American Association of Equine Practitioners (AAEP)

Biosecurity Guidelines for Control of Venereally Transmitted Diseases

Introduction

These guidelines are intended to serve as recommendations. They 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 to be taken 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 available to members on the AAEP website (e.g., Vaccination Guidelines) are recommended reading. Even though not strictly speaking correct, in this document, the term horse will be used to refer to all equids (horse, donkey, mule, and pony).

The emphasis of the Biosecurity Guidelines for Control of Venereally Transmitted Diseases is to control the transmission of:

Taylorella equigenitalis (Contagious Equine Metritis Organism; CEMO)
Equine arteritis virus (EAV)
Equine herpesvirus-3 (EHV-3; 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 is a venereally transmitted disease of equids (i.e., horses, donkeys, mules). This organism can be recovered from the uterus in acutely infected female equids that have genital discharges. Edema is also a characteristic of the disease, and development of edematous ‘silver dollar plaques’ is considered pathognomonic for the disease. Transmission of dourine takes place almost exclusively by coitus, principally from an infected stallion to a mare. While spread of the causal agent by artificial Insemination (AI), has not been confirmed this could potentially occur since *T. equiperdum* is present in seminal fluid. Appropriate samples for the diagnosis of

dourine include blood, serum, seminal fluid, mucous exudate on the penis/sheath of stallions, and vaginal secretion of infected mares. If the disease is suspected, state and federal health authorities must be notified. Treatment is not permitted. Since dourine is a transboundary disease in the US, any confirmed cases must be euthanized. Moreover, even if permitted, treatment would not necessarily eliminate *T. equiperdum* from the reproductive tract and infected animals would remain inapparent carriers.

Other infectious agents, such as the bacteria *Pseudmomonas aeruginosa* and *Klebsiella pneumonia*e) have been incriminated as venereally transmissible pathogens; however, there is a lack of scientific information on the pathogenicity of different strains of these bacteria and their ability to cause disease. For example, there is evidence that only a few capsule types of *Klebsiella pneumoniae* and only a few serotypes of *Pseudomonas aeruginosa* are truly venereal pathogens. Accordingly, simply recovering either of these two organisms using standard aerobic culture techniques is not of itself sufficient proof that either bacterium is inherently pathogenic and a cause of venereal disease. Very few laboratories currently offer capsule typing or serotyping services for either bacterium. Nevertheless, mares susceptible to infection can develop endometritis when exposed to pathogenic organisms at the time of breeding.

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 AI program, some of these diseases are highly contagious and have been shown to be transmissible between animals by direct horse-to-horse contact, through use of contaminated semen, or by indirect venereal contact by the use of contaminated semen collection and processing equipment (including 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 consequences 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.

Natural service (live cover) and AI are associated with inherent infectious disease risks. Advanced reproductive procedures (e.g., embryo transfer, oocyte transfer and intracytoplasmic sperm injection followed by embryo culture and transfer) may also be mechanisms for transmission of infectious disease agents. For example, viruses (particularly Equine Arteritis Virus) can be very difficult, if not impossible, to remove from equine embryos despite being subjected to multiple washes; accordingly, this may result in infection of recipient mares upon transfer.

Although horses used for breeding are the focus of these guidelines, non-breeding equids must also be considered when evaluating biosecurity. For example, when a mare is exposed to an infectious agent at a breeding facility and returns to its farm of residence with its foal, the foal may be at risk of acquiring infection from its dam. Therefore, a biosecurity plan for an equine breeding farm must also 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. Live cover breeding has generally been thought to provide greater risk of venereal disease transmission than AI. However, AI with contaminated fresh, cool-transported or frozen semen can also be responsible for transmission of venereal pathogens.

Infectious diseases that are important in the equine breeding industry are not limited strictly to agents transmitted during coitus or insemination. Stallions, mares and foals are at risk of disease from a wide variety of infectious organisms (viruses, bacteria, parasites). The mode(s) of transmission and propensity to spread to other horses on a farm vary with each organism and the susceptibility of the at-risk population.

Internal parasites continue to be a problem at horse facilities, including breeding farms. Resistance to certain anthelmintics is a problem of increasing importance in many geographical areas. Appropriate identification of parasitized horses and development of a strategic deworming program should be a high priority and an integral part of 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 measures employed should depend on potential risk, geographic location, animal density, horse traffic, ages of resident horses, breeding management procedures (e.g. live cover versus AI) and other factors, such as importation of embryos or preserved semen. Biosecurity protocols should be understood by all facility personnel and

reviewed regularly. State and federal animal health authorities must be notified should a reportable venereal disease be suspected.

Managers of breeding operations, 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) and procurement of a health certificate from an accredited and licensed veterinarian prior to transport, as well as pre-breeding testing and vaccination of serologically-negative stallions against Equine Viral Arteritis (EVA) or testing for *Taylorella equigenitalis* (CEMO) where required (e.g., following initial importation, or upon return from breeding in a USDA listed CEM-affected country). The window of time given for collecting samples for testing and reporting can be limited. To verify testing requirements in your area, please check with your state/provincial animal health office (see U.S. State Veterinary Offices), (see Canadian Provincial Veterinary Offices), and USDA-APHIS animal health regulations.

While this communication specifically addresses venereal diseases, the potential modes of transmission of any disease agent on a breeding farm include:

1. Direct horse-to-horse, 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 AI.
5. Fomite exposure, including contaminated semen collection/processing/insemination 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 aspiration or 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 can reduce the risk of bacterial contamination of the female reproductive tract by eliminating physical contact between the mare and

stallion and by incorporating antimicrobial drugs (AMD) in the semen extender. Artificial insemination with shipped semen allows the mare (and foal) to remain at home and thus minimize risk of exposure 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 AMD cannot be expected to kill or eliminate all bacteria in a semen sample. Furthermore, viruses are not killed by AMD nor are they eliminated by cooling or freezing.

2. Consider whether the stallion is at risk for carrying an infectious disease agent. Determine if the horse has been tested for Equine Infectious Anemia, CEM (*Taylorella equigenitalis*) and EVA (serology, agent detection). Testing is strongly recommended if the stallion is at risk of infection. Check whether there are potentially pathogenic bacteria or viruses on the external genitalia or in the semen.

3. If a mare has to be moved to a breeding facility other than its farm of usual residence, assess whether the mare and/or the foal by its side are at risk of contracting a disease. Quarantine of the mare (and foal) for an appropriate time, preferably 21 to 28 days, is recommended upon their return to the home farm.

Recommendations for a biosecurity program for horses on a breeding facility:
Additional information on general biosecurity guidelines is available to AAEP members on the AAEP website here.

1. Allow only healthy horses to enter the facility. Entrance will 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 herd of origin and the premises.

2. Examine all new arrivals for signs of contagious disease and to verify that the CVI, including individual animal identification, vaccination history, other tests required by the destination 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 premise. Unless the disease status and test results are known for leased mares or teaser stallions, appropriate quarantine and diagnostic testing should be performed to ensure they are not carriers of communicable disease(s) prior to exposing the breeding population on the 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 possibly increasing this to 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 an 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 vacated stall the horse resided in is essential. Procedures for caretakers, housing, manure disposal, stall disinfection, etc. are available within the AAEP Biosecurity Guidelines.

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 daily 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 (i.e. no shared fencing or nose-to-nose contact) to reduce cross contact until all the mares in a group have foaled. This will limit the on-facility spread of a disease if it occurs in an individual horse (i.e., EHV-1 abortion).

8. Vehicles and people are potential sources of infectious organisms. 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. Strategies for 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. Use of disposable gloves is recommended. Otherwise, personnel should wash their hands prior to contacting resident horses and prior to departure from a group of animals or the facility. This shall include thorough hand cleansing with soap and water or the use of an alcohol based-hand sanitizer. In addition, use of foot baths for human traffic in barns or between paddocks/farms may help prevent dissemination of infectious organisms.

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 infectious agents prior to use. All stallions should be tested annually for EIA. Some states (e.g., Kentucky and New York) require breeding stallions to be vaccinated yearly against EVA. Maintaining permanent medical records confirming that a breeding stallion was seronegative for antibodies to equine arteritis virus prior to vaccination against EVA is very important.

Evaluating stallions prior to each breeding season for venereally transmissible diseases is a highly recommended practice since most major causes of venereal disease give rise to an asymptomatic carrier state in the stallion. Detection of the persistently infected stallion requires laboratory examination of semen or other specimens from the reproductive tract. Venereal organisms can easily be transmitted unknowingly within and from breeding centers using natural service or AI. Regulatory officials must be notified if a stallion is identified as carrying CEMO, and treatment/control measures must be supervised by state/federal animal health officials. For confirmed EAV shedding stallions, follow current recommendations (see Recommendations for Transported Semen as it is related to Equine Viral Arteritis (EVA) and Vaccination Schedule for Mares in the EVA discussion of this document for breeding a mare to a known carrier/shedder stallion. *See page 18*).

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 only with 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 potential pathogens.

To minimize the risk of cross-contamination between stallions, the external genitalia should be cleaned using a dedicated water bucket if possible. Alternatively, the bucket should be cleaned and disinfected between stallions. It is recommended that a disposable plastic liner be used in the bucket and changed between stallions. Ensure that the water source is not contaminated with potentially pathogenic bacteria, such as *Klebsiella pneumoniae* and/or *Pseudomonas aeruginosa*. 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 AI should be examined to ensure that they are in the correct stage of the estrous cycle and are free of any clinical evidence of infection with potentially pathogenic microorganisms. For imported mares, a series of diagnostic tests must be performed in a prescribed manner. All regulatory requirements must be followed. To verify requirements in your area, please check with your state/provincial animal health office (see State Veterinary Offices in the AAEP Resource Guide and Membership Directory).

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 prior to breeding reduces the likelihood of infection of the mare's reproductive tract. The person washing the mare's external genitalia 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. Fresh cotton or paper towels should be used for each mare to minimize chances of contamination; if possible, and depending on circumstances, a separate bucket, or bucket with disposable plastic liner, will further minimize chances of cross contamination. In addition, appropriate washing procedures should be used to minimize contamination of the clean water source 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 (up to 6 inches) 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. It is important to gently part the vulvar labia and pass a moist paper towel or cotton pledget between the vulvar lips to ensure 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.

Protocol for breeding via live cover: Before a mare is allowed to enter a shed for breeding, a veterinary certificate should be required by a stallion station to confirm that a mare has no physical signs of genital tract infection and that a recent uterine culture yielded no significant growth of potentially pathogenic bacteria. 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 (see State Veterinary Offices in the AAEP Resource Guide and Membership Directory). 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 also be required.

Mares may also be required to have received specific vaccine(s) during an approved interval prior to visiting the breeding shed. Depending on the initial breeding status of the mare (maiden, foaling, barren), the stallion station may require an additional culture for each heat period in which the mare is bred (i.e., cultures carried out more than 30 days previously may not be acceptable). Negative cultures and complement fixation (CF) tests for *Taylorella equigenitalis/asinigenitalis* may be required prior to breeding of imported mares.

A vaginal speculum examination, using a sterile (preferably disposable) equipment, 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 cleaning the stallion's penis and directing it into the mare's vagina. 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. Disposable gloves should also be used by personnel holding the tail and/or breeding roll, and lead rope (and twitch if used). 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 prior to use on another horse. Before each breeding session, all facilities used to handle and breed mares, as well as the breeding shed itself, should be cleaned and disinfected as necessary.

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 an artificial vagina should wear disposable gloves/sleeves and change these between each cleaning procedure. One accepted method of cleaning an artificial vagina, 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 large bottle-, flask- or beaker-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. Since soaps or disinfectants (other than alcohol) may permeate the latex rubber and subsequently leach into semen, their use is not recommended.
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, ensuring the entire latex liner is covered. Rinsing can easily 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.

It would be ideal to have a latex artificial vagina liner dedicated for each individual stallion. It would also be ideal to clean used artificial vagina liners in a sink separate from the one used during filling of liners. 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 if the stallion will tolerate its use.

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 or rinsed with 70% isopropyl alcohol and allowed to air dry.

Protocol for maintenance of breeding phantoms: Contaminated breeding phantoms have a high potential for horizontal transmission of venereal pathogens. 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 after use. The phantom can be washed with soap and water, if visible debris is present, and then disinfected by applying 70% alcohol. To avoid damage to semen during collection, disinfectants should only be used on the breeding phantom if any residue will not contaminate the semen during collection and impair its quality (once dried, alcohol leaves no residue, in contrast to some other disinfectants). Reusable and washable covers can be made for individual stallion use. Where only farm horses previously confirmed free from venereal disease are involved, modification of this cleaning regimen can be considered.

Protocol for AI: Routine evaluation of the semen prior to insemination may reveal signs that an infectious agent may be present (e.g., numerous neutrophils, contaminating debris). Further diagnostic testing should be done if deemed necessary.

It is recommended that all semen be diluted in a semen extender containing appropriate antimicrobial drugs (AMD), which may limit bacterial growth and transmission of potentially pathogenic bacteria by AI. Most commercial equine semen extenders contain AMD, or an AMD can be added to the base ingredients. In some cases, AMDs are not present in commercial extenders at desirable concentrations, in which case further AMD may be added. Be aware that, bacterial or fungal pathogens may survive in extended semen containing AMD and might still result in infection of a mare following insemination.

Consider the contents of a transported-semen shipping container as potentially contaminated. While there is no way to recognize the presence of CEMO or EAV in transported or frozen semen (other than culture or PCR testing), be aware that both diseases have been transmitted by breeding with infective semen from shedding stallions, and even recipient mares have been infected with EAV following transfer of embryos derived from donor mares bred with infective semen. Wear disposable gloves when opening a shipping container and handling packages of shipped semen. Clean all surfaces of the laboratory area in contact with the semen container and semen packages. Wash hands thoroughly with soap and water after removing disposable gloves.

Use sterile disposable equipment for insemination, including all-plastic syringes and insemination pipettes, and use sterile tubes or individual sachets of obstetrical lubricant and obstetrical sleeves. Discard all disposable equipment promptly and properly. Wash hands with soap and water once all procedures are completed. If possible, clean and disinfect entire area, including examination equipment and flooring, after breeding/insemination procedure(s) is/are completed.

Disease-specific Information

Contagious Equine Metritis; *Taylorella equigenitalis*

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

Contagious equine metritis is considered a foreign animal or transboundary disease making it reportable 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.

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

A taxonomically closely related bacterium, *T. asinigenitalis*, was first discovered in a Mammoth donkey jack in California in 1997. Primarily an inhabitant of the distal reproductive tract of donkey jacks, this organism has not yet been confirmed as a cause

of CEM in horses or donkeys. *T. asinigenitalis* has been infrequently reported in the U.S. and is not known to cause clinical disease. There is currently no regulatory action taken when *T. asinigenitalis* is isolated and its main significance is in confirming diagnostically that the *Taylorella* organism isolated is not *T. equigenitalis*.

Several incursions of CEM caused by *T. equigenitalis* into the U.S. have occurred since 2006. A significant CEM outbreak occurred in 2008-2010 in which over 1,000 exposed horses in 48 states were required to be tested and 23 contaminated stallions and 5 infected mares were ultimately identified and treated. The source of that outbreak was determined to be a stallion imported to the U.S. from a CEM-affected country in 2000. The extended duration before detection of the primary infected individual in this instance emphasizes the need to maintain vigilance and follow protocols proven effective in preventing the introduction and spread of venereal disease organisms.

Transmission: Although venereal transmission of *T. equigenitalis* by live cover has historically been the most emphasized route of infection, extensive stallion-to-stallion transmission occurred in the 2008-2010 outbreaks through contaminated fomites at multiple semen collection facilities. Additionally, transmission to mares has occurred via fresh, cool-transported semen in which semen extender containing antibiotics was used. During the investigation of an incursion of CEM into the U.S in 2013, live *T. equigenitalis* was cultured from the frozen semen of a carrier stallion in that incident. These recent findings have highlighted that the risk of CEMO transmission is not limited to venereal transmission by live cover and that veterinarians need to be alert to the potential risk of future incursions of this foreign animal disease (FAD). Although uncommon, CEMO transmission from mare to foal either *in utero* or during parturition has also been documented.

Clinical Signs: The stallion is an asymptomatic carrier of the organism in which the latter exists as a commensal bacterium on the external genitalia. Infection in the mare is confined to the reproductive tract. Mares bred to a carrier stallion by natural cover or by AI with contaminated semen can develop a mucopurulent vaginal discharge that typically lasts 2-3 weeks. Infection is characterized by a degree of inflammation (often marked) of the oviducts, endometrium, cervix and vagina. The organism can be recovered from a variable percentage of mares for about three months after primary infection in mares with or without clinical signs. The clitoris (clitoral sinuses and fossa) is the most important site of persistence of the organism. A small number of mares become intrauterine carriers of *T. equigenitalis*. Salpingitis may occur that can persist for a longer period than endometritis, cervicitis or vaginitis. A mare bred with contaminated semen may not conceive on the first or second estrus after exposure. Subsequent breeding(s) will often result in establishment of a normal 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 shortened estrous cycles with an early return to estrus. Most mares eventually clear the infection, and do not become long-term carriers. However, some mares can remain persistently infected for several months or years and can be a source of the organism for at-risk stallions.

Testing Recommendations: Some recommend that all stallions being bred or subjected to semen collection at a given facility have a minimum of 1 set of four direct swab cultures (described below) that are culture negative for *T. equigenitalis* prior to the start of the breeding season. The test results for the prophylactic annual testing, with identification of the stallion, time and date of sampling, should be provided by the veterinarian who collected and submitted the swabs to a USDA approved VSL CEM laboratory for testing. This would mean that 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.

Diagnostic Testing: Prophylactic, annual testing of stallions prior to the breeding season has been proposed, but has not been mandated, by USDA: APHIS: VS as a means of reducing the risk of spread of CEM. Mares that develop genital discharges or that 'short-cycle' after breeding to an untested or imported stallion should be considered for evaluation for *T. equigenitalis* infection.

It is acknowledged that screening domestic (non-imported) stallions for CEM by testing a single set of direct swab cultures is not equivalent to the USDA testing protocol mandated on all post-entry male and female equines above 731 days of age (i.e., being imported into the U.S. from USDA listed CEM-affected countries). Specifically, during the 2008-2010 U.S. outbreaks, it was documented that out of the 23 infected stallions ultimately found, 18 stallions were identified positive on the first set of direct swab cultures, 1 stallion on the second set, and 1 stallion on the third set of cultures. Three (3) of the 23 infected stallions were not positive on direct swab cultures and were found positive only after breeding susceptible test mares by live cover. However, one single annual testing (point-in-time sampling) could aid in screening the stallion (or mare) resident population in the U.S. and Canada. Such testing could 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.

Transport media and shipping of samples: *Taylorella equigenitalis* can be detected by directly swabbing specific sites in the mare and the stallion, placing each swab in a separate tube of Amies transport medium with charcoal, marking each tube with stallion

identification, site of swab sample and time and date of collection, and sending the swab set to a USDA approved laboratory for testing for CEM. Samples must be shipped on ice packs to arrive cool at the laboratory and be inoculated onto appropriate media within 48 hours of collection. Therefore, samples should either be hand delivered or sent by overnight courier Monday through Thursday to the approved laboratory. The collection of these samples should be performed either by a regulatory veterinarian, or an accredited veterinarian who has been instructed how to collect, handle and transport such samples by a state or USDA/APHIS/VS veterinarian. These are similar to requirements of the Canadian Food Inspection Agency (CFIA).

Please consult this USDA/APHIS list of CEM approved laboratories.

***Collection of direct swab samples from a stallion for prophylactic annual testing*:** It is recommended that swabs are obtained, wearing disposable gloves, from four sites on the unwashed external genitalia of the stallion: the shaft of the penis and prepuce; dorsal diverticulum of the fossa glandis (urethral sinus); the fossa glandis; and the distal urethra.

***Collection of direct swab samples from a mare*:** Direct swab samples should be obtained, while wearing disposable gloves, from at least two sites, the clitoral fossa and clitoral sinuses. A standard-sized swab should be used to collect samples from the clitoral fossa, while mini-tipped swabs small enough to enter the clitoral sinuses should be used to sample those sites. One mini-tipped swab can be used to sample multiple clitoral sinuses. Collecting more than one set of swab samples at 72hour intervals (as required for mares imported from CEM-affected countries) can increase the chances of detecting the presence of *T. equigenitalis* in mares. A third sample site, a deep cervical or endometrial swab, can also be collected. Washing of the vulva in preparation for a deep cervical or endometrial swab collection should be performed after all clitoral samples have been collected. Additionally, unlike stallions, mares produce a short-lived systemic antibody response to *T. equigenitalis* infection, so a serum sample should also be collected on clinical or recently exposed mares for complement fixation testing (CFT) for *T. equigenitalis.*

Where there are strong grounds for suspecting the carrier status of a particular stallion (or mare), this must be reported to the USDA: APHIS: VS veterinarian and state veterinarian. All testing procedures, under their supervision, may then be required according to previously established guidelines. Any regulatory work should be reviewed in the Code of Federal Regulations (CFR) prior to initiation of any sample collection to ensure that they are in keeping with the most up-to-date requirements.

Specific Control Measures – Semen Collection: The following recommendations are for stallions used for semen collection for any reason:

- Swab samples should be taken prior to the onset of a stallion's breeding season
  - A minimum of one set of direct swab samples (procured from each of 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 transported to a USDA approved laboratory as previously described.
  - Where possible, stallions should not be subjected to semen collection for insemination of mares or natural cover while awaiting results of laboratory testing for CEM.

- Teaser stallions
  - Teaser stallions should be cultured for *Taylorella equigenitalis* prior to the beginning of each breeding season. Teaser stallions pose a potential risk of disease transmission due to their frequent exposure to multiple mares on a premise; thus, their health status should be monitored regularly, and they should receive the same preventative care such as vaccinations as 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 if the stallion is trained to a phantom. Jump mares and teaser mares should be tested for CEM prior to the beginning of each breeding season (culture swabs from the clitoral fossa, clitoral sinuses 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 quickly 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
    - Direct swab samples (from each of the four designated sites as described above) should be negative for *T. equigenitalis* on bacteriological culture prior to breeding the stallion. *Where possible, semen collection for insemination of mares or breeding by natural cover should be postponed until the results of laboratory testing for CEM are known.*

- 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% chlorhexidine 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 can be appropriately disposed of after use.

- Consider pre-breeding CEM testing of high-risk mares as described above.

- 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 hand-held 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)

Background: 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, and experience has shown that carrier stallions and EAV infective semen are principally responsible for the international spread of EVA.

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 thorax/abdomen, lower limbs, scrotum and prepuce or mammary glands)
- Localized or generalized urticaria
- Supra- or peri-orbital edema
- Conjunctivitis
- Serous to mucoid nasal discharge

Abortion is a frequent sequel to infection in pregnant mares and can occur from 2 months gestation to term in the unprotected mare. Abortion due to EAV only occurs in mares that are already pregnant at time of exposure to the virus. 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 the accessary sex glands (particularly the ampulllae) 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: Laboratory confirmation is required for diagnosis of EVA. 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 blood samples should be kept cold but not frozen in transit to the laboratory.

Sampling for serologic titers: 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. When used in pregnant mares, recommendations are to vaccinate before 9 months gestation. 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 (one ten-thousandth) than that observed with natural infection. Nasopharyngeal shedding, if it occurs, is frequently intermittent and of less than 1-week duration. The occasional stallion may shed very low concentrations of vaccine virus in barely detectable quantities in its semen for a limited period.

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 need to be serologically tested and confirmed to be negative for antibodies to EAV.
- Testing should be performed immediately prior to 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 (as described above for breeding stallions) against EVA is highly 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:

- To enable the vaccinated mare adequate time to develop immunity against the virus before potentially being exposed to EAV infection during natural breeding. Retesting to confirm seroconversion prior to breeding can be done.
- To 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 the above recommended protocols.

Vaccination Schedule for Foals: The manufacturer does not recommend the use of the MLV 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. Colt foals should be vaccinated annually in accordance with the manufacturer's recommendations.

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. Serologic 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 USDA-APHIS National Import and Export Service Center for the state of origin 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 and proof that immunized horses are vaccinated annually in accordance with vaccine manufacturer's recommendations.

Equine Herpes Virus 3 (EHV-3)

Background: 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. Lesions are usually limited to the penile skin of stallions and the vulva/perineum of mares. The virus initially causes vesicular lesions that heal spontaneously and leave whitish plaque-like scars.

Clinical Signs: An incubation of 5 to 9 days is typical; the disease starts with development of fluid filled vesicles and progresses 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). To avoid transmission to susceptible horses, infected stallions or mares should ideally 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: Presumptive diagnosis is based on the presence of typical pox-type lesions. A diagnosis of EHV-3 may be confirmed by virus isolation, 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. Suitable specimens for virus detection can be swabs taken from some of the lesions or fragments of tissue from ruptured vesicles.

Specific Control Measures: To avoid horizontal transmission of the virus, 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 lesion 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 semen collection. Additionally, changing sleeves between palpations, and covering ultrasound probes with fresh sleeves, will help to reduce the chance of inadvertent transmission between mares if lesions are not noticed. Stallions requiring tease mares for phantom collection of semen should be restrained to avoid nose-to-nose or nose-to-vulva contact with these mares. Mounting of tease mares should be avoided. Tease mares should be visually inspected for EHV-3 lesions prior to each use. The virus is easily destroyed by common disinfectants, heat, and sunlight and drying.

Disclaimer - *AAEP guidelines are created simply to serve as guidelines for the practitioner and the equine industry. As such, they do not have the force of law. All guidelines issued by the AAEP should be regarded as one of several tools or resources, which a practitioner may take into consideration in the context of his or her practice. All practitioners are encouraged first and foremost to understand and comply with the laws, regulations and standard of care of their appropriate jurisdiction. While guidelines are intended to promote a standard for veterinary practice, lack of adherence to any specific AAEP guideline does not constitute grounds for disciplinary action. The AAEP can exercise disciplinary action only in connection with its own members and its action is limited to denial of membership in the AAEP. The AAEP shall have no liability whatsoever for any of its guidelines.*

A subcommittee of the AAEP board reviews all of the AAEP guidelines and position statements every five years. Any proposed revisions are approved by a vote of the full board. Dates listed in parenthesis indicate either the date the original statement was approved or the approval date of the latest revision.