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Evidence of Herpesvirus Infection in Woodland Caribou in Saskatchewan

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ABSTRACT: Sera were collected from 40 female and two male woodland caribou (*Rangifer tarandus caribou*) in Saskatchewan (Canada) from March 1992 to January 1995, inclusive. The samples were examined for antibodies against smooth *Brucella* spp., five serovars of *Leptospira interrogans*, bovine viral diarrhea virus, and bovine herpesvirus 1 (BHV-1). Twenty-two (52%) of 42 sera exhibited positive reactions to BHV-1 by a modified serum neutralization test, and the prevalence correlated positively with the age of the animals. No antibodies were detected against the other pathogens. This is the first reported evidence of herpesvirus infection in isolated populations of woodland caribou in western Canada.

Key words: Bovine herpesvirus 1, bovine viral diarrhea, *Brucella* spp., cervid herpesvirus, *Leptospira interrogans*, *Rangifer tarandus caribou*, serologic survey, woodland caribou.

In 2000, the boreal population of woodland caribou (*Rangifer tarandus caribou*) in Canada was designated as threatened by the Committee on the Status of Endangered Wildlife in Canada, and this was recently confirmed in May 2002 (www.coseic.gc.ca). Several subpopulations, including those in Saskatchewan (Canada), have shown continued decline in numbers (Rettie et al., 1998). Sera were obtained from isolated populations of woodland caribou during the early 1990s as part of a major study of factors influencing these animals in Saskatchewan. The samples were tested to determine whether some common pathogens or antigenically-related agents of livestock and wild ungulates were present that might contribute to the decline in the number of these animals.

The caribou were tested for brucellosis because *Brucella suis* biovar 4 is enzootic

and pathogenic in barren ground caribou (*R. tarandus groenlandicus*) populations in Canada (Tessaro and Forbes, 1986). In addition, the caribou were tested for exposure to *Leptospira interrogans* because there is little information about the occurrence in wildlife and ecology of this potential pathogen within the ecosystem inhabited by woodland caribou. We were also particularly interested to determine whether these animals had antibodies against bovine herpesvirus 1 (BHV-1) and bovine viral diarrhea virus (BVDV) because a high prevalence of antibodies against these viruses has been reported in woodland caribou in Quebec (Canada) (El-Azhary et al., 1979, 1981) and because European reindeer (*R. tarandus tarandus*) are hosts of cervid herpesvirus 2 (Ek-Kommonen et al., 1986).

Sera were collected from 40 female and two male woodland caribou in Saskatchewan (approximately 54–56°N, 104–108°W), from March 1992 to January 1995, inclusive. Ages of 31 of the females and both males were determined by cementum annuli counts (Miller, 1974). Samples were collected during the winter, between December 13th and March 14th.

The buffered plate agglutination test (BPAT; Stemshorn et al., 1985) was used to test sera for antibodies against *Brucella* spp. (*B. suis*, *B. abortus*, and *B. melitensis*). This assay cannot differentiate between antibodies produced to these three species. The microagglutination test (MAT; Gale and Howard, 1997) was used to detect antibodies against *L. interrogans* ser-

TABLE 1. Prevalence, by age of animal and titer, of antibodies to bovine herpesvirus 1 (BHV-1) in 40 female woodland caribou from Saskatchewan.

Age (yr)	Negative	Number of caribou with BHV-1 antibody titer							Positive/total
		1:2	1:4	1:8	1:16	1:32	1:64	1:128	
<3	9	0	0	1	0	0	0	0	1/10 ^a (10%)
3–6	3	3	1	0	2	0	0	1	7/10 ^a (70%)
>6	1	0	0	5	3	2	0	0	10/11 ^a (91%)
Unknown	5	0	1	1	0	0	0	2	4/9 (44%)
Total	18	3	2	7	5	2	0	3	22/40 (55%)

^a Difference in prevalence among age groups is significant (Chi-square, $P < 0.001$).

ovars pomona, copenhageni, canicola, hardjo, and grippotyphosa.

Serum neutralization (SN) tests were used to detect antibodies to BVDV and BHV-1. For these tests, sera were heat inactivated at 56 C for 30 min prior to testing. For detection of antibodies to BVDV, serial two-fold dilutions of sera were incubated for 1 hr with 100 50% tissue culture infectious doses (TCID₅₀) of cytopathic Singer strain of BVDV (Agriculture and Agrifood Canada, Diagnostic Virology Laboratory, Hull, Quebec) prior to addition of Madin-Darby bovine kidney cells (MDBK; Dr. H. Bielefeldt-Ohman, Veterinary Infectious Diseases Organization, Saskatoon, Saskatchewan). Plates were incubated at 37 C in 5% CO₂ for 3 days and then examined microscopically. Antibodies to BHV-1 were detected by a modified SN test in which sera were incubated with the challenge virus (Colorado strain BHV-1, 100 TCID₅₀; Agriculture and Agrifood Canada, Diagnostic Virology Laboratory) for 24 hr prior to addition of trypsinized cells (Deregt et al., 1993; Van Oirschot, 1996). For the SN tests, antibody titer was determined to be the highest dilution of sera (final dilution after addition of virus) which completely inhibited viral cytopathic effect (Deregt et al., 1992). A titer of 1:2 was the threshold for positive/negative determinations.

Chi-square analysis (Microsoft Excel 97 SR-2 software, Microsoft Corporation, Redmond, Washington, USA). Following a significance test for differences in antibody prevalence among age groups, the data

were partitioned and tested for differences in the prevalence of antibodies between successively older age groups (Siegel and Castellan, 1988).

The woodland caribou ranged in age from 8 mo to 15 yr. No antibodies were detected against *Brucella* spp., the five serovars of *L. interrogans*, or BVDV. Antibodies against BHV-1, with titers ranging from 1:2 to 1:128, were found in 22 (55%) of 40 female caribou (Table 1), but both yearling males were negative. Prevalence of antibodies to BHV-1 differed significantly ($\chi^2 = 14.95$, $df = 2$, $P < 0.001$) with age in the 31 females where age data were available (Table 1). Prevalence of antibodies to BHV-1 was higher in animals older than 3 yr than in animals younger than 3 yr ($\chi^2 = 14.00$, $df = 1$, $P < 0.001$).

These data indicate that either BHV-1 or another herpesvirus with antigenic similarity to BHV-1 is present in the Saskatchewan woodland caribou population. The latter explanation seems more probable because these animals historically have been isolated from livestock and caribou with antibody to BHV-1 in this study came from several subpopulations that became relatively isolated from each other by forest fragmentation after the mid-1960s (Rettie and Messier, 1998). Cervid herpesvirus 1 of European red deer (*Cervus elaphus*) and cervid herpesvirus 2 of European reindeer (*Rangifer tarandus*) both demonstrate significant antigenic cross-reactivity with BHV-1 in neutralization tests and enzyme-linked immunosorbent assays (Nixon et al., 1988; Lyaku et al., 1992).

Thus, the possibility of there being a unique herpesvirus in woodland caribou cannot be precluded. It is also possible that woodland caribou in the study area have been exposed to a virus found in other ungulates in this area, which include elk (*Cervus elaphus*), moose (*Alces alces*), and white-tailed deer (*Odocoileus virginianus*).

Antibodies to BHV-1 have been reported in reindeer in Alaska (Dieterich, 1981) and woodland caribou in Quebec (El-Azhary et al., 1979, 1981). However, the identity of the herpesvirus(es) responsible for antibodies in these subspecies of *R. tarandus* has not been determined. Ek-Kommonen et al. (1982), using a 24 hr incubation test, reported that 69 (23%) of 300 domestic reindeer in Finland, but not cattle in the same area, had SN antibodies to BHV-1. Shortly thereafter, they isolated a reindeer-specific herpesvirus (cervid herpesvirus 2) from these animals (Ek-Kommonen et al., 1986). In Canada, antibodies to BHV-1 have been reported in other species of cervids including elk in southwestern Alberta (Kingscote et al., 1987) and white-tailed deer on Anticosti Island, Quebec (Lamontagne et al., 1989), but not in a sample of moose from Alberta (Thorsen and Henderson, 1971). Prior to this study, elk from Elk Island National Park in central Alberta were translocated to woodland caribou habitat in Saskatchewan (Arsenault, 1998) and there is serologic evidence of exposure to BHV-1 in elk from elsewhere in Alberta (Kingscote et al., 1987). A herpesvirus with similarities to equine herpesvirus 4 was isolated from captive fallow deer (*Dama dama*) in Alberta (Thorsen et al., 1977; Kinyili and Thorsen, 1979). Recently a unique herpesvirus showing antigenic relatedness to BHV-1 was isolated from an elk in Saskatchewan (Deregt et al., 2000). Hence, one or more species-specific herpesviruses, possibly related to cervid herpesvirus 2, may occur in woodland caribou. If so, one might expect that all vestigial subpopulations of woodland caribou in North America could be infected.

Whether the herpesvirus has any effect on the health of individual woodland caribou or their populations is not known. Clinical disease has not been reported in reindeer infected with cervid herpesvirus 2. Cervid herpesvirus 1 has caused outbreaks of ocular disease in farmed red deer (Inglis et al., 1983; Nettleton et al., 1986). In cattle, bovine herpesvirus 1 is associated with respiratory disease, genital disease, abortion, conjunctivitis and, rarely, systemic disease (Radostits et al., 2000).

The taxonomy and biology of herpesviruses in Cervidae may be more complex than previously considered. We speculate that a unique herpesvirus is enzootic in woodland caribou populations. This would be best determined by antigenic and/or genetic analysis of a virus isolated from a woodland caribou.

The age-related increase in the prevalence of antibodies to BHV-1 in the woodland caribou is likely due to increased opportunity for infection and recrudescence of latent infection in older animals. Maternal antibodies to herpesvirus may occur in woodland caribou calves but this could not be assessed because the animals were ≥ 8 mo of age. In domestic cattle, maternal antibody titers have generally decayed to undetectable levels by the modified IBR-SN test by that age (Cho et al., 2002).

None of the woodland caribou we sampled had antibodies to BVDV. In Quebec, woodland caribou in the George River herd had antibodies to BVDV (El-Azhary et al., 1979). Unpublished data suggest the occurrence of BVDV in Alaskan caribou and reindeer (University of Alaska, unpubl. data, cited in Dieterich, 1981). In other cervids, antibodies to BVDV have been reported in moose in southeastern Alberta (Thorsen and Henderson, 1971), elk in southwestern Alberta (Kingscote et al., 1987) and, in Britain, fallow deer, red deer, roe deer (*Capreolus capreolus*), and sika deer (*Cervus nippon*) (Lawman et al., 1978).

Absence of antibodies to *Brucella* spp. is interesting because *B. suis* biovar 4 is

enzootic in Canadian barren ground caribou populations (Tessaro and Forbes, 1986), including the Beverly herd which historically migrated into Saskatchewan during winter months. Zarnke and Yuill (1981) did not find *Brucella* antibodies in sera from 22 woodland caribou in the Fort McKay area of northern Alberta. Perhaps woodland caribou populations are free of the disease, which would be important in understanding the ecological history, biogeography, and epidemiology of *B. suis* biovar 4 in North America.

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