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EPIDEMIOLOGY OF *Herpesvirus sylvilagus* INFECTION IN COTTONTAIL RABBITS

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Abstract: The epidemiology of *Herpesvirus sylvilagus* infection in wild cottontail rabbits was studied in a defined, natural cottontail population over a period of 13 months. Spread of this virus showed significant correlation with seasonal variation as well as with the sex and age of the host. The highest rate of infection occurred during the winter and spring seasons with males over the age of 4 months sustaining a significantly greater percentage of infections than younger males or females of all age groups.

INTRODUCTION

Herpesvirus sylvilagus, a new member of the herpesvirus group, has been shown to produce a lymphoproliferative disease in its natural host, the cottontail rabbit, *Sylvilagus floridanus*.[4,6] Description of the characteristics of the virus, as well as the pathologic changes induced by *H. sylvilagus* have been documented.[2–8,15] Information concerning the epidemiology of this agent in nature, however, is limited. The present study was designed to examine the occurrence and distribution of *H. sylvilagus* infection, as indicated by the presence of specific antibody, in a defined, natural cottontail population. This work was carried out as part of a cottontail disease-ecology survey conducted on the Thunderbird Recreation Area, Walworth County, in southeastern Wisconsin from January, 1970, through January, 1971. Data are presented herein on the relationship of season, age, and sex on the prevalence of *H. sylvilagus* infection.

MATERIALS AND METHODS

Thunderbird is a private recreation area and shooting preserve located 3.2 km northwest of Genoa City, Wisconsin. The 134 ha study area was composed of 40% wooded upland, 27% open upland, 27% water and islands, 6% marshy lowland and 1% multiflora rose briers.

All animals were captured in unbaited, single door, live traps[2] (18 x 18 x 50 cm. Blood was collected by cardiac puncture. Sex, weight, hind foot length, and exact location of each animal captured were recorded while in the field. Prior to release each animal was earmarked with a rotary tattoo. A second or third blood sample was taken from those animals subsequently recaptured. Approximate age of each rabbit was determined by comparing weight and hind foot length with data from known-aged cottontails,[3][10] Animals were put in the 1-year or older age group if they were recaptured 11 months or more after their initial capture.

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[3] Rongstad, O. J., unpublished report.

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Infection with *H. sylvilagus* was determined by the appearance of specific antibody to this virus in the serum. Antibody was detected by 50% plaque reduction neutralization in tissue culture using methods previously described.[5] All samples were tested at a serum dilution of 1:10 using 50 to 100 plaque-forming units of virus. Studies in this laboratory have shown that antibody may be detected at this level within one week after virus infection of weanling and adult cottontails and persists in the blood for the remainder of their life.[5]

Statistical examination of the data was conducted by an analysis of variance using orthogonal individual degrees of freedom to examine both the independent and combined influence of season, sex and age on prevalence of infection.

RESULTS

Sera from 135 juvenile and adult cottontails of both sexes were collected during the study period. Thirty-seven animals were recaptured and sampled twice and six of these were recaptured and sampled a third time. Neutralizing antibody against *H. sylvilagus* was detected in 24% (32 of 135) of the animals during the entire observation period. Examination of the influence of season on the prevalence of infection (Table 1) showed that the greatest number of infections occurred during the winter and spring months. The prevalence of infection in the population was at a low of 2% during the summer but rose to 12% in the fall, 29% in winter and a peak of 41% in spring. In the 37 animals sampled more than once there were 10 conversions

TABLE 1. Monthly prevalence of antibody to **H. sylvilagus** in cottontail rabbits from January, 1970 to January, 1971.

	Month												
	J	F	M	A	M	J	J	A	S	O	N	D	J
No. Tested	34	0	5	22	0	7	0	13	33	20	7	25	12
No. Pos.*	6	0	2	9	0	1	0	0	0	1	0	6	7
% Pos.*	18	0	40	41	0	14	0	0	0	5	0	24	58

*Pos. = neutralizing antibody.

TABLE 2. Influence of age and season on prevalence of antibody to **H. sylvilagus** in cottontail rabbits.

Age (Months)	Spring		Summer		Fall		Winter	
	M*	F	M	F	M	F	M	F
No. Tested								
1-3	0	0	10	10	4	3	0	0
4-11	15	11	11	11	19	25	16	25
>11	1	0	2	3	2	6	2	2
No. Pos.**								
1-3	0(0)**	0(0)	0(0)	0(0)	1(25)	0(0)	0(0)	0(0)
4-11	7(47)	3(27)	1(9)	0(0)	2(11)	2(8)	6(38)	3(12)
>11	1(100)	0(0)	0(0)	0(0)	2(100)	0(0)	2(100)	2(100)

*M = male, F = female.

**Pos. = neutralizing antibody.

***Figure in parentheses is percent with positive antibody.

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from negative to positive antibody. Fifty percent of these conversions occurred between 22 January and 4 April.

The combined influence of seasonal variation, age and sex on the prevalence of infection is shown in Table 2. Seasonal variation exerted a significant influence on the prevalence of infection in both males ($P < 0.01$) and females ($P < 0.05$) with each sex showing a peak of infection during the spring season. During the seasons of maximum virus transmission, winter and spring, males showed a significantly higher rate of infection than females ($P < 0.05$). An analysis of the influence of age on the prevalence of antibody in males and females showed a direct correlation between increasing age and prevalence of infection in males ($P < 0.01$) but not in females ($P > 0.05$).

Most of the sera (80%) included in this study were collected in two areas where the fall population densities of cottontails were similar, i.e., more than 5 rabbits per ha. The remaining 20% of the samples were collected in an area of population density less than 2.5 rabbits per ha. Population estimates in these areas were determined by the Lincoln-Peterson Index and the capture-efficiency method[1] as modified by Meslow and Keith.[13] Comparison of the rate of infection between the areas of high and low population densities showed no significant difference ($P > 0.05$).

DISCUSSION

The natural spread of *H. sylvilagus* among cottontail rabbits appears to be coordinated with seasonal variations. In animals captured during summer and fall the prevalence of antibody to this virus was significantly lower (8 of 106) than during winter and spring (24 of 72). This may be explained, in part, by the recruitment of young seronegative animals into the population since the age distribution of animals captured during August and September indicated that the majority of cottontails in the population at this time were less than 4 to 5 months old.

The winter home range of male cottontails in the study area is larger than that of females[9] and would account for a correspondingly greater probability for contact and virus transmission between males. The mode of transmission of *H. sylvilagus* among rabbits is not presently known; however, the results reported here suggest that age and season may be significant factors. Two mechanisms for transmission warrant consideration in light of this study. One possibility involves an insect vector, the common rabbit flea (*Cediopsylla simplex*). This ectoparasite is found on cottontails throughout the year; however, the rate of flea infestation is highest in cottontail populations during the winter and spring seasons.[14] The concentration of cottontails during this time of year into habitats offering escape cover and a source of food also would increase the chance of contact and possible exchange of fleas. A second possible mechanism of transmission for this virus is by direct contact. Interactions between male-male and male-female cottontails are high during the late winter and spring breeding season.[11,12] This increase in social activity during the breeding season could also enhance the possibility of exchange of virus between rabbits.

Hinze[6] found the incidence of severe lymphoma-like disease among juvenile cottontails inoculated with *H. sylvilagus* was 27% compared to 10% of all inoculated adults. The significance of a high rate of mortality among young animals infected with this virus has not been determined, but may also lead to the rapid elimination of a greater percentage of that age group and their consequent exclusions from such a survey.

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