state.
- Immunize seronegative mares before being bred with EAV-infective semen.
- Curtail outbreaks in non-breeding populations.

NOTE: It is not possible to differentiate a vaccine-induced antibody response from that due to natural infection. It is strongly recommended therefore, that *prior to vaccination*, serum from all first-time intact males to be vaccinated against EVA be tested and confirmed negative for antibodies to EAV by a USDA/CFIA- approved laboratory. Mares intended for export should be similarly tested.

Vaccination Schedule for Stallions: Breeding stallions that were previously vaccinated should receive a booster vaccination against EVA every 12 months and not less than three weeks prior to the start of each breeding season.

For breeding stallions that are first-time vaccinates:

- Prior to initial vaccination, all stallions undergo serologic testing and are confirmed to be negative for antibodies to EAV.
- Testing should be performed shortly prior to or preferably at the time of vaccination.
- Certification of seronegative test is of high importance should a vaccinated stallion be considered for export at a later date.
- All first-time vaccinated stallions should be isolated from any other seronegative horses for not less than three weeks following vaccination and before being used for breeding.

Teaser stallions can play a role in the introduction and dissemination of EAV within a breeding population. Vaccination against EVA is recommended on an annual basis.

Vaccination Schedule for Mares: Mares to be bred to carrier stallions or to be bred with virus-infective semen should first be tested to determine their serological status for EAV antibodies. Seronegative mares should be vaccinated against EVA and isolated from any other seronegative horses for three weeks. The purpose of the isolation period is twofold:

- Enable the vaccinated mare adequate time to develop immunity against the virus before potentially being exposed to EAV infection during natural breeding.
- Afford ample opportunity for cessation of possible post-vaccinal viral shedding via the respiratory tract.

Following insemination, first-time vaccinated mares must be isolated for an additional three-week period as they are likely to experience a limited re-infection cycle with the strain of EAV present in the semen. Should such mares fail to become pregnant, they can be bred back to a carrier stallion or with infective semen without the need for revaccination or an additional three-week isolation period post insemination.

Mares testing seropositive for antibodies to EAV can be bred to a carrier stallion or with infective semen for the first time without the need for prior vaccination against EVA. After breeding, such mares should be physically separated from unvaccinated or unprotected horses for 24 hours to avoid possible risk of mechanical transmission of virus from voided semen.

The manufacturer does not recommend use of the MLV vaccine in pregnant mares, especially in the last two months of pregnancy. Under circumstances of high risk of natural exposure to infection, however, the vaccine has been administered to pregnant mares in order to restrict spread of the virus and to control outbreaks of the disease. Based on experimental studies and extensive field experience using this vaccine, the last 1-2 months of pregnancy represent the time of greatest risk for a possible adverse effect on pregnancy. This was most recently illustrated in the aftermath of the 2006 multi-state occurrence of EVA when a very limited number of abortions caused by the vaccine virus were confirmed in mares vaccinated within the final two months of gestation but not during the first two trimesters of pregnancy.

Nurse mares can play a role in the introduction and spread of EAV among resident equine populations and should be vaccinated annually according to recommended protocols.

Vaccination Schedule for Foals: The manufacturer does not recommend the use of this vaccine in foals less than six weeks of age unless under circumstances of high risk of natural exposure to infection.

Male foals (colts), especially in EAV-endemic breeds, should be vaccinated between 6-12 months of age to protect against the risk of their becoming carriers later in life. Colts should be confirmed seronegative for antibodies to EAV prior to vaccination as described above and kept isolated for three weeks following vaccination. Because foals of EAV- seropositive mares can carry colostral derived antibodies for up to six months, testing and vaccination should not be performed prior to six months of age.

Outbreak mitigation: For the non-breeding population, vaccination is an effective strategy in containing outbreaks, particularly in closely congregated groups of horses where isolation may be problematic. Serology testing, as described above, should be performed on intact males and females that may be intended for future breeding purposes and/or export.

For breeding populations, outbreaks of EVA can be complex and can have far-reaching implications. Vaccination is a component of outbreak management but should be performed only after consultation with and under the direct supervision of a veterinarian.

Equine Infectious Disease Outbreak: AAEP Control Guidelines

Vaccination and Exporting of Horses: In instances where there is uncertainty or concern over whether vaccination against EVA could prevent the export of a horse to a particular country, it is advisable to consult the federal area veterinarian in charge in the state to determine the specific import requirements of that country. A number of countries bar entry of any equid that is serologically positive for antibodies to EAV, regardless of vaccination history. Countries that do accept EVA-vaccinated horses, regardless of gender, typically require stallions or colts to have a certified vaccination history and confirmation of pre-vaccination negative serological status.

Equine Herpes Virus 3 (EHV-3)

Definition: Coital exanthema is a venereally transmitted disease of horses caused by Equine Herpesvirus 3 (EHV-3). It is not currently a reportable disease in the U.S. or Canada. It is usually limited to the penile skin of stallions and the vulva/perineum of mares. It initially causes vesicular lesions that heal spontaneously and leave whitish plaque-like scars.

Clinical Signs: An incubation of 5 to 9 days is typical, starting with fluid filled vesicles and progressing to crater-like ulcerative lesions on the epithelial surface of the penis and skin of the perineum or vulva. Systemic signs are rare. Reactivation of latency can occur under condition of stress. This herpesvirus has not been associated with abortion.

Transmission: Equine herpesvirus-3 is highly contagious and may be passed between horses by nose-to-nose contact and by contaminated fomites, in addition to breeding by natural cover or artificial insemination (AI). Infected stallions or mares should not be used for natural breeding or semen collection until lesions are fully healed. Lesions are considered to be healed when the crater-like lesions are filled in, resulting in smooth whitish scars, and there are no signs of acute inflammation or discharge.

Diagnostic Testing: Diagnosis is based on the presence of typical pox-type lesions. A diagnosis of EHV-3 may be confirmed by virus isolation or by PCR from active lesions, by negative contrast electron microscopy and possibly by testing paired serum samples with the objective of demonstrating a four-fold or greater rise in neutralizing antibody titers to EHV.

Specific Control Measures: Breeding of affected stallions, including semen collection for AI, should not occur until the lesions have completely resolved. Lesions are considered to be healed when the crater-like lesions are filled in, resulting in smooth whitish scars, and there are no signs of acute inflammation or discharge.

Biosecurity Guidelines: Dedicated artificial vaginas, barrier procedures and gloves used in semen collection should be successful in preventing horizontal transmission and contamination of breeding equipment in latent carriers or stallions discovered to have lesions after collection. Stallions needing tease mares for phantom collection of semen should be restrained to avoid nose-to-nose or nose-to-vulva contact with theses mares. False mounting tease mares should be avoided. Tease mares should be visually inspected for EHV-3 lesions at each use. The virus is easily destroyed by common disinfectants, heat, sunlight and drying.

Disclaimer

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