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Herpesvirus sylvilagus IN COTTONTAIL RABBITS: ATTEMPTED LABORATORY TRANSMISSION BY TWO INSECT SPECIES[1]

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Abstract: The vector potential of the rabbit flea (*Cediopsylla simplex*) and a mosquito (*Aedes triseriatus*) was investigated for *Herpesvirus sylvilagus* transmission among cottontail rabbits (*Sylvilagus floridanus*). Twelve groups of 12-50 fleas were fed on three viremic cottontails for 2-21 days before transfer to 12 susceptible rabbits. Standard interrupted feeding trials employed five groups of 6-12 mosquitoes, two viremic donor cottontails and five healthy recipients. No evidence of virus was detected from recipients' blood nor did they develop specific antibody. Virus acquisition and persistence in the insects was evaluated by attempting to recover the virus from 19 pools of mosquitoes engorged on viremic blood and 36 pools of engorged fleas or those living on viremic hosts for 1-21 days. Results were negative.

INTRODUCTION

Herpesvirus sylvilagus is a carcinogenic agent which establishes a chronic, low-titer viremia in cottontails, probably for life.[4] Pilot experiments by Hinze (pers. comm., 1972) failed to demonstrate that cottontail herpesvirus (CHV) was transmitted by close contact of caged cottontail rabbits. Recent serologic studies of two cottontail populations in Wisconsin indicated that CHV transmission was occurring during winter[5] (H. C. Hinze, pers. comm.). Concomitant increase of rabbit flea burdens in winter have suggested fleas as potential vectors. It is unknown whether mosquitoes are capable of spreading or maintaining the virus in summer. This study tested the ability of *Cediopsylla simplex* and *Aedes triseriatus* to transmit CHV from viremic cottontails in laboratory feeding trials. Attempts were made to recover the virus from pools of engorged insects.

MATERIALS AND METHODS

Cell culture methods for virus and antibody assay and information on sources of CHV and host cottontails were reported previously.[5]

A preliminary test for flea-borne transmission of CHV was done. *C. simplex* fleas were collected from a winter population of cottontails. Viremia in the donor rabbit measured 15 plaque-forming units (PFU) per 0.5 ml 30 days following CHV inoculation. Identity of the recovered virus was confirmed by neutralization with specific antiserum. The donor was exposed to 100 starved (two days) *C. simplex* fleas. Two, 4, 8, and 15 days later, 83 exposed fleas were recovered and divided into groups of 12, 13, 25 and 33 fleas (Table 1). Each group was transferred to a susceptible (CHV antibody negative) cottontail. A control cottontail was infested with 12 fleas which had no known contact with the virus. Experimental and control recipients were held in screened cages. Blood samples from each rabbit were tested for CHV and specific antibody 14, 30 and 60 days following flea infestation.

The above experiment was repeated using two viremic donors with viremias of 19 and 20 PFU per 0.5 ml. Pools of 25 and 50 fleas were placed on these donor rabbits for 7, 14 and 21 days. A

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total of 300 fleas were exposed to the rabbits. After removal from the donor rabbits, fleas were placed on 5 susceptible (CHV antibody-negative) cottontails. Fifty fleas were fed on three CHV antibody-free cottontail controls. Recipients were held for 60 days and sampled as above.

The ability of mosquitoes to transmit CHV was tested using standard interrupted feeding technique. Colony-reared, eight-day-old *A. triseriatus* were divided into three groups of 12, two groups of six and a control group of three specimens (Table 1). Mosquitoes were individually fed on a viremic donor cottontail doe (viremia: 23 PFU per 0.5 ml) through the nylon mesh of 3 x 2.5 x 16 cm Horsfall feeding cages.[6] Each group finished its bloodmeal on a separate CHV-negative recipient cottontail. After 14, 30 and 60 days, recipients were tested for evidence of CHV infection as in the flea trials.

TABLE 1. Attempts to transmit cottontail herpesvirus with fleas (**Cediopsylla simplex**) and mosquitoes (**Aedes triseriatus**).

Infected donor no. and sex	No. days on host	Recipient no. and sex	No. insects
Flea experiment 1			
29F[a]	2	3M	13
29F	4	2F	13
29F	8	53F	12
29F	15	6M	25
None (control)	15	9F	12
Flea experiment 2			
13F[b]	7	8M	50
19M[c]	14	17M	50
19M	14	23F	50
19M	21	25F	50
13F	21	9F	50
None (control)	14	25F	25
None (control)	21	9F	25
Mosquito experiment	Feeding Route		
290F[d]	Ear	1F	12
290F	Thigh	5M	12
290F	Thigh	4F	12
290F	Ear	29M	6
290F	Ear	39M	6
None (control)	Thigh	7F	3

[a] viremia: 15 PFU per 0.5 ml
[b] viremia: 20 PFU per 0.5 ml
[c] viremia: 19 PFU per 0.5 ml
[d] viremia: 23 PFU per 0.5 ml

TABLE 2. Attempts to recover cottontail herpesvirus from fleas (**Cediopsylla simplex**) and mosquitoes (**Aedes triseriatus**) fed on viremic cottontails.

		Flea trials		Mosquito trials[b]			
Host no. and sex	Viremia PFU/0.5 ml	No. days on host	Pool N	No pools tested	Days post feeding	Pool N	No pools tested
291 F	14	4	6	3	4	6	2
290 F	23	1	6	3	1	6	2
290 F	23	0[a]	4,1	5,7	0(1hr)	6	2
13 F	15	14	4	3	4	2	1
13 F	15	7	4	4	1	2	2
19 M	20	3	4	3	0(1hr)	2,1	3,7
19 M	20	1	3,1	3,5	control-0 (1hr)	4	2
20 M	0(control)	7	4			4	2

[a] triturated immediately after they engorged

[b] cottontail nos. 290, 291 used as viremic hosts, no. 20 as control

To investigate CHV uptake and viability in arthropods, inocula were prepared from engorged mosquitoes and fleas exposed to viremic cottontails (Table 2). Group pools of the insects were triturated using individual Ten Broeck tissue grinders, each in two ml Medium 199 with antibiotics (1000 units penicillin, 500 μg streptomycin, 50 μg Fungizone,®[2] all amounts per ml) and inoculated onto rabbit kidney cell culture.

RESULTS AND DISCUSSION

No evidence of CHV transmission was detected, nor was virus recovered from engorged insects in these experiments.

This study is similar to an unsuccessful attempt to transmit *Herpesvirus saimiri* (HSV) by four blood-sucking insect species.[2] Attempts to transmit two other herpes viruses, Marek's disease,[1] and bovine herpes mammalitis[3] viruses, by arthropods also were reported as unsuccessful.

The failure of the mosquitoes and fleas to transmit HSV may have been due to the relatively low viremia present in the infected cottontails. CHV viremias at the time of insect feeding were 28 to 46 infectious units per ml. This is similar to the viremias found in chronic HSV of the natural host, the squirrel monkey. The small bloodmeal volume taken by *A. triseriatus* (approximately 1.8 mg) and by fleas (approximately 0.1 mg) indicates that only 1 in 14 mosquitoes and 1 in 235 fleas would have acquired an infectious unit of CHV. The feeding frequency of *C. simplex* is unknown. They were on the infected cottontails for up to 21 days, and any refeeding would have increased the probability of virus ingestion. Since 76 mosquitoes and 412 fleas were used in these experiments, a few of them should have obtained the virus. Failure to recover CHV from these insects or to demonstrate transmission suggests that virus replication in the arthropods did not occur, nor was the virus transmitted mechanically. Thus, the likelihood that these arthropods play a significant role in the natural transmission of CHV seems to be negligible, and other arthropods or an alternative mechanism to account for transmission must be sought.

[2] E. R. Squibb and Sons, Inc., Princeton, New Jersey 08540, USA.

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