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EVIDENCE FOR ARBOVIRUS INFECTIONS IN A POPULATION OF SNOWSHOE HARES: A POSSIBLE MORTALITY FACTOR*

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Abstract

Studies of arbovirus infections of snowshoe hares in central Alberta were carried out in 1961 through 1967, in an attempt to determine which arboviruses were importantly associated with the hare population. These studies were carried out during a period of marked decline of the hare population. Neutralizing antibodies to California encephalitis virus and Silverwater virus were present in the population throughout the study with prevalence ranging from 59% to 97% and from 0% to 68%, respectively. Antibody prevalence rates to western equine encephalomyelitis virus were generally low except for two epizootics during the spring and early summers of 1963 and 1965, when rates of over 80% were observed. The significance of these findings is discussed. Data are presented which show a rapid, significant decline in neutralizing antibody prevalence which suggests that hares convalescent from these arbovirus infections experienced higher mortality than did uninfected animals.

Introduction

Over 100 years of fur records show that the snowshoe hare *(Lepus americanus)* undergoes dramatic population fluctuations every 9-11 years.[1] In 1961 a study of the biology of the snowshoe hare and its unexplained cycle of abundance and decline was begun in central Alberta, Canada, by University of Wisconsin personnel. Included in these studies were investigations (1) to determine which viruses of animal and human health importance were associated with the hare population and (2) to assess the effect of these viruses on the hare population. This communication reports occurrence and possible effects of certain arbovirus infections of the hare population.

Materials and Methods

The study area is located in the Rochester area in central Alberta, approximately 60 miles north of Edmonton. Although somewhat agricultural, the area contains extensive forested tracts comprised of black spruce *(Picea mariana)*—*Sphagnum* moss bogs in the lowlands and of upland ridges covered with aspen *(Populus tremuloides)*, balsam poplars *(Populus balsamifera)*, white spruce *(Picea glauca)* and jack pine *(Pinus banksiana)*.

Most of the hares were captured alive in traps, although some were shot. Blood was taken from the animals and various observations and measurements were

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made. Live hares were marked by ear tattooing or toe clipping, and released.

Blood clots and ticks were frozen on dry ice and stored in a mechanical —65C freezer prior to virus isolation attempts in suckling mice. Sera were similarly frozen and then stored at —20C prior to serological testing. In 1961, blood specimens were collected on paper discs and then dried.[4] Sera or paper disc eluates were tested for neutralizing antibody in the HeLa cell metabolic inhibition test,[2] in embryonating hen's eggs[5] or in suckling mice.[1]

Population data, including estimates of population size, age and sex composition, mortality and natality, and distribution on the area, were gathered by project wildlife ecologists.

Results

California encephalitis virus (CEV), western equine encephalomyelitis virus (WEEV) and Silverwater virus (SWV) appeared to be the principal arboviruses infecting the hare population during the study. Of these, CEV was the most prevalent. Neutralizing antibody was detected in the sera of 59% to 97% of the adult hares tested in 1961 through 1966 but declined to 23% in the spring of 1967.

SWV neutralizing antibody was also detected regularly in hares (Table 1). Antibody prevalence rates varied from 0% in June and July of 1961 to 68% in June of 1963. Antibody prevalence appeared to be highest in spring and summer. SWV was recovered from the blood of snowshoe hares captured in June of 1962 and May of 1965. This virus was also recovered from 15 pools of rabbit ticks, *Haemaphysalis leporis - palustris,* collected from hares during the spring and summer, 1962 through 1965.

WEEV infected the hare population in two epizootic waves, one in 1963[11] and the other in 1965 (Figure 1). In early spring (April), antibody prevalence was less than 10%, but this rate soared to over 80% when the hare population was resampled in early summer (June). Following both epizootics, antibody prevalence rates rapidly declined to pre-epizootic levels. In 1963, there was a significant decline two months after the peak.

TABLE 1. *Percent Silverwater virus antibody prevalence in adult hares.*

Year	Low-Month		High-Month		Mean
1961	0	June-July	8	Aug	4
1962	16	Nov-Dec	50	Apr & June	36
1963	8	Apr	68	June	38

Discussion

Continuing association of CEV, SWV and WEEV with the study area hare population has been established. Infection of the hare population with CEV is supported by these and previous[11] serological findings, and by the recovery of CEV from the blood of a snowshoe hare. The hare-CEV relationship will be described in detail in the following paper.[9]

SWV infection of hares during the study was quite common. The presence of SWV neutralizing antibody in hares, the recovery of SWV from the blood of hares on two occasions and the repeated isolation of SWV from rabbit ticks collected from hares supports this contention. The association of hares, rabbit ticks and SWV was first established in Ontario.[7,10] Our findings extend these observations both biologically and geographically.

The WEEV serological study is of particular interest because it again documents the occurrence of late spring epizootics.[11] In both years, the epizootics preceded, by about two months, outbreaks of WEEV infecting both man and horses (Morgante, pers. comm.) in the transitional aspen parklands and prairie to the south. There was also an increase in WEEV antibody prevalence among Franklin's ground squirrels, *(Citellus franklinii),* suggesting that the epizootic was not limited to hares. The absence of neutralizing antibody to eastern equine encephalomyelitis (EEE) virus in sera neutralizing WEEV suggests that the observed WEEV neutralizations were specific for that virus.

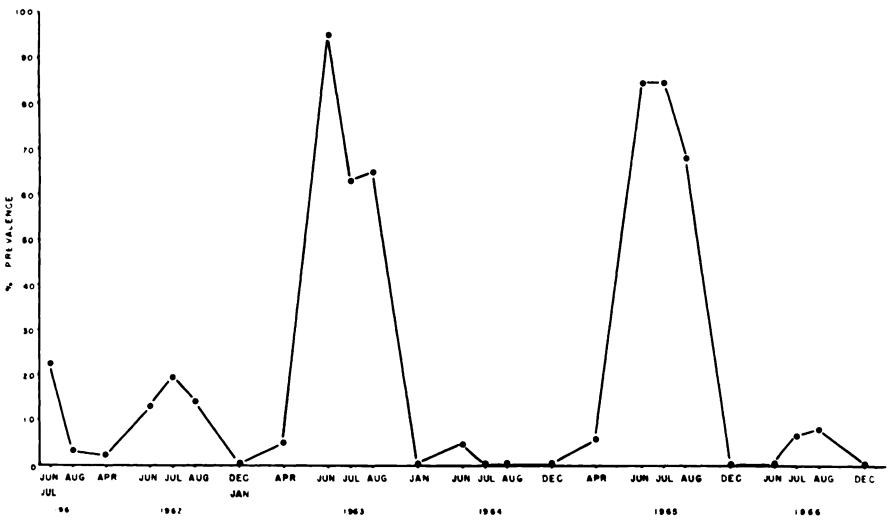

FIGURE 1. *Neutralizing antibody prevalence rates for western equine encephalomyelitis virus in adult snowshoe hares.*

During the course of the study, the hare population declined dramatically from a high of 622 animals per square mile in April, 1962, to a low of three in July, 1965.[10] Despite this decline, there appeared to be continuing infection of the hare population with CEV and SWV, as well as the epizootics of WEEV. How dependent these viruses were on numbers of susceptible hares for propagation and dissemination in nature is not known.

There was also some evidence for infection of the hare population by other arboviruses. Powassan, St. Louis Encephalitis and EEE viruses were neutralized by hare sera, and EEE virus was recovered from two hares. The evidence for specificity of these serological reactions or for continuing infection of the hare population by them is far less complete than for CEV, WEEV or SWV.

It has been far easier to associate viruses with hares than to assess the impact of the more prevalent viral infections on the hare population. Despite extremely high hare mortality, dead or ill individuals were very rarely found. In most instances, the animals simply vanished from the population. There is some circumstantial evidence for increased mortality of hares following CEV, WEEV and SWV infections, however. For each of these viruses, there is an instance of decline of neutralizing antibody prevalence within a comparatively short period of time (Figure 2). SWV and WEEV antibodies in adult hares declined within two summer months to significantly lower levels. Since no juvenile hares were added to the adult cohort during this time, this can only be explained by (1) a decline in neutralizing antibody to undetectable levels or (2) higher mortality among hares convalescent from these infections than ones which had never been infected.

The decline in CEV antibody took place over a 9-month period. The observed antibody prevalence (23%) in March and April was lower than the expected prevalence (52%). The expected adult hare March-April prevalence rate was adjusted to include immune and susceptible animals born the previous summer. This adjustment eliminated prevalence reduction by "dilution" of old adult immunes with susceptible yearlings and established that an actual decline in

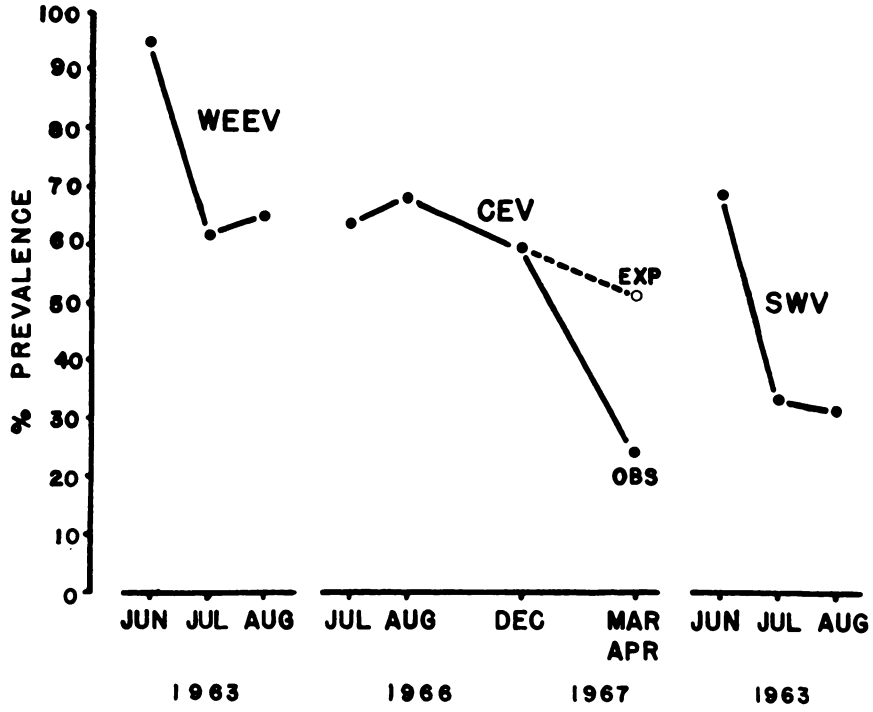

FIGURE 2. *Two-month declines in western equine encephalitis virus (WEEV) and Silverwater virus (SWV) neutralizing antibody prevalence and a nine-month decline of observed (OBS) from expected (EXP) prevalence for California encephalitis virus (CEV) antibody.*

TABLE 2. *Persistence of arboviral neutralizing antibody in wild adult hares resampled at 1-4 month intervals.*

Interval (Months)	California [1]			Western [2]			Silverwater [3]		
	+ to — [4]	— to + [5]	+ to + [6]	+to—	—to+	+to+	+to—	—to+	+to+
1	2	2	7	3	1	4	0	1	0
2	4	3	14	0	11	7	0	1	1
3	0	1	5	1	1	0	0	0	1
4	1	0	3	0	2	0	0	1	0

[1] California encephalitis virus
[2] Western equine encephalomyelitis virus
[3] Silverwater virus
[4] Seropositive hares which became seronegative
[5] Seronegative hares which became seropositive
[6] Hares which remained seropositive

antibody prevalence had taken place, which could only be explained by the alternatives given above for WEEV and SWV antibody prevalence declines.

Several hares were recaptured 1 to 4 months after their initial capture, and their serum was tested for neutralizing antibody (Table 2). Some seropositive hares were subsequently seronegative, including 7 of 42 for CEV, 4 of 30 for WEEV and none of 5 for SWV. Thus, antibody in some individuals did decline to undetectable levels, but the percentage of individuals in which this occurred was not sufficient to explain the magnitude of antibody prevalence decline observed in the population. These data also indicate that the observed changes were not due to inherent variability of the serologic tests, per se. This suggests, then, that mortality among animals with antibody was higher than among those without. Why such mortality may have occurred, at least long enough after infection for antibody to have been produced, we can not say. There are several possible explanations. First, infection may have produced some residual damage in the hares which enhanced their susceptibility to other mortality factors. Second, some of the hares may have remained chronically infected, despite the production of antibody, and mortality may have resulted from viral recrudescence, as appears to be the case in *Herpesvirus hominus* encephalitis in man. There is no evidence that arboviruses act this way in non-hibernating animals, however. Third, arbovirus infections may activate other infectious agents which are latent. Fourth, it is possible that transmission to hares occurred in focal areas which happened, coincidentally, to be ones in which other mortality factors were operating at a high level, thus, removing a disproportionately large number of immune animals. The absence of foci of arbovirus infection argues against this, however. As yet we have no data to support any of these possibilities. We feel, however, that these data are suggestive enough of arbovirus-associated mortality to warrant further investigation. Current plans for studies of hares in the field employing radio telemetry will hopefully result in examination of sufficient numbers of freshly dead or moribund hares to determine if a disproportionate number have been infected with arboviruses, and if so, what the mechanism of mortality might be.

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