A rare case of persistent testicular infection causes shedding of infectious virus in semen

M. Daniel Givens,a Kathy Toohey-Kurth,b Yan Zhang,c Bruce W. Brodersen,d Benjamin Newcomer,a Yijing Zhang,a Patricia K. Galik,a Kay P. Riddell,a Peter W. Christophersona
aCollege of Veterinary Medicine, Auburn University, AL; bWisconsin Veterinary Diagnostic Laboratory, University of Wisconsin-Madison, WI; cAnimal Disease Diagnostic Laboratory, Ohio Department of Agriculture, Reynoldsburg, OH; dUniversity of Nebraska Veterinary Diagnostic Center, Institute of Agriculture and Natural Resources, Lincoln, NE

Recently, a dairy bull in the United States was diagnosed as the second confirmed case of persistent testicular infection with bovine viral diarrhea virus (BVDV). This clinical case report characterizes the bull’s infection, humoral immune response, and epidemiologic significance.

Virus neutralization assays were performed to detect anti-BVDV antibodies in serum. To detect BVDV, (1) virus isolation, antigen capture ELISA, and various PCR assays were performed using serum; (2) PCR assays were performed using whole blood samples; and (3) direct immunoperoxidase staining, PCR, titration with subsequent immunoperoxidase monolayer assay and passage with subsequent virus isolation were performed using cryopreserved semen. Dual and serial immunohistochemical staining was performed to detect BVDV in germ and Sertoli cells, respectively, within testicular biopsies obtained at 33 months of age. Centrifugal separation with PCR assay of the supernatant and cell pellet was performed to determine if virus was cell associated and/or detectable free in seminal plasma. Sequencing of 248 nucleotides was performed to determine viral subgenotype. Epidemiologic investigation involved sequential virus neutralization assays of serum obtained from bulls and steers before, during, and after contact with the infected bull.

Between 6 and 24 months of age, this bull lacked BVDV in seven sequential serum samples and two peripheral white blood cell samples. The bull was seropositive to type 1, BVDV strains with serologic endpoints of 256, 2048, and 4096 at 8, 19, and 22 months of age, respectively. At 24 and 29 months of age, the bull exhibited serum neutralizing antibody titers of 4096 and 16384, respectively, to the strain isolated from his semen. The bull produced 25 collections of semen from 14 to 22 months of age that consistently contained BVDV as determined by direct immunoperoxidase staining, PCR and virus isolation when semen was shipped to the laboratory in a liquid nitrogen dry shipper. The concentration of infectious virus in semen ranged from < 250 to 6250 CCID50/mL with a median of 1250 CCID50/mL. Virus was detected in association with Sertoli and germ cells within some seminiferous tubules. Virus was not detected free in seminal plasma but was readily detected in association with pelleted cells. Sequencing revealed a 1a subgenotype of BVDV. Virus was not transmitted to directly contacted bulls and steers.

In conclusion, natural exposure to a 1a strain of BVDV can cause persistent testicular infection of at least nineteen months duration. This infection of a seropositive, non-viremic bull can cause contamination of semen with infectious virus.

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