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Source: Journal of Wildlife Diseases, 33(3) : 567-573

Published By: Wildlife Disease Association

URL: https://doi.org/10.7589/0090-3558-33.3.567

Journal of Wildlife Diseases, 33(3), 1997, pp. 567–573

EXPERIMENTAL INFECTION OF DEER WITH BOVINE VIRAL DIARRHEA VIRUS

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ABSTRACT: In order to determine the susceptibility of deer to infection with bovine viral diarrhea virus (BVDV), four mule deer (*Odocoileus hemionus*) fawns and one white-tailed deer (*O. virginianus*) fawn were inoculated intranasally with the New York-1 strain of BVDV originally isolated from cattle. None of the animals developed clinical signs of illness. Virus was isolated from white blood cells from four fawns on one or more occasions from day 2 through day 15 post-inoculation (PI) indicating that infection and systemic spread of BVDV had occurred. In addition, virus was isolated from nasal swabs from three fawns, one to three times, from day 2 through day 8 PI. Four fawns had virus neutralizing antibody titers to two strains of BVDV prior to inoculation and all developed greater than four-fold increases in virus neutralizing antibody titers by 3 wk PI. No gross lesions of bovine viral diarrhea were detected at necropsy approximately 3 mo PI. A variety of nonspecific lesions were detected by histopathology. Based on these findings, mule and white-tailed deer are susceptible to infection with BVDV. Isolation of virus from nasal swabs is evidence that BVDV could be transmitted by deer via direct contact.

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

INTRODUCTION

Bovine viral diarrhea (BVD) is a common and economically important viral infection of cattle worldwide. Estimates of 70 to 80% seroprevalence of BVD in the cattle population of the United States have been made (Ames, 1986). Acute infection of immunocompetent cattle with bovine viral diarrhea virus (BVDV), a pestivirus, usually results in subclinical disease (Wilhelmsen et al., 1990). Primary infections with BVDV also can result in transient to severe diarrhea, a hemorrhagic syndrome in calves, and peracute death in adult cattle (Corapi et al., 1989; Bolin and Ridpath, 1992; Pellerin et al., 1994).

Free-ranging North American wild ruminants found to be seropositive to BVDV include mule deer (*Odocoileus hemionus*) (Stauber et al., 1977; Couvillion et al., 1980), white-tailed deer (*Odocoileus virginianus*) (Kahrs et al., 1964; Friend and Halterman, 1967), moose (*Alces alces*) (Thorsen and Henderson, 1971; Kocan et al., 1986), caribou (*Rangifer tarandus*) (Elazhary et al., 1981), pronghorn antelope (*Antilocapra americana*) (Barrett and Chalmers, 1975), and bison (*Bison bison*) (Williams et al., 1993). Results of surveys of wild ruminants have varied from no seropositive animals (Sadi, 1991) to 69% seroprevalence in caribou (Elazhary et al., 1981). Seropositive wild ruminants have also been detected in captivity (Doyle and Heuschlele, 1983); some of these animals may have been vaccinated with bovine vaccines or had contact with domestic ruminants. Clinical disease in cervids rarely has been reported (Karstad, 1981). The source of infection and epizootiology of BVDV and other pestiviruses of free-ranging animals are not known.

Only a few experimental BVDV infections of wild ruminants have been reported and these provide few details. McMartin et al. (1977) infected three red deer (*Cervus elaphus*) with a bovine strain of BVDV which did not result in clinical disease. Two experimentally infected reindeer (*Rangifer tarandus*) developed mild clinical disease (Morton et al., 1990). Experimental infections of mule deer, white-tailed deer and pronghorn antelope were conducted in the 1950s (Richards et al.,

1956), but the severe disease produced may not have been due to BVDV (Karstad, 1981). Recent reports of infections of mule and white-tailed deer have not been published. Both species share range with cattle in Wyoming and in many parts of North America. To determine the susceptibility of deer to acute infection with BVDV and assess the potential for these animals to transmit virus, we infected mule and white-tailed deer fawns with a bovine strain of BVDV.

MATERIALS AND METHODS

Four male mule deer fawns and one female white-tailed deer fawn were submitted to the Wyoming Game and Fish Department's Sybille Conservation Education and Wildlife Research Unit (Wheatland, Wyoming, USA) as orphans, when <2-wk-old. They were hand-raised on evaporated cow's milk supplemented with vitamins and weaned onto grain mix, high quality alfalfa hay and mineralized salt block. Fawns were housed in a pen with inside shelter and outside access to grass and other vegetation. They were not vaccinated for BVD. There was no direct contact with domestic cattle, but other wild ruminants were held in the facility. Fawns were intranasally inoculated on 28 November 1994 with 1.6×10^5 50% tissue culture infectious doses ($TCID_{50}$) of New York (NY-1) BVDV in 1 ml culture medium when they were 5 to 6 mo of age.

Blood was collected by jugular venipuncture with manual restraint three times in the month prior to inoculation, every other day through day 8 post-inoculation (PI), then weekly through day 50 PI, and finally approximately 3 mo PI. Blood was collected into tubes with sodium heparin, and potassium ethylenediaminetetraacetic acid (EDTA), as well as serum collection tubes (Terumo Medical Corporation, Elkton, Maryland, USA). Blood for serum was allowed to clot for approximately 6 hr and serum harvested and used in virus neutralization tests. Buffy coats from heparinized blood were used for virus isolation. Complete blood counts using an automated cell counter (Coulter Counter, Hialeah, Florida, USA) and differential white blood cell counts were conducted on blood collected in EDTA. Nasal secretions were sampled by swabbing each nostril with a cotton swab, vortexed in 1.0 ml OptiMEM (Gibco, Grand Island, New York, USA) culture medium containing 200 IU penicillin, 200 μg streptomycin, and 2% horse serum filtered through a 0.45 μm syringe filter (Whatman Laboratory Division, Clifton, New Jersey, USA) and frozen at −70 C until cultured. Body temperature was determined using a digital rectal thermometer.

Fawns were killed with an intravenous overdose of approximately 0.3 ml/kg body weight of 26% sodium pentobarbital (Sleepaway, Fort Dodge Laboratories, Incorporated, Fort Dodge, Iowa, USA) following deep anesthesia with approximately 300 mg ketamine hydrochloride (Ketaset, Fort Dodge Laboratories, Incorporated) and 60 mg xylazine hydrochloride (Rompun, Mobay Corporation, Animal Health Division, Shawnee, Kansas, USA) approximately 3 mo PI. Animals were examined post-mortem and portions of liver, kidney, lung, heart, rumen, reticulum, omasum, abomasum, multiple sections of small intestine, spiral colon, colon, skeletal muscle, gonad, adrenal gland, thyroid gland, pituitary gland, pancreas, urinary bladder and brain were sampled. Lymphoid tissues collected included mediastinal, mesenteric, internal iliac, and retropharyngeal lymph nodes, spleen and thymus. Tissues were fixed in 10% buffered formalin, embedded in paraffin and 5 to 6 μm sections were stained with hematoxylin and eosin for examination by light microscopy.

Stocks of noncytopathic (ncp) NY-1 BVDV and cytopathic (cp) National Animal Disease Laboratory (NADL)-BVDV obtained from the National Veterinary Services Laboratory (Ames, Iowa, USA) were grown on bovine turbinate (BT) cells (NY-1 BVDV) or Madin-Darby bovine kidney (MDBK) cells (American Type Culture Collection, Rockville, Maryland, USA) (NADL-BVDV) maintained in cell culture medium (OptiMEM) supplemented with 2% horse serum. Three passes of all cell cultures were examined by IFA techniques as described to ensure that the cells were free of adventitial BVDV. Lots of γ-irradiated fetal bovine serum (FBS) (Summit Biotechnologies, Ft., Collins, Colorado, USA) were tested for BVDV contamination by inoculating BeTs with samples of FBS and examining the cells for BVDV by IFA.

Bovine embryonic testicle cells were used for virus isolation. Bovine embryonic testicle cells in 25 cm^2 flasks were inoculated with 1.0 ml of spleen, liver, kidney, lymph nodes and lung homogenates, white blood cells, and nasal swab fluid followed by incubation for 1 hr at 37 C. Following incubation, BeT cells were rinsed and maintenance medium composed of 199E (Gibco) plus 2% FBS was added; the cells then were incubated for 4 to 7 days. The BeT cells were sampled by treating the monolayer with trypsin-EDTA (Sigma Chemical Company, St. Louis, Missouri, USA) for 5 min and aliquots

of the loosened cells were placed in duplicate wells of printed microscope slides (Cel-line Associates, Inc., Newfield, New Jersey). Media containing 10% FBS was added to each well and the slides incubated at 37 C until the cells adhered to the slide. The medium was then blotted from the wells and the slides were air-dried. Following fixation of the cells in 10% acetone at 4 C, the cells were stained using monoclonal antibody (Mab) 20.10.6 (provided by E. Dubovi, Cornell University, Ithaca, New York, USA) and fluoroscein isothiocyanate-conjugated goat anti-mouse immunoglobulin (Ig)-G, heavy and light chains (Zymed Laboratories, South San Francisco, California, USA). New York-1 BVDV infected and uninoculated cells served as positive and negative virus controls. The cells were examined for characteristic cytoplasmic fluorescent-staining with a fluorescent microscope. Each sample was passed twice.

The amount of virus in the virus stocks and inoculum was determined by a modification of a microtiter virus isolation enzyme-linked immunosorbent assay (ELISA) developed by Ed Dubovi (Cornell University). Briefly, serial 10-fold dilutions were made in medium and 100 µl of each dilution placed into each of eight wells in a column on a 96-well plate (Evergreen Scientific, Los Angeles, California, USA). One-hundred µl of a suspension of BT cells (1×10^5 cells/ml) was added to each well and the plate incubated for 4 days. The medium was decanted and the plates were air-dried. The cells were fixed for 10 min in 20% acetone:80% phosphate buffered saline (PBS) (Becton Dickinson Microbiology Systems, Cockeysville, Maryland, USA) and allowed to air-dry overnight. Noncytopathic BVDV was detected using Mab 20.10.6 diluted 1:1600 in binding buffer (PBS, 0.05% Tween 20, and 2.95% sodium chloride) (Sigma). Following a 30 min incubation at 37C with 50 µl of diluted Mab, the wells were washed three times with a wash buffer (PBS and 0.05% Tween 20) incubated for 30 min with biotinylated rabbit anti-mouse IgG (Zymed Laboratories; 1:500 in 60% chicken serum (Sigma):40% PBS), washed and incubated for 30 min with strepavidin-horseradish peroxidase (Gibco) (1:1000 in wash buffer). The wells were washed again and 50 µl of substrate (2 mg/ml amino-ethyl-carbazole (Sigma) in dimethyl sulfoxide (Sigma) and 0.05 M sodium acetate (Sigma) buffer, pH 5.0 and 2 µl 30% hydrogen peroxide (Sigma)) was added to each well. The plates were rinsed with tap water after 10 to 40 min incubation and the wells scored for cytoplasmic staining indicative of BVDV infection. The $TCID_{50}$/ml was determined using the method of Reed and Muench (Reed and Muench, 1938).

To determine virus neutralizing antibody titers in deer sera to the reference cp NADL-BVDV and ncp NY-1 BVDV, two-fold serial dilutions of each serum was made in 96-well microtiter plates. All sera were complement-deactivated at 56 C for 30 min. One hundred $TCID_{50}$ of each virus strain was added to duplicate wells containing diluted sera. After 1 hr incubation at 37 C, MDBK cells (1×10^4 cells/well) were added and the plates incubated at 37 C for 4 days. For ncp BVDV, the media were decanted and the cells were air-dried for 1 hr in a laminar flow hood. The cells were fixed, air-dried, and the ncp BVDV detected using the microtiter ELISA system as described. Virus neutralization antibody titers to cp BVDV strains were performed similarly except that observation for neutralization of plaque formation using an inverted light microscope after a 3-day incubation was used. The virus neutralizing antibody titer recorded was the reciprocal of the highest antibody dilution which completely neutralized infection of the indicator cells by the virus.

RESULTS

None of the fawns developed clinical signs of illness during 98 days PI. With considerable fluctuation, body temperatures were considered within normal range for manually restrained deer (38 to 39.5 C). White blood cell and lymphocyte counts were highly variable between deer and between samples from each individual and consistent changes in these parameters were not noted during the observation period. Noncytopathic bovine viral diarrhea virus was isolated from white blood cells of four deer during the first 15 days and from nasal swab samples of three deer from day 4 to 8 PI (Table 1). No BVDV was isolated from tissues of deer collected at post-mortem examination.

Unexpectedly, four of five deer had virus neutralizing antibody titers in pre-inoculation serum samples (Fig. 1). All deer developed virus neutralizing titers to both NY-1 and NADL BVDV by day 8 to 15 PI. The three mule and one white-tailed deer that were seropositive pre-inoculation rapidly developed increased virus neutralizing titers to NY-1 BVDV compared to

TABLE 1. Isolation of bovine viral diarrhea virus from white blood cells and nasal swabs ( ) collected from experimentally infected mule and white-tailed deer.

Animal number	Day post-inoculation								
	0	2	4	6	8	15	22	29	98
Mule deer									
M1	−[a]	− (−[a])	+[b] (+[b])	+ (+)	+ (+)	+ (−)	− (−)	−	−
M2	−	− (−)	− (+)	− (−)	− (+)	− (−)	− (−)	−	−
M3	−	+ (−)	− (−)	+ (−)	− (−)	− (−)	− (−)	−	−
M4	−	− (−)	− (−)	+ (+)	− (−)	− (−)	− (−)	−	−
White-tailed deer									
W	−	− (−)	+ (−)	− (−)	− (−)	− (−)	− (−)	−	−

[a] Negative for bovine viral diarrhea virus.
[b] Positive for bovine viral diarrhea virus.

those of the fawn seronegative at the time of inoculation. Virus neutralizing antibody titers to NY-1 BVDV (the homologous virus) increased more rapidly in all deer than those to NADL-BVDV.

Deer were judged to be in fair to good body condition based on the amount of subcutaneous and visceral fat. No gross lesions were observed at post-mortem examination. Microscopic lesions were varied and included minimal nonsuppurative meningoencephalitis, mild to moderate chronic interstitial nephritis, mild acute multifocal suppurative rumenitis, mild eosinophilic enterocolitis, and lymphoid hyperplasia in one or more of the deer. Virus was not isolated from any of the tissues collected post-mortem.

DISCUSSION

Both mule deer and white-tailed deer were susceptible to infection with a BVDV isolate derived from cattle but did not become clinically ill. Similarly, red deer calves inoculated with a bovine BVDV did not become clinically ill (McMartin et al., 1977). In contrast, experimental infection of two reindeer calves with cp Singer strain BVDV induced mild diarrhea and laminitis or coronitis (Morton et al., 1990). Differences in response to infection among these cervid species might be related to differential species susceptibility, differences in the pathogenicity of the virus used for infection, the presence of antibodies in four of the five deer which could have provided a degree of protection from clinical disease, or may be an artifact of the small numbers of animals in these studies. Severe mucosal disease-like illness was reported by Richards et al. (1956) in mule deer inoculated with an isolate derived from mule deer showing signs of BVD. However, the literature in BVD prior to 1984 is subject to interpretive errors due to the problem of maintaining cells in vitro free of adventitial BVDV, the inability to detect noncytopathic BVD viruses as contaminants of cytopathic isolates and the possibility of the presence of undetected viruses; this confused the dependability of the results of these experimental inoculations.

The microscopic lesions found in mule deer were mild and nonspecific but similar lesions occasionally have been reported from domestic ruminants infected with pestiviruses (Barker et al., 1993). Minimal to mild nonsuppurative meningoencephalitis is relatively common in adult free-ranging mule deer from Colorado and Wyoming (USA) surveyed for chronic wasting disease but the cause has not been determined (E. S. Williams, unpubl. data). Mild multifocal rumenitis may be due to concentrates fed to the deer (Barker et al., 1993). The eosinophilic enteritis was likely due to parasitic infection (Barker et al., 1993). Lymphoid depletion, rather than the lymphoid hyperplasia observed in

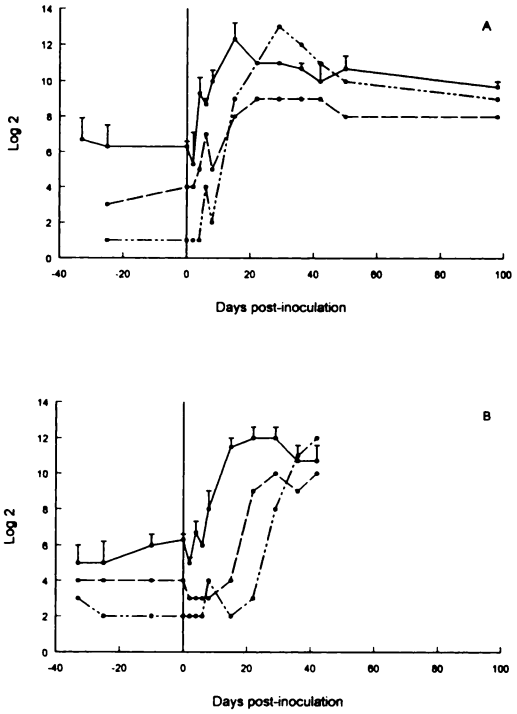

FIGURE 1. Virus neutralizing antibody titers against bovine viral diarrhea virus (BVDV) in deer experimentally infected with NY-1 BVDV. A. Neutralizing antibody against NY-1 BVDV. Mean (+SE) antibody titer of three mule deer fawns (m2, m3, m4) seropositive at the start of the experiment (———); antibody titer of mule deer fawn (m1) seronegative at the start of the experiment (— —); antibody titer of white-tailed deer (w1) (— —). B. Neutralizing antibody titers against National Animal Disease Laboratory BVDV. Mean (+SE) antibody titer of three mule deer fawns (m2, m3, m4) seropositive at the start of the experiment (———); antibody titer of mule deer fawn (m1) seronegative at the start of the experiment (— —); antibody titer of white-tailed deer (w1) (— —).

some of the deer, is the expected result of BVDV infection in cattle (Barker et al., 1993). Peribronchial accumulations of neutrophils and mononuclear inflammatory cells, periportal inflammatory cell aggregates in liver, and mononuclear inflammatory infiltrations in the kidney were described in experimentally infected reindeer (Morton et al., 1990). Failure to isolate virus from tissues from the experimentally infected fawns at necropsy makes association of the mild microscopic lesions with BVDV infection tenuous.

The source of infection for the fawns that were seropositive prior to experimental inoculation is not known. All fawns were obtained as wild individuals from different parts of Wyoming and could have had exposure to BVDV prior to arrival at Sybille. Once at Sybille, they were fed the same feed and housed together throughout the experiment. The presence of a seronegative fawn in the group at the time of inoculation is evidence the fawns were not exposed to BVDV during the time they were housed at Sybille. It is unlikely that the serum antibodies reflected maternal antibody given the 5 to 6 mo age of the fawns at the start of the experiment. Serologic surveys of deer in Wyoming for BVD have not been done; based on opportunistic testing of deer sera, we detected animals with antibodies but do not know the seroprevalence in our free-ranging deer (H. Van Campen, unpubl. data).

The deer responded with neutralizing antibody production more rapidly to NY-1 BVDV, the virus used in the infections, than to NADL-BVDV. Based on the dynamics of the antibody production to NY-1 BVDV, we believe an anamnestic response occurred; possibly the virus to which the seropositive deer had been exposed was antigenically more similar to NY-1 BVDV than to NADL-BVDV. The dynamics of the antibody response were similar in both mule and white-tailed deer.

The presence of antibodies in four deer did not prevent systemic infection by the NY-1 BVDV. Thus, the antibodies probably were to another strain of BVDV or pestivirus, or that the antibodies were not protective. Antibody-positive animals have been detected in serologic surveys of wild ruminants in many parts of the world (Kahrs et al., 1964; Friend and Halterman, 1967; Thorsen and Henderson, 1971; Stauber et al., 1977; Couvillion et al., 1980; Elazhary et al., 1981; Williams et al., 1993; Kocan et al., 1986; Frölich, 1995), but the identity of the virus or viruses responsible for these antibodies has not been determined. Only a few BVDV have been

isolated from deer in North America (Doyle and Heuschele, 1983) but the epizootiology of these infections is not known. Pestiviruses other than BVDV may be circulating in free-ranging cervid populations as has been recently reported in roe deer (*Capreolus capreolus*) in Germany (Frölich and Hofmann, 1995). Additional study will be required to determine the nature of the viruses in deer giving rise to virus neutralizing antibodies.

Bovine viral diarrhea is maintained in cattle through two transmission cycles. First, the virus can be transmitted from a persistently infected individual to other susceptible hosts through nose-to-nose contact with virus-laden secretion (Traven et al., 1991). The presence of virus in nasal secretions from three mule deer is evidence that transmission by this route to susceptible animals in contact with infected deer may be possible. It is not known if this method of transmission could serve to maintain BVDV within deer populations or if deer could serve as a reservoir of BVDV infection for cattle.

Bovine viral diarrhea also is transmitted readily from the blood of an infected cow to her fetus, possibly resulting in persistently infected calves (Brownlie et al., 1984; McClurkin et al., 1984). The virus continues to be replicated within these animals and is shed in high titers in the nasal secretions, urine and feces throughout life. The prolonged period in which these persistently infected cattle can transmit BVDV enhances the probability that the virus can be maintained in cattle herds. Persistently infected cattle also could serve as sources of infection for deer in situations of close contact between the species. It is not known if deer can become persistently infected with BVDV or other pestiviruses.

There is little evidence that BVD is an important disease of free-ranging deer in North America. However, the presence of seropositive animals detected in surveys, the susceptibility of deer to BVDV infection, and the lack of information on the pestiviruses that may be circulating in wild cervid populations, indicate the need for a better understanding of the epizootiology of these viruses in free-ranging deer.

ACKNOWLEDGMENTS

We thank Tom Thorne, Ed Dubovi, Dave Zieler, Julia Ridpath, and Valerie Welch, Hank Edwards, Cathey Lynn, Jackie Cavender, Karen Hill, Mindy Wells, Brad Meyer and Eika Rohr for assistance with this project. This research was funded by Animal Health Funds, the Department of Veterinary Sciences, University of Wyoming, and the Wyoming Game and Fish Department.

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Received for publication 22 January 1996.