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ISOLATION OF BOVINE VIRAL DIARRHEA VIRUS FROM A FREE-RANGING MULE DEER IN WYOMING

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ABSTRACT: A noncytopathic type 1a bovine viral diarrhea virus (BVDV) was isolated from a free-ranging yearling female mule deer (*Odocoileus hemionus*) from northwestern Wyoming (USA). The mule deer was emaciated, weak, and salivating, and *Arcanobacterium pyogenes* was cultured from lung abscesses. Bovine viral diarrhea virus was isolated from lung, however, BVDV antigen was not detected by immunohistochemistry. The BVDV genotype was determined by reverse transcriptase polymerase chain reaction and the RNA sequences from the 5'UTR and E2 genes compared with sequences of a type 1a BVDV isolated from cattle from the same area as the deer. The sequences from the deer BVDV were distinct from those of the bovine type 1a BVDV, but similar to other bovine type 1a BVDVs. Seventy-four (60%) of 124 sera collected from mule deer in this area had serum neutralizing antibody titers to type 1a BVDV of $\geq 1:32$. The high prevalence of seropositive mule deer and isolation of BVDV suggests that this virus circulates in the mule deer population. The isolate described in this report is the second reported BVDV isolate from free-ranging deer in North America and the first from a mule deer.

Key words: Bovine viral diarrhea virus, case report, mule deer, *Odocoileus hemionus*, serologic survey.

INTRODUCTION

Bovine viral diarrhea virus (BVDV) is the most common infectious cause of reproductive failure in beef herds in the western United States (Woodard, 1994). Horizontal transmission of BVDV in cattle results in a range of diseases including subclinical infections, hemorrhagic syndrome, immunosuppression, secondary infections, and peracute death. Vertical transmission of BVDV from the dam to the bovine fetus results in infertility, abortions, stillbirths and weak, non-viable calves (Baker, 1995). A key feature of infection during the first trimester of pregnancy is the development of immunotolerance to the virus by the fetus and persistent, life-long infection of the calf (McClurkin et al., 1984). If these persistently infected (PI) calves survive, they serve as an important source of infection for other cattle. Control of BVDV infection in an infected herd includes removal of PI animals and vaccination to reduce the severity of

acute BVDV infections. Because fetal infections can have devastating economic consequences in beef herds, prevention of the entry of BVDV into uninfected herds is paramount. Wild ruminants share range, feed, and water sources with range cattle, therefore, development of adequate control programs depends on knowing whether there are reservoirs of BVDV on the ranges other than domestic cattle.

Bovine viral diarrhea viruses have been isolated from free-ranging roe deer (*Capreolus capreolus*) (Romvary, 1965; Fröllich and Hofmann, 1995), fallow deer (*Dama dama*) (Edwards et al., 1988), a giraffe (*Giraffa camelopardis*) (Cay et al., 1989) and eland (*Taurotragus oryx*) (Anderson and Rowe, 1998; Vilcek et al., 2000). Surveys of wild ruminants in North America have found a wide range in seroprevalence to BVDV (Kahrs et al., 1964; Friend and Halterman, 1967; Thorsen and Henderson, 1971; Barrett and Chalmers, 1975; Stauber et al., 1977; Couvillion et

al., 1980; Elazhary et al., 1981; Kocan et al., 1986; Aguirre et al., 1995). Clinical signs reported for reindeer (*Rangifer tarandus*) experimentally inoculated with a cytopathic BVDV included mild diarrhea, coronitis and laminitis (Morton et al., 1990). Neither red deer (*Cervus elaphus*) nor elk (*Cervus elaphus*) developed clinical disease when inoculated with an untyped BVDV (McMartin et al., 1977) or a type 1 or type 2 BVDV (Tessaro et al., 1999), respectively. Mule deer (*Odocoileus hemionus*) and white-tailed deer inoculated with a noncytopathic BVDV became viremic and shed virus in nasal secretions, but did not develop clinical signs of disease (Van Campen et al., 1997). These studies have established the susceptibility of wild ruminant species to BVDV infection. Serologic and virologic data indicate that BVDV is sustained in eland populations (Anderson and Rowe, 1998). However, it is not known in most cases if wild species serve as a reservoir for BVDV or whether infections occur due to contact with cattle.

MATERIALS AND METHODS

A yearling female mule deer from Pinedale, Wyoming, USA (109°55'W, 42°52'N) was reported to be emaciated, weak, and foaming at the mouth when killed on 19 November 1997. The deer was necropsied in the field. Formalin-fixed brain, lung and heart and chilled, fresh lung tissue containing large abscesses were submitted to the Wyoming State Veterinary Laboratory (University of Wyoming, Laramie, Wyoming, USA) for examination. *Arcanobacterium pyogenes* was cultured from a swab of the nasal passages and lung on blood agar using standard techniques (Carter and Cole, 1990). Fixed tissues were embedded in paraffin, sectioned at 6 µm, and stained with hematoxylin and eosin. Microscopically, lung was characterized by marked bronchial epithelial hyperplasia with degenerative changes in scattered epithelial cells including swelling, granularity, and cytoplasmic clearing. Inclusion bodies were not observed. In some bronchi, there was mild sub-epithelial edema and vessels and bronchi were often cuffed by lymphocytes and macrophages; plasma cells were occasionally numerous. Some of these lymphoid accumulations contained depleted germinal centers. Mild hypertrophy of

alveolar type II epithelial cells occurred and these bulged into the alveolar lumina which contained abundant neutrophils and macrophages. Abscesses were scattered in the lung sections and these contained inspissated necrotic debris. Finally, the pleural surfaces were fibrotic. Multiple sections of brain including hippocampus, cerebral cortex, thalamus, and hypothalamus were characterized by scattered light perivascular lymphoid cuffs. A few aggregates of lymphocytes and scattered plasma cells also were present in the epicardium and myocardium. Sarcocysts were scattered in the myocardium without inflammatory reaction.

Sections of brain, lung, and heart were stained for BVDV antigen by immunohistochemistry (IHC) (Haines et al., 1992) using a monoclonal antibody, 15.C.5, obtained from E. J. Dubovi (Cornell University, Ithaca, New York, USA) as were positive and negative controls. Bovine viral diarrhea virus antigen was not detected. This may have been due to prolonged formalin-fixation, the limited number and type of tissues examined, or inability of the IHC to detect small amounts of antigen. A 10% homogenate of lung in Bovarnick's solution (0.218 M sucrose, 3 mm KH₂PO₄, 7.2 mm K₂HPO₄, 5.4 mm L-glutamic acid, 0.001% phenol red, pH 7.2) was prepared and used to inoculate primary bovine embryonic testicle cells (BeTs) in a 24 well plate (Corning Inc., Corning, New York, USA). Bovine embryonic testicle cells were inoculated with 0.3 ml of homogenate, incubated for 1 hr at 37 C, rinsed and 1.0 ml of media composed of 199E (Gibco, Grand Island, New York, USA) and 2% γ-irradiated, BVDV-free fetal bovine serum. Inoculated BeTs were incubated at 37 C with 5% CO₂ for 9 days. The BeTs were found to be infected with a noncytopathic virus. The isolate (97W10745) was identified as a pestivirus by indirect immunofluorescent antibody staining with the monoclonal antibody 20.10.6 (provided by E. J. Dubovi) and rabbit anti-mouse immunoglobulin-FITC conjugate (Zymed Laboratories, Inc., South San Francisco, California, USA) as previously described (Van Campen et al., 1997).

RNA templates were prepared from BVDV infected cells as described previously (Ridpath and Bolin, 1998). Reverse transcriptase-polymerase chain reaction (RT-PCR) was used to amplify sequences from the 5' untranslated region (UTR) and regions coding for structural and nonstructural proteins (Ridpath and Bolin, 1997). Primers were selected from genomic regions conserved between BVDV genotypes and within genotypes using the Primer 2 program (Scientific and Educational Software, State Line, Pennsylvania, USA). The viral genomic

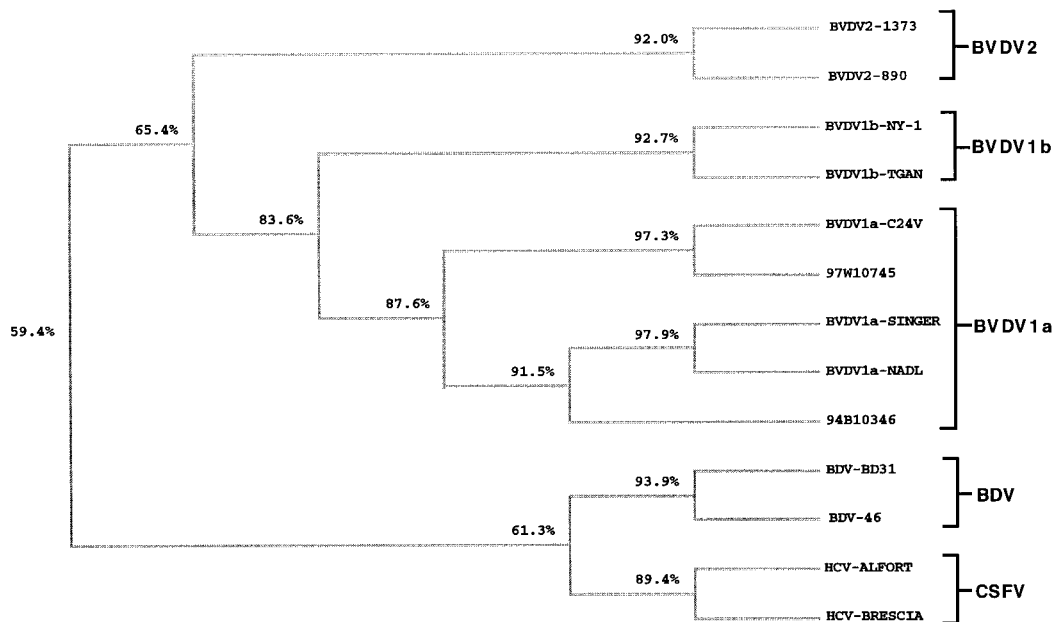


FIGURE 1. Genotyping of Wyoming deer and cattle isolates based on comparison of 5' UTR sequences. The two isolates 97W10745 and 94B10346 were segregated to the BVDV 1a subgenotype. Phylogenetic analysis was performed as described by Ridpath and Bolin (1998) based on comparison of sequences from the highly conserved 5' UTR.

sequences (5'UTR and E2) were derived by direct sequencing of PCR amplification products as described by Ridpath and Bolin (1997). All sequencing reactions were done in duplicate and all sequences were confirmed by sequencing both strands. Sequences were aligned and compared using Align Plus (Scientific and Educational Software) and MacDNASIS (Hitachi Software Engineering Co., Ltd., San Bruno, California, USA). The sequence of 97W10745 was compared to corresponding sequences of a noncytopathic type 1a BVDV (94B10346) isolated from a persistently infected, 2-yr-old heifer (Van Campen et al., 1998) on a ranch located 48 km southeast of the location where the deer was found. The 5'UTR sequences of 97W10745 and 94B10346 demonstrate that both isolates belong to the type 1a BVDV subgenotype (Fig. 1). The E2 sequence of 97W10745 indicate that this virus is similar to other type 1a BVDVs and is not the same as Deer-NZ1, a type 1b BVDV previously isolated from deer (Becher et al., 1999) (Fig. 2). These findings are evidence that the BVDVs circulating among deer are very much like the viruses circulating among cattle.

One hundred twenty-four sera were obtained from mule deer captured in the same area as the deer from which the BVDV isolate was made. Helicopter net-gunning techniques

(Barrett et al., 1982) were used to capture adult deer in February 1998. Deer were physically restrained while blood samples were collected. For comparison, sera from 50 farm-raised mule deer and nine white-tailed deer were obtained from H. Lehmkuhl (National Animal Disease Center, Ames, Iowa, USA). The farm-raised deer originated from northeastern Michigan (USA) and were raised apart from cattle. Sera were complement-deactivated at 56 C for 30 min, and tested for serum neutralizing (SN) antibodies to NADL-BVDV (National Veterinary Services Laboratory, Ames, Iowa, USA), a cytopathic type 1a BVDV. Two-fold serial dilutions of serum were made in triplicate wells in a 96-well microtiter plates. One hundred TCID₅₀ of NADL-BVDV in 50 µl were added to duplicate columns of wells and the plates were incubated for 1 hr at 37 C. The third column of diluted serum served as the serum control. Madin-Darby bovine kidney (MDBK) cells (1 × 10⁴ cells/well) were added and the plates incubated at 37 C. After 3 days, the MDBK cells were examined for cytopathic effect of NADL-BVDV using an inverted light microscope. Serum neutralizing antibody titers for each serum sample were the reciprocal of the highest dilution at which NADL-BVDV was completely neutralized (Carbrey et al., 1971). Serum neutralization titers ranged from

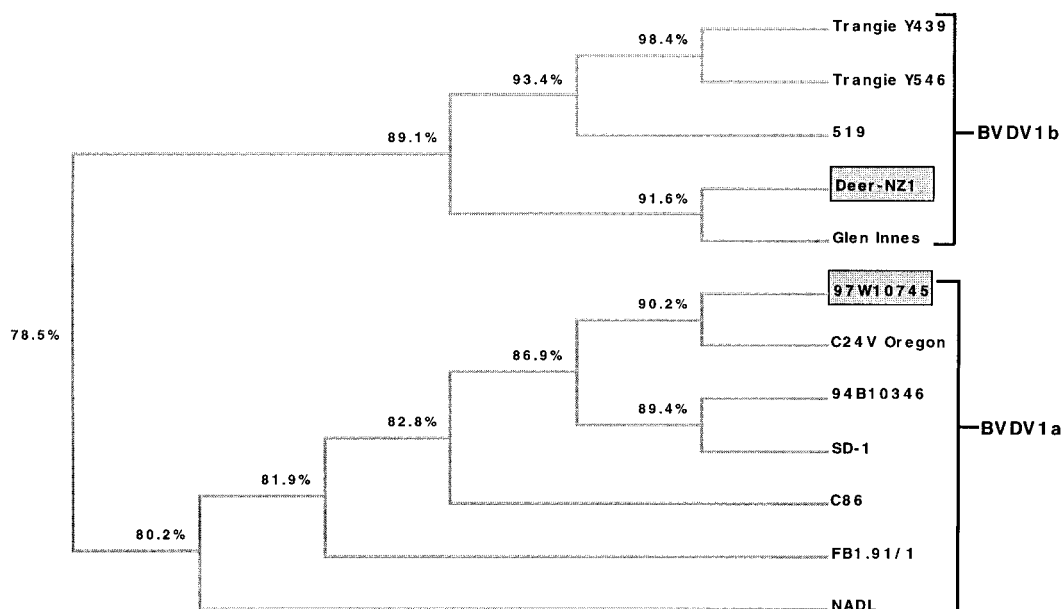


FIGURE 2. Comparison of the Wyoming deer isolate to other BVDV1 strains isolated from cattle and deer. Nucleotide sequences derived from the region coding for the E2 viral structural protein of isolate 97W10745 were compared to E2 coding sequences submitted to Genbank. Strains Trangie Y439 (Acc. #AF049224), Trangie Y546 (Acc. #AF049223), 519 (Acc. #AF144610), Glen Innes (Acc. #AF049226), C24V Oregon (Acc. #AF091605), 94B10346, SD-1 (Acc. #M96751), C86 (Acc. #AF144611), FB1.91/1 (Acc. #L38697) and NADL (Acc. #M31182) were isolated from bovine sources in the U.S. and Europe. Strains Deer-NZ1 (Acc. #AF144614) and 97W10745 were isolated from deer, in Europe and the USA, respectively. Sequences compared correspond to nucleotides 2834 through 3094 of SD-1 (Deng and Brock, 1992).

<1:4 to 1:512, and 74 of 124 (60%) samples had SN titers of $\geq 1:32$ to type 1a BVDV. The mule deer and white-tailed deer sera from NADL were seronegative to NADL-BVDV.

RESULTS AND DISCUSSION

This report describes a type 1a BVDV isolated from free-ranging deer in North America. Bovine viral diarrhea virus is not generally considered to be a serious pathogen of deer. Experimentally, cervids are susceptible to infection with noncytopathic BVDV, become viremic, shed virus in nasal secretions for a short time, and develop antibodies to the virus, but apparently seldom develop clinical signs of disease (Van Campen et al., 1997). Fetal infection and production of PI deer have not been reported; and, it is not known whether PI deer are produced and can survive under free-ranging conditions.

Prior to the use of BVDV vaccines, seroprevalence of 60% in infected cow herds

was reported (Malmquist, 1968). The comparable prevalence of SN titers to BVDV in mule deer from Pinedale, Wyoming suggest that deer may also maintain the virus in their populations. The seropositive animals were evenly distributed across the 3,072 km² winter complex where the deer were captured. Approximately 30,000 deer from five different mountain ranges in western Wyoming gather in this winter range. The implications for viral transmission between domestic livestock and deer are unknown. Since deer and cattle share range, feed, and water sources, transmission of BVDV between species is possible.

The impact of BVDV on the health of free-ranging deer populations is unknown and there is minimal evidence to suggest that the virus causes significant disease. Limited experimental infection of wild cervids with BVDV suggests that they are

subclinically affected or develop mild clinical signs similar to those reported in cattle (Morton et al., 1990; Van Campen et al., 1997; Tessaro et al., 1999). Bovine viral diarrhea virus is recognized for its immunosuppressive properties and role in respiratory disease in cattle (Baker, 1995). It is possible that BVDV played a role in the clinical presentation and lesions in the mule deer in this report. The high seroprevalence in mule deer from the Pinedale herd suggests that although exposure to BVDV is common, many animals survive the infection.

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