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Herpesvirus Infection in Woodland Caribou in Alberta, Canada

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ABSTRACT: Sera and genital swabs collected from 121 adult woodland caribou (*Rangifer tarandus caribou*) in five subpopulations in northern Alberta, Canada, between December 1997 and October 1999, were examined for evidence of infection with herpesviruses or pestiviruses. No virus was isolated from sera or swabs, and no antibodies against bovine viral diarrhea virus were detected. However, 63 (52%) of the 121 animals had neutralizing antibody titers against bovine herpesvirus 1. There was sufficient serum from 37 of the 121 caribou to allow parallel testing for antibodies against a new alphaherpesvirus isolated from an elk (*Cervus elaphus nelsoni*), and 20 animals had antibodies against this virus. Paired sera collected 11 mo apart from 14 caribou showed seroconversion in seven animals, indicating that an active herpesvirus infection was present. Virus neutralization data suggest that these caribou are infected with a distinct alphaherpesvirus.

Key words: Cervidae, herpesvirus, pestivirus, *Rangifer*, serology, woodland caribou.

The boreal population of woodland caribou (*Rangifer tarandus caribou*) in Canada was designated "threatened" in 2000 (COSEWIC, 2004). Several subpopulations have shown continued decline in numbers (Rettie and Messier, 1998; Dzus, 2001; McLoughlin et al., 2003). As part of a comprehensive ecologic study of these animals in Alberta, efforts were made to evaluate their health status. One objective was to look for the presence or absence of infection with herpesviruses and pestiviruses.

Sera and vaginal or preputial swabs were obtained from 121 adult woodland caribou from five subpopulations across northern Alberta, Canada, from December 1997 to February 1998, October 1998 to January 1999, and in October 1999. The geographic range of these subpopulations has been described (Dzus, 2001). The geographic name and approximate central coordinates of each subpopulation are: A La Peche (53°30′N, 118°50′W); Caribou Mountains (58°55′N, 115°40′W); Cold Lake Air Weapons Range (CLAWR) (55°05′N, 110°45′W); Redrock (53°50′N, 119°45′W); and West Side of Athabasca River (WSAR) (56°00′N, 113°10′W).

Sera were tested in serum neutralization (SN) tests to determine whether the caribou had antibodies against bovine herpesvirus 1 (BHV-1) and bovine viral diarrhea virus (BVDV). A 24 hr incubation SN test without complement was used to test for antibodies against BHV-1 (Colorado strain) and a standard SN test was used to test for antibodies against BVDV (Singer strain), as described previously (Deregt et al., 1992, 1993). Antibody titers were expressed as final dilutions. Sera were also tested for the presence of BVDV by an immunoperoxidase monolayer (microisolation) assay (Deregt and Prins, 1998). For herpesvirus isolation, swabs were placed into tubes containing 2 ml of medium, vortexed, and stored at −80 C. Two hundred microliters of the supernatants were inoculated onto Madin–Darby bovine kidney cells in 24-well plates. After 1 hr, the inoculum was removed and cells were incubated in fresh medium for 7 days at 37 C. A second passage was performed and cells were again observed for cytopathic effect.

No virus was isolated from sera or genital swabs from any caribou. All sera were negative for antibody against BVDV, unlike woodland caribou in Quebec where 69% of the animals had antibodies (Elazhary et al., 1979). However, a high proportion (52%) of the Alberta woodland caribou had antibodies against BHV-1 (Table 1), similar to their counterparts in

TABLE 1. Prevalence, by regional subpopulation and titer, of antibodies against bovine herpesvirus 1 (BHV-1) in 121 woodland caribou from northern Alberta.

Region	Number of caribou with BHV-1 antibody titer								
	Negative	2	4	8	16	32	64	128	Positive/Total
A La Peche	5	1	1	0	0	1	0	0	3/8
Caribou Mountains	3	1	2	1	1	2	2	0	9/12
CLAWR[a]	21	1	3	4	6	6	4	0	24/45
Redrock	11	0	0	1	4	1	2	1	9/20
WSAR[b]	18	3	1	2	5	6	1	0	18/36
Total	58	6	7	8	16	16	9	1	63/121 (52%)

[a] Cold Lake Air Weapons Range.
[b] West Side of Athabasca River. For 14 animals in this subpopulation, two serum samples, obtained 11 months apart, were tested. The titers recorded in the table are the highest titers obtained for individual animals.

Quebec and Saskatchewan (ElAzhary et al., 1979; Jordan et al., 2003). Antibody titers against BHV-1 were as high as 128 and were observed in caribou from each subpopulation in Northern Alberta. We obtained paired serum samples, collected 11 mo apart, from 14 caribou in the WSAR subpopulation. The paired antibody titers against BHV-1 indicated an active herpesvirus infection in the subpopulation, as seroconversion (negative to positive antibody titer) occurred in seven of the 14 animals.

At the time of this study, we isolated and characterized an alphaherpesvirus from elk (*Cervus elaphus nelsoni*) which was discovered to be serologically related to BHV-1 (Deregt et al., 2000). Sufficient sera from 37 caribou (a subset of the 121 animals) were available to test for the presence of antibodies against the elk herpesvirus (ElkHV) in parallel with BHV-1 antibody testing. The same SN test procedure was performed except that ElkHV was used as the challenge virus. All animals that were negative for antibodies to BHV-1 ($n=17$) were also negative to ElkHV. However, antibody titers in positive caribou ($n=20$) were typically higher against ElkHV than against BHV-1: two animals had equal titers, 12 had titers that were twofold higher, and six had titers that were fourfold higher (Table 2).

Previously, we found that bovine antisera against BHV-1 efficiently neutralize ElkHV in cross-neutralization SN tests with titers within twofold of those achieved against BHV-1, whereas bovine antisera against ElkHV neutralize BHV-1 poorly with 16-fold differences in titer (Deregt et al., unpubl. observations, 2000). However, we did not observe this pattern of neutralizing activity against these viruses with any of these caribou sera. This observation, together with the fact that Caribou Mountains, CLAWR, and WSAR subpopulations of woodland caribou continue to be segregated geographically or ecologically from cattle and elk, suggests that the infection in caribou is neither that of BHV-1 nor ElkHV. Instead, it is likely that Canadian woodland caribou are infected with a different alphaherpesvirus. However, the A La Peche and Redrock subpopulations

TABLE 2. Prevalence, by titer, of antibodies against bovine herpesvirus 1 (BHV-1) or elk herpesvirus (ElkHV) in 37 woodland caribou from northern Alberta.

Virus	Number of caribou with BHV-1 or ElkHV antibody titer									
	Negative	2	4	8	16	32	64	128	256	Positive/Total
BHV-1	17	2	1	2	6	5	3	1	0	20/37
ElkHV	17	0	3	0	3	3	5	3	3	20/37

of woodland caribou do share their range with elk. This may lead to cross-species transmission of cervid herpesviruses, although we do not yet see any serologic evidence of this from our data.

The likelihood that Canadian woodland caribou are infected with another herpesvirus is supported by the fact that European reindeer (*Rangifer tarandus tarandus*) are hosts to cervid herpesvirus-2, which causes an inapparent genital infection (Ek-Kommonen et al., 1986; Nettleton et al., 1988). As with ElkHV, this virus demonstrates significant antigenic cross-reactivity with BHV-1 (Ek-Kommonen et al., 1986).

Whether Canadian woodland caribou are infected with an alphaherpesvirus that is the same as, or similar to, the one that infects European reindeer awaits isolation of the virus from woodland caribou and its complete characterization. Failure to isolate the virus in this study may have been because of the time of the year that samples were collected. Virus shedding and transmission may occur during early autumn breeding or spring calving if this is a genital herpesvirus infection. Thus, additional effort to obtain the virus in samples collected during these seasons is merited.

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