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SEROLOGICAL EVIDENCE OF BOVINE HERPESVIRUS 1-RELATED VIRUS INFECTION IN THE WHITE-TAILED DEER POPULATION ON ANTICOSTI ISLAND, QUEBEC

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ABSTRACT: High white-tailed deer (*Odocoileus virginianus*) population densities and the occurrence of harsh environmental conditions are present on Anticosti Island, located in the Gulf of Saint-Lawrence (Quebec, Canada). This island is the northernmost region of white-tailed deer distribution in northeastern North America. The aim of this work was to determine whether a herpesvirus serologically related to bovine herpesvirus 1 (BHV1) may occur in a stressed white-tailed deer population. One hundred one deer sera were collected from apparently healthy animals during the hunting season from September to late November 1985. Fifty-three percent of tested deer were positive to the seroneutralization test using Colorado strain of BHV1 virus. Higher percentages of seropositivity were observed in animals of both sexes >4-yr-old. Analysis of antibody titers in seropositive animals according to age suggests that BHV1-related viral infection is endemic in the Anticosti Island deer population. It is postulated that environmental stress may induce immunosuppression of certain infected and/or carrier animals in their population that shed virus for long periods of time.

Key words: White-tailed deer, *Odocoileus virginianus*, bovine herpesvirus 1, serological survey, environmental stress, immunosuppression, field study.

INTRODUCTION

Various diseases and reproductive problems may be encountered in wild deer populations under stressed conditions, such as high population densities or the occurrence of harsh environmental conditions (Halls, 1984). Anticosti Island, located in the Gulf of Saint-Lawrence (Quebec, Canada), is the northernmost extent of white-tailed deer (*Odocoileus virginianus*) distribution in northeastern North America (49°25′W, 62°45′N). Approximately 200 deer were introduced in 1896; presently, the spring population of this 7,900-km^2 island varies between 80,000 and 100,000 deer depending upon the severity of the winter. This island is exempt from any predation except a 3 mo controlled hunt in the fall. The Anticosti Island deer population is known to have had no direct or indirect contact with domestic ruminants for at least 50 yr. The structure of the boreal vegetation (Pimlot, 1963) on the island, once dominated by balsam fir (*Abies balsamea*), changed due to the intensity of browsing by the deer. Now, most brush species have disappeared from the island as well as young balsam fir because the saplings have been completely eaten. Abundant forbs and herbs predominate in the summer diet of the deer. During the long winter, deer live on lichens from fallen trees or on their own fat reserves because snow covers other forage for 5 to 6 mo. Anticosti Island deer weigh about 20% less than continental deer, and the reproductive rate is assumed to be lower (Goudreault, 1980).

Bovine herpesvirus 1 (BHV1) infection, known as bovine rhinotracheitis, is ubiquitous in cattle and causes diverse clinical manifestations. It is primarly involved in respiratory disease characterized by tracheitis, rhinitis and fever but it also is associated with reproductive problems including abortions and weak calves (Karhs, 1977). Evidence of herpesvirus infections in wild ruminants from North America have been demonstrated by serologic surveys (Friend and Halterman, 1967; Barrett and Chalmers, 1975; Elazhary, 1979) or by virus isolation (Thorsen et al., 1977). Serological evidence of exposure of white-tailed or mule deer (*Odocoileus hemion-*

us) to a virus related to BHV1 has been reported in a small number of animals (Karstad, 1970; Elazhary, 1979). The BHV1 virus induced a benign respiratory disease in experimentally infected mule deer (Chow and Davis, 1964). Recently, a virus serologically related to BHV1 was isolated from a healthy reindeer (*Rangifer tarandus*) following an immunosuppressive treatment (El-Kommonen et al., 1986). This procedure was succesfully used to isolate a herpesvirus unrelated to BHV1 from fallow deer (*Cervus dama*) (Thorsen et al., 1977).

This study is part of a long term investigation on deer adaptation to a harsh environment. Its purpose was to determine whether a herpesvirus infection serologically related to BHV1 may occur in this white-tailed deer population under naturally stressed conditions.

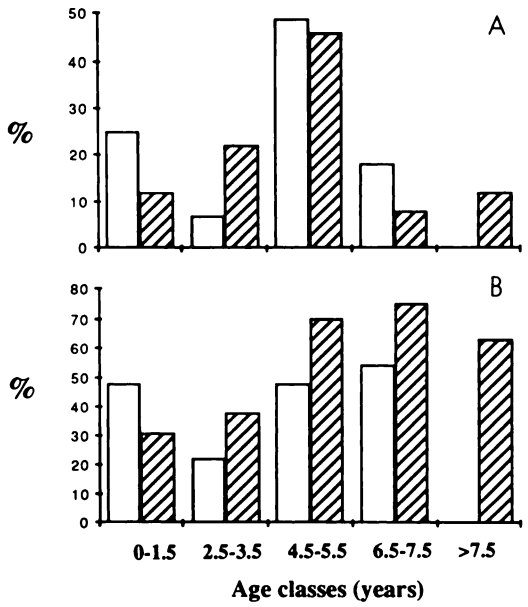

FIGURE 1. Age classes and sexes (male □, female ▨) in the total deer population (A) and percentages of BHV1 seropositive deer (B) from Anticosti Island.

MATERIALS AND METHODS

Serum samples

One hundred one deer sera were collected from apparently healthy deer from the eastern population on Anticosti Island during the 1985 hunting season (September to late November). Animals were 0.5- to 10.5-yr-old. Ages were determined by size for fawns (<6 mo), by premolar eruption for the 1.5-yr-old group, and by the dental annuli technique for the older group (Lockard, 1972). Blood samples were centrifuged at 2,000 *g* for 15 min, the sera removed, stored at −20 C, and shipped in frozen containers to the laboratory.

Virus

Bovine herpesvirus 1 (Colorado strain), was obtained from American Type Culture Collection (ATCC, Bethesda, Maryland 20852, USA) adapted to grow in embryonic bovine turbinate (EBTr) cells also obtained from ATCC. Cells were cultured in minimum essential media (MEM) with 10% gamma globulin-free fetal calf sera (Flow Laboratories, Missisauga, Ontario, Canada L4T 1A3) and antibiotics. Cells were infected with the virus at a multiplicity of infection (ratio virus/cell) of 0.01. Infected cells were frozen-thawed three times when cytopathic effect (CPE) reached 75%, centrifuged at 2,000 *g* for 30 min and the supernatant was used as a virus source for seroneutralization tests.

Seroneutralization test

Antibody titers to BHV1 were determined by seroneutralization test in microtiter plates (Carbrey et al., 1971). The samples were tested in triplicate using two-fold serum dilutions prepared in MEM. An equal volume of diluted virus supernatant containing 100 tissue culture infecting doses ($TCID_{50}$) was mixed with the serum dilutions and incubated for 1 hr at 37 C. These were then added to cultured EBTr cells. Cells were incubated for 4 days at 37 C in a 5% CO_2 humidified atmosphere until occurrence of typical CPE. Antibody titers were expressed as the reciprocal of the highest serum dilution showing neutralization of CPE activity. Titers <1:8 were recorded as negative. Antibody titer data according to age classes or sex were compared using chi-square tests.

RESULTS

Fifty-three deer sera of 101 tested (53%) were positive for antibodies against BHV1 virus. The prevalence of seropositive deer was similar in males (48%) and females (58%). Seronegative animals remained negative even when sera were tested at a lower dilution (1:4).

To determine if the viral infection occurred in epidemic or endemic forms in

TABLE 1. Distribution of antibody titers to bovine herpesvirus 1 (BHV1) across different classes of white-tailed deer from Anticosti Island.

Age (yr)	Number of deer showing antibody titers (%)					Total
	<8	8	16	32	64	
0–1.5	12 (57)	2 (10)	3 (14)	3 (14)	1 (5)	21
2–3.5	10 (67)	0	2 (13)	2 (13)	1 (7)	15
4–5.5	19 (43)	7 (16)	9 (20)	5 (12)	4 (9)	44
6–7.5	5 (33)	6 (40)	2 (13)	1 (7)	1 (7)	15
>7.5	2 (33)	1 (17)	2 (33)	0	1 (17)	6
Total	48 (48)	16 (16)	18 (18)	11 (11)	8 (8)	101

the deer population, percentages of seropositive animals were compared according to age. Median age was between 4- and 5.5-yr-old in males and females (Fig. 1A). Serum samples were not collected from males >7.5-yr-old because of the extremely low survival rate of older males in this deer population on Anticosti Island. Higher percentages of seropositive animals were observed in older animals (>4-yr-old) of both sexes (Fig. 1B) ($P < 0.05$). The levels of antibody titers observed in seropositive animals were analyzed according to age class (Table 1). Similar levels of antibody titers (>1:16) were observed in all age classes. In addition, the percentage of seropositive fawns (<6-mo-old) was similar to that observed in the age class of >0.5- to 1.5-yr-old.

DISCUSSION

We found the highest prevalence of BHV1 antibodies reported in a wild ungulate population (Chow and Davis, 1964; Friend and Halterman, 1967; El-Kommonen et al., 1982). The antibody prevalence and age distribution of seropositive animals suggests that BHV1 infection is endemic in the white-tailed deer population on Anticosti Island. The high prevalence of serologic reactors in older animals may be a function of the greater opportunity for exposure to the virus.

The high prevalence of antibodies to BHV1-related virus in the Anticosti Island deer population also may suggest a low virulence BHV1-related viral strain inducing a subclinical disease or transient clinical signs in deer without high rates of morbidity or mortality. Under controlled conditions, BHV1 infection in mule deer induced mild clinical signs such as transient anorexia, depression, excessive salivation, increased respiratory rate, dyspnea and occasionnal cough (Chow and Davis, 1964). Virus was shed from the nasal secretions for 4 to 5 days after exposure by intratracheal inoculation. Virus was isolated from rectal swabs and from ocular exudates at 4 wk postinoculation in two deer. Furthermore, all experimentally infected deer recovered and showed immune protection when challenged 5 wk postinfection. It appears that BHV1 may be less virulent for cervids than for domestic bovids.

The above two cases suggest that deer can carry the virus for a long period of time and could be potential spreaders of the disease. Shedding of herpesviruses by cervids may be induced experimentally following immunosuppressive treatment with dexamethasone and the viruses were isolated from specimens collected from vagina, nasal secretions and prepuce from fallow deer and reindeer (Thorsen et al., 1977; El-Kommonen et al., 1986). The occurrence of an endemic infection in white-tailed deer on Anticosti Island suggests that deer are the reservoir for BHV1 because there are no domestic ruminants on the island. It is postulated that the shedding of the virus may occur for a prolonged period of time in infected deer on Anticosti

Island since stress due to environmental conditions could induce an immunosuppressive state and thereby facilitate the shedding of the virus.

Studies are in progress to isolate the BHV1-related viral agent involved in the induction of the serologic responses in the white-tailed deer population on Anticosti Island and to develop a surveillance program for the viral infection in the herd.

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