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PREVALENCE OF NEUTRALIZING ANTIBODY TO JAMESTOWN CANYON VIRUS (CALIFORNIA GROUP) IN POPULATIONS OF ELK AND MOOSE IN NORTHERN MICHIGAN AND ONTARIO, CANADA

Paul R. Grimstad,¹ Stephen M. Schmitt,² and Diane G. Williams^{1,3}

ABSTRACT: Blood samples were collected from free-ranging elk (*Cervus elaphus*) harvested in Michigan's northern Lower Peninsula, from moose (*Alces alces*) relocated from Ontario's Algonquin Provincial Park to Michigan's Upper Peninsula, and from moose from Michigan's Isle Royale National Park. Sera were tested by serum dilution neutralization tests in Vero cell culture for neutralizing antibody to California serogroup viruses, in particular Jamestown Canyon (JC), La Crosse/snowshoe hare (LAC/SSH), and trivittatus (TVT) viruses. Specific neutralizing antibody to JC virus was detected in 71% of 31 and 65% of 20 moose from Algonquin and Isle Royale, respectively. An additional six moose from Algonquin and five from Isle Royale showed evidence of multiple infection. One juvenile moose from Isle Royale had specific neutralizing antibody to TVT virus. Specific neutralizing antibody to JC virus was detected also in 54% of 50 elk from Michigan; 20 of the 50 elk showed evidence of multiple infection. While no single serum sample showed specific neutralizing antibody only to LAC/SSH virus, its presence in sera from some animals may have been masked by the high prevalence of antibody to JC virus.

INTRODUCTION

Jamestown Canyon (JC) virus, a subtype of Melao virus of the California (CAL) serogroup, is distributed widely throughout temperate North America. Recent evidence of widespread infection of humans (Grimstad, 1983) including cases with severe central nervous system illness (Grimstad et al., 1982; Deibel et al., 1983; Srikongse et al., 1984; Grimstad et al., 1986) has prompted new studies of this agent's natural history.

Numerous species of aedine mosquitoes and several tabanid flies have yielded isolates of JC virus throughout North America (Grimstad, 1983); however, only a single isolate has been made from a wild vertebrate, a white-tailed deer (*Odocoileus virginianus*) (Issel, 1973). Results of

serologic surveys suggest that JC viral infection of white-tailed deer is widespread (Grimstad, 1983). Adult white-tailed deer in upper midwestern populations often have a high (>65%) prevalence of neutralizing antibody for JC virus (Boromisa and Grimstad, 1987). White-tailed deer are considered to be the primary vertebrate host for JC virus (Issel et al., 1972a, b; Issel, 1973; Watts et al., 1979, 1982). However, other wild vertebrates are naturally infected also including the opossum (*Didelphis virginiana*) (Pinger et al., 1975), bison (*Bison bison*), dall sheep (*Ovis dalli*), snowshoe hare (*Lepus americanus*), arctic fox (*Alopex lagopus*) (Zarnke et al., 1983) and moose (*Alces alces*). While these latter workers found no neutralizing antibody to JC virus in moose in Alaska, McFarlane et al. (1981) reported that 2% (6/280) of moose killed in Nova Scotia in the 1977 and 1978 hunting seasons and 3% (4/127) of New Brunswick moose sera collected in 1979 (McFarlane et al., 1982) had neutralizing antibody to JC virus. Other serologic surveys of large wild vertebrates, including moose, have employed a single CAL group virus other than JC in neutralization tests (Trainer and Hoff,

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1971; Zarnke and Yuill, 1981) or have assayed animals outside the known range of JC virus (Brummer-Korvenkontio, 1973). We are aware of no report of a serologic survey for specific neutralizing antibody to JC virus in populations of elk (*Cervus elaphus*) in North America.

Detection of a 35–42% prevalence of neutralizing antibody in human residents of Michigan's northern Lower Peninsula (LP) and throughout the Upper Peninsula (UP) (Grimstad et al., 1986) prompted our survey of populations of large wild and domestic animals to determine the host range of the virus in the upper Midwest. This report summarizes our findings of neutralizing antibody to JC virus in several populations of moose and elk.

MATERIALS AND METHODS

Serum collections

Moose sera were collected by one of us (S. M. Schmitt) on Isle Royale in May 1984 (Seal et al., 1985) and during the transfer of 31 moose from Ontario's Algonquin Provincial Park to Michigan's UP in January 1985 as part of a reintroduction program (Burgoyne, 1985). In the Isle Royale project, moose were darted from the ground with a mixture of Carfentanil® and Rompun® as they came into a lick, and were bled where they fell. The Algonquin Provincial Park moose were darted from a helicopter (by S. M. Schmitt) with Carfentanil® and Rompun®, and bled just before placing them into transport crates. Blood samples were collected by hunters from free-ranging elk harvested at a private ranch and in a special hunt in Michigan's northern LP in December 1984 (Schmitt et al., 1985).

Serologic testing

All sera were tested initially at a single dilution (1:2) in a screening microtiter neutralization test that we have used successfully for large numbers of human sera for antibody to JC virus and other CAL group agents (Grimstad et al., 1984). We used only JC virus in the screening test since our previous experience had indicated that use of only JC virus and an initial 1:2 serum dilution resulted in the detection of virtually all sera having neutralizing (N) antibody to JC virus and the vast majority of sera with antibody to La Crosse (LAC) and trivittatus (TV) viruses (we have also detected N an-

tibody to Keystone virus in white-tailed deer and human sera by this method). Sera neutralizing JC virus in the screening test were further assayed in a serum dilution neutralization (SDN) test (Grimstad et al., 1984) that employed representative serotypes of the three CAL group viruses: JC virus (prototype strain; representative of Melao virus), TVT virus (prototype strain; representative of TVT virus), and LAC virus (an Indiana isolate—Pinger et al., 1983—representative of California encephalitis virus). Since snowshoe hare (SSH) virus is a variety of LAC, we assumed that any sera with N antibody to SSH would cross-react also with LAC virus and thus be readily detected. These three representative viruses were used for all SDN assays. Sera at an initial dilution of 1:4 or greater that neutralized a 100 median tissue culture infectious dose (100 TCID₅₀) of virus in African Green Monkey kidney (Vero) cells were considered positive. In addition, we did not consider any serum to have specific N antibody to one of the three viruses unless there was a four-fold or greater difference between the highest titer and the next highest heterologous titer. Sera having less than a four-fold difference between two heterologous titers were classified as having nonspecific CAL group antibody only. The minimal serum titer chosen (1:4) as positive was the lowest titer observed in captive white-tailed deer in Michigan known to have had a JC virus infection in the past 10 mo (Grimstad et al., 1987).

RESULTS

Elk

Forty-eight sera were obtained from elk during the hunt in the northern LP of Michigan and two additional sera came from adult elk from a private herd near the hunt sites. Evidence of extensive prior JC virus infection was seen in these animals. More than half (57%) of adult elk had specific N antibody to JC virus (Table 1); all 46 of the adult animals had N antibody to one or more CAL group virus. However, only one of the four juvenile animals had specific N antibody to JC virus (Table 1).

Moose

The 1984 moose relocation effort initiated at Algonquin Provincial Park, Ontario, yielded 31 sera from adult animals of

TABLE 1. Prevalence of neutralizing antibody to California group viruses in moose and elk from three sites in upper midwestern North America.

Species and site	Age class (yr)	Number tested	Number of animals positive (%) for neutralizing antibody			No. of animals seronegative for CAL group antibodies
			JCV ^a	CAL group	Total CAL group	
Elk						
Michigan ^b	<1	4	1 (25)	1 (25)	2 (50)	2 (50)
	>1	46	26 (57)	20 (43)	46 (100)	0
Moose						
Algonquin	>1	31	22 (71)	6 (19)	28 (90)	3 (10) ^c
Isle Royale	<1	2	0	1 (50) ^d	1 (50)	1 (50)
	>1	18	13 (72)	5 (28)	18 (100)	0

^a Prevalence = number positive/number tested (% positive); categories listed are as follows: JCV represents the total number of sera with specific antibody to JC virus in each population sampled; CAL group represents those sera where there was less than a four-fold difference in titer between JC and TVT or JC and LAC viruses; seronegatives were those where the serum dilution neutralization (SDN) titer for all three viruses was <1:4.

^b The elk in Michigan were harvested primarily in two northern LP counties (Montmorency and Otsego); a few animals also came from Cheboygan and Presque Isle counties. In addition, two of the JCV-seropositive adult animals came from a private herd in Cheboygan County near Gaylord.

^c These three adult moose all had a SDN titer of 1:2 to JC virus but <1:2 for TVT and LAC viruses. These may represent JC viral infections in the distant past or may represent low level nonspecific neutralization.

^d This serum sample represented a TVT virus infection with a SDN titer of 1:32.

both sexes (Table 1), 29 of which survived relocation to the UP. No juvenile animals were sampled in that effort. Of these 31 adults, 22 (71%) had specific N antibody to JC virus; 2/22 had cross-reactive N antibody to LAC/SSH virus, while 8/22 had cross-reactive N antibody to TVT virus. The cross-reactive N antibody titer pattern was almost always JC > TVT > LAC. Six additional animals showed highest titers to JC virus with only a two-fold lower titer to TVT virus, and were considered to be evidence of a prior CAL group infection (Table 1).

Twenty sera were obtained from moose which were tagged on Isle Royale. Neither of the two juvenile animals had specific N antibody to JC virus, however, one animal showed evidence of a prior TVT virus infection (Table 1). Seventy-two percent (13/18) of adult animals showed evidence of prior infection with JC virus and an additional 28% (5/18) of the adults had high titers to JC virus with a two-fold less titer to TVT virus (Table 1). As with the Algonquin moose, we saw no specific

evidence of prior infection with LAC/SSH virus in these animals.

DISCUSSION

Serologic results reported above (Table 1) from the population of wild elk in the northern LP mark the first serologic testing of this species for specific N antibody to JC virus in Michigan and perhaps elsewhere in North America. Our results establish that (1) these animals are infected frequently by JC virus and produce detectable N antibody, and that (2) the low seropositive prevalence in the juvenile population (elk <1.0 yr old) may reflect passive protection by maternal antibody from a primary infection similar to that occurring in white-tailed deer fawns (Isel, 1974).

Elk calving occurs in late spring and early summer in Michigan coincident with the apparent transmission of JC virus by mosquitoes to deer in the upper Midwest (Boromisa, 1985; Grimstad and Mandracchia, 1985). The effect of a primary infection of JC virus on susceptible elk is

unknown; we have had no opportunity as yet to observe viremic elk. However, experimentally and naturally infected white-tailed deer apparently show no ill effects (Issel et al., 1972a).

Most elk sera with specific N antibody to JC virus showed cross-reactive neutralization with LAC/SSH and/or TVT viruses. In all instances of cross-reactors, however, the TVT SDN titer was greater than that of the LAC/SSH SDN titer. This is a pattern seen in virtually all sera from white-tailed deer tested for N antibody to CAL group viruses in Indiana (Boromisa and Grimstad, 1987) and the moose sera reported in this paper.

Among the adult elk, 43% (20/46; Table 1) had nonspecific N antibody to both JC and TVT or LAC viruses. These animals apparently have experienced either multiple serotype infections in the past (i.e., JC and TVT or JC and LAC/SSH) or may have experienced repeated exposure to strains of JC virus that represented varying antigenic topotypes, thus producing the "group-specific" immune response we observed. While we have found no evidence of a primary infection with TVT (or LAC/SSH) virus in these elk as we did with the moose (Table 1), it is probable that the overwhelming prevalence of specific N antibody to JC virus masks the less frequent occurrence of primary TVT and/or SSH viruses in the elk population. The LAC serotype apparently does not circulate in Michigan (Grimstad et al., 1986), however, both TVT virus and especially SSH virus might be expected to occur within the elk range in northern Michigan (Calisher, 1983).

Moose

The 71% and 72% N antibody prevalences to JC virus in adult moose bled at Algonquin in Ontario and on Isle Royale, respectively (Table 1), are by far the highest reported to date to that CAL group serotype. In contrast, McFarlane and co-

workers reported N antibody prevalences to SSH virus of 68% and 74% in sera collected from moose in Nova Scotia (McFarlane et al., 1981) and New Brunswick (McFarlane et al., 1982), respectively. These workers additionally noted that 13% of sera from moose in Nova Scotia were JC-SSH cross-reactors while only 3% of the sera had specific N antibody to JC virus (McFarlane et al., 1981); interestingly, no white-tailed deer in Nova Scotia had N antibody to JC virus.

Our sample of two juvenile moose from Isle Royale lacked specific antibody to JC virus; this sample size was too small to suggest that maternal antibody protected either animal from a JC virus infection. Indeed, one of the two Isle Royale moose calves had specific N antibody to TVT virus (Table 1). McFarlane and coworkers did detect fewer SSH reactors in the young moose calves in Nova Scotia (19% of 0.5-yr-olds) compared to older animals (33% to 80% of moose 1.5 to 16.5 yr old were SSH reactors) sampled there (McFarlane et al., 1981). These data suggest that perhaps maternal antibody protects moose calves from an SSH virus infection during their first summer since they are born in the late spring prior to the major transmission of SSH virus (Artsob et al., 1983; Mokry et al., 1984).

Our record of specific N antibody to TVT virus in moose calf from Isle Royale is the first documentation we are aware of that this CAL group agent infects moose. The report of antibody to Inkoo virus in moose in Finland (Brummer-Korvenkontio, 1973) and reports of antibody to Northway virus in moose from Alberta and Alaska (Zarnke and Yuill, 1981; Zarnke et al., 1983) brings to five the number of specific mosquito-borne arboviruses known to infect moose.

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BOOK REVIEW . . .

Mammalian Diseases and Arachnids. William E. Nutting (ed.). CRC Press, Inc., Boca Raton, Florida, USA. 1984. **Volume I, Pathogen Biology and Clinical Management.** 277 pp. \$80.00 US. **Volume II, Medico-Veterinary, and Laboratory and Wildlife Diseases, and Control.** 280 pp. \$83.50 US.

The format of each volume is planned so that each chapter is a self contained unit with a list of references. Arachnid biology, evolution and clinical management of disease caused by the arthropods is presented in Volume I. In Volume II, diseases transmitted and/or caused by arachnids are discussed under the headings of global distribution, specific practitioner concerns with an endemic organism account which summarizes practical problems of diagnosis, control and treatment. Chapter 1 and the appendices (1 to 4) are repeated in each volume so each tome may be used as an independent working unit. The illustrations, for the most part, are excellent.

Chapter 8 in Volume II, "Diseases of Wildlife," deals more extensively with mites than

with ticks, spiders, or scorpions. The mite discussion is primarily limited to the Nearctic Region. Certain diseases, such as tularemia, and numerous references in European (Russian) literature are omitted. In one chapter, no one could adequately discuss wildlife diseases associated with arachnids.

This two volume work is intended to "stimulate curiosity and produce enthusiasm for research" in the problems posed by arachnid-caused or transmitted diseases of man and other mammals. Pragmatically, it is an ambitious attempt at retrieval of pertinent information for the various subjects discussed. In this last mentioned regard, it has been successful. Overall, it is a worthy reference for physicians and public workers; that is, perhaps, its strongest contribution. It is not viewed as a major contribution in wildlife diseases, nor was that probably the original intent.

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