

Review of breeding shed biosecurity and disinfection agents

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Introduction

A complete biosecurity plan for an equine facility hinges upon a current understanding of disease risk, disease entrance, disease propagation, and disease recognition. All reproduction facilities need to be aware of the acute disease risks for our patients, areas of disease entrance and incubation, protocols for disease testing, routine disease mitigation, and specific action protocols to follow in the face of a disease breakthrough. Furthermore, an adequate biosecurity plan and strategy requires 100% buy-in from staff, technicians, veterinarians, and front office personnel. This buy-in includes client understanding of the risks to their horses and their willingness to help provide a safe and disease free veterinary or breeding center. Clients, veterinarians, and veterinary staff are ultimately responsible for adhering to adequate disease testing schedules, routine and specific facility cleaning, horse movement management, monitoring for disease breakthrough, and providing information for traceability in the event of biosecurity breakdown.

Currently in the US and other North American countries, there are no regulations, certifications or licenses required to collect, process, and distribute semen and to operate a breeding or boarding center; as such, the possibility for venereal disease transmission as well as contagious disease transfer between equines, remains a major veterinary and economic threat. Recent occurrences of both equine viral arteritis (EVA) and contagious equine metritis (CEM) in the US have illustrated the potential for the spread of such venereal diseases in the equine breeding population. Moreover, a lack of sufficient quarantine and entrance requirements for many breeding farms allows for a high likelihood of contagious disease to be spread rapidly as numbers of breeding horses spike during the busy times of the year.

Areas of concern

This author identifies biosecurity risks to our facility into three distinct categories. There is certainly crossover between all categories, but this classification scheme helps to outline the best management strategies to implement and to control exposure. In all areas, the goal is to limit the possibility of direct (horse-to-horse contact and venereal transmission) and indirect (environmental and fomites) passage of disease in the following categories:

1. Facility oriented
2. Venereal transmitted
3. Contagious etiologies

Briefly, facility oriented diseases come from an individual location and disease history for a farm. Is *Rhodococcus* present? Is there history of *Clostridium* in foals? Is rabies prevalent in the area? Is leptospirosis or tickborn disease a concern? Others might be concerned about transmission of equine protozoal myeloencephalitis and viral encephalopathy in some areas of the country.

Possible venereal diseases of concern include:

- Equine viral arteritis virus
- Contagious equine metritis
- Equine herpes virus 3 (coital exanthema)
- West Nile virus
- Equine infectious anemia
- Piroplasmiasis
- Vesicular stomatitis
- African horse sickness

Venereal diseases of the horse are discussed elsewhere, see Metcalf and Timoney in suggested readings. Exposure to these venereal diseases can be from outside stallions or mares shipped into the facility, cooled or frozen semen sources, horizontal transmission from fomites, and from horses housed at

the facility such as tease mares and recipient mares. Contagious areas of concern include equine herpes virus, *Streptococcus equi*, influenza, and respiratory or gastroenterological disease of various etiologies that remain a concern for many breeding farms and stallion stations throughout the country.

Specific action areas

Each area of the facility has unique and specific requirements in the overall biosecurity plan. However, every facility has the following areas to address in a biosecurity plan:

1. Long term horse population
 - a. Resident horses, tease mares, recipients, and stallions
2. Incoming/departing horses
 - a. Short term boarders, foaling mares/foals, and non-resident stallions
3. Barn and outdoor environment
 - a. Pasture turnouts, communal areas, and round pen/exercise area
 - b. Environmental concerns
 - c. Vectors
4. Breeding shed
 - a. Phantom
 - b. Tease mare
 - c. Equipment
5. Laboratory
 - a. Collection supplies
 - b. Outside sample handling from other centers
 - c. Microbiology
6. Quarantine
 - a. Import and export qualification
 - b. Sick or hospitalized animals

This presentation will focus primarily on a biosecurity plan on the laboratory and breeding shed.

Collection equipment should be new and disposable for each individual stallion. If reusable artificial vaginas (AV) are utilized, each stallion should have a specific AV that is properly cleaned and disinfected prior to each use or undergo appropriate disinfection protocols as outlined in the American Association of Equine Practitioners Guidelines in between stallions. Other types of AVs can be utilized but must undergo a specific and thorough cleaning, disinfection, and drying period in between uses. Wash buckets, lip chains, twitches, and breeding halters should be cleaned and disinfected between uses as well as having appropriate disposable barrier protection applied to the rear of the breeding phantom and appropriate cleaning of the entire phantom, especially near the bite strap, before each use. Shed floors should be washable and sprayed with environmental disinfectants to keep contaminant and infectious agents at manageable levels.

Collection and processing personnel should wear appropriate barrier protection including smock, two layers of disposable gloves, and use appropriate footbaths placed at the entrance/exit of the breeding shed and laboratory entrances, as well as wash hands and use alcohol based sanitizing hand wash frequently and before handling collected semen or laboratory equipment.

Lastly, laboratory areas should be routinely disinfected with appropriate tabletop laboratory sprays, and areas of semen processing, water baths, and sinks/water supplies, should be cleaned and monitored as areas for possible bacterial and fungal contamination. A common source of contamination is laboratory water baths. Easily contaminated with bacteria from hands, collection bottles, tubes, and labware, these water baths must be cleaned and disinfected regularly.

For a semen collection shed and laboratory, there is the added concern of residues that could have a toxic on the semen. Therefore, it is imperative to have protocols in place with agents that leave no detergent, soap, or antimicrobial residue that may have an undesired effect on the collection equipment or areas in close proximity to the collection vessel. It is important to have an in-house quality control program to document disinfection protocols. It should also include periodic surveillance cultures of

surfaces, water baths, counters and tap water used to wash the stallion. Hoses attached to water faucets can also contain *Pseudomonas spp.* and other bacteria and when used to wash the stallion's penis can result in repeated inoculation of the penis just prior to collection.

Overview of common disinfectants

Antimicrobial pesticides as defined by the Environmental Protection Agency can be very effective agents to control and sometimes to destroy microorganisms on inanimate and hard, non-porous surfaces. Classified as a sanitizer, disinfectant or sterilant, certain agents have patterns of efficacy that can be used to help develop appropriate biosecurity measures. Sanitizers reduce the microorganism load, but do not eliminate all microorganisms. Sanitizers help bring bacterial or viral load into a more manageable level, but do not act as a germicide that removes all microorganisms. A disinfectant, when applied to surfaces, destroys or inactivates most bacteria and some viruses. Spores and hardy viruses remain viable even in the presence of a disinfection protocol. The most extreme agents act as sterilants, and by definition, remove all forms of microbial life. Using extreme heat or chemicals, surfaces are sterilized of all microbial life. Detergents are additives that help disinfectants and sanitizers remove organic debris and dirt to allow access of the disinfectant to the microorganisms. Detergents, based upon their cationic or anionic nature have different properties for use in disinfectants, and can affect their use in a breeding shed environment.

The following table provides a basic outline for choosing a disinfectant for various locations throughout the breeding shed and stallion center. Of note, specific concerns such as relative humidity, application temperature, hard/soft water, and pH can affect the efficacy of each agent. All agents require that the surface be free of organic debris, therefore adequate washing with water for equipment and pressurized hose for the shed is required to remove the organic debris and dirt that will prevent the mechanism of action of the applied disinfectant.

Implementation and conclusions

Overhauling or implementing a new biosecurity plan is not without difficulty. However, a biosecurity plan is one of the most important plans in place at the clinic, hospital, or collection center. The cost of routine and effective biosecurity is far less than the cost of a total shutdown of operations and cleanup following a disease outbreak or biosecurity breach. To successfully implement an effective biosecurity plan, each member of the team has an important and definable role, from the receptionist confirming vaccination history of a patient, to the technician performing a visual inspection of the horse, to the attending veterinarian providing physical examinations, and to the barn staff who interact with the animals daily. Staff must follow checklists, between collections, daily, weekly, and monthly to insure the biosecurity plan is being followed. Sometimes, it takes a dedicated staff to slow down during the busiest times of the year and perform extensive cleaning to prevent any disease spread and to create a "go-back point" to know that the facility was adequately cleaned and disinfected. With the advancements in reproductive technology and the increased use of cooled and frozen semen, the potential for devastating consequences from a disease breakthrough is real. As seen recently, a disease outbreak can easily spread to multiple breeding centers very quickly even when separated by vast physical distances. Simple biosecurity steps can help mitigate and reduce these risks, but only when all staff follow proper cleaning techniques, use effective disinfectants, and appropriate application technique.

Suggested reading

Timoney PJ: Diseases potentially transmitted with frozen or cooled semen. In: McKinnon AO, Squires EL, Vaala WE, et al, editors. Equine reproduction. 2nd ed. Hoboken: Blackwell Publishing Ltd; 2011.

Biosecurity guidelines for control of venereally transmitted diseases. American Association of Equine Practitioners. Available at: <http://www.aaep.org/info/breeding-guidelines>

Metcalf ES: Venereal disease. In: McKinnon AO, Squires EL, Vaala WE, et al, editors. Equine reproduction. 2nd ed. Hoboken: Blackwell Publishing Ltd; 2011.

Dvorak G: Disinfection 101. May 2008. <http://cfsph.iastate.edu>

<i>Disinfectant</i>	<i>Alcohol</i>	<i>Aldehydes</i>	<i>Biguanides</i>	<i>Hypochlorite</i>	<i>Iodine Compounds</i>	<i>Oxidizing Agents</i>	<i>Phenols</i>	<i>Quaternary Ammonium Compounds (QAC)</i>
Trade Name	70% Isopropyl Alcohol	Formaldehyde Glutaraldehyde	Chlorohexidine Nolvasan	Bleach	Betadine Providone	Hydrogen Peroxide Virkon-S	One-Stroke Environ Tek-Trol	Rocal DiQuat
Mechanism of Action (MOA)	-Protein precipitation -Denatures lipids	-Denatures Proteins -Alkylates nucleic acids	-Alters membrane permeability	-Denatures proteins	-Denatures proteins	-Denatures lipids and proteins	-Alters membrane permeability -Denatures proteins	-Denatures proteins -Binds cell membranes
Advantage	-Short contact time -No Residues	-Broad efficacy -Non corrosive	-Broad efficacy	-Broad efficacy -Cost -Short contact time	-Safety	-Broad efficacy	-Active with organic matter -Non-corrosive	-Non irritating -Efficacy at high temp and high pH
Disadvantage	-Rapid evaporation -Damage to some rubber	-Safety (Carcinogens) -Wear Protective equipment -Irritating to mucus membranes	-Specific pH required -Limited use with fungi, viruses, and spores	-Inactivated by sunlight -Corrodes metal -Tissue irritant	-Inactivated by QAC compounds -Staining -Corrodes metals	-Corrodes metals	-Eye irritant	
Precautions	Fire Risk	Carcinogens		Chlorine gas				

Table. Summary of common disinfectants