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Source: Journal of Wildlife Diseases, 44(3) : 753-759

Published By: Wildlife Disease Association

URL: https://doi.org/10.7589/0090-3558-44.3.753

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Journal of Wildlife Diseases, 44(3), 2008, pp. 753–759
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Bovine Viral Diarrhea Virus Multiorgan Infection in Two White-Tailed Deer in Southeastern South Dakota

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ABSTRACT: The susceptibility of wild ruminants, especially cervids, to bovine viral diarrhea virus (BVDV) has remained an enigma. Two white-tailed deer (*Odocoileus virginianus*) were submitted to the Animal Disease Research and Diagnostic Laboratory (ADRDL) in the fall of 2003 by the South Dakota Game Fish and Parks for chronic wasting disease (CWD) testing. Both animals were CWD negative. The animals were necropsied and histopathology, viral antigen detection, and virus isolation were performed. A noncytopathic (NCP) BVDV was isolated from the lungs and several other tissues of both animals. Formalin-fixed ear notches from both animals were positive for BVDV antigen by immunohistochemistry. The BVDV isolates were typed with the use of polymerase chain reaction in 5′ untranslated region (UTR) and one isolate was typed a Type 2a and the other a Type 1b. Future field surveys to determine the incidence of BVDV along with experimental studies to determine if white-tailed deer fawns can be persistently infected with BVDV are needed.

Key words: Bovine viral diarrhea virus, infection, *Odocoileus virginianus*, white-tailed deer.

Bovine viral diarrhea virus (BVDV) infection results in a wide range of severe clinical diseases that range from respiratory to enteric to reproductive (Evermann and Barrington, 2005). In spite of intensive vaccination control programs, the incidence of BVDV remains high, in part due to the ability of the virus to cause persistent infections (Houe, 1999). These persistently infected animals are a continual risk for the spread of BVDV. Free-ranging cervid populations, particularly white-tailed deer (*Odocoileus virginianus*; WTD) are frequently in contact with domestic cattle in the USA. The risk that BVDV persistently infected cattle pose in the transmission of BVDV to WTD and other free-ranging wildlife species is unknown. This possible transfer of BVDV between cattle and cervids has significant implications for the voluntary BVDV eradication programs proposed by the Academy of Veterinary Consultants (AVC, 2007) and the National Cattlemen's Beef Association (NCBA, 2007).

Bovine viral diarrhea viruses have been isolated from free-ranging and captive deer. A noncytopathogenic (NCP) BVDV was isolated from a roe deer (*Capreolus capreolus*) in Hungary (Romvary, 1965), and cytopathogenic BVDV-like virus was isolated from roe deer in Germany (Frolich and Hoffmann, 1995; Fischer et al., 1998). Noncytopathic BVDV has been isolated from a fallow deer that died in England (Edwards et al., 1988), from a free-ranging emaciated mule deer (*Odocoileus hemionus*) in Wyoming (Type 1a; Van Campen et al., 2001), and from a captive lesser Malayan mousedeer (*Tragulus javanicus*) in Denmark (Type 1f; Grondahl et al., 2003; Uttenthal et al., 2005). A female mousedeer was shown to be persistently infected and transmitted the virus to her offspring (Grondahl et al., 2003; Uttenthal et al., 2005). In this article, we describe gross and microscopic lesions and virologic findings in two free-ranging white-tailed deer infected with BVDV.

Two white-tailed deer were submitted to the Animal Disease Research and Diagnostic Laboratory (ADRDL) in the fall of 2003 by the South Dakota Game Fish and Parks for chronic wasting disease (CWD) testing. The two deer were submitted from adjoining counties in southeastern South Dakota and were found within ~30 km of each other. Chronic wasting disease was suspected

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because the animals, as described by the conservation officers who submitted them, appeared "weak and disoriented" when captured. The first case (ADRDL Case No. 03-20663; Deer 1) was submitted on 16 October 2003; age was estimated at 1.5 yr by dentition, and the animal was in good body condition. Deer 1 had difficulty standing and would throw its head back immediately before falling down. The animal was euthanized by gunshot to the chest by the conservation officer in the field. At necropsy, a single lesion was observed: a large (8 cm) chronic abscess in the right caudal lung lobe. The second animal, a fawn (ADRDL Case No. 03-24272; Deer 2) was submitted on 18 December 2003. It appeared stunted, unthrifty and in poor body condition. This animal was euthanized by gunshot to the chest by the conservation officer in the field. On gross examination, Deer 2 had feces on the tail and hindquarters and the carcass was thin. Multifocal abscesses were present in mandibular lymph nodes and multifocal ulcers were present in the abomasum. Tissues from both animals were collected for bacteriology, histopathology, viral antigen detection and virus isolation. Both animals were CWD negative (based on the United States Department of Agriculture–approved immunohistochemistry test).

Fixed tissues were embedded in paraffin, sectioned at 6 μm, and stained with hematoxylin and eosin. On histologic examination of Deer 1, brain had minimal spongiosis with prominent astrogliosis in the obex region, proliferation of the endothelial cells lining blood vessels adjacent to the central canal of the brain stem, and rare lymphocytic perivascular cuffs in the cerebellum and cerebrum. No lesions were observed in the hippocampus, lung, colon, rumen, liver, ileum, heart, or kidney. Deer 2 had lymphoid depletion of the spleen; the mandibular lymph nodes had lesions typical of actinobacillosis (mulitifocal pyogranulomatous lymphadenitis with sulphur granules at the center of the pyogranulomas). A focal ulcer was present in the abomasal mucosa. Segments of the ileum, colon, and rumen were autolyzed. No lesions were observed in the lung, liver, heart, kidney, or brain. Immunohistochemistry (IHC) was performed on fixed tissue sections with the use of a monoclonal antibody, 15C5, which specifically binds the Erns region of BVDV (Haines et al., 1992). Control slides consisting of known BVDV-positive and -negative bovine ear notch samples were stained concurrently, to monitor quality control. To distinguish nonspecific binding, serial sections of each block were treated with an irrelevant monoclonal antibody. The IHC slides were evaluated and the sample was considered positive for BVDV if cell-specific staining was present (Haines et al., 1992; Njaa et al., 2000). Additionally, the IHC-stained slides were submitted blindly for evaluation to Dr. Debbie Haines, Prairie Diagnostics Services, Saskatoon, Saskatchewan, Canada.

Fresh tissues (lung and intestine from Deer 1 and abomasum from Deer 2) were used for BVDV immunofluorescence (IF) staining or homogenized for virus isolation. For IF, tissues were mounted in OCT compound (Miles, Elkhart, Indiana, USA), stored at −70 C and cryostat sectioned onto microscope slides. Tissues were stained for BVDV antigen with the use of fluorescein isothiocyanate (FITC)–labeled polyclonal anti-BVDV antibody (NVSL, Ames, Iowa, USA). Control slides, consisting of known BVDV-positive and -negative bovine samples, were stained concurrently for positive and negative controls. Location and intensity of fluorescence was compared to the controls. Tissues (Deer 1–lung; Deer 2–kidney, abomasum, and lung) placed in approximately 35 ml of Hanks media were homogenized with the use of a stomacher (Brinkman Instruments Inc., Westbury, New York); samples were homogenized for 1–5 min, decanted into a 30 ml centrifuge tube, and centrifuged at 1,300 × G at 4 C. The supernatant was decant-

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ed into another tube, and 0.5 ml was inoculated directly on BVDV-free primary bovine lung (BL) cells or BVDV-free primary bovine turbinate (Bt) cells in tissue-culture tubes and incubated for 1 hr at 37 C. The inoculum was removed and replaced with 1.0 ml of growth media (Minimal Essential Media [MEM, Gibco Invitrogen Cell Culture, Carlsbad, California, USA], 5% BVDV-antibody and virus-free fetal bovine serum [Hyclone, Logan, Utah, USA], 5% lacto albumin, penicillin/streptomycin, and amphotericin B). Inoculated cell cultures were incubated for 7 days to allow viral growth and were observed for any changes in the following 2–3 days. If the inoculum appeared to be toxic, it was filtered with the use of a 0.45-μm syringe filter and isolation was attempted on a new cell culture. Inoculated cell cultures were observed under an inverted light microscope daily to determine if cytopathic effect (CPE) was expressed. The cells were scraped, fixed to slides, and stained with fluorescein isothiocyanate (FITC) -labeled polyclonal anti-BVDV antibody (National Veterinary Services Laboratory) and examined for immunofluorescence (IF). Control slides consisting of BVDV-infected (positive control) or noninfected cells (negative control) were stained concurrently. The location and amount of fluorescence present was noted. If the primary cell culture was BVDV IF negative, a second passage was performed with the use of the first passaged inoculated cell cultures that had been incubated for 7 days. The first-passage cells were frozen, thawed, and clarified, and the supernatant was inoculated onto fresh cells. The 7-day incubation period and detection process was repeated.

To confirm the presence of BVDV and genotype isolates, multiplex reverse transcriptase–polymerase chain reaction (RT-PCR) was used to amplify either a 223–base-pair (bp) product from Type 1 or a 448-bp product from Type 2 with the use of primers from the BVDV Erns region (Sullivan and Akkina, 1995). The RNA templates were prepared from virus isolation positive cell cultures, as described previously (Ridpath et al., 2006). Control samples consisting of BVDV plasmid-spiked control cell samples (positive control) or noninfected cells (negative control) were used for quality control. A second RT-PCR was performed on the virus isolates to further subtype the virus with the use of the 5′ untranslated region (5′UTR; Ridpath et al., 2006). The primary virus isolates were passaged once in BVDV-free Madin Darby bovine kidney cell line (MDBK) and characterized by direct sequencing and phylogenetic analysis of 5′UTR sequences as described previously (Ridpath et al., 2006). All sequencing reactions were done in duplicate and all sequences were confirmed by sequencing both strands. Sequences were aligned and compared with the use of Align Plus (Scientific and Educational Software) and MacDNASIS (Hitachi Software Engineering Co. Ltd., San Bruno, California, USA).

BVDV was detected in Deer 1 in several different tissues (skin, intestine, lung) with the use of two different techniques (IHC and VI). Ear skin from Deer 1 tested positive by IHC (Fig. 1A). The distribution and location of antigen in the hair follicular cells and the dermis was consistent with the distribution of BVDV antigen in persistently infected cattle (Njaa et al., 2000). The epidermal cell staining was prominent. The darkest staining was in the hair follicles and was diffuse within positive hair follicles. Positive-stained keratinocytes were also present. The intestine was IHC positive but IF negative. The lung tissues from Deer 1 were IF and IHC negative for BVDV. A NCP BVDV was isolated from the lungs. Multiplex RT-PCR produced a 448-bp product indicating the presence of a Type 2 BVDV. Based on 5′UTR sequencing, the isolate was a Type 2a BVDV (Fig. 2). This isolate was given the identifier, SDSU03-20663. Bacteriology results from Deer 1 identified

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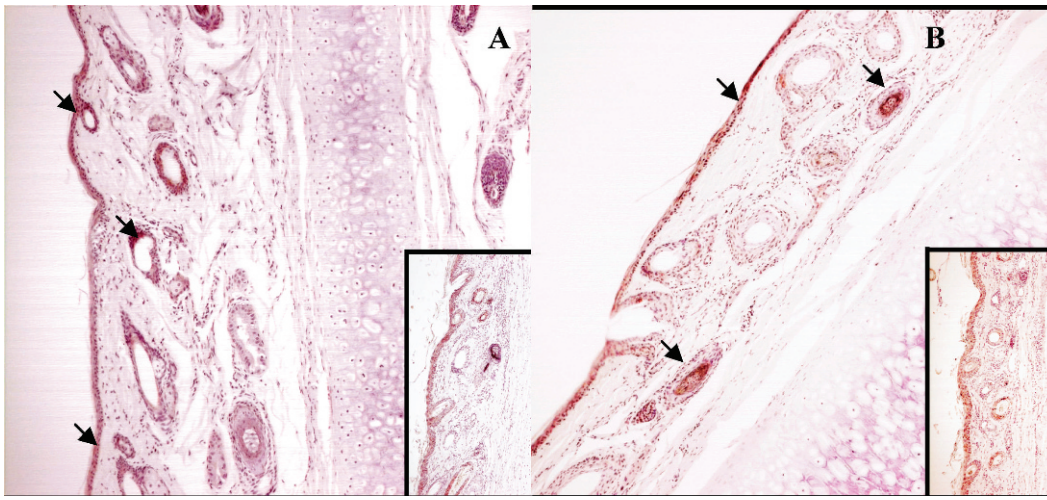

FIGURE 1. Bovine viral diarrhea virus (BVDV) immunohistochemistry (IHC) staining of the ear notches of two white-tailed deer. (A) Deer 1. (B) Deer 2. Insets are positive-control BVDV IHC bovine ear notches. Arrows indicate the presence of IHC staining of skin and hair follicles. Magnification 40×.

Arcanobacterium pyogenes from the lung abscess.

Bovine viral diarrhea virus was detected in Deer 2 in several different tissues (skin, kidney, lung, heart, lymph nodes, liver, lung, abomasum, small intestine, colon, spleen, salivary gland, and rumen) with the use of three different techniques (IHC, IF, and VI); ear skin from Deer 2 also was IHC positive (Fig. 1B). Like Deer 1, the distribution and location of antigen in the hair follicular cells and the dermis was consistent with the distribution of BVDV antigen in persistently infected cattle (Njaa et al., 2000). Additional tissues, including the kidney, heart,

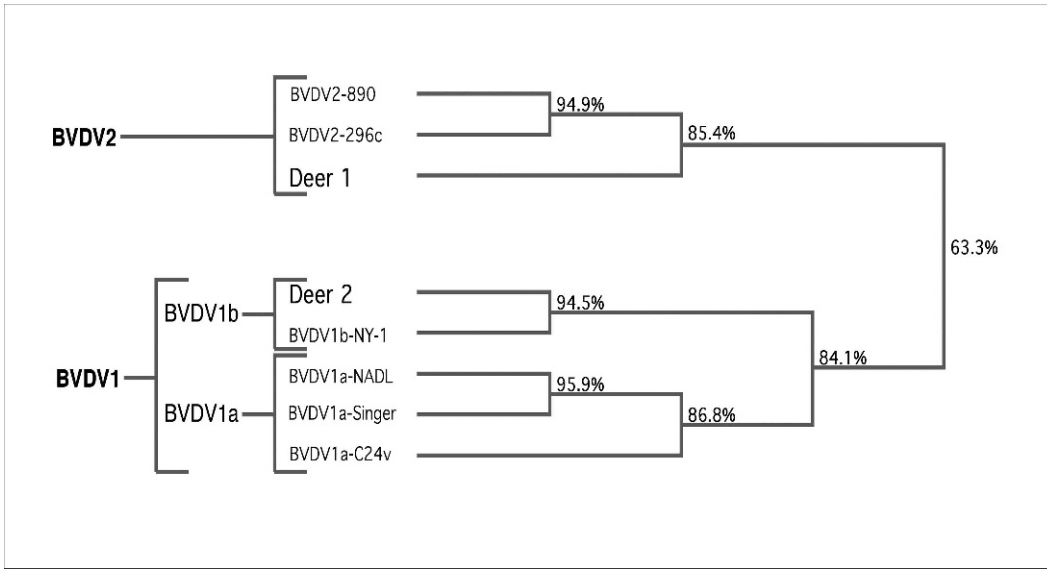

FIGURE 2. Dendrogram of the two white-tailed deer bovine viral diarrhea virus isolates based on 5′UTR sequence. The percentage of nucleotide sequence homology is indicated.

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lymph nodes, liver, lung, abomasum, small intestine, colon, spleen, salivary gland, and rumen were also IHC positive. The lung tissue from Deer 2 was also IF positive for BVDV (data not shown). A NCP BVDV was isolated from the kidney, abomasum, and lung. Multiplex RT-PCR produced a 223-bp product, indicating the presence of a Type 1 BVDV; 5′UTR sequencing indicated that the isolate was a Type 1b BVDV (Fig. 2). This isolate was given the identifier SDSU03-24272.

This report describes the detection of BVDV from two free-ranging white-tailed deer in southeastern South Dakota. The deer exhibited clinical signs of weakness. Although not proven, a focal *Arcanobacterium pyogenes* infection was suspected as the cause of ataxia and CNS signs in Deer 1. Bovine viral diarrhea virus antigen was present in the skin, hair follicles, and dermis with a staining pattern consistent with that seen in cattle persistently infected with BVDV, and the virus was detected in multiple tissues in both animals. Deer 2 had abomasal ulcers that contained abundant BVDV antigen consistent with lesions seen in BVDV-infected cattle. Different genotypes were isolated from each animal: One animal had a Type 1b infection and the other animal had a Type 2a infection.

This is the first report of BVDV isolation and BVDV antigen detection from free-ranging white-tailed deer. It is impossible to determine whether these animals were persistently infected, but one could speculate on the significance of these findings. The IHC staining pattern of the ear skin was consistent with that seen in persistently infected cattle (Njaa et al., 2000) and white-tailed deer fawns (Passler et al., 2007; Duncan et al., in press). The pattern of IHC staining by itself is not 100% definitive of a persistent infection, as three nonpersistently infected calves were reported to have a similar staining pattern, but were virus negative (Cornish et al., 2005). However, BVDV-infected cattle (Njaa et al., 2000) or fawns (Ridpath et al., 2007) have had this pattern in experimental studies. In addition, the three calves with this staining pattern were virus-isolation negative, which would be characteristic of an acute infection, whereas the two deer described in this study were virus positive, which could be the result of either an acute or persistent infection. In addition, persistent infection has been demonstrated in a white-tailed fawn born from a pregnant white-tailed doe that was infected with two cattle isolates of BVDV (Passler et al., 2007). Although this animal lived 100 days, its ability to transmit BVDV was not tested and conclusions relating to the biologic significance of persistently infected WTD cannot be made. There is a single report of a BVDV transmission from a persistently infected captive mousedeer to her progeny (Grondahl et al., 2003; Uttenthal et al., 2005). However, the initial infection in the mousedeer occurred in captivity and in a species with limited contact with domestic animals in the wild. The significance of this finding in mousedeer on free-ranging deer species with high population densities in rural areas in the USA in the transmission of BVDV can only be speculated.

Based on clinical signs of disorientation in both animals and wasting in one of the animals, it is possible that these animals could have been in the terminal stages of BVDV infection. Clinical signs in the BVDV-infected mule deer in Wyoming also included emaciation and weakness; a lung abscess also was reported (Van Campen et al., 2001).

The susceptibility of free-ranging wild ruminants to BVDV and their potential role in transmission has been difficult to assess. Experimental infections with cattle strains of BVDV in wild ruminants have been reported, but in these studies there was no clinical disease (Van Campen et al., 1997; Tessaro et al., 1999). Transient viremias and viral shedding from the nasal passages were demonstrated, and the animals developed BVDV antibody re-

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sponses. There have been many serological studies demonstrating BVDV exposure in wild ruminants throughout the world (Couvillion et al., 1980; Frolich, 1995; Frolich and Flach, 1998; Cuteri et al., 1999; Tessaro et al., 1999; Nielsen et al., 2000; Van Campen et al., 2001; Lillehaug et al., 2003; Krametter et al., 2004). Although many of these studies were done in areas where BVDV had been isolated from cattle or other wild ruminants, no conclusion on BVDV susceptibility or role of the wild ruminants in transmission could be made.

Further studies are needed to demonstrate the infectivity of these white-tailed deer BVDV isolates in both cattle and deer. Based on the close phylogenic relationship between cattle and the white-tailed deer isolates (Fig. 2), it is likely that these isolates will infect cattle, and it has been demonstrated that isolates derived from wild ruminant can infect cattle (Romvary, 1965; Uttenthal et al., 2005). Future studies need to consider both acute infection and fetal infection. Only by demonstrating fetal infections in wild cervids will we be able to produce evidence that persistent infections can occur in wild cervids. Although we have infected a pregnant deer with the SDSU03-20663 isolate and produced persistently infected fawns (Duncan et al., in press) further studies need to been done to determine transmission efficiency in white-tailed deer.

Although much more work needs to be done on BVDV diagnostics in wildlife, these findings indicate that surveillance of white-tailed deer populations for BVDV could be done with the use of tests and reagents currently being used for cattle. Since the time that these two deer were identified as BVDV infected, we have done two additional BVDV surveys. The first study was done testing retropharyngeal lymph nodes that had been submitted for CWD between July 2003 and the end of June 2004. Over 2,400 samples were submitted, and we randomly sampled approximately 10% of the lymph nodes (204 samples). There were 107 white-tailed deer, 55 mule deer, and 42 elk. All the lymph-node samples were negative with the use of BVDV IHC. From the 2004 hunting season (September–December 2004) approximately 500 white-tailed deer and less than a dozen each of mule deer and elk from South Dakota were tested for BVD virus by IHC staining of ear notches from animals submitted for CWD testing (data not shown). None of the ear notches were positive. In both of these studies more than 90% of samples came from the western portion of the South Dakota, where deer and cattle density is much lower.

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Received for publication 6 November 2006.

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