

# Review: Risks of disease transmission through semen in cattle

M. D. Givens<sup>†</sup>

College of Veterinary Medicine, Auburn University, Auburn, AL 36849-5536, USA

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*The purpose of this paper is to review scientific evidence concerning pathogens that could potentially be transmitted via bovine semen. As a result of a careful analysis of the characteristics of infections that may cause transmission of disease through semen, effective control procedures can be identified that provide minimal constraint to the introduction of new bulls into herds for natural breeding and importation of valuable novel genetics through artificial insemination. The potential for transmission through bovine semen and corresponding effective control procedures are described for bovine herpesvirus 1, bovine viral diarrhoea virus, bovine leukaemia virus, lumpy skin disease virus, bluetongue virus, foot-and-mouth disease virus, and Schmallenberg virus. Brief consideration is also provided regarding the potential for transmission via semen of *Tritrichomonas foetus*, *Campylobacter fetus venerealis*, *Brucella abortus*, *Leptospira spp.*, *Histophilus somni*, *Ureaplasma diversum*, *Mycobacterium avium subsp. paratuberculosis*, *Chlamydiaceae*, *Mycobacterium bovis*, *Coxiella burnetii*, *Mycoplasma mycoides ssp. mycoides* and *Neospora caninum*. Thoughtful and systematic control procedures can ensure the safety of introducing new bulls and cryopreserved semen into cattle production systems.*

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**Keywords:** cattle, pathogen, semen, venereal, infertility

## Implications

The characteristics of viral, bacterial, protozoal, and parasitic infections and infestations of cattle vary significantly. Understanding these infections allow effective control procedures that minimally impede optimal cattle production to be thoughtfully enacted.

## Introduction

Some viral, bacterial, protozoal, and parasitic organisms may be transmitted through bovine semen. Infections of a bull's testicle, epididymis, vas deferens, ampulla, seminal vesicle, prostate, urethra, penis, or prepuce or the migration or leakage of infected blood cells into the male reproductive tract can readily contaminate semen. In some but not all instances, these conditions may result in (a) detection of DNA or RNA of the pathogen in semen, (b) detection of infectious pathogen in the semen or (c) detection of infectious pathogen in semen sufficient to result in transmission to heifers or cows via natural breeding and/or artificial insemination. Detection of DNA or RNA of the pathogen in semen does not consistently imply that venereal transmission would result from natural breeding or insemination. Often, testing blood samples for absence or presence of antibodies or absence of pathogen will provide confidence that semen from

the bull is free of contamination. Unfortunately, this assessment of blood samples will not always provide clarity regarding the status of semen. Conditions of reactivated, persistent, or prolonged infections increase concern for pathogen transmission via semen as the duration of shedding of infectious pathogen may significantly increase the potential for pathogen transmission. Thus, understanding infections of the male reproductive tract provides clarity on how best to prevent disease transmission while facilitating cattle production.

## Viral pathogens

Transmission via semen has been scrutinized for important viral pathogens including bovine herpesvirus 1 (BoHV-1), bovine viral diarrhoea virus (BVDV), bovine leukaemia virus (BLV), lumpy skin disease virus (LSDV), bluetongue virus (BTV), foot-and-mouth disease virus (FMDV) and Schmallenberg virus (SBV). Prevention of venereal transmission of these viruses involves understanding the characteristics of various viral infections, appropriate assessments of risk and consistent application of control procedures to mitigate or abolish the potential for viral transmission.

### *Bovine herpesvirus 1*

Bovine herpesvirus 1 (synonym infectious bovine rhinotracheitis/infectious pustular vulvovaginitis virus) can cause

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<sup>†</sup> E-mail: givenmd@auburn.edu

clinical disease of the male reproductive tract (infectious balanoposthitis) and be shed in seminal plasma due to replication in the mucosa of the prepuce, penis, and urethra (Weiblen *et al.*, 1992; van Oirschot *et al.*, 1995; van Oirschot, 1995; Vogel *et al.*, 2004; Wrathall *et al.*, 2006). While BoHV-1 may not affect sperm motility or acrosomal status (Tanghe *et al.*, 2005), others have described an effect on semen quality most likely due to generalized illness rather than a direct effect of the virus on sperm (van Oirschot *et al.*, 1995; van Oirschot, 1995; Vogel *et al.*, 2004). The virus may inhibit sperm-zona binding by interacting with sperm (Tanghe *et al.*, 2005). Bovine herpesvirus 1 can be transmitted through semen and result in infection, reduced conception rates, endometritis, abortion and infertility (Bielanski *et al.*, 1988; van Oirschot, 1995). Once infected, bulls may shed the virus in semen intermittently throughout life though remaining clinically normal (van Oirschot, 1995). However, results suggest that BoHV-1 seropositive bulls can be free of virus for long periods of time if bulls are well managed in a low-stress environment (Eaglesome and Garcia, 1997).

To prevent BoHV-1 from contaminating semen, bulls should be maintained in isolation or in a BoHV-1-free herd or artificial insemination center during collection and blood should be tested negative for antibody to BoHV-1 at least 21 days after the last collection of semen that is to be used for insemination. Alternatively, when a bull is seropositive or of unknown serologic status, an aliquot of semen from each collection should be tested negative for virus using an assay validated for semen samples (virus isolation or PCR; Chapter 11.8 of the Office International des Epizooties (OIE) Terrestrial Animal Health Code) (OIE, 2017a). To prevent BoHV-1 in semen from being transmitted, 0.25% trypsin could be added to semen for 5 min at room temperature before processing to inactivate the enveloped virus (Bielanski *et al.*, 1988; Silva *et al.*, 1999; Bielanski, 2007). Alternatively, treatment of BoHV-1 contaminated semen with gamma globulins from hyperimmune serum can neutralize the virus and reduce the risk of viral transmission via artificial insemination without affecting fertility (Wrathall *et al.*, 2006).

#### *Bovine viral diarrhea virus*

Another important reproductive pathogen, BVDV, can replicate throughout the bull including within the seminal vesicles, prostate gland and epididymis (Kirkland *et al.*, 1991). In addition, BVDV antigen has been detected in sertoli cells, spermatogonia and epithelial cells of the urethra

(Borel *et al.*, 2007; Newcomer *et al.*, 2014). Bovine viral diarrhea virus can be present in semen from bulls with persistent infection, acute (transient) infection, persistent testicular infection and prolonged testicular infection (Table 1). Bulls previously exhibiting acute infections (at up to 28 days after initial infection), persistent testicular infections (at up to 42 months of age), and prolonged testicular infections (at up to 33 months after initial infection) may subsequently produce semen that is free of BVDV (Gard *et al.*, 2007; Givens *et al.*, 2009; Newcomer *et al.*, 2014). Virus has been transmitted to inseminated animals through semen collected from bulls exhibiting persistent infections, acute infections and persistent testicular infections. The defining difference between cases termed 'prolonged testicular infections' and those termed 'persistent testicular infection' has not been the assessed duration of the testicular infection but the ease with which virus could be isolated from semen and the associated consistency of viral transmission to inseminated cattle (Givens *et al.*, 2009; Newcomer *et al.*, 2014). Extended isolation (quarantine) in association with routine periodic monitoring from presence of detectable virus in semen might be advisable for some valuable bulls diagnosed with persistent testicular infection. Bovine viral diarrhea virus in semen may be associated with sperm and cannot be removed by centrifugation through a percoll gradient, swim-up techniques, glass wool filtration or glass bead filtration (Bielanski *et al.*, 1992).

The OIE recommended standards (Chapter 4.6 of the Terrestrial Animal Health Code) require bulls to be free of BVDV in blood on entry into artificial insemination centers with ongoing testing for BVDV and anti-BVDV antibodies during semen collection procedures (Gard *et al.*, 2007). When admitting bulls through an isolation and quarantine procedure, groups of bulls should be assembled, tested and cleared based on all-in and all-out management after assurance that BVDV is not circulating within the group based on serologic evidence. The OIE recommendations necessitate that seropositive bulls produce semen free of BVDV as determined by virus isolation or virus antigen testing. To meet Certified Semen Services (CSS; a subsidiary of the National Association of Animal Breeders to establish industry self-regulation) standards in the United States, bulls must be non-viremic on entry and maintain the non-viremic status. If these non-viremic bulls test seronegative, no semen testing is required (Gard *et al.*, 2007). If bulls are seropositive, at least one sample of semen that is completely

**Table 1** Characteristics of infectious with bovine viral diarrhea virus that may result in the contamination of bovine semen with virus

Infection	Serologic status to infecting strain	Transmission via insemination	Viral concentration in semen	Duration of viral shedding in semen
Acute infection	Seroconversion	Demonstrated	8 to 200 CCID <sub>50</sub> /ml	2 to 28 days after infection
Persistent infection	Seronegative	Consistently demonstrated	1 × 10 <sup>5</sup> to 3.98 × 10 <sup>7</sup> CCID <sub>50</sub> /ml	Throughout life
Persistent testicular infection	Seropositive	Demonstrated	≤ 6.25 × 10 <sup>3</sup> CCID <sub>50</sub> /ml	Up to 42 months of age
Prolonged testicular infection	Seropositive	Not demonstrated	Detectable only by virus isolation after ultracentrifugation or by RT-PCR	Up to 33 months after infection

extended, frozen, and transported to the diagnostic lab at cryogenic temperatures must test negative using a PCR assay (CSS, 2014). Research does clearly indicate that straws of semen should be transported to diagnostic laboratories at cryogenic temperatures (i.e. liquid nitrogen or liquid nitrogen vapor tank) to optimize either PCR or virus isolation assays to detect BVDV in semen (Newcomer *et al.*, 2014).

#### *Bovine leukemia virus*

Bovine leukemia virus (BLV; synonym enzootic bovine leucosis virus) produces a benign persistent lymphocytosis and infections may eventually cause lymphatic tumors in some adult cattle. A seropositive status is considered evidence for ongoing infection with this *Deltaretrovirus*. The virus is rarely found in semen though possibly present in association with virus-infected lymphocytes in the genital tract (Choi *et al.*, 2002; Wrathall *et al.*, 2006). While substantial evidence demonstrates that BLV is rarely – if ever – transmitted by artificial insemination, transmission was detected when infected lymphocytes were inoculated into the uterus of seronegative cows (Van Der Maaten and Miller, 1978; Wrathall *et al.*, 2006). To ensure that semen is free of BLV, Chapter 11.6 of the OIE terrestrial animal health code recommends that semen donor bulls be residents of BLV-free herds and come from a serologically negative dam (if less than 2 years of age), or that blood samples from the bull test negative for antibodies against BLV on two occasions, the first being at least 30 days before and the second at least 90 days after collection of semen (OIE, 2017a).

#### *Lumpy skin disease virus*

Lumpy skin disease virus, a member of the genus *Capripoxvirus*, causes acute, subacute or subclinical disease in cattle. The disease is characterized by firm, circumscribed skin nodules, necrotic plaques in the mouth and nares, fever, and generalized lymphadenopathy. This virus is reported to be endemic in sub-Saharan Africa and Egypt with the potential for spread to other parts of Africa, the Middle East, and Europe (Irons *et al.*, 2005; Annandale *et al.*, 2014). After experimental infection, LSDV could be isolated from semen up to 42 days after infection while viral DNA was detected in semen using PCR for up to 5 months (Irons *et al.*, 2005). Another study involving experimental intravenous inoculation with a large dose of LSDV suggested that the testis and epididymis are sites of viral persistence within bulls that exhibit prolonged viral shedding (Annandale *et al.*, 2010). The potential for viral transmission via semen remains to be clearly demonstrated though semen that was experimentally spiked with a notably high dose of LSDV did result in viral transmission to inseminated heifers (Annandale *et al.*, 2014). To ensure that semen is free of LSDV, Chapter 11.9 of the OIE terrestrial animal health code recommends that semen donor bulls not show clinical signs of lumpy skin disease (LSD) and reside in an LSDV-free country or zone for at least 28 days before semen collection (OIE, 2017a). Alternatively, the code recognizes the assurance of safety of bovine semen collected from bulls in countries or zones not free of LSD if (a) bulls do

not show signs of LSD, (b) bulls reside in an artificial insemination center where no case of LSD occurred during 60 days before semen collection, (c) semen to be exported was tested negative for LSDV by PCR, and either (1) bulls were vaccinated according to vaccine manufacturer's instructions at least 60 days before first semen collection and exhibited antibodies against LSDV at least 30 days after vaccination, or (2) bulls tested negative using PCR for LSDV in blood samples at commencement of, conclusion of, and at least every 28 days during semen collection and tested seronegative for antibodies to LSDV at least every 28 days during semen collection and at 21 days after the final semen collection of the consignment (OIE, 2017a).

#### *Bluetongue virus*

Controlled scientific clinical studies and field investigations focused on the excretion of bluetongue virus (BTV) in semen of bulls has significantly influenced regulatory policy regarding the movement (or lack of movement) of both live cattle and semen for many years (Thibier and Guerin, 2000; Wrathall *et al.*, 2006). Bluetongue virus is considered the prototype of the genus *Orbivirus* in the family *Reoviridae* with the viral genome consisting of ten linear double-stranded RNA segments. Bluetongue viruses can be categorized into various serotypes, of which 27 distinct serotypes are currently proposed. This robust diversity among BTV often leads to contradictory research findings and notable consternation when clear and specific regulatory guidelines pertaining to BTV are pursued (Gu *et al.*, 2014). Epizootic hemorrhagic disease virus is a closely related *Orbivirus* that cross-reacts with BTV and sometimes causes clinical disease in cattle. These viruses are transmitted between ruminants by biting midges in the *Culicoides* species. Cattle usually experience a subclinical BTV infection though they serve as a reservoir host due to prolonged, low level cell-associated viremias that can last up to several months (Vanbinst *et al.*, 2010). Clinical signs in sheep are more notable than those in cattle in most circumstances and include fever, excessive salivation, nasal discharge and rare cyanosis of the tongue due to vascular compromise. Some sheep may develop respiratory distress from pulmonary edema and notably painful inflammation of the coronary bands. Depending on the strain involved, early embryonic deaths, abortions, malformed calves or lambs, temporary infertility in bulls and rams, and shedding of BTV in semen may occur (Vanbinst *et al.*, 2010).

The sporadic detection of BTV in semen of individual bulls has generally been attributed to BTV-associated red blood cells and mononuclear cells leaking into the semen through microvascular injuries due to inflammation within the male reproductive tract (Wrathall *et al.*, 2006). However, with some BTV serotypes, the hypothesis that contamination of semen results from something other than leakage of BTV-carrying blood cells into semen is supported by: (a) the detection of concentrations of BTV in semen that equal concentrations detected in blood, (b) the shedding of BTV in semen of young and old bulls regardless of the perceived

propensity for inflammation of the reproductive tract and (c) the absence of detectable blood cells in semen that tested positive for BTV (Vanbinst *et al.*, 2010). The presence of live, virulent BTV serotype 8 in extended semen from naturally infected bulls has been clearly demonstrated (Vanbinst *et al.*, 2010). Natural infections of bulls with BTV serotype 8 have been demonstrated to precede a transient decrease in the post-thaw motility of sperm and a brief increase in the percentage of sperm exhibiting morphologic abnormalities (Muller *et al.*, 2010).

Though scientific claims were published in the late 1970s and early 1980s of transplacental BTV infections resulting in the production of immunotolerant, persistently viremic bulls, a number of attempts to confirm the findings resulted in strong refutation in 1993 of the existence of persistent BTV infection within immunotolerant cattle (Wrathall *et al.*, 2006). More recent research has demonstrated that BTV serotype 8 can cause a transplacental infection of developing fetuses that results in the birth of virus-positive, specific antibody-negative calves (De Clercq *et al.*, 2008). However, attempts to follow up on these five detected calves after one month demonstrated that the calves were not maintaining a detectable persistent infection (De Clercq *et al.*, 2008). Thus, concerns regarding the persistent infection of a seronegative, post-pubertal bull that consistently or sporadically produces semen contaminated with BTV appear to lack substantial scientific support.

To prevent shipment of semen containing BTV, Chapter 8.3 of the OIE terrestrial animal health code recommends that semen should be collected from bulls which show no clinical signs of bluetongue on the day of semen collection and have been – for at least 60 days before commencement of and during collection of semen – (a) kept outside a restricted zone, (b) protected against viral vectors or (c) kept during the seasonally vector-free period in a BT seasonally-free area. Bulls may also be subjected to diagnostic tests with negative results to provide assurance of uncontaminated semen: (a) lack of detected antibodies to the BTV group at least every 60 days throughout the semen collection period and between 28 and 60 days after the final collection for this consignment, or (b) virus isolation from blood samples collected at commencement, at least every 7 days during, and at conclusion of semen collection for this consignment with negative results or (c) PCR test on blood samples collected at commencement, at least every 28 days during, and at conclusion of semen collection for this consignment with negative results (OIE, 2017a).

#### *Foot-and-mouth disease virus*

Foot-and-mouth disease (FMD) is an extremely contagious disease that affects all cloven-hoofed animals. Though the mortality rate of FMD is generally low, the morbidity rate can be very high in naïve populations of cattle (Meyer *et al.*, 2017). In acutely infected cattle, this *Aphthovirus* in the family *Picornaviridae* is shed in all secretions and excretions including expired air, saliva, skin lesions, urine, feces, and semen. After experimental infection, FMDV has been

detected in the semen of naïve bulls up to 4 days before the development of oral vesicles (Sellers *et al.*, 1968).

Experimental infection studies involving a low number of vaccinated bulls (21 total) have failed to detect FMD virus in semen of vaccinated bulls subsequent to experimental infection (Meyer *et al.*, 2017). Infection of cows through artificial insemination with semen collected from FMDV-vaccinated bulls has not been reported. In some clinically and subclinically infected cattle, FMDV can be isolated from oropharyngeal fluids and/or tissues >28 days after infection (Hayer *et al.*, 2017). This condition is referred to as persistent FMDV infection and these animals are referred to as 'carriers'. Transmission of FMDV from carrier cattle by direct contact to naïve cattle is extremely difficult to reproduce under experimental conditions and is considered not to be epidemiologically significant (Hayer *et al.*, 2017). Accordingly, the potential for seropositive carrier bulls to transmit FMDV via semen has been considered negligible by some authors (Meyer *et al.*, 2017). Federal regulations require that all vaccinated donor bulls whose semen shall be imported to the United States exhibit a negative serology test for antibodies against nonstructural proteins of FMDV – vaccination does not induce such antibodies – and a negative test for virus from an esophageal–pharyngeal sample (Callis, 1996). To prevent transmission of FMDV in shipped semen, Chapter 8.8 of the OIE terrestrial animal health code recommends that semen donors (a) show no clinical signs of FMD on the day of collection, (b) were kept for at least 3 months before collection in a FMD-free country, zone, or compartment and (c) were kept in an artificial insemination center where no animals had a history of infection with FMDV (OIE, 2017a). If being shipped from areas where vaccination is practiced, bulls should additionally either be vaccinated at least twice with the last vaccination  $\geq 1$  month but  $\leq 6$  months before collection or test free of antibodies against FMDV not <21 days after collection of the semen (OIE, 2017a).

#### *Schmallenberg virus*

Schmallenberg virus may cause stillbirths and musculoskeletal and central nervous system malformations of developing fetuses in naïve dams infected during pregnancy (Hoffmann *et al.*, 2013). This *Orthobunyavirus* is transmitted by biting midges of the *Culicoides* genus and mosquitoes. The viremia in SBV infected cattle is very short (1 to 5 days; Hoffmann *et al.*, 2012; OIE, 2017b) though SBV RNA has been detected – sometimes intermittently – up to almost 3 months after seroconversion (Hoffmann *et al.*, 2013; Ponsart *et al.*, 2014). This virus can be detected in seminal plasma early in acute infections (which may clear without serial positive semen samples) and be associated with the seminal cell fraction in serial positive samples weeks after seroconversion (Hoffmann *et al.*, 2013). A single insemination dose of semen can contain SBV sufficient to infect naïve cattle through experimental subcutaneous injection though transmission of SBV by natural breeding or artificial insemination remains to be demonstrated (Schulz *et al.*, 2014). To declare semen free of SBV, testing of semen samples for SBV

RNA using PCR is recommended unless the bull tests negative for SBV-specific antibody at least 28 days after semen production (Van Der Poel *et al.*, 2014).

### Bacterial, protozoal and parasitic pathogens

*Tritrichomonas foetus* and *Campylobacter fetus venerealis* are sexually transmitted diseases that do not cause detectable disease in the bull (Peter, 1997; BonDurant, 2005). These organisms reside on the epithelium of the preputial cavity of infected bulls. The epithelial crypts of the prepuce provide a microaerophilic environment that supports replication of these microbes. Accordingly, bulls may develop a life-long infection. While the organisms are generally associated with the glans penis and proximal prepuce, semen can become contaminated during collection. *Tritrichomonas foetus* and *Campylobacter fetus venerealis* can survive cryopreservation of semen (Peter, 1997). Trichomonads have been shown to adhere to sperm causing a decrease in sperm motility, sperm agglutination, and phagocytosis (Benchimol *et al.*, 2008). Transmission of *T. foetus* or *C. fetus* to the female can result in vaginitis, cervicitis, endometritis, infertility, delayed return to estrus, early embryonic death and rarely abortion (up to 4 months of gestation, *T. foetus*; 4 to 7 months of gestation, *C. fetus*) (Peter, 1997; BonDurant, 2005). Occasionally, postcoital pyometra can result from uterine infection. Infection of the female reproductive tract consistently leads to a notable humoral immune response that commonly clears infections within 90 days. A reliable vaccine is available for campylobacteriosis that can stimulate the prevention and elimination of infections in cows and bulls. The killed, whole cell vaccine available for *T. foetus* has not been demonstrated to stimulate immunity that consistently clears infections in bulls though vaccination of heifers and cows before the breeding season will significantly improve reproductive performance in the event of venereal transmission (Edmondson *et al.*, 2017). For natural breeding, only virgin bulls should be introduced to cattle operations to minimize concerns regarding introduction of *T. foetus* or *C. fetus*.

*Brucella abortus* is an organism that can also localize in the reproductive tract of the bull. The cells of the genital tract contain high concentrations of erythritol which enhances the growth of this zoonotic pathogen. Infection can lead to orchitis, epididymitis, seminal vesiculitis, ampullitis, decreased libido and infertility. The organism can also be present in collected semen (Eaglesome and Garcia, 1997).

Other bacterial organisms can be transmitted in semen and might be associated with infertility or transmission of the disease. Furthermore, several of the organisms are infectious following cryopreservation of semen. *Leptospira* spp. can be isolated from the genital tract of subclinical bulls and transmitted in semen (BonDurant, 2005). The organism can also survive cryopreservation. *Histophilus somni* can be isolated from the reproductive tract of normal bulls and be present in semen (Humphrey *et al.*, 1982). While the organism is sensitive to antimicrobials, it is not known if transmission via processed semen would result in infection of susceptible

cows. Likewise, *Ureaplasma diversum* can be transmitted in semen and induce endometritis, salpingitis and cervicitis, but can also be isolated from unaffected animals (BonDurant, 2005). *Mycobacterium avium* subsp. *paratuberculosis* can also be present in the semen of subclinical bulls (Ayele *et al.*, 2004). This organism is capable of surviving antibiotics and cryopreservation. In addition, *Chlamydiaceae* can cause infection of the reproductive tract of the bull (Teankum *et al.*, 2007). These gram-negative intracellular pathogens can be present in semen and survive cryopreservation. Other organisms which might be transmitted via semen include *Mycobacterium bovis*, *Coxiella burnetii* and *Mycoplasma mycoides* ssp. *mycoides* (Kruszewska and Tylewska-Wierzbanska, 1997; Wentink *et al.*, 2000). Though DNA of *Neospora caninum* has been detected in semen, studies indicate that the possibility of venereal transmission is very low to non-existent (Ferre *et al.*, 2008).

### Conclusion

Based on understanding specific viral, bacterial, protozoal, and parasitic infections that may result in contamination of bovine semen, prudent and practical control measures can be effectively developed and implemented for each farm, region, state or country. While several pathogens can potentially be transmitted through cryopreserved bovine semen, following disease control recommendations provided by the World Organization for Animal Health (OIE) and CSS will ensure that the risk of pathogen transmission through semen is negligible.

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### Declaration of interest

None.

### Ethics statement

None.

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