

SHEDDING & TRANSMISSION OF BOVINE VIRAL DIARRHEA VIRUS IN SEMEN

M. Daniel Givens, DVM, PhD
Diplomate American College of Theriogenologists
Diplomate American College of Veterinary Microbiologists

INTRODUCTION

Bovine viral diarrhea virus (BVDV) is an economically significant pathogen of cattle that can be shed in the semen of persistently and acutely infected bulls.¹⁻⁴ This pathogen causes gastrointestinal, respiratory and reproductive disease in cattle.⁴ While gastrointestinal and respiratory disease due to highly pathogenic strains of BVDV are more clinically dramatic, reproductive losses due to BVDV can be much more economically significant. The importance of BVDV as a pathogen is manifested by the large number (>140) of available licensed vaccines⁵ and the efforts in some European countries to eradicate the virus.⁶

Bovine viral diarrhea virus is a small, positive-sense, single stranded, RNA virus in the family Flaviviridae and genus *Pestivirus*, with antigenic similarity to hog cholera in swine and border disease in sheep. Two biotypes of BVDV, cytopathic (CP) and noncytopathic (NC), have been described based on the presence or absence of visible cytopathic effect in vitro when susceptible cell monolayers are infected.⁷ The noncytopathic biotype is isolated from field outbreaks in a vast majority of cases. Bovine viral diarrhea virus strains can also be categorized into 2 separate species (i.e. genotypes), type 1 and type 2, based on substantial differences within the viral RNA. Bovine viral diarrhea viruses survive cryopreservation and processing of semen for artificial insemination; thus, BVDV in semen of bulls can infect susceptible, inseminated cows.⁸ Sequelae may include reduced pregnancy rates, early embryonic death, abortion and birth of persistently infected offspring.^{1,2,8}

DISEASE CONDITIONS RESULTING IN SEMEN CONTAMINATED WITH BVDV

Persistent infection: Persistent infection with BVDV occurs when a bovine fetus is infected with a noncytopathic strain of BVDV before 125 days of gestation. Persistently infected animals develop immunotolerance to the strain with which they have been infected, act as a pathogen reservoir and commonly shed large quantities of virus in milk, urine, vaginal mucous, feces, semen, saliva, tears and nasal mucous throughout life.⁹ Persistently infected bulls actually shed a higher concentration of virus ($10^{7.6}$ cell culture infective doses (50%)/mL) in seminal fluid than is found in serum.³ The high concentration of BVDV in semen from persistently infected bulls is likely due to replication of the virus in the prostate and seminal vesicles.¹⁰ While evaluation of semen from some persistently infected bulls revealed poor motility of spermatozoa and many reverse tails and flattened spermatid heads,¹¹ evaluation of semen from another persistently infected bull revealed normal concentration, motility and morphology of spermatozoa.² Research indicates that cryopreserved semen from a persistently infected bull can produce significantly lower fertilization, cleavage, and blastocyst development rates when used for in vitro embryo development.¹² In vivo research indicates that artificial insemination of

seronegative heifers or cows with cryopreserved semen from a persistently infected bull can result in low (38%)² or high (100%; 12/12)¹ first service conception rates.

Acute infection: Acute or “transient” infection with BVDV occurs when an immunocompetent animal is exposed to a cytopathic or noncytopathic strain of BVDV. While subclinical infection is most common, signs such as depression, inappetance, oral erosions and ulcerations, decreased milk production, diarrhea and death might be observed.^{13,14} An acutely infected immunocompetent animal can transmit the virus to susceptible animals, but much less efficiently than persistently infected animals.¹ An acutely infected bull can shed virus in semen with acceptable concentration, motility and morphology of spermatozoa.^{3,15} In contrast, a decrease in motility of spermatozoa and an increase in diadem defects, small spermatozoal heads and proximal droplets have been reported to coincide with acute infection.¹⁰ While BVDV can be isolated from semen of some acutely infected bulls after viremia subsides, the ability to isolate virus from semen ceases when serum antibodies are detectable (18 to 28 days).^{3,10,16} Cryopreserved BVDV-contaminated semen collected prior to seroconversion (12 days post-inoculation) from an acutely infected bull produced a first service conception rate of 65%.¹⁵

Persistent testicular infection: Persistent testicular infection with BVDV has been identified in 1998 in the testes of a seropositive, nonviremic bull at an AI center.¹⁷ This bull gained entry into the AI center because required attempts to isolate BVDV from blood were negative. The persistent testicular infection resulted from an unknown exposure to BVDV. Despite absence of viremia, the bull continuously shed infectious BVDV in semen throughout his life. The concentration of virus in this bull’s semen ($<2 \times 10^3$ cell culture infective doses [50%; CCID₅₀]/mL) was lower than exhibited by bulls with classical persistent BVDV infections (10^4 to 10^7 CCID₅₀/mL), but it was much higher than exhibited by bulls with transient, acute infections (5 to 75 CCID₅₀/mL).^{3,17} Evaluation of semen from this bull revealed normal motility and morphology of spermatozoa. Insemination of heifers with cryopreserved BVDV-contaminated semen from this bull resulted in a 74% (17/23) pregnancy rate.¹⁷ This bull displayed a consistently high concentration of circulating serum antibodies that neutralized the specific viral strain that was persistently shed in the semen. After slaughter, virus could only be isolated from the testes.

In 2002, persistent, localized testicular infections with BVDV in post-pubertal, non-viremic bulls were experimentally produced in our research facilities.¹⁶ After experimental acute infection of seronegative, post-pubertal bulls, BVDV persisted within testicular tissue of 2 of 3 bulls for at least 7 months.¹⁶ For months after acute infection, BVDV was detected in semen by reverse transcription-nested polymerase chain reaction (RT-nPCR) but could not be isolated. Despite this inability to isolate virus, BVDV in semen collected 5 months after inoculation proved to be infectious when administered by the intravenous route to a seronegative calf.¹⁶ Although the prevalence of bulls that shed BVDV in semen in the Southeastern United States as determined by RT-nPCR is very low,¹⁸ the potential for persistent testicular infections that result from acute infections has been identified under controlled experimental conditions.¹⁶

Persistent, localized, testicular infections with BVDV were maintained during our research despite lack of viremia.¹⁶ However, differences existed between these bulls and the bull from an AI center that was discovered to have persistent, localized, testicular infection after an unknown

exposure to BVDV.^{17,19} The bulls in our research developed anti-BVDV antibody titers of 1:5,120 to 1:10,240 against the infecting virus. The bull described in those other reports had antibody titers of > 1:100,000 against the infecting strain. Furthermore, in the study in our research facilities, BVDV could not be isolated from semen after bulls seroconverted, even when the more sensitive roller bottle virus isolation technique was used.¹⁶ In comparison, virus was consistently isolated from semen of the infected bull at the AI center despite the detection of neutralizing antibodies.¹⁷

DETERMINANTS OF VIRAL TRANSMISSION VIA INSEMINATION

The potential for transmission of BVDV in semen is determined by characteristics of the semen and characteristics of the inseminated cow or heifer. Qualities of the semen that are believed to impact the potential for pathogen transmission include quantity of semen deposited, concentration of virus and anti-BVDV antibodies, biotype and genotype of the contaminating virus, site of deposition and the association of virus with seminal fluid or spermatozoa. Qualities of the inseminated cow or heifer that are believed to impact the potential for pathogen transmission include innate immunity, antigen-specific humoral immunity, antigen-specific cell-mediated immunity and stage of the estral cycle. Cows in anestrus or diestrus may be slightly more susceptible than cows in estrus to infection with BVDV via insemination.¹⁹

POTENTIAL FOR TRANSMISSION OF BVDV IN SEMEN

Persistent infection: As semen from persistently infected bulls lacks anti-BVDV antibodies and contains a very high concentration of virus that is associated with seminal fluid and spermatozoa, BVDV in semen of persistently infected bulls consistently infects susceptible, inseminated cows.⁸ Insemination of twelve heifers that were seronegative for BVDV with extended semen from a persistently infected (PI) bull, containing $10^{5.0}$ to $10^{7.5}$ TCID₅₀ /ml, resulted in twelve clinically normal calves at term.¹ One of the 12 calves sired by the PI bull was determined to be persistently infected. Attempts to isolate virus from 61 calves sired by a PI bull in another study resulted in identification of two PI calves.² All other calves in this retrospective study were normal and uninfected. The method by which the persistently infected semen produced PI calves is intriguing since researchers have clearly reported protection of the embryo from BVDV infection by the intact zona pellucida.^{20,21} This protection has allowed the production of normal calves by washing and transfer of embryos flushed from persistently infected cows.^{20,21}

Acute infection: Semen from an acutely infected bull contains a low concentration of virus that is associated with seminal fluid and spermatozoa and lacks anti-BVDV antibodies prior to seroconversion of the donor. Thus, BVDV in semen of acutely infected bulls infrequently infects susceptible, inseminated cows. Virus in semen collected prior to seroconversion (12 days post-inoculation) from an acutely infected bull infected 5% of inseminated heifers.¹⁵ Subsequent horizontal transmission of virus from these infected heifers to pregnant animals resulted in the production of persistently infected fetuses.¹⁵

Persistent testicular infection: The bull in an AI center that exhibited a persistent testicular infection produced semen that contained a low concentration of type Ia virus that could be detected by virus isolation despite the presence of anti-BVDV antibodies.^{17,19} Virus in the semen

of this bull resulted in infection and subsequent seroconversion of 1 of 3 inseminated seronegative heifers.¹⁹

Bulls that were experimentally infected with type Ia BVDV and sustained a persistent testicular infection secondary to an acute infection produced semen that contained a low concentration of virus that could not be isolated after seroconversion due to presence of anti-BVDV antibodies.¹⁶ Despite the inability to isolate virus, BVDV in semen collected 5 months after experimental inoculation proved to be infectious when administered by the intravenous route to a seronegative calf.¹⁶ Research to determine the potential for transmission of BVDV in this semen via intrauterine insemination is yet to be performed.

PREVENTING TRANSMISSION OF BVDV IN SEMEN

Persistent infection: While persistently infected bulls have been identified in artificial insemination (AI) centers, these bulls are currently prevented from entering an AI center by requirements that specimens of blood be negative by virus isolation.^{2,22-24} Similar requirements are prudent for bulls purchased for natural breeding purposes.

Acute infection: Acute infection of bulls within an AI center is prevented by initially screening blood for BVDV and by subsequently quarantining all nonviremic animals for 4 to 6 weeks prior to entrance.¹⁵ Artificial insemination center health programs also include regular and repeated surveillance of animals by both clinical observation and specific diagnostic tests. Similar requirements are prudent for bulls purchased for natural breeding purposes.

Persistent testicular infection: Many authors prudently recommend testing of semen from bulls prior to admittance to an AI center;^{4,6,17,25} however, diagnostic tests on semen have significant limitations.²⁶ Unprocessed, raw semen is a challenging sample for detection of BVDV because seminal plasma exhibits virucidal properties, cell culture cytotoxicity and inhibition of reverse transcriptase enzyme.^{27,28} Reverse transcription nested polymerase chain reaction (RT-nPCR) allows rapid detection of a small quantity of BVDV in partially extended semen after removal of the seminal inhibitors but is easily susceptible to cross contamination.^{26,29} Although the prevalence of bulls that shed BVDV in semen in the Southeastern United States as determined by RT-nPCR is very low ($\leq 0.54\%$),¹⁸ the potential for persistent testicular infections that result from acute infections has been identified under controlled experimental conditions.¹⁶ Future research should evaluate the likelihood that BVDV can be transmitted in semen collected from seropositive, non-viremic bulls that exhibit a persistent testicular infection secondary to acute infection.

CONCLUSION

Bulls exhibiting a persistent infection, acute infection or persistent testicular infection can transmit BVDV via semen. Application of prescribed measures can prevent transmission of BVDV in semen from bulls with a persistent or acute infection. As the current prevalence of persistent testicular infections within beef bulls of breeding age in the Southeastern United States is known to be $\leq 0.54\%$, the risk of transmission from this source is limited.

Reference List

- (1) Meyling A, Jensen AM. Transmission of bovine virus diarrhoea virus (BVDV) by artificial insemination (AI) with semen from a persistently- infected bull. *Vet Microbiol* 1988; 17:97-105.
- (2) Kirkland PD, Mackintosh SG, Moyle A. The outcome of widespread use of semen from a bull persistently infected with pestivirus. *Vet Rec* 1994; 135:527-529.
- (3) Kirkland PD, Richards SG, Rothwell JT et al. Replication of bovine viral diarrhoea virus in the bovine reproductive tract and excretion of virus in semen during acute and chronic infections. *Vet Rec* 1991; 128:587-590.
- (4) Houe H. Epidemiological features and economical importance of bovine virus diarrhoea virus (BVDV) infections. *Vet Microbiol* 1999; 64:135-144.
- (5) Ridpath JF, Neill JD, Frey M et al. Phylogenetic, antigenic and clinical characterization of type 2 BVDV from North America. *Vet Microbiol* 2000; 77(1-2):145-155.
- (6) Lindberg AL, Alenius S. Principles for eradication of bovine viral diarrhoea virus (BVDV) infections in cattle populations. *Vet Microbiol* 1999; 64:197-222.
- (7) Perdrietz JA. Diseases of the alimentary system, bovine virus diarrhea(BVD); mucosal disease;(BVD/MD). In: Smith BP, editor. *Large Animal Internal Medicine*. St Louis: Mosby, 1990: 731-737.
- (8) McGowan MR, Kirkland PD. Early reproductive loss due to bovine pestivirus infection. *Br vet J* 1995; 151:263-270.
- (9) Brock KV, Grooms DL, Ridpath J et al. Changes in levels of viremia in cattle persistently infected with bovine viral diarrhea virus. *J Vet Diagn Invest* 1998; 10:22-26.
- (10) Paton DJ, Goodey R, Brockman S et al. Evaluation of the quality and virological status of semen from bulls acutely infected with BVDV. *Vet Rec* 1989; 124:63-64.
- (11) McClurkin AW, Coria MF, Cutlip RC. Reproductive performance of apparently healthy cattle persistently infected with bovine viral diarrhea virus. *J Am Vet Med Assoc* 1979; 174:1116-1119.
- (12) Guerin B, Chaffaux St, Marquant Le Guienne B, Allietta M, Thibier M. IVF and IV culture of bovine embryos using semen from a bull persistently infected with BVD. *Theriogenology* 37[1], 217. 1992.
- (13) Baker JC. Bovine viral diarrhea virus: a review. *J Am Vet Med Assoc* 1987; 190:1449-1458.

- (14) Pellerin C, van den Hurk J, Lecomte J et al. Identification of a new group of bovine viral diarrhoea virus strains associated with severe outbreaks and high mortalities. *Virology* 1994; 203:260-268.
- (15) Kirkland PD, McGowan MR, Mackintosh SG et al. Insemination of cattle with semen from a bull transiently infected with pestivirus. *Vet Rec* 1997; 140:124-127.
- (16) Givens MD, Heath AM, Brock KV, Brodersen BW, Carson RL, Stringfellow DA. Detection of bovine viral diarrhoea virus in semen after infection of seronegative, post-pubertal bulls. *Am J Vet Res*. In press.
- (17) Voges H, Horner GW, Rowe S et al. Persistent bovine pestivirus infection localized in the testes of an immuno-competent, non-viraemic bull. *Vet Microbiol* 1998; 61:165-175.
- (18) Givens MD, Heath AM, Carson RL, Brock KV, Edens MSD, Wenzel JGW et al. Validation and application of assays for detection of bovine viral diarrhoea virus in semen. *Vet Microbiol*. Submitted.
- (19) Niskanen R, Alenius S, Belak K et al. Insemination of susceptible heifers with semen from a non-viraemic bull with persistent bovine virus diarrhoea virus infection localized in the testes. *Reprod Domest Anim* 2002; 37(3):171-175.
- (20) Bak A, Callesen A, Meyling A et al. Calves born after embryo transfer from donors persistently infected with BVD virus. *Vet Rec* 1992; 131:37.
- (21) Brock KV, Lapin DR, Skrade DR. Embryo transfer from donor cattle persistently infected with bovine viral diarrhoea virus. *Theriogenology* 1997; 47:837-844.
- (22) Howard TH, Bean B, Hillman R et al. Surveillance for persistent bovine viral diarrhoea virus infection in four artificial insemination centers. *J Am Vet Med Assoc* 1990; 196(12):1951-1954.
- (23) Polak MP, Zmudzinski JF. Prevalence of bovine viral diarrhoea virus infection in bulls in artificial insemination centers in Poland. *Vet Microbiol* 1999; 64:253-257.
- (24) Anonymous. Bovine semen. In: Anonymous, editor. *International Animal Health Code-2000*. Paris: Office International Des Epizooties, 2000: 1-11.
- (25) Fray MD, Paton DJ, Alenius S. The effects of bovine viral diarrhoea virus on cattle reproduction in relation to disease control. *Anim Reprod Sci* 2000; 60-61:615-627.
- (26) Da Silva N, Zardoya R, Santurde G et al. Rapid and sensitive detection of the bovine viral diarrhoea virus genome in semen. *J Virol Methods* 1995; 55:209-218.
- (27) Schultz RD, Adams LS, Letchworth G et al. A method to test large numbers of bovine semen samples for viral contamination and results of a study using this method. *Theriogenology* 1982; 17(2):115-124.

- (28) Revell SG, Chasey D, Drew TW et al. Some observations on the semen of bulls persistently infected with bovine virus diarrhoea virus. *Vet Rec* 1988; 123(5):122-125.
- (29) Hooft van Iddekinge BJ, van Wamel JL, van Gennip HG et al. Application of the polymerase chain reaction to the detection of bovine viral diarrhoea virus infections in cattle. *Vet Microbiol* 1992; 30:21-34.