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Selected Microbial Agents in Snowshoe Hares and Other Vertebrates of Alberta

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Abstract

Serologic surveillance of populations of snowshoe hares and other vertebrate species of north-central Alberta from 1961 to 1969, revealed activity of one bacterial and eight viral agents. The most prevalent agents infecting the snowshoe hare were California encephalitis and Silverwater viruses, while in other vertebrates California encephalitis and Western equine encephalomyelitis viruses were the most common. The role of the snowshoe hare in the natural history of the agents is considered as is the effect of the agent on the hare ten-year cycle of abundance.

Introduction

From 1961 through 1969, investigations have been conducted of selected viral and bacterial agents in the snowshoe hare and other vertebrate species near Rochester, Alberta, Canada.[4,5,6,9,16,17] During this period eastern equine encephalomyelitis (EEE),[16] Silverwater (SIL),[5] and two serotypes of California encephalitis (CE)[7,8] viruses have been isolated from the blood of snowshoe hares and from various species of arthropods. The cycle of abundance of the Rochester hare population has been under study by the Department of Wildlife Ecology, University of Wisconsin, since 1961.[11] During that period the population declined to approximately 3 adult hares per square mile in 1965 and subsequently increased to a pre-peak abundance of approximately 113 adult hares per square mile in 1969.[12,15]

This investigation has been directed towards determining, 1) the role of the snowshoe hare as a reservoir of pathogens of human and animal health significance, and 2) the effects of certain infectious diseases on the periodic fluctuations of snowshoe hare populations. Although the hare has been emphasized in these studies, some work has been done on other vertebrates of the area. The present paper summarizes, 1) serology of the snowshoe hare population through 9 years of a "ten-year" cycle, 2) serology of associated wild vertebrates, and 3) serology of man and domestic animals during that same period.

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Materials and Methods

In general, all wild animals were live trapped, subjected to examination and were bled. The species sampled included: snowshoe hare, *Lepus americanus;* red squirrel, *Tamiasciurus hudsonicus;* Richardson's ground squirrel, *Citellus richardsonii;* Franklin's ground squirrel, *C. franklinii;* chipmunk, *Eutamias minimus;* woodchuck, *Marmota monax;* red-backed vole, *Clethrionomys gapperi;* field vole, *Microtus pennsylvannicus;* white-footed mouse, *Peromyscus maniculatis;* jumping mouse, *Zapus hudsonicus;* pocket gopher, *Thomomys talpoides;* weasel, *Mustela frenata* and *M. rixosa;* muskrat, *Ondatra zibethicus;* porcupine, *Erethizon dorsatum;* lynx, *Lynx canadensis;* coyote, *Canis latrans;* ruffed grouse, *Bonasa umbellus,* sharp-tailed grouse, *Pedioecetes phasianellus;* spruce grouse, *Canchites canadensis;* red-tailed hawk, *Buteo jamaicensia;* Cooper's hawk, *Accipiter cooperii;* red-winged blackbird, *Agelaius phoeniseus;* mallard duck, *Anas platyrhynchos;* blue-winged teal, *A. discors;* pintail duck, *A. acuta tzitzihoa;* American golden eye duck, *Bucephala clangula americana;* buffle-head duck, *B. albeola;* baldpate duck, *Mareca americana;* canvas-back duck, *Aythya valisisneria;* redhead duck, *A. americana;* ring-necked duck, *A. collaris;* toad, *Bufo cognatus;* and frog, *Rana sylvatica.* In addition to the wildlife sampled, blood was collected from local cattle, horses, rabbits, poultry and from both resident and non-resident people of the Rochester area.

Stock virus preparations of 10% and 20% infected suckling mouse brain were maintained in a mechanical freezer at —65°C. The viruses used in the neutralization tests were the following: California encephalitis (CE) of the snowshoe hare serotype,[2] provided by the Rocky Mountain Laboratory, Hamilton, Montana and used as nineteenth mouse passage; Western equine encephalomyelitis (WEE), Fleming isolate from a fatal human case in California provided by the National Communicable Disease Center (NCDC), Atlanta, Georgia, and used as eighth mouse passage; Eastern equine encephalomyelitis (EEE), Rochester II isolate[18] from the rectal swab of a snowshoe hare collected near Rochester, Alberta and used as fourth mouse passage; St. Louis encephalitis (SLE), CDC-904 isolate from a flicker in Kentucky in 1955, provided by NCDC and used as sixth mouse passage; Venezuelan equine encephalomyelitis (VEE), the attenuated "Trinidad" vaccine, provided by the U.S. Army Biological Laboratories, Fort Detrick, Maryland and used as eighty-first tissue culture passage; Powassan (POW) from a fatal human case in Ontario[11] provided by NCDC and used as seventh mouse passage; Buttonwillow (BUT) isolated from a cottontail rabbit, *Sylvilagus audubonii,* from California in 1964, provided by the Yale Arbovirus Research Center, Connecticut and used as ninth mouse passage; Silverwater (SIL), isolated from *Haemaphysalis leporispulustris* ticks removed from snowshoe hares in Ontario,[13] provided by Dr. D. M. McLean, University of British Columbia and used as third mouse passage, and also another stock was obtained from the Yale Arbovirus Research Center and used as fifth mouse passage; and Encephalomyocarditis (EMC), CDC-862 isolate from a Florida squirrel was provided by NCDC and used as eighth mouse, first tissue culture passage. The various types of virus neutralization procedures used in this investigation included mouse inoculation, the metabolic inhibition test and tube neutralization.[6,16]

The presence of antibodies to *Francisella tularensis,* the causative agent of tularemia, was demonstrated by means of the rapid plate agglutination test (Difco Laboratories, Inc., Detroit, Michigan). Only those sera with a reaction titer of 1:80 or greater were considered positive. *Brucella abortus* strain 119-3 tube agglutination antigen (National Animal Disease Laboratories, Ames, Iowa) was used to test for the presence of antibodies to *Brucella* spp. The decimal dilution method of the standard tube agglutination test (USDA) was used. The tests were read at 24 and 48 hours and only titers of 1:100 or greater were considered to be positive.

Results

In snowshoe hares, neutralizing antibodies were detected to CE, WEE, EEE, POW, BUT, SIL and EMC viruses but not to VEE virus (Table 1). Some hare sera reacted with SLE virus but whether this represents infection with SLE or cross reactivity with some other group B arboviruses is not presently clear. Sera which in 1967, 1968 and 1969 neutralized SLE did not neutralize POW and vice versa. Of the viruses included in this study, the most persistently active in the snowshoe hare population were CE and SIL. Low levels of activity of WEE virus were detected throughout the study, with epizootics sweeping through the hare population in 1963 and 1965. EEE virus was detected in 1961 and 1969, the two years in which the hare population was near its peak abundance. Serological reactors to SLE, POW, BUT and EMC viruses were few during this study. Only two snowshoe hares of 1543 tested, reacted positively for tularemia and none of 1194 hares possessed agglutinating antibodies to brucella antigen.

Among the wild vertebrate species, excluding the snowshoe hare, no sero-positive reactors were detected to VEE, SLE, BUT, and brucella antigen (Tables 2 and 3). Ten of the 32 species yielded sero-positive reactors to one or more of the following: CE, WEE, EEE, POW, SIL, and *Francisella tularensis*. The two agents infecting the most number of species were CE and WEE.

Among the domestic animals tested, only antibodies to CE, WEE and SIL viruses were detected (Table 4). The sera of human residents of Alberta reacted with CE, WEE, SLE, EMC and *Francisella tularensis*.

TABLE 1. *Serology of snowshoe hares, Rochester, Alberta, 1961-1969.*

	Viruses								
Year	CE[1]	WEE[2]	EEE[3]	VEE[4]	SLE[5]	POW[6]	BUT[7]	SIL[8]	EMC[9]
1961	46/61	8/60	5/185					3/140	
1962	196/274	25/247	0/331		4/271			72/234	2/272
1963	124/214	111/234	0/296		14/150			43/175	
1964	42/78	2/72	0/51	0/40	0/72			46/122	0/51
1965	26/58	41/101	0/85	0/60	0/101			8/32	0/85
1966	33/60	3/62	0/49	0/38	0/62			16/61	0/49
1967	31/155	2/155	0/155	8/155	8/155	1/129	0/136	15/100	0/155
1968	49/226	7/221	0/221	0/221	4/226	8/214	6/219	61/197	
1969	30/136	32/135	24/135		0/88	5/92	5/93	28/125	

[1] California encephalitis
[2] Western equine encephalomyelitis
[3] Eastern equine encephalomyelitis
[4] Venezuelan equine encephalomyelitis
[5] St. Louis encephalitis
[6] Powassan
[7] Buttonwillow
[8] Silverwater
[9] Encephalomyocarditis

TABLE 2. *Serology of mammals and amphibians, Rochester, Alberta, 1962-1968.*

	Viruses							
	CE[1]	WEE[2]	EEE[3]	VEE[4]	SLE[5]	POW[6]	BUT[7]	SIL[8]
Red squirrel	1/49	0/49	0/49	0/49	0/28			2/23
Franklin ground squirrel	3/64	12/64	0/64	0/34	0/42			0/46
Richardson ground squirrel	0/94	0/93	0/93		0/40	2/78	0/88	0/43
Chipmunk	0/1	0/1	0/1					1/1
Red-backed vole	0/33	3/33	0/33		0/33	0/12	0/15	0/4
Field vole	0/12	0/7	0/3	0/3				
White-footed mouse	0/37	0/38	0/26	0/24				0/3
Jumping mouse	0/1	0/1	0/1					
Weasel	0/10	1/10	0/10		0/3	0/1	0/1	0/1
Pocket gopher	0/1	0/1	0/1	0/1				
Porcupine	6/10	0/10	0/6		0/8			0/12
Muskrat	0/4	0/4		0/4				
Lynx	2/4	0/4	0/3		0/4			
Coyote	3/6	0/6	0/6		0/4			2/5
Toad	0/3	0/3	0/3					
Frog	0/3	0/3	0/3					

[1] California encephalitis
[2] Western equine encephalomyelitis
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[4] Venezuelan equine encephalomyelitis
[5] St. Louis encephalitis
[6] Powassan
[7] Buttonwillow
[8] Silverwater

TABLE 3. *Avian serology, Rochester, Alberta, 1962-1968.*

	Viruses					
	CE[1]	WEE[2]	EEE[3]	VEE[4]	SLE[5]	SIL[6]
Ruffed grouse	4/27	2/28	4/27	0/4	0/9	0/9
Sharp-tailed grouse	0/7	0/7	0/7	0/3	0/6	0/1
Spruce grouse	0/1	0/1	0/1			0/1
Cooper's hawk	0/2	0/2	0/2		0/2	
Red-tailed hawk	0/8	0/8	0/8			
Red-winged blackbird	0/8	0/8	0/8			
Mallard duck	0/6	0/6	0/6	0/6	0/6	
Blue-winged teal	0/5	0/5	0/5	0/5	0/5	
Pintail duck	0/1	0/1	0/1	0/1	0/1	
American golden-eye duck	0/10	0/10	0/10	0/10	0/10	
Buffle-head duck	0/1	0/1	0/1	0/1	0/1	
Baldpate duck	0/7	0/7	0/7	0/7	0/7	
Canvas-back duck	0/7	0/7	0/7	0/7	0/7	
Redhead duck	0/1	0/1	0/1	0/1	0/1	
Ring-necked duck	0/1	0/1	0/1	0/1	0/1	

[1] California encephalitis
[2] Western equine encephalomyelitis
[3] Eastern equine encephalomyelitis
[4] Venezuelan equine encephalomyelitis
[5] St. Louis encephalitis
[6] Silverwater

TABLE 4. *Serology of man and domestic animals, Alberta, 1961-1968.*

	Viruses							
	CE[1]	WEE[2]	EEE[3]	SLE[4]	POW[5]	BUT[6]	SIL[7]	EMC[8]
Man	102/368	32/369	0/208	3/208			0/20	4/227
Cattle	5/51	1/10					4/37	
Horse	2/24							
Rabbit	2/39	2/39	0/39	0/39	0/32	0/32	7/35	
Chicken	2/46	1/46	0/46				0/14	
Turkey	0/7	0/7	0/7					
Goose	0/5	0/5	0/5					
Duck	0/9	0/9	0/9					
Guinea fowl	0/1	0/1	0/1					

[1] California encephalitis
[2] Western equine encephalomyelitis
[3] Eastern equine encephalomyelitis
[4] St. Louis encephalitis
[5] Powassan
[6] Buttonwillow
[7] Silverwater
[8] Encephalomyocarditis

Discussion

Of the microbial agents studied in this investigation, the two most prevalent in the snowshoe hare population have been SIL and CE. Silverwater virus and the two serotypes of CE, Jamestown Canyon and Snowshoe hare, known to be present in the Rochester area are not known to be of human or veterinary significance.[8,9] The low level of activity of tularemia, POW, BUT, SLE, EEE, and EMC suggests that the snowshoe hare either plays little role in their maintenance cycles or that the recovery rate of the hares after infection is low. In 1963 and 1965, epizootics of WEE virus swept through the hares and the Franklin ground squirrels, preceding by about two months outbreaks in man and horses in the southern part of the province.[17] Therefore, the hares may have served as short term amplifying hosts for this virus, and could conceivably do the same for any of the other agents under the proper conditions. This then suggests that continued surveillance of the hare population would be desirable from both a public health and veterinary standpoint.

The effect of the various agents on the hare population is unclear. Of the agents studied, only *F. tularensis* is known to be capable of producing mortality in snowshoe hares. However, the majority of the hares infected with tularemia survive infection.[1,3] Other workers have also maintained that tularemia is a minor factor in the biology of the snowshoe hare.[10] Experimental infection studies with EEE, SIL and the snowshoe hare serotype of CE virus failed to produce clinical disease in the hares tested.[5,16,18] In the experiments, adult hares were challenged with these viruses and infection in juveniles or pregnant females may yield different results. Serological findings have suggested that hares who have recovered from CE, WEE, and SIL virus infections may be predisposed to other mortality factors.[17] It is hoped that radiotelemetry studies may shed more light on this proposed arbovirus-associated mortality.

As the hare population declined and subsequently increased, several hare-microbe relationships seem to have

emerged. Neutralizing antibody prevalence rates to SIL and CE group viruses appear to be related to hare population dynamics, the highest rates being observed during the population decline.[4,6,16] During this period, there was a shift in the age distribution of the population which became comprised of largely old, immune animals.[15] It is difficult to assess the amount of antibody carry-over from year to year which may be reflected in the observed rates. Subsequent bleedings of retrapped adult hares have shown that they are capable of retaining detectable antibody titers to CE group viruses for up to four months and possibly even longer, a similar pattern of antibody retention has also been observed with SIL.[17] After 1964, the population has shifted to a younger age distribution and beginning with 1966, the shift has accelerated. The lower antibody prevalence rates observed with the increasing population and the shift in the age distribution, may represent a dilution of the immune cohort by addition of young susceptibles. Perhaps the high prevalence rates of CE and SIL viruses will again be observed when the population declines in the early 1970's.

The incidence of WEE virus, on the other hand, does not seem to be related to hare numbers but to other factors, perhaps vector density. The two observed epizootics occurred while the hare population was declining or at its low point. Of the remaining agents investigated, none seem to be related to hare population dynamics with the exception of EEE virus. This virus has only been detected when the hare population was near its 1960 and 1970 peaks.

In attempting to assess the effect of infectious disease on the ten-year cycle of the snowshoe hare, unfortunately, little attention has been given to the ruffed grouse. This species also has a ten-year cycle in Alberta that is almost in synchrony with that of the hare. In general, grouse populations peak or decline slightly before hare populations do,[11] as happened at Rochester.[12] Serology has shown the grouse to have been exposed to CE, WEE, and EEE viruses, all of which have been demonstrated in the hares. It is of interest that the four grouse which reacted to EEE virus were sampled in the spring of 1968, one year prior to the reappearance of EEE virus in the hares. This could be a very significant observation and should be investigated further.

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