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SEROLOGIC SURVEY OF WHITE-TAILED DEER ON ANTICOSTI ISLAND, QUEBEC FOR BOVINE HERPESVIRUS 1, BOVINE VIRAL DIARRHEA, AND PARAINFLUENZA 3

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ABSTRACT: In 1985 unusual mortality was observed among the 3- to 4-yr-old white-tailed deer (*Odocoileus virginianus*) on Anticosti Island, Québec (Canada). A viral pathogen was suspected to be the cause of the deaths. Thus, a serologic survey for bovine herpesvirus 1 (BHV-1), bovine viral diarrhea (BVD) virus and parainfluenza-3 (PI-3) virus was conducted. We examined 396 deer sera from 1985. Results indicated that the high mortality mainly afflicted 3- to 4-yr-old deer. In 1985, 57% of deer sampled were seropositive for viral neutralizing antibodies against BHV-1. Prevalences decreased over the next 2 yr of the survey. Prevalence of antibodies against PI-3 virus, determined by hemagglutination inhibition test, remained high (82% to 84%) for the 3 yr period. No deer were seropositive for neutralizing antibodies against BVD virus during the survey period. Analysis of antibodies against BHV-1 and PI-3 viruses according to sex, age and antibody titers revealed that an epizootic BHV-1 infection occurred in 1985; PI-3 infection appears to be enzootic in Anticosti deer.

Key words: White-tailed deer, *Odocoileus virginianus*, prevalence, bovine herpesvirus 1, bovine viral diarrhea virus, parainfluenza 3 virus, serosurvey

INTRODUCTION

Diseases, high mortality and decreased productivity may be pervasive among white-tailed deer (*Odocoileus virginianus*) when stressed by high population density or the occurrence of harsh environmental conditions (Halls, 1984). Anticosti Island, located in the Gulf of Saint-Laurent (Québec, Canada: 62°45′W; 49°25′N), is the northernmost range extension of white-tailed deer in northeastern North America. Approximately 200 deer were introduced on the island in 1896 (Newson, 1937). Presently, the spring population on this 7,900-km² island varies between 80,000 and 100,000 depending upon the severity of winter. Wolves (*Canis lupus*), coyotes (*Canis latrans*) or black bears (*Ursus americanus*) are not present on Anticosti Island (Huot et al., 1984). Predator species occurring on the island, including mustelids, red foxes (*Vulpes vulpes*), great horned owls (*Bubo virginianus*), bald eagles (*Haliaetus eucocephalus*) and ospreys (*Pandion haliaetus*), are not known to have any effect on deer population. A 3-mo controlled hunt occurs in the fall. The Anticosti Island deer population has had no direct or indirect contact with domestic ruminants for at least 50 yr.

We previously observed a high prevalence of antibodies against bovine herpesvirus 1 (BHV-1) in this deer population during 1985 (Lamontagne et al., 1989). Bovine herpesvirus 1 infection in cattle may cause a respiratory disease and abortion (Rosner, 1968). Little is known of BHV-1 infection in deer but, under controlled conditions, viral infection in mule deer (*Odocoileus hemionus*) induced mild respiratory clinical signs (Chow and Davis, 1964). Bovine herpesvirus 1 infection in reindeer (*Rangifer tarandus*) and fallow deer (*Cervus dama*) remained asymptomatic and shedding of herpesviruses was experimentally induced following immunosuppressive treatments (Thorsen et al., 1977; Ek-Kommonen et al., 1986). Evidence of herpesvirus infections in wild ruminants from North America has been demonstrated by serologic surveys (Friend and Halterman, 1967; Barrett and Chalmers, 1975; Elazhary et al., 1981) or by virus isolation (Thorsen et al., 1977; Reid et al., 1986). Two herpesviruses, which are antigenically related to BHV-1, also have

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been isolated from wild ruminants: herpesvirus of Cervidae 1 (HCV-1) (Reid et al., 1986), and a reindeer herpesvirus, isolated in Scandinavia (Ek-Kommonen et al., 1982).

Bovine viral diarrhea (BVD) virus induces a highly contagious disease in cattle. The most frequent form of BVD virus infection in cattle is subclinical or mild disease characterized by transient fever and inappetence, with or without mild diarrhea (Mills et al., 1965; Malmquist, 1968). Although naturally occurring clinical disease has been occasionally reported in wild ruminants (Richards et al., 1956; Karstad, 1981), serum neutralizing antibodies have been demonstrated in various deer species in Canada and USA (Richards et al., 1956; Kahrs et al., 1963; Friend and Halterman, 1967; Thorsen and Henderson, 1971; Stauber et al., 1977). Cattle may also be persistently infected expressing no neutralizing antibodies against BVD virus, but may appear healthy and have no gross lesions associated with clinical BVD viral infection (Coria and McClurkin, 1978).

Parainfluenza-3 (PI-3) infection may induce extensive pneumonia in domestic cattle in association with *Pasteurella haemolytica* (Lopez et al., 1976; Jericho et al., 1982). Serologic surveys of several species of wild ruminants have provided evidence of PI-3 infection (Kahrs et al., 1963; Stauber et al., 1977; Riemann et al., 1979; Ingebrigtsen et al., 1986) although the virus has only been isolated from nasal swabs in corticosteriod-treated deer (Thorsen et al., 1977). PI-3 virus has also been isolated from such other ruminant species as bigshorn (*Ovis canadensis cremnobates*) (Turner and Payson, 1982) or bison (*Bison bison*) (Zarnke and Erickson, 1990).

The purpose of this serologic survey was to determine the prevalence of antibodies against BHV-1, BVD, and PI-3 viruses during a 3 yr period from 1985 to 1987, among the white-tailed deer population in Anticosti Island.

MATERIALS AND METHODS

Male or female deer were collected during the 1985, 1986, and 1987 hunting seasons held in September to late November on the eastern part of Anticosti Island. Young deer, including fawns (less than 12-mo-old) and yearlings (12- to 23-mo-old) were aged in the field using the tooth replacement and wear technique (Severinghaus, 1949). Older deer (24-mo-old and more) were aged by the cementum annuli method (Gilbert, 1966; Lockard, 1972; Rice, 1980).

One hundred-eight, 185 and 103 blood samples were collected from apparently healthy hunter-killed deer during 1985, 1986, and 1987, respectively. Some deer sera, collected in 1985, have been previously studied for antibodies against BHV-1 (Lamontagne et al., 1989). The 244 male and 172 female deer were 0.5-yr-old to 10.5-yr-old. Blood samples were centrifuged at 2,000 *g* for 15 min, the sera removed, stored at −20 C, and shipped frozen to the laboratory.

Female age-specific mortality rates (q_x) were calculated for 271 does, including those selected for serologic studies, for the year 1985–86 using the formula given by Caughley (1977):

$$q_x = \frac{(f_{x,t} - f_{x+1,t+1})}{(f_{x,t})}$$

The number of deer aged x in 1985 was compared with the number of these that subsequently died before attaining age x + 1 in 1986. The number of dead divided by the number alive at the beginning of the interval provided the estimate of q_x. Frequency of the fawn age class (f_0) was estimated from the fecundity rates (m_x) in using the formula: $f_0 = \sum_{x=1}^{x=i} f_x m_x$. Frequencies of 9-yr-old deer in 1985 and 10-yr-old deer in 1986 were grouped because of low sample size. Frequencies in the 1986 sample were adjusted to account for sampling variations since the rate of increase was approximately equal to zero during the study and the proportion of does harvested from the sample population did not differ between 1985 and 1986 (8% and 6%, respectively) (St-Georges, 1989). When the calculated q_x for those age classes was negative, the mortality rate was arbitrarily fixed at 0.10. Male mortality rates were not calculated since the age structure, constructed from a hunter harvest sample, could be biased due to selection of bucks with trophy-sized antlers (Coe et al., 1980).

Bovine herpesvirus 1 (Colorado strain), BVD (NADL strain), and PI-3 (SF-4 strain) viruses were obtained from the American Type Culture Collection (ATCC, Rockville, Maryland 20852, USA). The viruses were adapted to grow in embryonic bovine turbinate (EBTr) cells which were also obtained from the ATCC. Cells were

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cultured in minimum essential media (MEM) with 10% gamma globulin-free fetal calf sera (GIBCO, Grand Island, New York 14072, USA) and antibiotics. Cells were infected with the viruses at a multiplicity of infection (ratio virus/cell) of 0.01. Infected cells were frozen-thawed 3 times when cytopathic effect (CPE) of the cell monolayer reached 75%. This suspension was centrifuged at 2,000 *g* for 30 min. The supernatant was used as a virus source for serologic tests.

Serum antibody titers against BHV-1 or BVD virus were determined by a neutralization test in microtiter plates (Carbrey et al., 1971). Samples were tested in triplicate using two fold serum dilutions prepared in MEM. Equal volumes of serum dilutions and virus suspension, containing 100 tissue culture infecting doses ($TCID_{50}$), were incubated for 1 hr at 37 C, and then added to cultured EBTr cells. Cells were incubated for 4 (BHV-1) or 7 (BVD virus) days at 37 C in a 5% CO_2 humidified atmosphere until occurrence of typical CPE. Antibody titers against PI-3 virus were determined by the hemagglutination inhibition microtiter method (Carbrey et al., 1971). Bovine erythrocytes, at a concentration of 1%, were used with four hemagglutinating units of virus. Antibody titers were expressed as the reciprocal of the highest dilution showing neutralization of CPE or hemagglutinating activity. Titers <8 were recorded as "negative" and considered as providing no evidence of exposure to the viral agent. All higher titers were referred to as "positive." Prevalences according to year of collection, age class or sex were compared using chi-square tests.

RESULTS

During 1985–86, mortality of female white-tailed deer was highest among fawns, 3- to 4-yr-old does, and oldest does >9-yr-old (Table 1). The observed biphasic mortality pattern was caused by unusually high mortality in 3- to 4-yr-old cohort, peaking at about 40%. Unfortunately, fresh carcasses were not found and examined to determine the cause of mortality. Viral disease was suspected since other factors such as winter weather condition, hunting, poaching, and road accidents could not account for the increased deer mortality (St-Georges, 1989).

Three hundred ninety-six deer sera were examined for antibodies against BHV-1, BVD and PI-3 viruses (Table 2). Antibody prevalence for BHV-1 was 57% in 1985,

TABLE 1. Mortality rates of female white-tailed deer on Anticosti Island in 1985–86.

Age (x) (years)	Frequency (f_x) 1985	Frequency (f_x) 1986	Mortality rate (q_x)
<1	(55)[a]		0.53
1	9	23 (26)[b]	0.10[c]
2	12	12 (14)	0.10[c]
3	30	15 (17)	0.43
4	37	15 (17)	0.38
5	17	20 (23)	0.06
6	8	14 (16)	0.10[c]
7	8	9 (10)	0.25
8	9	5 (6)	0.00
9+	15	8 (9)	0.60
10+		5 (6)	
Total	145	126 (145)	

[a] Frequency of fawns estimated from age-specific fecundity rates (m_x) and frequency of age class.
[b] Adjusted frequencies used in the calculation.
[c] Arbitrary low mortality rate to account for sampling variations leading to a negative calculated mortality rate.

but declined significantly over the next 2 yr ($P < 0.005$). No specific difference in prevalence was found between males and females in 1985 or 1986. In 1987, prevalence in females (0/44, 0%) was significantly lower than in males (7/59, 12%) ($P < 0.025$). Unexpectedly, all sera were negative for BVD antibodies. High prevalence of anti-PI-3 antibodies, however, was observed in both males and females during the survey.

Availability of data for three consecutive years permitted us to follow the evolution of viral exposure in the Anticosti deer population. Percentages of deer with antibodies against BHV-1 varied during the 3 yr according to age and sex (Table 3). In 1986, prevalence in young females (0.5- to 1.5-yr-old) was significantly higher than in the same age male cohort ($P < 0.005$), but lower in 2.5- to 3.5-yr-old cohort ($P < 0.025$). Numbers of positive animals were similar in older male and female deer in 1985 to 1987. Parainfluenza-3 serologic prevalence was significantly higher in the 2.5- to 3.5-yr-old female cohort (16/17, 94%) during 1985 as compared to the same age male cohort (nine of 13, 69%) ($P < 0.01$). Prevalences for PI-3 virus were sim-

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TABLE 2. Prevalence of antibodies against bovine herpesvirus 1, bovine viral diarrhea and parainfluenza-3 viruses in white-tailed male and female deer (*Odocoileus virginianus*) on Anticosti Island, 1985 to 1987.

Viruses*	Years	Number positive deer/total number tested (%)		
		Males	Females	Total
Bovine herpes virus 1	1985	34/60 (57)	28/48 (58)	62/108 (57)
	1986	19/125 (15)	11/60 (18)	30/185 (16)*
	1987	7/59 (12)	0/44 (0)**	7/103 (7)*
Bovine viral diarrhea virus	1985	0/60 (0)	0/48 (0)	0/108 (0)
	1986	0/125 (0)	0/60 (0)	0/185 (0)
	1987	0/59 (0)	0/44 (0)	0/103 (0)
Parainfluenza-3 virus	1985	47/60 (78)	42/48 (88)	89/108 (82)
	1986	104/125 (83)	51/60 (85)	155/185 (84)
	187	48/59 (81)	38/44 (87)	86/103 (84)

* Serum antibody titers against bovine herpesvirus 1 or bovine viral diarrhea virus were determined by neutralization tests, and for parainfluenza 3, by hemagglutination inhibition method.

* $P < 0.005$: compared to prevalence in 1985.

** $P < 0.025$: compared to the same male age cohort.

ilar for male and female deer in other age cohorts during the 3 yr period under study (results not shown). Distribution of antibody titers was studied according to time, sex and age cohorts. Generally, lower antibody titers against BHV-1 (8 and 16) were observed in 1985 in fawns and in >3.5-yr-old deer whereas higher antibody titers (32 and 64) were found in 1.5- to 4.5-yr-old male or >3.5-yr-old females (Fig. 1A, D). Antibody titers against BHV-1 generally decreased during the next 2 yr (Fig. 1B and E, and 1C and F). Only young males were BHV-1 negative in 1986, but some young deer expressed low antibody titers (8 to 32) in 1987 (Fig. 1B, C). Bovine herpesvirus 1 antibodies were not detected in female deer in 1987 (Fig. 1E, F). Low (8 to 64) or high (128 to 512) antibody titers against PI-3 virus were seen in both males (Fig. 2A) and females (Fig. 2D) in 1985. Antibody titers decreased without regard to age or sex in 1986 (Fig. 2B, E). During 1987, however, antibody titers increased in most males (Fig. 2C) and females (Fig. 2F) in all age cohorts.

DISCUSSION

The analysis of mortality pattern in the white-tailed deer population on Anticosti Island in 1985–86 revealed a peculiar W-shaped pattern instead of the classic U-shaped described by Caughley (1966). St-Georges (1989) measured a marked increase in pregnancy rates among 3- to 4-yr-old does suggesting that higher frequency of contact between individuals occurring in the female cohort during estrus favors transmission of pathogen agents.

The presence of antibodies against BHV-1 and PI-3 viruses indicated that some deer had been exposed to viral agents which are related or identical to the livestock pathogens. We have previously reported a high prevalence of BHV-1 antibodies among the deer from Anticosti Island, in 1985 (Lamontagne et al., 1989). Such high prevalence rate has been reported only among mule deer (*Odocoileus hemionus*) (Stauber et al., 1977). Prevalences of BHV-1 antibodies, however, decreased in 1986 and 1987 but remained higher than those reported in other deer populations (Chow and Davis, 1964; Friend and Halterman, 1967; Thorsen and Henderson, 1971; Ingebrigsten et al., 1986).

Analysis of deer with antibodies against BHV-1 according to age, sex and antibody titer revealed that 1.5- to 4.5-yr-old male deer were infected during the year 1985, whereas fawns (<6-mo-old), expressed low antibody titers probably resulting from residual maternal antibodies derived from the colostrum. Maternal antibodies rather

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TABLE 3. Sex-specific and age-specific prevalence of antibodies against bovine herpesvirus 1, in white-tailed deer (*Odocoileus virginianus*) on Anticosti Island, 1985 to 1987.

Years	Age	Number positive deer*/total number tested (%)		
		Male	Female	Total
1985	0.5–1.5	11/16 (69)	3/8 (38)	14/24 (58)
	2.5–3.5	6/13 (46)	10/17 (59)	16/30 (53)
	4.5–5.5	12/22 (55)	9/13 (66)	21/35 (60)
	>6.5	5/9 (56)	6/10 (60)	11/19 (58)
1986	0.5–1.5	0/33 (0)	4/14 (29)*	5/47 (11)
	2.5–3.5	9/40 (23)	0/17 (0)**	9/57 (16)
	4.5–5.5	5/34 (15)	4/14 (29)	9/48 (19)
	>6.5	4/18 (22)	3/15 (20)	7/33 (21)
1987	0.5–1.5	3/16 (19)	0/15 (0)	3/31 (10)
	2.5–3.5	3/28 (11)	0/13 (0)	3/41 (7)
	4.5–5.5	0/4 (0)	0/6 (0)	0/10 (0)
	>6.5	1/11 (9)	0/10 (0)	1/21 (5)

* Serum antibody titers against bovine herpesvirus 1 were determined by neutralization test.
* $P < 0.005$, ** $P < 0.025$: compared to the same age male cohort.

than a naturally acquired infection were the source of immunity in the suckling fawns. In addition, passive immunity provided to the fawns assures a sizeable cohort of susceptible 1-yr-old deer during spring time for potential virus amplification and dissemination (Gramstad et al., 1987). Higher viral titers for BHV-1 virus were found in 3.5- to 4.5-yr-old male or female deer. Higher mortality rate was demonstrated in this age cohort by analysis of mortality pattern (Table 1). Bovine herpesvirus 1 infection occurred during the following years only in some fawns, but not in adult deer as demonstrated by no or low antibody titers. The results suggest the occurrence of an epizootic BHV-1 infection on Anticosti Island, in 1985.

The relationship between mortality and high prevalence of antibodies against BHV-1 in 3.5- to 4.5-yr-old cohort was not expected. Clinical signs associated with BHV-1 in white-tailed deer are not well-known, but Chow and Davis (1964) and Reid et al. (1986) have demonstrated under controlled conditions that BHV-1 induced only mild clinical signs in deer, characterized by a transient anorexia, depression, excessive salivation, increased respiratory rate, dyspnea and occasional cough or asymptomatic infection. Some BHV-1 have been isolated from apparently healthy reindeer (*Rangifer tarandus*) (Ek-Kommonen et al., 1982) or red deer (*Cervus elaphus*) (Inglis et al., 1983; Reid et al., 1986) or in corticosteroid-treated deer without clinical signs (Thorsen et al., 1977), thereby indicating the occurrence of healthy carrier animals following BHV-1 infection.

Parainfluenza-3 viral infection was also observed in the Anticosti deer population. Although the prevalence of antibodies against PI-3 virus in this deer population was comparable to those observed among mule deer (*Odocoileus hemionus*) (Stauber et al., 1977), it was higher than those reported in other North American deer populations (Kahrs et al., 1963; Ingebrigtsen et al., 1986). While prevalences remained stable during our study, higher antibody titers in male or female deer in 1985 and 1987 suggest frequent exposure to PI-3 virus.

The maintenance of the BHV-1 and PI-3 viral infections in Anticosti deer may be explained by ability of these viruses to persist after primary infection and to shed when the animals are under stressed conditions. Thorsen et al. (1977) have isolated herpesviruses from vaginal and preputial swabs collected from fallow deer (*Cervus*

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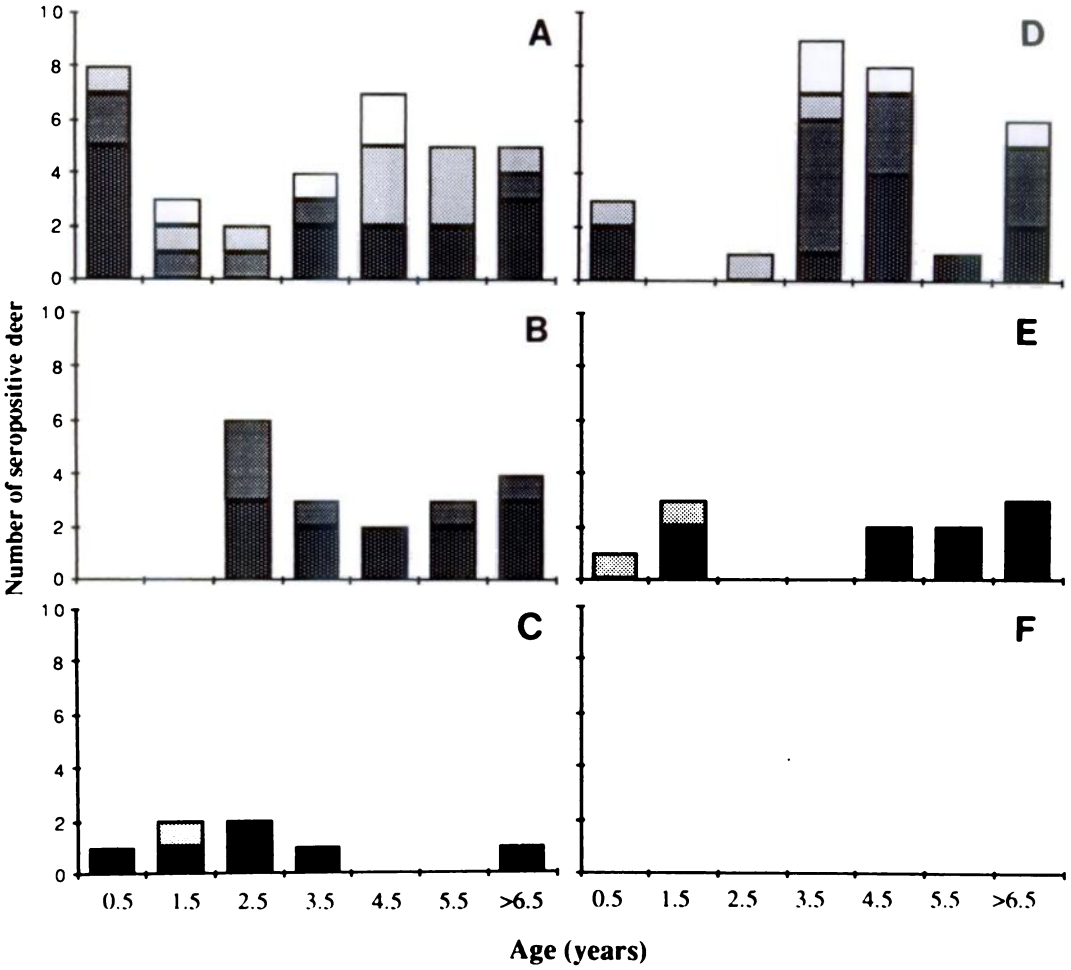

FIGURE 1. Distribution of positive antibody titers for bovine herpesvirus 1 in male (A, B, C) or female (D, E, F) deer on Anticosti Island, in 1985 (A, D), 1986 (B, E), and 1987 (C, F). 8 (■); 16 (■); 32 (■); 64 (□).

dama) prior to dexamethasone treatment and subsequently from nasal swabs from the same deer after 5 days of treatment. Other herpesvirus and PI-3 virus were isolated from nasal swabs of injured or corticosteroid-treated deer (Ek-Kommonen et al., 1986; Thorsen et al., 1977). The effect of corticosteroids as inducers of virus shedding has been previously reported (Sheffy and Davies, 1972; Gaskell and Povey, 1973). Latency or viral persistence would allow these viruses to be maintained in a restricted host population for a long period. It is then postulated that the viral shedding may occur in latently infected deer on Anticosti Island as a result of an immunosuppressive state induced by stress due to environmental conditions. On Anticosti Island, winters are long (averaging 156 days) with heavy snow fall (Huot, 1982). Boreal forest offers relatively good sheltering but winter food is scarce because of overbrowsing by deer which leads to a virtual eradication of all the deciduous bush species that occur on the Island (Victorin and Germain, 1969). Deer's winter survival strategy is based on maximal reduction of energy expenditures. Body reserves supply one-third of the energy needs of a fawn and 20 to 25% for an adult doe

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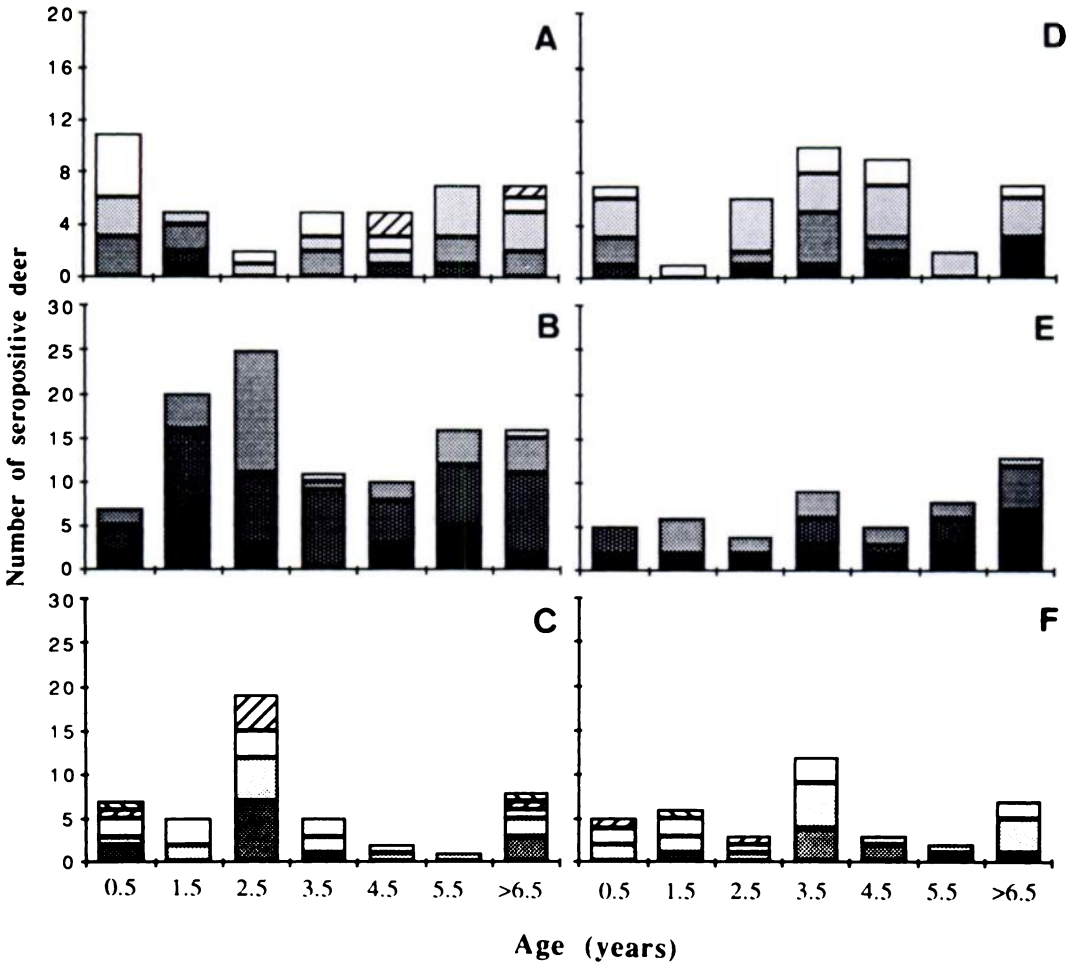

FIGURE 2. Distribution of positive antibody titers for parainfluenza 3 virus in male (A, B, C) or female (D, E, F) deer on Anticosti Island, in 1985 (A, D), 1986 (B, E), and 1987 (C, F). 8 (■); 16 (■); 32 (■); 64 (■); 128 (□); 256 (▨); 512 (▧).

during winter (Huot, 1982). Anticosti Island deer must use all their energy to stand a chance to survive winter.

In contrast to previous studies on the prevalence of antibodies against BVD virus in deer (Kahrs et al., 1963; Friend and Halterman, 1967; Stauber et al., 1977; Couvillion et al., 1980), no antibodies against BVD virus were found in deer sera. This result may reflect the fact that no contact with domestic ruminants occurred in Anticosti Island for at least 50 yr.

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