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Update on testicular infections with bovine viral diarrhea virus

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Bovine viral diarrhea virus (BVDV) is the prototypical member of the *Pestivirus* genus of the *Flaviviridae* family and is a significant cause of disease and economic losses in cattle herds worldwide. Clinical disease is manifested in several body systems including the respiratory, gastrointestinal, nervous and immune systems. However, the effects of infection on reproduction likely have the largest economic impact for the producer. In the female, the consequence of BVDV infection depends largely on the immune status of the dam and the stage of gestation at the time of infection. Conception rates may be negatively impacted if infection occurs near the time of breeding and abortion can occur at any time during gestation. Infection of susceptible cattle with a noncytopathic strain of the virus between 18 and 125 days of gestation may result in immunotolerance in the fetus and persistent infection. Persistently infected (PI) animals serve as a viral reservoir and consistently shed high amounts of infectious virus throughout their lifetime. Infection of the pregnant dam may also result in several congenital defects, including cerebellar hypoplasia, hydranencephaly and microphthalmia if the event occurs during critical times of organogenesis.

Infection in the bull is not without consequence and can be classified into four distinct syndromes; transient infection, persistent infection, persistent testicular infection (PTI) and prolonged testicular infection. In addition to systemic clinical signs seen in cows and heifers, transient and persistent infection in bulls results in shedding of the virus through the semen. Viral shedding in the raw semen of transiently infected bulls has been detected at concentrations of 5 to 75 CCID$_{50}$/mL and persists until the development of serum neutralizing antibodies (14 to 28 days post-infection). Concentrations of 100 to 200 CCID$_{50}$/mL have been detected in extended semen. Viral shedding through semen in PI bulls occurs at much higher concentrations than in transient infections, with reported ranges of 10$^4$ to 10$^7$ CCID$_{50}$/mL in both raw and extended semen. Virus shed in the semen is infectious and capable of being transmitted to susceptible animals.

Persistent testicular infection was first reported after a localized, persistent BVDV infection was documented in the testes of a seropositive, nonviremic bull at an artificial insemination (AI) center. The infection resulted in the continuous shedding of infectious BVDV in semen throughout the life of the bull despite the presence of neutralizing antibodies to BVDV in serum. The concentration of virus detected was intermediate between the level of viral shedding commonly seen in transiently (5 to 75 CCID$_{50}$/mL) and persistently (10$^4$ to 10$^7$ CCID$_{50}$/mL) infected bulls. When used to inseminate a seronegative heifer, semen from the bull resulted in infection of the heifer and subsequent seroconversion. After euthanasia of the bull at the age of nearly 22 months, virus was isolated only from testicular tissue.

Localized testicular infections have been produced experimentally in post-pubertal seronegative, non-viremic bulls following intranasal inoculation or subcutaneous vaccination with a noncytopathic type 1a BVDV. Infections persisted for at least seven months in some bulls and infectious virus could be isolated from testicular tissue for over one year following challenge. Viral nucleic acid could be detected in semen by reverse-transcription nested PCR for at least 33 months post-inoculation. However, live virus was undetectable in semen, even when using the more sensitive roller bottle virus isolation technique. Challenge studies using semen from bulls with prolonged testicular infection failed to yield evidence of transmission when seronegative heifers were inseminated. Consequently, this syndrome was termed prolonged testicular infection to differentiate it from other longstanding BVDV infections in cattle known to have significant epidemiologic consequences. In other words, the distinguishing characteristic of persistent and prolonged testicular infections is not the duration of infection but the presence or absence of infectious virus isolated from semen.

A second case of PTI was subsequently reported, also identified in a bull at an AI center. Like the first bull, this second case was found to shed infectious virus in the semen while demonstrating no viremia. Viral concentration in processed straws of semen obtained from the bull between the ages of 16 and 22 months were similar to concentrations (10$^2$ to 10$^3$ CCID$_{50}$/mL) seen in semen from the first bull.
However, the detected concentration of BVDV was lower when semen samples were transported on ice as opposed to in liquid nitrogen. Live virus was shed in the semen despite consistent serum antibody titers to BVDV1 ≥ 1:256 from the age of eight months onward, illustrating the immune privileged status of the testicles. Immunohistochemistry (IHC) staining of testicular tissue obtained by testicular biopsy at the age of 33 months confirmed the presence of BVDV within the tissue in association with Sertoli and germinal cells.

What is perhaps most notable about the second bull with PTI is that the bull was apparently able to clear the infection. Semen collected from the bull between the ages of 36 to 41 months was PCR positive for the presence of BVDV. Routine isolation techniques failed to detect live virus from any samples obtained during this period although one sample collected from the bull at 36 months of age was virus isolation positive using the more sensitive roller bottle technique. All subsequent semen samples obtained from the age of 41 months until the bull was euthanized at 48 months of age were virus isolation and PCR negative. Additionally, IHC staining of testicular tissue following euthanasia failed to detect viral antigen, in contrast to the clear association of virus with Sertoli and germinal cells 16 months earlier.

The apparent clearance of a PTI by this bull is surprising based on our current understanding of persistent BVDV infections. One obvious difference between persistent infection and PTI is the production of serum neutralizing antibodies to the infecting strain of the virus. Persistently infected animals are capable of mounting a serologic response to heterologous strains of the virus or strains closely related to the infecting strain but not to the infecting strain itself. Both bulls demonstrating PTI exhibited a serum neutralizing antibody titer to the infecting strain causing the testicular infection. However, the blood-testis barrier (BTB), formed between Sertoli cells of the seminiferous tubules at the time of puberty prevents the entry of serum antibodies to the site of spermatogenesis although trauma may allow the barrier to be breached. In this case, there was no known trauma to the testicles and the tissue was normal on histological examination. A testicular biopsy was performed on only one testicle; yet, at the time of the procedure, infectious virus could no longer be detected from semen using routine isolation techniques. Thus, the mechanism by which the bull resolved the testicular infection is unclear.

Consequently, extended isolation with routine monitoring for the presence of infectious virus in the semen could be considered for valuable animals diagnosed with PTI in the future.

The pathophysiology of PTI in bulls is unknown and remains a topic of speculation. The BTB only becomes functional at the onset of puberty. Therefore, it has been speculated that a bull could become infected just prior to closure of the BTB, allowing entry of the virus into the immune privileged site but blocking the subsequent entry of neutralizing antibodies. Transient BVDV infections in post-pubertal bulls can result in the isolation of virus from semen for < 21 days after infection but a consistent shedding of infectious virus over a sustained period (as with PTI) is not seen. The length of viral shedding in semen following transient infection appears to be inversely related to the rapidity and magnitude of the host immune response. However, both bulls with PTI maintained high neutralizing antibody titers to the infecting strain and yet shed high levels of virus in the semen.

It is unknown if the fact that both recorded cases of PTI have occurred in dairy bulls is a reflection of management factors important to the pathogenesis of infection, a reflection of sampling bias or random variation. Colostrum management of dairy bull calves is often less of a concern than that of dairy heifers. Thus, it is possible that a dairy bull calf with low levels of passive immunity is infected with the virus through exposure in milk from a PI animal or through the respiratory tract. If the virus were able to elude the host immune response until the time of puberty it could be protected in the testes after formation of the BTB.

In summary, though much has been learned about testicular infections with BVDV, questions remain. Development and pathogenesis of testicular infections with BVDV remain to be clearly understood. Though testicular BVDV infections are believed to occur relatively infrequently, the incidence is likely underreported due to lack of testing or reliance on testing for the detection of PI animals that will not detect persistent or prolonged testicular infections (e.g., ear notch IHC, serial virus isolation). Semen containing infectious virus is capable of viral transmission to susceptible animals and...
thus, out of caution, any semen testing positive for BVDV, regardless of testing method, should not be used for artificial insemination. Isolation and repeated testing of valuable animals diagnosed with PTI may be warranted given the reported clearance of PTI in a single bull.

References
