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SNOWSHOE HARES AND THE CALIFORNIA ENCEPHALITIS VIRUS GROUP IN ALBERTA, 1961-1968 [1]

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Abstract

The relationship between the California encephalitis (CEV) group of arboviruses and a snowshoe hare population near Rochester (54N, 113W), Alberta has been studied since 1961. The neutralizing antibody prevalence to the CEV group of viruses in adult hares was high, 58% to 95%, during the 6 years of snowshoe hare population decline, when the population dropped from over 600 hares per square mile to 3 hares per square mile. In the 2 years of population recovery thus far observed, the antibody prevalence has been low, 0% to 43%. The prevalence rates in juveniles have been lower than in the adults throughout the study. At least 2 strains of the virus group are present and a total of 7 isolations from *Aedes* mosquitoes and 1 from a snowshoe hare have been made, representing the northern most known limit of the CEV froup in North America. Experimental viremia studies with the Montana snowshoe hare strain of virus resulted in titers up to 10^4 mouse LD_{50} per ml. of whole blood and viremia lasted up to 3 days with an increase in length of viremia with age.

Introduction

The 10-year cycle of abundance of the snowshoe hares in North America has been summarized by Keith.[5] The nature of this cycle and its relationship to disease agents within the hare population itself has been under study near Rochester, Alberta since 1961. Among the disease agents investigated were the California encephalitis (CEV) group of arboviruses. Since the isolation of the first member of this mosquito-borne group of viruses in 1943,[2] they have been shown to have world-wide distribution and several have been associated with human disease.[13,8] The first evidence of natural CEV group infection of snowshoe hares was presented in 1959, when a new strain of the virus was isolated from the blood of a snowshoe hare in Montana and neutralizing antibodies were detected in the sera of snowshoe hares in Michigan.[1] Subsequent serological surveys demonstrated neutralizing antibodies in snowshoe hares from Montana, Michigan, Ontario, British Columbia,[11,9] and Alberta.[15] In all of the surveys, except western Montana, antibody prevalence was 50% or more. The Alberta hare studies provided a unique opportunity to investigate the occurrence of this group of viruses in a well described wild-host population.

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Materials and Methods

The study area has previously been described[10,4] as has the hare population dynamics through 1967.[10]

In general, the hares were live trapped and bled by cardiac puncture. The sera were separated from the clots and held on dry ice or in a —65C Revco refrigerator until all tests had been completed. Inactivated, unhemolysed sera were tested for neutralizing antibodies by means of the HeLa cell metabolic inhibition test. Only those sera which neutralized $10^{1.5}$ to $10^{2.0}$ LD_{50} of the Montana snowshoe hare strain of CEV, as determined in HeLa cells, were considered positive.[7] Virus neutralization tests with hemolysed sera or sera toxic for tissue cultures were performed intracerebrally in suckling mice and only those sera which neutralized 100 to 150 suckling mouse LD_{50} of virus were considered positive.[14]

To ascertain the specificity of the CEV group neutralizing antibodies and to ascertain which of the virus strains the snowshoe hare population might have been exposed, complement fixation (CF) titers were determined on 16 neutralization positive and negative sera from wild caught snowshoe hares[15] and Ouchterlony immunodiffusion tests were conducted on 13 neutralization positive sera from sentinel domestic rabbits.[3] The CEV group reference strains employed included BFS-283, *Trivitattus,* Montana snowshoe hare, Jamestown Canyon and LaCrosse.[12]

Blood clots from various vertebrate species and various types of hematophagous arthropods were collected for virus isolation attempts. The collection and identification of the mosquitoes has been previously described.[4] All materials for isolation attempts were stored on dry ice or at —65C until testing. The blood clots were dispersed by aspiration and expulsion into and from a tuberculin syringe. Ticks were sorted into pools consisting of larvae, nymphs and adults. They were then ground in M-199 containing 10% hypogamma calf serum, penicillin (250 units/ml.) and streptomycin (125 μg/ml.) with a porcelain mortar and pestle. After identification, the mosquitoes were separated into pools of approximately 45 individuals and suspended in media of the same composition as used for the tick pools. The mosquitoes were ground in glass vials with sterile glass beads. The resulting homogenates were inoculated intracerebrally into 1 to 4 day old suckling mice. The inoculated mice were observed for 14 days. Identification of the isolates was by means of neutralization of the infectivity in mice with reference hyperimmune serum or mouse ascitic fluids and /or by means of the Ouchterlony immunodiffusion technique[1,3,12] with reference hyperimmune mouse ascitic fluids. The Ouchterlony technique and the CF test were also used to determine relationships between the isolates and other members of the CEV group.

In order to determine duration and amplitude of viremia, 10 snowshoe hares 16 to 32 weeks old, free of detectable CEV neutralizing antibodies, were inoculated subcutaneously with 10^3 LD_{50} of virus as determined in HeLa cells.[15] Experimental hares were bled from the marginal ear vein at 24 hour intervals for 5 days. Whole blood dilutions were inoculated intracerebrally into 2 to 4 day old suckling mice minutes after drawing. Mice were observed for 10 days. Viruses, when present in brain suspensions prepared from infant mice, were identified by neutralization with reference CEV antisera.

Results

Serological evidence of CEV group activity in adult hares was detected every year from 1961 to 1968, in spite of a marked decrease in the adult population from over 600 hares on a square mile study area in 1961, to 3 by the summer of 1965, then an increase to about 60 in 1968.[10] The prevalence of CEV group neutralizing antibody remained above 58% between 1961 and 1966, and then decreased significantly in 1967 and 1968 (Figure 1). During the first 6 years of the study, the highest monthly prevalence rates occurred in June and July, reaching

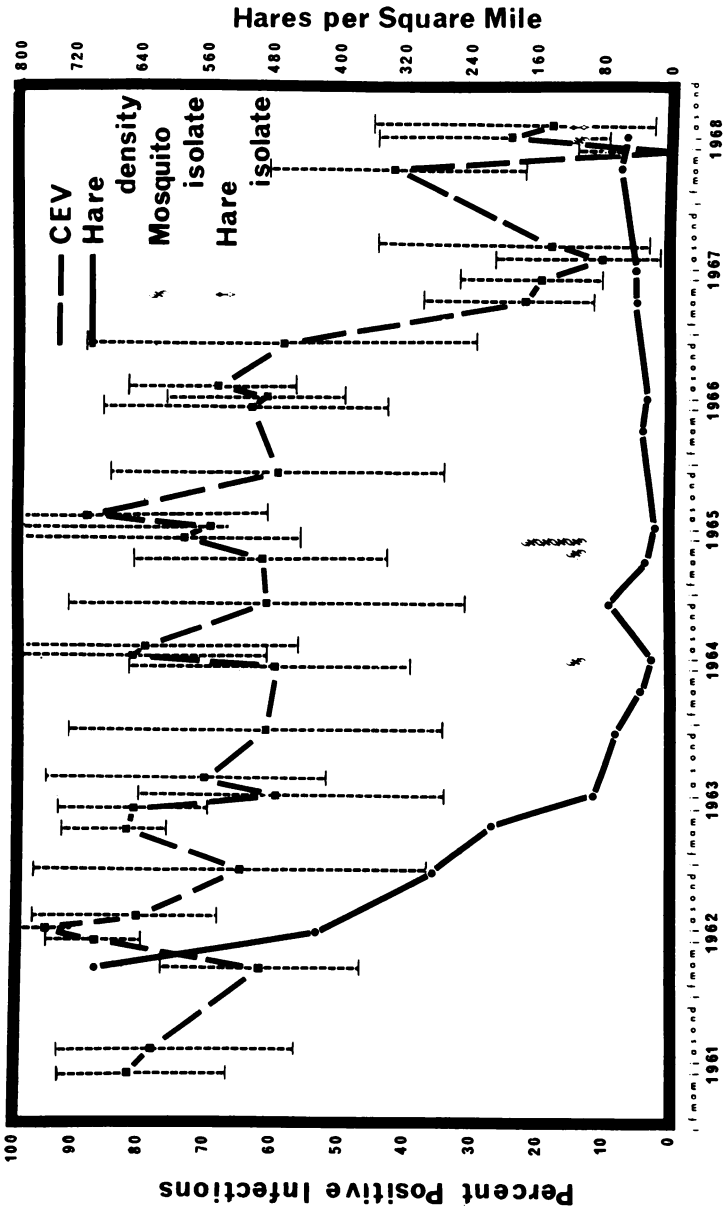

FIGURE 1: Prevalence in adult hares (with 95 percent confidence limits) of California encephalitis virus group neutralizing antibodies

as high as 95%. However, in 1967 and 1968 the highest monthly rates occurred in April and were 25% and 43% respectively. In June 1968, none of the adult hares sampled had demonstrable antibody titers.

Throughout the study, CEV group neutralizing antibody prevalence was lower in juvenile than in adult hares, except in June 1968. Antibody prevalence in juvenile hares appeared to be highest in June and July, although infection of sentinel domestic rabbits in 1965 and 1967 demonstrated virus transmission had occurred throughout the summer (Table 1). There was no detectable increase or decrease in prevalence rates observed with age within litter group cohorts throughout the summer. The effect of maternal antibodies on these rates is not known.

The results of the CF tests on the hare sera and the Ouchterlony immunodiffusion tests on the sentinel rabbit sera suggested that the virus present on the study area was most closely related to the Montana snowshoe hare strain of virus.[15,3]

A total of 8 CEV group viruses have been isolated in 1964, 1965 and 1968[4,3] (Table 2). Seven have been isolated from boreal *Aedes* mosquitoes and 1 from the blood of a snowshoe hare approximately 24 days old. Six of the viruses are most closely related to the Montana snowshoe hare strain while the other 2 are more closely related to the Jamestown Canyon strain. The isolates have been characterized elsewhere.[4,3]

In the experimentally infected snowshoe hares, virus was detected in the blood of 9/10 of the hares with titers up to 10^4 suckling mouse LD_{50} per ml. of whole blood.[15] Virus was recovered up to 3 successive days following exposure. There appeared to be an increase in the length of viremia with age.

TABLE 1. *Cumulative sero-conversions by week of month for CEV in sentinel domestic rabbits, Rochester, Alberta, 1965, 1967.*

Month	June				July				August			
Week	1	2	3	4	1	2	3	4	1	2	3	4
1965	0/4	0/4	1/4	NS*	1/5	NS	2/5	2/5	2/3**	3/3	3/3	NS
1967	NS	NS	0/16	NS	0/16	6/16	NS	7/13	NS	9/11	NS	NS

*NS = not sampled

**denominator decreases due to deaths of sentinel rabbits

TABLE 2. *Isolates of CEV by species; by collecting date; by relationship to other CEV strains; and by investigator; Rochester, Alberta; 1964-1965 and 1968.*

Isolate No.	Species	Collection date	Ouchterlony diffusion relationship	Investigator
M-49	*Aedes* ssp.	13 July, 1964	Jamestown Canyon	Iversen
2188	*A. communis* gr.	27 May, 1965	Snowshoe hare	Iversen
0864	*A. communis* gr.	10 June, 1965	Snowshoe hare	Iversen
1003	*A. communis* gr.	14 June, 1965	Snowshoe hare	Iversen
1792	*A. stimulans* gr.	14 June, 1965	Snowshoe hare	Iversen
0607	*A. communis* gr.	17 June, 1965	Jamestown Canyon	Iversen
F6807-251	*Lepus americanus*	25 July, 1968	Snowshoe hare	Hoff
H57	*A. communis* gr.	8 August, 1968	Snowshoe hare	Hoff

Discussion

The monthly fluctuations in the prevalence rates within years and the possible effects of the viruses on the hare population were discussed in detail previously.[10] It is interesting that while the hare population was declining, the monthly prevalence rates were high, ranging from 58% to 95%, and in the first years of the population recovery, the rates were low, ranging from 0% to 43% (Figure 1).

The decline of the hare population was associated with decreased survival of the adult and especially juvenile rabbits. It seems unusual that high antibody prevalence rates occurred throughout the sharp population decline and then decreased as the hares became more abundant. The high rates associated with the declining population suggest (1) that hare-mosquito-hare transmission remained efficient despite low hare numbers, (2) that the high observed mortality resulted in a very low recruitment of young susceptible animals[10] with a resulting old and largely immune population, (3) that other hosts were the principal reservoirs and hare infection just represented "spill-over," or (4) all or any of the above. That the viruses were present at the low point in the hare cycle is shown by the 6 isolations of CEV group viruses from *Aedes* mosquitoes in 1964 and 1965.[4] The high prevalence rates observed in 1964-1966 may then be largely the product of antibody carry-over plus occasional infection of the susceptible animals.

The low prevalence rates observed with the increasing hare population and the absence of seropositive hares in June 1968, suggests (1) that the virus was no longer present on the study area, (2) that the virus present was not transmitted to the hares, or (3) that recruitment of susceptible animals into the population took place at a rate much greater than that of virus transmission. That the CEV group viruses were present and actively infecting the hares has been shown by the isolation in 1968 of a virus from the blood of a hare less than a month old and a virus from a pool of *Aedes* mosquitoes.[3] It has also been established that juvenile survival from birth to the following April in 1966-1967 and 1967-1968 was 9- to 10-times that which occurred during the first 2 years of the population decline.[6] In 1967 and 1968, the animals born the previous summer comprised 90% to 94% of the population,[10] thereby lending support to the hypothesis that prevalence rates remained low because susceptible hares were being added to the population at a rate faster than that which the virus could be transmitted to them. This would then account for the lower observed antibody prevalence rates in the increasing hare population.

From the experimental viremia studies and the isolation of a virus from the blood of a naturally infected hare, it seems likely that the hares can provide infectious blood meals to mosquitoes. The increase in the length of viremia with age as seen in the experimentally infected hares may be due to either an increase in susceptibility with age or to the disappearance of low levels of non-detectable maternal antibody. The young hares studied were born in captivity to formerly CEV infected wild females.

Thus, snowshoe hares are involved in the maintenance cycle of CEV on the study area. The presence of specific antibody in the population over a long period of time, and the recovery of the virus from a wild caught hare is evidence of frequent natural infection. The isolation of virus from the snowshoe hare by ourselves and others, and the experimental infection trials, suggest that hares serve as sources of infectious blood meals for hematophagous arthropods. Since the snowshoe hare is often one of the most abundant mammals in the boreal forest, it seems likely that it is one of the major reservoir hosts of CEV.

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