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DURATION OF BLUETONGUE VIREMIA IN EXPERIMENTALLY INFECTED AMERICAN BISON

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ABSTRACT: Six yearling American bison (*Bison bison bison*) bulls and one yearling ewe (*Ovis aries*) were inoculated intradermally and subcutaneously with 2×10^5 plaque forming units (pfu) of bluetongue (BT) virus serotype 11. Two uninoculated yearling bison bulls served as negative controls. Blood samples were collected for serology and virus isolation on 0, 4, 7, 11 and 14 days post-inoculation (dpi) and every 2 wk thereafter to 127 dpi. Every 4 wk a new ewe was inoculated with a pooled sample of whole blood from the six infected bison, and each sheep was monitored for 28 days for clinical signs of BT and seroconversion. Bluetongue viremia was detected in all six inoculated bison starting at 4 to 28 dpi and was no longer detectable from 42 dpi onward. Pooled blood samples collected at 28, 56, 84 and 112 dpi from the six infected bison were not infectious for sheep. The six infected bison seroconverted by 11 to 28 dpi on a competitive enzyme-linked immunosorbent assay and by 28 dpi on the serum neutralization test, and all remained seropositive thereafter. No clinical signs or lesions attributable to BT were observed in the infected bison or controls. There was evidence that a small amount of epizootic hemorrhagic disease virus type 2 had been present in the BT virus inoculum; reasons are given for concluding that this did not affect the results of the BT study.

Key words: American bison, *Bison bison*, bluetongue virus, epizootic hemorrhagic disease virus, experimental infection.

INTRODUCTION

Bluetongue (BT) virus is an orbivirus that occurs in some countries and is naturally transmitted by certain species of blood-feeding insects. The virus primarily causes disease in species of sheep and deer and in pronghorn antelope (*Antilocapra americana*), and BT has been one of the main factors resulting in restriction of movement of ruminants around the world (Roberts et al., 1992). Nations with defined freedom from BT attempt to prevent the introduction and potential establishment of BT by imposing restrictions on the importation of hoofstock, including wild ruminants, from nations that either have the disease or share a common border with a country that has BT (Office International des Épizooties, 1997).

Although there are no reports of clinical BT in bison (*Bison bison*), in the USA some bison have developed BT antibodies indicating exposure to the virus (Vestweber et al., 1991). The risk of importing BT virus into Canada in U.S. bison depends upon the duration of viremia in infected bison relative to the time of year when insect vectors might be present to spread the virus. Uniform measures to control BT could be applied to the importation of both U.S. cattle and bison into Canada if the duration of BT viremia in bison is not longer than it is in cattle. The purpose of this research project was to evaluate the duration of BT viremia in bison.

MATERIALS AND METHODS

Facilities and biocontainment

All handling of the BT virus inoculum was done in a level 3 biocontainment laboratory (Best, 1996), and experimentation with the virus in bison and sheep (*Ovis aries*) was done in a level 3 animal biocontainment facility, as required by the Canadian Food Inspection Agency (Winnipeg, Manitoba, Canada). The animal experimentation was conducted between 12 January 1998, and 19 May 1998, to further ensure the absence of potential arthropod vectors. Shipment of samples was in accordance with Transport of Dangerous Goods requirements.

Bluetongue virus inoculum

The inoculum of BT virus serotype 11, passaged in *Culicoides variipennis,* was obtained from Dr. W.C. Wilson at the Arthropod-borne Animal Disease Research Laboratory (United States Department of Agriculture, Laramie, Wyoming). Upon receipt, the inoculum was titrated by infectivity assay in BHK-21 cells and the serotype confirmed using serotype-specific antiserum in a virus neutralization test. Serotype 11 was chosen because it occurs in the USA and was implicated in a past incursion of bluetongue in the Okanagan Valley of British Columbia, Canada (Dulac et al., 1988). In experimentally infected sheep, the duration of BT serotype 11 viremia is similar to that of other serotypes (Katz et al., 1993). The dose of BT virus (see below) was similar to that used in other experimental studies of BT (e.g., Hoff and Trainer, 1974; Richards et al., 1988; MacLachlan et al., 1994; Koumbati et al., 1999).

To test the inoculum prior to the start of the bison experiment, one seronegative yearling ewe from a BT-free flock was inoculated intradermally and subcutaneously with a total dose of 1×10^5 plaque forming units (pfu) of the virus. The ewe developed a measurable BT viremia from 3 days post-inoculation (dpi) to 18 dpi, and a small, transient elevation in rectal temperature (40.1 C) at 5 dpi. The competitive enzyme-linked immunosorbent assay (cELISA) (Afshar et al., 1987, 1989) detected BT antibodies in the serum of the ewe starting at 7 dpi and continuously thereafter until the ewe was killed at 24 dpi. This pilot study provided assurance that the bluetongue virus inoculum was viable and infective after transport.

Experimental animals

Ten yearling bison bulls were provided by the Canadian Bison Association, and five yearling ewes were provided from the specific pathogen-free (SPF) flock at the Animal Diseases Research Institute (Lethbridge, Alberta, Canada). All animals were seronegative for BT and epizootic hemorrhagic disease (EHD) at the start of the experiment. The bison and sheep were given a diet of cubed alfalfa and fresh drinking water *ad libitum.* Animal care was in accordance with the guidelines of the Canadian Council on Animal Care (Ottawa, Ontario, Canada).

Experimental design

Six yearling bison bulls were each inoculated intradermally or subcutaneously at four sites with a total of 2 ml of inoculum (total dose of 2×10^5 pfu of BT-11 virus) on day 0. A positive control ewe in a separate room was similarly inoculated. Four other yearling bison bulls were kept in a separate biocontainment room: Two as negative controls and two held in reserve. All bison were observed at least twice daily for signs of clinical disease or behavioral problems. Heparinized blood and serum samples were collected by jugular puncture from the six inoculated and two control bison on 0, 4, 7, 11, and 14 dpi and every 2 wk thereafter until the experiment was ended at 127 dpi. Blood samples were also collected from the inoculated ewe on the same days until the ewe was euthanized at 28 dpi. The rectal temperature of each bison and the ewe was monitored each time blood samples were collected.

Every 4 wk (28, 56, 84, and 112 dpi) 2 ml of heparinized whole blood from each of the six inoculated bison were pooled and inoculated intradermally and subcutaneously into a new yearling ewe which served as a bioassay. Serum samples were collected on the day of inoculation and weekly thereafter from each of the four ewes inoculated in this manner until each was euthanized at 28 dpi.

At the end of the experiment (127 dpi for all bison, 28 dpi for each ewe) all animals were euthanized by an intravenous overdose of pentobarbital and a complete necropsy was performed. Tissues were collected in 10% neutral buffered formalin for histology.

Analytical methods

Samples from each collection were tested for BT antibodies using the cELISA, which had been evaluated previously for use in bison (Afshar et al., 1995). The quantal microtiter assay developed by Parker et al. (1975) was used to determine the serum neutralizing (SN) titer in sera from the bison and ewes.

Each sample of whole blood collected in heparinized tubes was washed three times in sterile phosphate buffered saline (PBS) by centrifugation at 420 × g for 10 min at 4 C. The pelleted red blood cells (RBCs) were then resuspended in an equal volume of sterile RBC resuspension medium [0.5% bovine serum albumin in PBS (0.01 M, pH to 7.2) with 50 μg/ml gentamicin]. The suspension was mixed gently to avoid hemolysis of RBCs and stored at 4 C until tested for bluetongue virus.

For each sample a 1/10 dilution of RBCs was made in MEM-alpha (Gibco, BRL) containing 50 μg/ml gentamicin (Sigma Chemical Co., St-Louis, Missouri, USA). This was sonicated using a 1/8″ microprobe on 10 ml samples by three cycles of 30 sec with 1 min cooling, using a Virsonic Digital 475 ultrasonic cell disrupter

(Virtis Co., Gardiner, New York, USA) at the control setting of 3 in a bath of ice water to prevent overheating during sonication. Diluted samples were stored at 4 C and used for virus isolation within a 24 h period.

A 1/10 RBC suspension was used to inoculate seven 10-day-old chicken embryos intravenously (IV) as previously described (Clavijo et al., 2000). Seven eggs were inoculated for each sample, where 5 to 6 eggs must survive the first 24 hr. The eggs were incubated at 33.5 C for 7 days and candled twice daily (morning and afternoon) for viability.

The chicken embryos that died between 2 to 7 dpi were refrigerated and each embryo was removed from the egg and placed in a sterile petri dish. Tissues (brain, liver, spleen, heart and lungs) were then removed and stored at 4 C. Tissues from embryos which died at 2 to 7 dpi were pooled and then emulsified in a Tenbroek grinder along with MEM-alpha to make a 10% W/V suspension. Viable eggs left at 7 dpi were refrigerated. However, this pool of embryo tissue was kept separate from tissues of dead embryos and processed as a 10% W/V suspension. Tissue suspensions from dead and live embryos were used for virus isolation in BHK-21 cell cultures.

For virus isolation, BHK-21 cells were seeded in 24 well plates (Costar Co., Cambridge, Massachusetts, USA) at a concentration of 40,000 cells/cm^2 in L15 (Gibco, BRL)/MEM-alpha (1:1), 10% V/V tryptose phosphate broth, 200 mM L-glutamine, 5% V/V fetal bovine serum (FBS), and 100 IU/ml penicillin-100 μg/ml streptomycin. At this seeding concentration, BHK-21 cells were 70 to 80% confluent when inoculated 24 hr later.

After 6 to 7 days, or when the extent of the cytopathic effect (CPE) was 90% or more, cells and media were removed with a pipette. Pools of the same sample (or wells showing CPE of the same sample) were made and stored at 4 C for further passage in cell culture. Samples were examined for BT virus by using an immunoperoxidase-staining procedure as described by Clavijo et al. (2000).

Bluetongue virus in blood samples was titrated in 10-day-old chicken embryos. Eggs were inoculated intravenously with 0.1 ml of each dilution. Seven eggs were used per dilution. Embryo that died between 2 to 7 dpi were used to calculate the 50% end-point of chicken embryo lethal doses per ml ($ELD_{50}ml^{-1}$) (Goldsmit and Barzilai, 1968).

RESULTS

All bison remained clinically normal throughout the experiment and none of the six inoculated bison showed any rise in rectal temperature after infection with BT virus. One negative control bison (#15) tore the lateral dew claw on its right fore foot during handling and was replaced on day 42 with one of the two spare bison (#35). The positive control ewe and four bioassay ewes remained clinically normal during the experiment, and none showed any rise in rectal temperature after inoculation. No gross or histologic lesions were seen in any of the bison or sheep at the time of necropsy.

Three of the six inoculated bison developed detectable antibodies to BT virus by 11 dpi, a fourth bison was positive by 14 dpi, and the remaining two were positive by 28 dpi, as determined by the cELISA (Table 1). All six bison remained positive on the cELISA thereafter for the duration of the experiment (127 dpi). The SN test first detected BT antibodies in all six bison at 28 dpi (Table 1). The SN titers increased until 84–98 dpi and thereafter declined, but all six inoculated bison remained seropositive for the remainder of the experiment. The positive control ewe was first seropositive on both cELISA and SN test at 11 dpi, and remained positive on both tests until euthanized at 28 dpi.

Both negative control bison remained seronegative on both cELISA and SN tests throughout the experiment. The four bioassay ewes remained seronegative on the cELISA and SN tests during the four wk after inoculation with whole blood from the six infected bison.

All six inoculated bison became viremic: BT virus was first detected in the blood of two bison (#30 and #90) at 4 dpi; in three others (#17, #73, and #87) at 7 dpi; and in the sixth bison (#94) at 28 dpi (Table 2). Only one of the inoculated bison (#94) still had evidence of BT viremia at 28 dpi, and no BT virus was isolated from blood of any of the six bison from 42 dpi onward. Titers of BT virus in bison blood increased from 10^3 at the earliest detection of viremia (4 dpi) to $10^{5.61}$ $ELD_{50}ml^{-1}$ at the peak of viremia (7 dpi), and declined to $10^{0.50}$

TABLE 1. Competitive enzyme-linked immunosorbent assay (cELISA) results and serum neutralizing (SN) antibody titers against bluetongue virus in six bison and one ewe inoculated with bluetongue virus serotype 11.

	Bison						Ewe
dpi[a]	#17	#73	#94	#30	#87	#90	#97040
0	1 (N)[b]	11 (N)	−3 (N)	−2 (N)	−4 (N)	1 (N)	6 (N)
4	16 (N)	19 (N)	8 (N)	11 (N)	14 (N)	11 (N)	12 (N)
7	11 (N)	27 (N)	10 (N)	27 (N)	27 (N)	33 (N)	56 (N)
11	48 (N)	82 (N)	38 (N)	76 (N)	29 (N)	59 (N)	87 (1:20)
14	52 (N)	85 (N)	38 (N)	76 (N)	48 (N)	70 (N)	89 (1:40)
28	90 (1:10)	98 (1:80)	91 (1:40)	98 (1:80)	98 (1:10)	98 (1:80)	99 (1:40)
42	96 (1:40)	99 (1:160)	97 (1:40)	98 (1:80)	98 (1:80)	99 (1:80)	ND[c]
56	96 (1:40)	98 (1:80)	97 (1:40)	98 (1:80)	98 (1:40)	98 (1:160)	ND
70	98 (1:80)	98 (1:160)	96 (1:80)	98 (1:160)	98 (1:80)	98 (1:160)	ND
84	98 (1:20)	98 (1:80)	98 (1:160)	98 (1:80)	98 (1:160)	98 (1:160)	ND
98	98 (1:80)	98 (1:160)	97 (1:320)	99 (1:320)	99 (1:80)	99 (1:320)	ND
112	91 (1:10)	99 (1:40)	98 (1:20)	99 (1:80)	99 (1:20)	99 (1:20)	ND
127	98 (1:20)	99 (1:80)	98 (1:20)	99 (1:40)	99 (1:80)	98 (1:80)	ND

[a] dpi = Days post-inoculation.
[b] cELISA percent inhibition, with reciprocal SN titer in brackets. The cELISA is considered positive when the percent inhibition is greater than 50. For SN testing, N = negative.
[c] ND = Not done (the ewe was euthanized at 28 dpi).

$ELD_{50}ml^{-1}$ at the last detection of viremia (28 dpi) (Table 2). The positive control ewe was viremic from 4 dpi until euthanized at 28 dpi. Bluetongue virus was never isolated from the blood of the uninoculated, negative control bison.

An isolate obtained from the blood of bison #30 at 28 dpi did not react with a group-specific monoclonal antibody (Mab) available for the identification of BT virus in the immunoperoxidase test. In order to investigate the nature of this isolate, a preparation of the virus was examined by electron microscopy. Observation by electron microscopy of infected BHK-21 cells indicated the presence of numerous viral

TABLE 2. Bluetongue virus isolation from the blood of six bison and one ewe inoculated with bluetongue virus serotype 11. Bluetongue virus titer ($ELD_{50}ml^{-1}$) from the blood of the six bison is given in brackets.

	Bison						Ewe
dpi[a]	#17	#73	#94	#30	#87	#90	#97040
0	N[b]	N	N	N	N	N	N
4	N	N	N	P[c] ($10^{3.00}$)	N	P ($10^{3.42}$)	P
7	P ($10^{3.36}$)	P ($10^{4.24}$)	N	P ($10^{5.33}$)	P ($10^{1.83}$)	P ($10^{5.61}$)	P
11	P ($10^{2.07}$)	P ($10^{3.93}$)	N	P ($10^{4.21}$)	N	P ($10^{2.93}$)	P
14	N	P ($10^{1.64}$)	N	P ($10^{2.79}$)	N	P ($10^{2.50}$)	P
28	N	N	P ($10^{0.50}$)	N	N	N	P
42	N	N	N	N	N	N	ND[d]
56	N	N	N	N	N	N	ND
70	N	N	N	N	N	N	ND
84	N	N	N	N	N	N	ND
98	N	N	N	N	N	N	ND
112	N	N	N	N	N	N	ND
127	N	N	N	N	N	N	ND

[a] dpi = Days post-inoculation.
[b] N = negative isolation.
[c] P = Positive isolation.
[d] ND = Not done (the ewe was euthanized at 28 dpi).

TABLE 3. Competitive enzyme-linked immunosorbent assay (cELISA) results and serum neutralizing (SN) antibody titers against epizootic hemorrhagic disease virus in six bison and one ewe.

	Bison						Ewe
dpi[a]	#17	#73	#94	#30	#87	#90	#97040
0	11 (N)[b]	22 (N)	11 (N)	13 (N)	24 (N)	16 (N)	24 (N)
4	15 (N)	19 (N)	13 (N)	14 (N)	20 (N)	16 (N)	17 (N)
7	12 (N)	14 (N)	10 (N)	17 (N)	22 (N)	16 (N)	28 (N)
11	14 (N)	22 (N)	13 (N)	15 (N)	20 (N)	23 (N)	40 (N)
14	11 (N)	25 (N)	11 (N)	11 (N)	30 (N)	22 (N)	36 (N)
28	11 (N)	24 (N)	15 (N)	42 (N)	25 (N)	19 (N)	32 (N)
42	16 (N)	46 (N)	16 (N)	46 (1:80)	26 (N)	33 (N)	ND[c]
56	16 (N)	31 (N)	14 (N)	50 (1:80)	24 (N)	35 (N)	ND
70	13 (N)	24 (N)	12 (N)	54 (1:160)	23 (N)	36 (N)	ND
84	17 (N)	22 (N)	11 (N)	59 (1:160)	21 (N)	34 (N)	ND
98	16 (N)	14 (N)	10 (N)	67 (1:640)	23 (N)	25 (N)	ND
112	15 (N)	16 (N)	17 (N)	72 (1:640)	22 (N)	38 (N)	ND
127	17 (N)	18 (N)	15 (N)	73 (1:640)	24 (N)	33 (N)	ND

[a] dpi = Days post-inoculation.
[b] cELISA percent inhibition, with reciprocal SN titer in brackets. The cELISA is considered positive when the percent inhibition is >25. For SN testing, N = negative.
[c] Not done (the ewe was euthanized at 28 dpi).

particles with characteristic orbivirus morphology. Large aggregates of double shelled, spherical viral particles of about 80 nm in diameter were evident. The viral particles appeared to have a dense core about 60–70 nm in diameter, surrounded by a less dense outer shell. The infected cells also contained extensive networks of tubules. None of these features were apparent in the uninoculated control cell cultures. The isolate was not neutralized by specific polyclonal antibodies against BT virus types 2, 10, 11, 13, and 17 or EHD virus type 1, but was neutralized by antibodies against EHD virus type 2.

Duplicates of bison blood samples that were not used in the BT virus isolation protocol were used to try to isolate EHD virus in tissue culture using BHK-21 cells. Cultures with and without CPE after 7 d in the second passage were used in an IPA using a VP7 EHD virus-specific Mab and a NS2 BT virus group-specific Mab. The EHD virus could only be isolated from the blood sample of bison #30 at 28 dpi. No EHD virus was detected by IPA in any other blood samples after two passages in BHK-21 cells.

Serum neutralization of EHD virus was performed as described for BT virus, using 100 $TCID_{50}$ of EHD virus type 2 (Alberta). Neutralizing antibodies were only found in bison #30, which developed a rising antibody titer starting at 42 dpi and lasting until the end of the experiment at 127 dpi (Table 3). Serologic evidence of infection with EHD virus infection in four of the six inoculated bison and positive control ewe was demonstrated by the c-ELISA (Afshar et al., 1997) which employs two Mab directed against VP7 (Zhou and Afshar, 1999) (Table 3).

DISCUSSION

The lack of clinical signs of disease and absence of lesions in the experimentally infected bison are compatible with the lack of reports of clinical BT in bison in the United States and other countries where bison and the virus co-exist. The experimental study did show that bison can become infected with BT virus; the quantity of virus and initial transient rise in virus titer in blood indicated that the virus did replicate for a short time in bison as is the case in sheep and cattle (MacLachlan et al., 1992).

In this study, the duration of BT viremia

in bison was determined to be at least 28 dpi and not more than 41 dpi based on virus isolation. The pattern of declining virus titers in bison blood, and the lack of infectivity for sheep of bison blood collected from 28 dpi onward, supported this determination. The length of BT viremia in the bison was highly comparable to that in sheep (Katz et al., 1993) and comparable to, or shorter than, the duration of BT viremia in cattle which is reported to range from 7 to 63 days based on virus isolation (Singer et al., 2001). The variability in duration of bluetongue viremia reported for cattle can depend on the virus serotype, blood fraction examined, and virus detection system used. Melville et al. (1996) studied the duration of detectable BT viremia in a sentinel herd of cattle near Darwin, Australia, over a period of 15 yr and found the duration of viremia to be <3 wk for most cattle (54%), <4 wk in 79% of infections, 4 to 6 wk in 17% of infections, and more than >6 wk in 3% of infections. These results are comparable with what was observed in bison: 5/6 were viremic for <3 wk and 1/6 was viremic for <6 wk.

The maximum duration of viremia in other species of wild ruminants has been reported to be 17 days in blesbok (*Damaliscus dorcas*), 22 days in white-tailed deer (*Odocoileus virginianus*), 10 days in North American elk (*Cervus elaphus*), and 35 days in mountain gazelle (*Gazella gazella*) (Hourrigan and Klingsporn, 1975).

In this study bison, like other susceptible hosts, seroconverted within four wk of exposure to BT virus and maintained detectable antibody titers against BT virus long after they had cleared the virus. The durability of the BT antibody response has implications for the health status of BT-free nations, and individual animal identification is critical for differentiating seropositive imports from native animals that may have been exposed indigenously to BT virus.

The highest BT virus titre of $10^{5.61}$ $ELD_{50}ml^{-1}$ at 7 dpi in viremic bison was comparable to the peak BT virus titres of $10^{4.4}$ to $10^{6.2}$ $ELD_{50}ml^{-1}$ reported in cattle during the second and third wk of infection (Bowen et al., 1985), and the mean peak BT virus titer of 10^{6} $ELD_{50}ml^{-1}$ reported in sheep at 6 dpi (Foster et al., 1991). In general, the peak of BT viremia occurs in the first two wk of infection, before the appearance of serum antibody, and then the virus titer drops rapidly to a low level when infection persist for a month or more. Bison #94 did not fit this pattern as BT viremia was not detected until 28 dpi. We think this relates to the very low titer of virus in this animal (the lowest of all infected bison) rather than delayed transmission of BT virus by unknown means from another bison. Viremia was probably present in bison #94 earlier than 28 dpi, but at a titer that was below the threshold of detection for the virus isolation system. If the inoculation had been ineffective in bison #94 and BT virus was transmitted subsequently by unknown means from another bison, then bison #94 would have seroconverted after the others. However, all six bison became seropositive on SN test at the same time (28 dpi) indicating that they were infected at the same time.

Bluetongue virus is highly cell-associated during viremia, and there is strong evidence that this cellular association protects circulating virus from elimination by neutralizing antibodies during prolonged infection (Barratt-Boyes and MacLachlan, 1994). In bison serum, antibodies were detected as early as 11 dpi, but neutralizing antibodies appeared between 14 and 28 dpi. The appearance of neutralizing antibodies in the circulation coincided with the decline in detectable virus in blood. However, we expect that viral RNA could be detected by polymerase chain reaction for a longer period in bison, as reported in cattle and sheep (Katz et al., 1994).

The timing of the isolation of, and seroconversion to, EHD virus indicated that this virus was introduced in the BT virus inoculum and not from any other source. The available evidence indicates that the

amount of EHD virus in the inoculum was small because (1) it could not be isolated directly from the inoculum by either of two reference laboratories; (2) it could only be isolated from one of the six inoculated bison and only from one blood sample collected on one day (28 dpi); and (3) only this one bison developed neutralizing antibodies against EHD virus. The positive control ewe remained BT viremic until euthanized at 28 dpi, which is comparable to the duration of BT serotype 11 viremia (29–36 dpi) in sheep reported by Katz et al. (1993). The duration of BT viremia in the bison was comparable to the 25 days duration of viremia in cattle inoculated with BT virus serotype 11 (Parsonson et al., 1994).

Immunity to EHD virus type 2 did not protect white-tailed deer against subsequent challenge with BT virus serotype 10 (Quist et al., 1997), just as prior infection with BT virus did not protect sheep from subsequent challenge with heterotypic serotypes of BT virus (Jeggo et al., 1983). For all of the above reasons, we believe that the presence of a very small amount of EHD virus did not unduly influence the duration of BT viremia in the bison.

The presence of both BT and EHD viruses simulated natural circumstances in that both viruses occur together in enzootic areas and are transmitted by the same insect vectors. During the 1987 incursion of BT virus serotype 11 in the Okanagan Valley of British Columbia, Canada, EHD virus type 2 also occurred and was isolated from bison in that area (Dulac et al., 1988).

From the data collected in this experiment, we conclude that the duration of BT viremia in bison is at least 28 d and not more than 41 d based on virus isolation, which is comparable to, or shorter than, the reported duration of bluetongue viremia in cattle.

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