Bull biosecurity: Diagnosing pathogens that cause infertility of bulls or transmission via semen
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Summary
Appropriate biosecurity measures to prevent bulls or cryopreserved semen from causing infertility or transmitting pathogens equates to performing an acceptable diagnostic test on an appropriate sample at an appropriate time to ensure the absence of particular pathogens. The goal of this review is to concisely describe appropriate biosecurity measures for bulls and semen that originate in the United States. Appropriate biosecurity measures to prevent the transmission of *Brucella abortus*, *Campylobacter fetus*, *Histophilus somni*, *Leptospira* species, *Mycobacterium bovis*, *Mycobacterium avium* subsp. *paratuberculosis*, *Tritrichomonas foetus*, bovine viral diarrhea virus, bluetongue virus, infectious bovine rhinotracheitis virus (bovine herpesvirus-1), and bovine herpesvirus-5 are described. While some determinants may cause producers to neglect desirable biosecurity measures when introducing bulls or semen to breeding herds, informed practitioners can often minimize disease risks by facilitating selection of sires or semen from low risk sources.

Keywords: Pathogen, diagnostics, venereal, infertility

Introduction
Introduction of novel genetics to improve the performance of cattle herds is most commonly and efficiently achieved through introduction of new sires. Unfortunately, the introduction of new sires via natural breeding or artificial insemination has the potential to introduce pathogens that may result in infertility or subfertility and transmit disease causing agents to naive cows or heifers that may exhibit additional morbidity or mortality. Pathogens that should receive consideration when introducing new bulls in the United States include *Brucella abortus*, *Campylobacter fetus*, *Histophilus somni*, *Leptospira* species, *Mycobacterium bovis*, *Mycobacterium avium* subsp. *paratuberculosis*, *Tritrichomonas foetus*, bovine viral diarrhea virus, bluetongue virus, infectious bovine rhinotracheitis virus (bovine herpesvirus-1), and bovine herpesvirus-5. When obtaining bulls or semen from outside of the United States, biosecurity measures to prevent introduction of foot-and-mouth disease, rinderpest, lumpy skin disease, and Rift Valley fever viruses should also be considered. Prudent use of resources may dictate that new bulls are only tested for some of these pathogens due to (a) low prevalence or total lack of the pathogen in the herd from which the bull originated, (b) a high likelihood that the pathogen is already present in the herd to which the bull will be introduced, (c) the lack of diagnostic sensitivity of available assays to detect particular pathogens in bulls, and (d) the time between when the owner first has access to diagnostic samples from the bull and when the bull needs to be introduced to optimize reproduction of the herd.

*Brucella abortus*
Localization of *Brucella abortus* in the reproductive tract of the bull can result in production of semen containing the bacteria. While the bull appears to play a minor role in the spread of *Brucella*, semen from infected bulls has been demonstrated to result in transmission to susceptible cows. The epididymis, seminal vesicle, and testicle (to a lesser degree) contain significant concentrations of erythritol, a polyhydric alcohol which enhances the growth of *B. abortus* resulting in localized inflammation and infertility of bulls. Bulls to be used for natural breeding should be obtained from herds or states that are certified as brucella-free and should not exhibit orchitis or epididymitis. To achieve greater biosecurity and fulfill the minimum requirements of Certified Semen Services (CSS), a serologic test to detect brucellosis—specifically a buffered antigen plate agglutination (BPAT) test, card test or complement fixation test—shall be negative within 30 days prior of entry into the isolation facility and at least 30 days after the pre-isolation test. While all three of these diagnostic tests exhibit acceptable sensitivities to detect brucellosis, the BPAT yields the highest sensitivity and highest specificity.
**Campylobacter fetus**

Infections of bulls with *Campylobacter fetus* subsp. *venerealis* are asymptomatic. Infections of heifers and cows cause infertility and early embryonic death. Transmission usually occurs via natural mating or artificial insemination using contaminated semen lacking antibiotics in extender.³ As vaccination of bulls up to five years of age using a bacterin in an oil emulsion adjuvant is considered both protective and curative, vaccination prior to natural mating may be considered the least expensive method of biosecurity against *C. fetus*. Minimum requirements for production of semen under CSS guidelines dictate that washings or scrapings of preputial smegma from bulls shall be negative for *C. fetus* using fluorescent antibody screening tests or cultures. To achieve this optimal biosecurity, the minimum number of negative tests at weekly intervals varies with up to six test negative results necessary to validate the specific pathogen free status of bulls over one year of age. Recently, PCR-based assays have been developed as rapid screening tests for the detection of *C. fetus*. Research involving a quantitative PCR assay for the 5' *Taq* nuclease of *C. fetus* subsp. *venerealis* indicates that PCR may be more analytically and diagnostically sensitive than culture.⁴ Notably, whether using a PCR assay or culture, collection of preputial samples using a 7.5-cm long, 8-mm diameter polyethylene corrugated scraper head ("bull rasper") increased the diagnostic sensitivity of the detection method by facilitating collection of more bacteria from infected bulls.⁴

**Histophilus somni**

The association of *Histophilus somni* (previously *Hemophilus somnus*) with bovine abortion and infertility is controversial and may depend on the production of specific virulence factors by the bacterial strain. This fastidious gram-negative rod was isolated from the prepuce, bladder, or accessory sex glands of 24 of 31 (77%) bulls from an Ontario slaughterhouse.⁵ Carrier bulls can infect cows as this commensal organism or opportunistic pathogen—depending on the host, environment, and one’s interpretation of prior research regarding this organism—readily spreads via natural breeding. Adding antibiotics to extended semen or treating bulls with appropriate antibiotics may control the spread of this potential pathogen. While administration of oxytetracycline to bulls has been implemented by some herds to prevent introduction of infected bulls, antibiotic sensitivity testing indicates some resistance of *H. somni* to oxytetracycline. Interpretation of serologic antibody titers to *H. somni* is difficult and may require assessment of paired serum samples. Titers between 1:256 and 1:512 in nonvaccinated cows have been attributed to early active or chronic infections while titers between 1:1040 and 1:4096 have been attributed to active infections.

**Leptospira species**

While pathogenic *Leptospira* species are mainly shed in urine, transmission of spirochetes is possible via semen.⁶ Similar to controlling *H. somni*, adding antibiotics to extended semen or rationally treating infected bulls with appropriate antibiotics may control the spread of this pathogen.¹ If leptospira vaccines have not been previously administered to bulls, serologic testing may indicate lack of exposure to the five most common pathogenic serotypes. Minimum requirements for production of semen under CSS guidelines dictate that bulls are seronegative within 30 days prior to entry or at least exhibit a stabilized low titer (≤ 1:400) on two tests at least two to four weeks apart before entering the isolation facility.

**Mycobacterium bovis**

Bovine tuberculosis caused by *Mycobacterium bovis* and rarely *M. tuberculosis* provides a significant risk for human infection. Transmission of this pathogen via semen is possible.⁶ Bulls to be used for natural breeding should be obtained from herds or states that are certified as bovine tuberculosis-free. To achieve greater biosecurity as is required by CSS guidelines, an intradermal tuberculin test shall be negative within 60 days prior to entry into the isolation facility and at least 60 days after the pre-isolation test.

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Paratuberculosis or Johne’s disease is caused by \textit{Mycobacterium avium} subsp. \textit{paratuberculosis} (MAP). Although the organism has been isolated from testicular tissue and semen of infected bulls, venereal transmission has been considered to be of negligible importance epidemiologically. Despite some investigation, transmission by contaminated semen or semen from contaminated bulls has never been demonstrated.\textsuperscript{6} Semen is considered to contain a low concentration of infectious MAP as only eight of 31 semen samples from a bull exhibiting clinical signs and one of 100 semen samples from a subclinically infected bull yielded MAP in culture.\textsuperscript{7,8} In contrast, semen collected over three years from a subclinically infected bull intermittently yielded high concentrations of MAP DNA which caused the authors to speculate that semen might be epidemiologically significant if the pathogen had entered the previously described state of being viable but non-cultivable.\textsuperscript{9} To achieve sufficient biosecurity for MAP in bulls that will be introduced into herds for natural breeding, a valid history of absence of paratuberculosis in the herd from which the bull originates is considered to be of equal or greater importance than negative results using currently available diagnostic tests on individual bulls less than two years of age.

\textit{Tritrichomonas foetus}

Similar to infections with \textit{Campylobacter fetus} subsp. \textit{venerealis}, infections of bulls with \textit{Tritrichomonas foetus} are asymptomatic. Infections of heifers and cows with \textit{T. foetus} cause transient infertility, early embryonic death, abortion, and pyometra. Transmission usually occurs via contact associated with natural mating; however, the protozoan may be present in semen and transmission via semen has been demonstrated.\textsuperscript{1} Practitioners and producers should clearly understand that the required regulatory testing for the sale of mature bulls in many states serves as a deterrent to selling known positive bulls but is not stringent enough to ensure a high degree of biosecurity. Minimum requirements for production of semen under CSS guidelines dictate that washings or scrapings of preputial smegma from bulls shall be negative for \textit{T. foetus} using microscopic examinations of cultured preputional material collected from the preputial fornix. To achieve this optimal biosecurity, the minimum number of negative tests at weekly intervals varies with up to six test negative results necessary to validate the \textit{T. foetus}-free status of bulls over one year of age. Recent research demonstrated that a gel-based PCR and microscopic examinations of cultured preputional material were functionally equivalent methods to detect \textit{T. foetus} if storage and transport temperatures can be appropriately controlled.\textsuperscript{10,11} Results suggested that when using cultured specimens for \textit{T. foetus} diagnostic purposes, a combination of culture and a gel-based PCR assay performed on three sequential preputial scrapings was the best method for identifying infected bulls during a naturally occurring herd outbreak.\textsuperscript{11} Trichomonads other than \textit{T. foetus} can be present in a preputial sample and may result in false positives when relying only on microscopic examination of cultured preputial samples.\textsuperscript{12} A staining technique or PCR assay can be useful in differentiating \textit{T. foetus} from other trichomonads observed in samples from virgin bulls.

\textbf{Bovine viral diarrhea virus}

Infections of cattle with bovine viral diarrhea virus (BVDV) can cause disease which ranges from subclinical to severe. From semen collected from bulls exhibiting a persistent infection, an acute infection, or a persistent testicular infection, the virus has been isolated and can result in transmission.\textsuperscript{13-15} At a minimum, biosecurity measures may include testing bulls for persistent infection via a validated PCR assay of ear notch tissues, serum or whole blood; a validated antigen capture ELISA assay of ear notch tissues, whole blood, or serum; or validated immunohistochemical staining of ear notch tissues.\textsuperscript{16-18} To prevent acute infections from spreading BVDV via semen, bulls should be isolated from contact with novel cattle for at least 21 days before entering the breeding herd. To prevent rare persistent testicular infections from transmitting BVDV to naïve heifers and cows, semen should be assayed for BVDV using validated PCR or virus isolation tests of semen. The CSS minimum requirements for preventing contamination of processed and cryopreserved semen with BVDV are summarized in Figure 1.
Figure 1. Certified Semen Services (CSS) minimum requirements for preventing bovine viral diarrhea virus contamination of semen produced for artificial insemination.

<table>
<thead>
<tr>
<th>Preventing persistent infections from contaminating semen</th>
<th>Preventing acute infections from contaminating semen</th>
<th>Preventing persistent testicular infections from contaminating semen</th>
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</table>
| 1 **Within 30 d prior to isolation entry.**  
**✓ negative by VI, PCR, or ACE of whole blood or serum (if ≥ 6 months of age).** | 2 **No sooner than 10 d after isolation entry, negative by**  
**✓ PCR on whole blood,**  
**or VI with one pass coupled with FA or IP on whole blood or serum (if ≥ 6 months of age).** | 7 **Before release of semen,**  
**test bulls by SN for antibodies to both types I and II.**  
**✓ If seropositive, must have negative test on processed semen by PCR or VI with one pass coupled with FA or IP viral detection.** |
| 2 **No sooner than 10 d after isolation entry, negative by**  
**✓ PCR on whole blood,**  
**or VI with one pass coupled with FA or IP on whole blood or serum (if ≥ 6 months of age).** | 3 **Only if # 2 is positive, then**  
**animal is isolated from all other cattle and retested**  
**✓ in not less than 21 d by**  
**PCR on serum**  
**or VI with one pass coupled with FA or IP on whole blood or serum (if ≥ 6 months of age).** | **Alt. Before release of semen,**  
**7 all bulls must have negative test on processed semen by PCR or VI with one pass coupled with FA or IP viral detection.** |
| 3 **Only if # 2 is positive, then**  
**animal is isolated from all other cattle and retested**  
**✓ in not less than 21 d by**  
**PCR on serum**  
**or VI with one pass coupled with FA or IP on whole blood or serum (if ≥ 6 months of age).** | 4 **If #3 is positive, then**  
**the animal is not eligible to enter the resident herd of the CSS-approved AI center.** | **8 If processed semen in 7 or Alt. 7 tests, positive by VI, then additional**  
**✓ processed semen should be tested to confirm persistent testicular infection.** |
| 4 **If #3 is positive, then**  
**the animal is not eligible to enter the resident herd of the CSS-approved AI center.** | **5 If #3 is negative, then**  
**animal remains isolated from all other cattle until it achieves**  
**2 negative tests at least 10 d apart by**  
**VI with one pass coupled with FA or IP on whole blood or serum (if ≥ 6 months of age).** | **9 Any bull exhibiting a persistent testicular infection for BVDV is not eligible for semen collection and is not permitted to remain in the resident herd.** |
| **6 If #5 is achieved, then**  
**any semen collected and processed within 30 days before or after the positive VI must be test negative by VI or PCR before distribution.** | **7** |  
| **VI = virus isolation; PCR = polymerase chain reaction; ACE = antigen capture ELISA (enzyme linked immunosorbent assay);**  
**FA = fluorescent antibody; IP = immunoperoxidase; AI = artificial insemination; SN = serum neutralization; Alt. = Alternate.** | | |

**Bluetongue virus**

Many infections of cattle with bluetongue fail to produce clinical signs of disease; however, viral infections may cause fever, facial edema, hemorrhages and ulceration of the mucous membranes. Some of the 26 serotypes of bluetongue virus such as BTV-8 are associated with more severe clinical signs. Bluetongue virus can be detected in the semen of viremic bulls and may result in viral transmission.¹⁶ Some researchers hypothesize that transmission via cryopreserved semen may have initiated the BTV-8 epizootic in north-western Europe in 2006.¹⁸ Therefore, the detection of circulating anti-BTV antibodies in bulls in semen collection centers may impede or prevent international trade of semen to countries free of BTV. To facilitate trade, the European Commission Regulation 1266/2007/EC states that semen may be imported if, for at least 60 days before and during collection of semen, (a) bulls are kept outside of an endemic zone, (b) bulls are protected against biting midges which spread the virus, or (c) bulls are kept during the seasonally midge-free period in a bluetongue seasonally-free area.²⁰ Semen may also be imported from bulls which test seronegative every 60 days or test free of bluetongue by PCR every 28 days according to the World Animal Health Organization (OIE) Terrestrial Animal Health Code.²⁰
Bovine herpes virus-1

Bovine herpes virus-1 causes economically significant respiratory and reproductive loss in cattle. This alphaherpesvirus can be detected in semen and can result in viral transmission. Insemination of naïve heifers and cows with semen containing BHV-1 can cause endometritis, shortened inter-estral intervals and reduced conception rates. Beyond the initial phase of infection, BHV-1 remains latent in sacral ganglia and a protracted course of intermittent virus excretion in seminal plasma may follow. As a general rule, the site of primary infection generally determines the site of latency in local sensory ganglia; thus, one would expect that BHV-1 from an intranasal infection usually would not lie latent in sacral ganglia. The intermittent shedding of BHV-1 in semen due to latent infections has caused some European countries to require that all bulls producing semen for import and all bulls in their domestic artificial centers must be seronegative for BHV-1. For bulls to be used for natural breeding purposes in the United States, quarantine of bulls for at least 21 days limits the risk of BHV-1 shedding to contacted cows and heifers due to an acute infection in the bull. Vaccination of bulls to be used for natural breeding in the United States is recommended at least 28 days prior to introduction into the breeding herd. To optimize trade opportunities, bulls from which semen may be shipped internationally are ideally maintained as seronegative and not vaccinated for BHV-1.

Bovine herpes virus-5

Bovine Herpes Virus-5 (previously BHV-1.3) shares antigenic similarity to BHV-1 but has been associated with a fatal meningoencephalitis in calves. This virus has been detected in semen using PCR and virus isolation techniques. Poor conception rates and pustular vulvovaginitis has been described in isolated cows and heifers artificially inseminated with semen containing BHV-5. Although genomic and pathogenic differences between BHV-1 and BHV-5 are quite consistent, the two related viruses display extensive serological cross-reactivity which can be evidenced in serum neutralization tests. Therefore, control measures to prevent contamination of semen with BHV-5 are the same as preventive measures for BHV-1.

Conclusions

The implementation of appropriate biosecurity measures for pathogens that exhibit the potential to cause infertility in cattle or may be transmitted via semen will consistently prevent the spread of animal disease. Bulls to be used in the United States should be prudently quarantined and tested appropriately for the described pathogens prior to introduction for natural breeding. Using semen collected under CSS minimum requirements for production provides assurance that appropriate biosecurity procedures have been applied. Appropriate biosecurity measures constitute using the least cumbersome management tools and diagnostic tests that will facilitate optimal trade in pathogen-free bulls and semen.

References