Arch Virol (1987) 94: 159–162

Archives of Virology
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Isolation and Partial Characterization of an Orthopoxvirus from a California Vole *(Microtus californicus)*

Brief Report

By

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With 1 Figure

Accepted November 7, 1986

Summary

A virus with the characteristics of an orthopox was isolated from a scab removed from the foot of a California vole. HAI antibody surveys indicated that the virus in enzootic in several different vole populations.

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Orthopoxviruses have been isolated from rodents indigenous to Germany (4), Russia (7) and West Africa (6) and serologic evidence suggests that viruses of this type have infected English (5) and Canadian (3) rodents. However, no cases of rodent orthopoxvirus isolations have been reported from the Western Hemisphere. In fact, the only well documented cases of orthopoxvirus infections of wild mammals in the New World involve raccoons *(Procyon lotor)* (1) and mule deer *(Odocoileus hemionus)* (8) as hosts. This paper presents a description of an orthopox isolated from a California vole *(Microtus californicus)* and evidence that the virus is enzootic in several vole populations.

The vole virus was first isolated in June, 1985 from a small scab removed from the hind foot of a healthy appearing, young (21 gram) vole. The scab was ground with sand in phosphate buffered saline and, after penicillin (10^8 units/ml) and streptomycin (.02 mg/ml) were added, the extract was inoculated on to the chorioallantois (CAM) of eggs that had been incubated for 12 days at 39° C. When the inoculated eggs were incubated at 35° C for an additional three days, small white pocks

developed. One of the pocks which was isolated from the others was removed along with a portion of the membrane and used as the initial source of vole virus for the remainder of this investigation.

At 35° C the pocks produced after three days on CAM were 0.7–1.0 mm in diameter and had concave surfaces. When cultured for three days at 39° C each pock was about 0.5 mm and dome shaped. At 40° C no pocks developed. Vole pox virus treated with ethyl ether for four hours at 4° C produced about half as many pocks on a CAM as did untreated controls.

Day-old *Mus musculus* (either C 3 H or Balb C) inoculated intracerebrally with 20–50 PFU of vole virus stopped eating on day two or three post inoculation and, when not euthanized, died a day or two later. Similar mice inoculated subcutaneously with the same dosage died on day five or six. No obvious signs were produced in five-day old mice that were inoculated subcutaneously with small amounts of vole virus.

A hemagglutinin extracted from infected mouse brains or chorioallantoic membranes agglutinated the same cardiolipin sensitive fowl cells as did the hemagglutinins of vaccinia (Mill Hill strain) or raccoon pox. Antibodies produced by experimentally or naturally infected voles inhibited the vole pox

Table 1. *Hemagglutination-inhibition antibody titers of individual vole sera using volepox, vaccinia, and raccoonpox viruses*

Vole sera			Hemagglutinins		
Capture site	Date	Vole No.	Vole pox	Vaccinia (VHM)	Raccoon pox
JR[a]	1984	79	5120[b]	20	40
JR	1986	202	2560	40	160
W	1985	40	640	80	80
CM	1985	1	640	20	40
CM	1985	2	640	20	40
JR	1984	60 A	320	80	40
JR	1984	75	320	20	0
JR	1984	19	320	10	20
JR	1984	85	320	0	20
PR	1986	1	160	80	80
JR	1984	86	160	80	40
ANP	1984	2	160	10	40
JR	1984	76	160	10	0
JR	1984	22	160	0	40
W	1984	2	160	0	0
ALV	1986	2003	80	10	40
JR	1984	26	80	0	40
JR	1986	1000 J	0	0	0

[a] *ALV* Alviso, *ANP* Año Nuevo Point, *CM* Corte Madera Creek, *JR* Jasper Ridge, *PR* Point Reyes, *W* Westridge

[b] Reciprocal of highest inhibiting dilution

hemagglutinin and to a lesser extent the hemagglutinins of vaccinia and raccoon pox (Table 1).

The intradermal injection of 0.1 ml of a 10^4 PFU/ml suspension of vole virus into a domestic rabbit resulted in the development of a slightly raised, pink lesion which by day four was 15 mm in diameter. The lesion gradually regressed so that by day nine the lesion was scarcely discernible.

Two types of experimental viral transfers between voles were performed. The first was achieved by probing a lesion with a sharp pin and then probing the foot or tail of a seronegative vole with the same pin. The object of this procedure was to simulate mechanical transmission by an arthropod. The second typed of transfer consisted of the subcutaneous injection of CAM-passaged virus into the tail and foot. With both types of transfers lesions developed six to nine days after the transfer attempt. In such experimentally infected voles hemagglutinin inhibiting (HAI) antibodies appeared between one and two weeks, increased to a maximum titer of 160–5120 by four weeks post-inoculation and gradually waned in the course of the next two months.

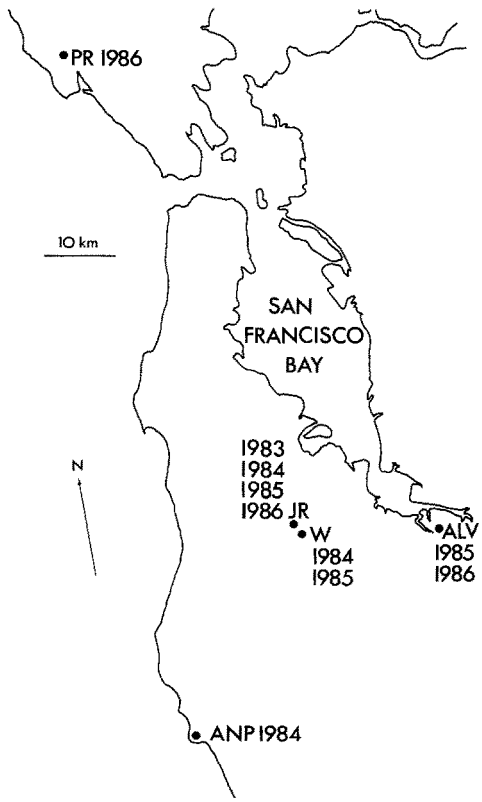

Fig. 1. Geographical and temporal distribution of voles with HAI antibody titers greater than 80. Abbreviations are the same as those in Table 1

The combination of characteristics of the vole-derived virus place it in the orthopox category. At the same time one or more features of the vole virus distinguish it from other known orthopoxes. It should prove interesting to determine the degree of relationship of the vole virus with other orthopoxes, particularly with such viruses as ectromelia (mouse pox) and the cowpox-like viruses of Russian rodents (7) and English cats (2).

As a result of previous serum surveys it was possible to test vole sera obtained at different times and places for antibodies to vole pox hemagglutinin. An analysis of these data will not be considered here expect to point out that antibodies were found in voles trapped in geographically separated populations and in some of those populations for two or more years (Fig. 1).

The widespread and disjunct distribution of infected voles suggests that there has been a long standing association between vole and virus. What role the virus plays in the population dynamics of the vole remains to be determined.

Acknowledgments

Many thanks are due to Adeline and Bruce Jessup who live-trapped several of the voles in which the infection was first noticed. Professor Hans Abplanalp's gift of fowl with cardiolipin sensitive erythrocytes was crucial to many facets of the study. Roberta Regnery prepared the plate. It was a pleasure to work with these individuals.

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Received September 28, 1986