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NATURAL AND EXPERIMENTAL ARBOVIRAL INFECTIONS IN A POPULATION OF BLACKTAIL JACKRABBITS ALONG THE SACRAMENTO RIVER IN BUTTE COUNTY, CALIFORNIA (1971-1974)[1]

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Abstract: A serologic survey of the blacktail jackrabbit *(Lepus californicus)* for infections with 10 arboviruses was conducted from 1971 through 1974 along the Sacramento River in Butte County, California. Of 325 animals captured and bled a total of 493 times, 40% were found positive for hemagglutination-inhibiting (HI) antibody to California encephalitis (CE) virus, 34% to western equine encephalomyelitis (WEE) virus, 20% to Buttonwillow virus, 15% to St. Louis encephalitis (SLE) virus and 12% to Main Drain virus. Only 5 and 2% of the animals had HI antibodies to Lokern and Turlock (TUR) viruses, respectively. There was no serologic evidence for infection of animals with Powassan, Modoc and Rio Bravo viruses. Differenles in monthly and yearly activities of some viruses were found by analyses of lata on antibody prevalence rates and immunologic conversions in recaptured animals.

Experimental studies revealed that subadult jackrabbits were highly susceptible by subcutaneous inoculation to infection with CE, WEE and SLE viruses but were refractory to infection with TUR virus. All animals infected with CE and WEE viruses developed viremia that persisted for 2 or 3 days after inoculation and then developed antibodies that were detectable from 7 through at least 56 days after infection. In contrast, only 2 of 7 animals that developed HI antibodies to SLE virus had viremia, and at barely detectable levels; and HI antibodies were undetectable in 3 of the 7 animals at 56 days after infection.

INTRODUCTION

Studies on the ecology of arboviruses in Kern County, California, documented the importance of the blacktail jackrabbit *(Lepus californicus)* in the primary transmission cycles of several *Culicoides*-transmitted viruses, including Buttonwillow (BUT), [13,15] Lokern (LOK)[1,8] and Main Drain (MD).[1] In contrast, the same studies indicated that jackrabbits played little or no role in the summer transmission cycles of 2 mosquito-borne viruses, i.e., western equine encephalomyelitis (WEE) and St. Louis encephalitis (SLE).[14,27] This latter conclusion was supported by the finding that *Culex tarsalis*, the epidemic vector of WEE and SLE viruses in the western United States,[27] fed infrequently on jackrabbits as compared to wild birds in Kern County.[30]

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More recent serological surveys for arboviral infections in wildlife of California have demonstrated that jackrabbits in the Sacramento Valley frequently were infected with WEE and California encephalitis (CE) viruses (authors' unpublished observations). In fact, considerable evidence was accumulated to indicate that an *Aedes melanimon* — jackrabbit transmission cycle of WEE virus coexisted along with the usual *C. tarsalis* — wild bird cycle.[12]

This paper reports an intensive capture-tag-release study that was undertaken to study mosquito-borne arboviral infections in a jackrabbit population along the Sacramento River. Experimental infection studies were also done to more clearly define the role of jackrabbits in the transmission cycles of selected arboviruses.

MATERIALS AND METHODS

Jackrabbits were collected from 1971 through 1974 at the Llano Seco Rancho, which is situated on the Lower Sonoran floodplain of the Sacramento River about 16 km southwest of Chico in Butte County, California. Animals were live-captured at night from a moving truck using a spotlight and salmon nets with 3.05-m handles. Netting usually was done during one week of each month from May through October in 1971 through 1973 and weekly in the summer of 1974. Animals were banded with ear tags upon initial capture, bled by cardiac puncture on each capture for serum samples (1 and 3 ml of blood from young and adult animals respectively), and released. Data on tag number, date of capture, sex and maturity status (young-of-year or adult) were recorded on all new and recaptured animals.

In 1974, pregnant does were taken to the field station in Chico where they were allowed to give birth to young in captivity. The does were released at the field capture site 2 weeks postpartum and the offspring were transported to Bakersfield in Kern County for experimental infection studies when 4 to 6 weeks of age.

Subadult animals were inoculated subcutaneously with an estimated 5,000 plaque forming units (PFU) of either WEE, SLE, CE or Turlock (TUR) virus. Control animals were inoculated similarly with the viral diluent which consisted of 20% heat-inactivated (56 C, 30 min) fetal bovine serum (FBS) in phosphate buffered saline at pH 7.4. All viruses were isolated originally from mosquito pools collected in 1971 or 1972 at the study site or nearby areas in the Sacramento Valley and had undergone 2 intracerebral passages in suckling mice. An aliquot of each viral inoculum was saved and stored at −70 C for back titration. Whole blood samples were obtained for viremia determinations by cardiac puncture from control and infected animals at daily intervals from 1 through 10 days after inoculation and stored at −70 C until tested. Undiluted serum samples were collected for antibody determinations at 0, 7, 14, 21, 28 and 56 days postinoculation.

Viremic bloods were titrated by plaque assay in primary cultures of duck embryonic (DE) cells for WEE and TUR viruses and in Vero cell cultures for SLE and CE viruses. Cell cultures were grown in 60 mm plastic petri dishes at 36 C in a humidified atmosphere of 5% CO_2 in air. Growth medium consisted of 5% FBS in autoclavable Eagle's Minimal Essential Medium prepared in Earle's balanced salt solution plus antibiotics. Overlay medium for plaque assay was similar except it contained only 2% FBS plus 1% purified agar to plaque CE, WEE and TUR viruses or 1.5% methylcellulose to plaque SLE virus. Diethylaminoethyl - dextran (0.02 gm%) was added to the agar overlay medium to improve plaquing of CE virus.

Antibodies in sera from animals collected in the field or experimentally infected with WEE and SLE viruses were measured by the microtiter hemagglutination-inhibition (HI) test using the basic procedure described by Clarke and Casals.[5] An HI titer of 1:20 or greater was considered positive.

Sera from animals experimentally infected with CE and TUR viruses were tested for serum-dilution neutralizing (SDN) antibodies by plaque reduction in microcultures of Vero and DE cells respectively.[7] The SDN endpoint titer was

taken as the highest dilution of serum giving an 80% or greater reduction of a challenge dose of 50 PFU of virus. A SDN titer of 1:5 or greater was considered positive.

RESULTS

Jackrabbit population analysis

From 1971 through 1974, 329 tagged jackrabbits were captured a total of 501 times during 36 sampling periods. The number of animals captured in each sampling period was small, ranging from 6 to 33 with an average of 14. Maturity status and sex were determined on 327 animals. Of these, 170 were classified as adults and 157 as young-of-year. The ratio of males to females was nearly 1:1 for young animals. The adults, however, included a significant preponderance of females (108 females to 62 males). Adult pregnant females were collected in each month with the peak proportion of pregnant females occurring in July (20/32 or 63%). Adult females apparently mated more than once each summer. Five of the females that were pregnant when captured in late May or early June of 1974 and released in the field 2 weeks after littering in captivity were pregnant again when recaptured later the same summer.

One hundred (or 30%) of the 329 tagged animals were recaptured at least once (Table 1) and 15 of these were retaken 3 to 8 times. The average number of recaptures for these 100 jackrabbits was 1.7. The length of time between the first and final captures varied from 1 week to 39 months. Of the 100 recaptured animals, 52 were banded prior to 1974 and 34 (or 65%) of these were recaptured 1 or more years after banding. Using only the data from the 34 jackrabbits that were known to survive at least 12 months, an estimated average annual survival rate of .486 was calculated by means of the FORTRAN computer program for maximum likelihood estimation developed by Roberts.[28] No statistically valid estimate of the total jackrabbit population could be made from the data obtained.

Arboviral infections in jackrabbits

Natural infections: Results of HI tests for antibodies to 10 arboviruses in 493 sera from 325 jackrabbits are summarized in Table 2 both as percent of sera and percent of animals positive. The ranking of viruses by percent positive samples was the same by both methods of analysis. Jackrabbits were infected most frequently with CE (40%) and WEE (34%) viruses followed by BUT (20%), SLE (15%) and MD (12%) viruses. Relatively few animals were positive for antibodies to LOK and TUR viruses. Three sera reacted with Powassan (POW), Modoc (MOD) or Rio Bravo (RB) antigens but each reacted at equal or higher

TABLE 1. Number of **Lepus californicus** tagged and later recaptured at the Llano Seco Rancho, Butte County, California.

Year	Number Tagged	Number Subsequently Recaptured	Number known to be alive* (and number actually captured) in		
			1972	1973	1974
1971	77	13	8(5)	4(2)	3(3)
1972	96	24	—	16(11)	8(8)
1973	50	15	—	—	10(10)
1974	106	48	—	—	—
Total	329	100			

*Determined by a recapture in a subsequent year.

titers with SLE antigen. As expected, antibody prevalences to most viruses were significantly higher in sera from adult than from young-of-year animals. No significant differences between males and females were observed for antibody prevalences to any virus.

Monthly and yearly antibody prevalences in sera from young-of-year animals were examined to obtain information on the times when each virus was active (Table 3). Concurrent studies had demonstrated that maternal antibodies to the arboviruses being studied were rarely demonstrable by HI tests in sera obtained from juvenile animals later than 14 days after birth (authors' unpublished data). Therefore, it was assumed that antibody in these sera represented a recent infection rather than transfer of maternal antibody since most young animals were more than 1 month old when initially captured. Interpretation of these data still must be tempered with caution since juvenile and subadult animals were not differentiated in this study.

The data suggested that CE virus was most active in 1971 and 1973 and that young animals first became infected with CE virus in late spring (Table 3). WEE viral activity in jackrabbits was highest in 1971. Antibodies to the other 2 mosquito-borne viruses, SLE and TUR, were found comparatively infrequently in sera from young animals.

Of the 3 *Culicoides*-transmitted viruses, BUT appeared to be the most active, albeit at low levels (Table 3), and young animals first became infected with BUT virus in late spring. MD viral activity was confined primarily to 1972, and in contrast to BUT virus, most young jackrabbits acquired infection in late summer. LOK viral activity occurred at minimal levels.

Fifty-nine jackrabbits that were initially HI negative were found to be HI positive to 1 or more arboviruses when subsequently recaptured. Of these, 29 were known to convert immunologically within a 2 month period. The data indicated that rabbits were being infected with SLE virus in late summer. Too few animals developed HI antibodies to the other viruses to warrant any conclusions.

Experimental infections: All experimental animals became infected (i.e., developed viremia and/or antibody) after inoculation with CE, SLE or WEE virus whereas none developed SDN antibody after inoculation with TUR virus. Viremia was detected in all 7 animals inoculated with WEE virus from 1 to 3 days after inoculation and peak viremia titers usually occurred on day 2 (Table 4). Similarly, viremia was demonstrated in all 7 animals inoculated with CE virus but usually was of shorter duration and lower titered than was observed with WEE virus. Although all 7 animals inoculated with SLE virus developed HI antibody, viremia was barely detectable and in only 2 animals.

HI and SDN antibodies to WEE and CE viruses respectively were demonstrable in sera from all animals at 7 days postinoculation and persisted in all animals for at least 8 weeks (Table 5). Peak WEE and CE antibody titers occurred at 21 to 28 days after inoculation and then decreased approximately 2-fold by 8 weeks after inoculation. HI antibody to SLE virus developed more slowly than antibodies to CE and WEE viruses, as only 1 of 7 animals was seropositive 7 days after inoculation, and was relatively low titered. Further, HI antibody to SLE virus was undetectable in 3 of 7 animals at 8 weeks after inoculation, which resulted in a 6-fold decrease in geometric mean antibody titers between 4 and 8 weeks after infection. Cross-reactions with heterologous flaviviruses (RB, MOD and POW) were low titered.

DISCUSSION

Data obtained from a serologic survey from 1971 to 1975 demonstrated that jackrabbits along the Sacramento River in Butte County, California, were being infected with at least 7 arboviruses. HI antibodies to CE and WEE viruses were detected in sera of 34-40% of the animals and to BUT, SLE and MD viruses in 12-20% of the animals. Jackrabbits rarely were infected with LOK and TUR viruses whereas there was no serologic evidence that they were being infected with POW, MOD or RB virus.

TABLE 2. HI antibodies to arboviruses in sera of **Lepus californicus** collected at Llano Seco Rancho, Butte County, California, 1971-1974*.

Viral Antigen	No. (%) Sera Positive** by HI Test			No. (%) Animals Positive**
	Total	Adults	Young-of-year	
Total Tested:	493	287	206	325
CE	169(34)	129(45)	40(19)	131(40)
WEE	143(29)	124(43)	19(9)	109(34)
BUT	71(14)	52(18)	19(9)	64(20)
SLE	59(12)	53(18)	6(3)	49(15)
MD	44(9)	36(13)	8(4)	40(12)
LOK	21(4)	19(7)	2(1)	17(5)
TUR	8(2)	4(1)	4(2)	8(2)

* Three sera that were positive for antibody to SLE virus were positive at lower or similar levels when tested against POW, MOD or RB antigens.

** HI titer of 1:20 or greater considered positive.

TABLE 3. Monthly and yearly antibody prevalence to 7 arboviruses in young-of-year **Lepus californicus** at Llano Seco Rancho, Butte County, California.

Viral Antigen	No. (%) Animals Positive by HI Test							
Month:	May	June	July	Aug.	Sept.	Oct.	Nov.	Total
No. of Animals:	10	17	33	60	58	5	3	186
CE	0	3(18)	10(30)	16(27)	9(16)	1(20)	0	39(21)
WEE	2(20)	0	3(9)	6(10)	6(10)	2(40)	0	19(10)
BUT	0	3(18)	6(18)	6(10)	3(5)	0	1(33)	19(10)
SLE	0	0	1(3)	3(5)	1(2)	1(20)	0	6(3)
MD	0	1(6)	0	0	6(10)	1(20)	0	8(4)
TUR	0	1(6)	1(3)	2(3)	0	0	0	4(2)
LOK	0	1(6)	0	1(2)	0	0	0	2(1)

Year:	1971	1972	1973	1974	Total
No. of Animals:	27	39	25	63	154
CE	13(48)	4(10)	11(44)	9(14)	37(24)
WEE	11(41)	4(10)	2(8)	2(3)	19(12)
BUT	3(11)	2(5)	2(8)	12(19)	19(12)
SLE	1(4)	2(5)	0	3(5)	6(4)
MD	1(4)	7(18)	0	0	8(5)
TUR	1(4)	1(3)	0	2(3)	4(3)
LOK	1(4)	0	1(4)	0	2(1)

TABLE 4. Viremia titers in subadult **Lepus californicus** inoculated subcutaneously with WEE, CE or SLE virus*.

Virus (Strain)	Animal Number	Viremia Titers by Days Postinoculation 1	2	3	4
WEE (BFN 3060)	1247	*1.9***	*5.0*	*3.8*	<1.0
	1248	*3.0*	*3.9*	*1.7*	<1.0
	1257	*4.7*	*5.4*	<1.0	<1.0
	1258	*4.2*	*6.6*	*3.9*	<1.0
	1259	*4.2*	*4.8*	*2.0*	<1.0
	1285	*4.3*	*5.6*	*3.2*	<1.0
	1286	*4.5*	*6.5*	*4.6*	<1.0
	$\bar{X}$ titer	*3.8*	*5.4*	*2.9*	<1.0
CE (BFN 3894)	1288	*1.2*	*3.7*	*2.6*	<1.0
	1293	*2.5*	*3.2*	*2.2*	<1.0
	1303	*2.5*	*3.2*	<1.0	<1.0
	1305	*2.3*	*3.6*	<1.0	<1.0
	1326	*2.9*	*3.0*	<1.0	<1.0
	1330	<1.0	*2.8*	<1.0	<1.0
	1348	*2.6*	*2.7*	<1.0	<1.0
	$\bar{X}$ titer	*2.0*	*3.2*	<1.0	<1.0
SLE (BFN 4585)	1132	<1.0	<1.0	*1.0*	<1.0
	1231	<1.0	<1.0	*1.0*	<1.0
	$\bar{X}$ titer***	<1.0	<1.0	<1.0	<1.0

* The $\log_{10}$ PFU of virus inoculated was 3.5, 4.5 and 3.0 for WEE, CE and SLE viruses respectively.

** Expressed as $\log_{10}$ PFU/0.1 ml.

*** Virus was not detectable in bloods of 5 other animals that were inoculated with SLE (BFN 4585) virus and that subsequently developed HI antibody to SLE virus.

TABLE 5. Geometric mean antibody titers in subadult **Lepus californicus** following subcutaneous inoculation with WEE, CE or SLE virus.

Virus (Strain) Inoculated	Type of Test	Antigen	GM Antibody Titers by Weeks Postinoculation 0	1	2	3	4	8
WEE (BFN 3060)	HI	WEE	0*	417	951	2100	2826	1560
CE (BFN 3894)	SDN	CE	0	780	951	1280	1280	580
SLE (BFN 4585)	HI	SLE	0	2	44	40	49	8
	HI	RB	0	0	6	9	9	2
	HI	MOD	0	0	3	6	3	7
	HI	POW	0	0	2	5	2	2

*Expressed as the reciprocal of the serum dilution.

We are reasonably certain that the antibody to the California group of arboviruses resulted from infections with a CE serotype virus.[18] This conclusion was supported by the isolation of 46 strains of CE serotype virus from pools of *Ae. melanimon* collected in or near the study area during the same time period and the finding that this mosquito frequently fed on jackrabbits (authors' unpublished data). A jackrabbit — *Ae. melanimon* transmission cycle for CE virus was further supported by evidence that subadult jackrabbits were susceptible to infection with a recent CE isolate from *Ae. melanimon* and that all animals developed viremia of moderate titers, which should be sufficient to infect susceptible mosquitoes.[26] This latter point, however, needs to be further documented to unequivocally establish this transmission cycle. Taken collectively, these data confirm and greatly extend the initial observations made with CE virus in Kern County some 30 years ago.[9,10] Further, and of considerable interest, is the fact that the results of our studies on CE viral infections in blacktail jackrabbits are remarkably similar to those obtained for infection of *Lepus americanus* in southern Canada and northern United States with snowshoe hare virus,[19,21,23,24,25,32,33] another serotype of the California group of arboviruses.[18]

The finding that jackrabbits in the Sacramento Valley were being infected with WEE virus was not unexpected. Antibody to WEE virus has been demonstrated with varying degrees of frequency in sera of blacktail jackrabbits from numerous areas of the western United States [2,6,14,20,22,27] as well as in sera from snowshoe hares in Alberta, Canada.[19,21,32,33] Also, WEE virus has been isolated from the blood of naturally infected jackrabbits.[2,16,17]

It was unexpected, however, that the prevalence of WEE viral antibody in jackrabbit sera in Butte County would be significantly higher than that observed previously in Kern County.[14] This was based on the low WEE viral infection rates found for *C. tarsalis* that were collected in the Sacramento Valley during the same time as jackrabbits were collected (authors' unpublished data). A difference observed between jackrabbit populations in Butte and Kern Counties was that young and presumably highly susceptible animals entered the population throughout the summer in Butte County but primarily in April and May in Kern County,[15] which was 2 to 3 months prior to peak *C. tarsalis* populations.[27] Further, one would expect that jackrabbits in the rice field-slough habitats of Butte County were more subject to vector attack than were jackrabbits in the arid desert habitat of Kern County. This seems evident if one compares the percent of *C. tarsalis* reported to feed on rabbits during the summer in Butte County (62%)[31] versus the percent in Kern County (3%).[30]

Another explanation for the higher prevalence of WEE viral antibody in jackrabbits in Butte County than in Kern County is that the jackrabbits in Butte County were being infected by *Ae. melanimon* as well as *C. tarsalis*. In concurrent studies *Ae. melanimon* was found to be a competent experimental vector of WEE virus, it frequently fed on jackrabbits and it was involved in the transmission cycle of WEE virus in the Sacramento Valley.[12] Smart *et al.*[29] reported the isolation of numerous strains of WEE virus from a taxonomically closely related *Aedes* species, *Aedes dorsalis*, collected in 1965 in west central Utah. More recently Hayes *et al.*[16] reported the isolation of WEE virus from *Ae. dorsalis* and *Aedes vexans* collected in Arizona and New Mexico. However, many pools of *Ae. melanimon* were collected in Kern County and tested for virus from 1943 to 1952 when WEE viral infection rates were high in *C. tarsalis* and only 2 strains of WEE virus were isolated from *Ae. melanimon*.[27] Thus, *Aedes* species do not seem to be as important vectors of WEE virus in Kern County as they appear to be in Butte County and perhaps in other areas of southwestern United States.

The prevalence of SLE viral antibody in jackrabbit sera was also higher in Butte County than was recorded earlier in Kern County.[14,27] Although no attempt was made to confirm HI results by SDN tests, we feel that the flavivirus infecting jackrabbits was SLE virus since cross-

reactions with 3 other flaviviruses were rarely observed. Further corroboration of SLE viral transmission to jackrabbits was provided by numerous isolations of SLE virus from *C. tarsalis* collected in Butte County during 1971-1973 and by the fact that this mosquito species was feeding on jackrabbits (authors' unpublished data). Also, subadult jackrabbits developed antibodies to SLE virus after experimental infection.

A question still remains, however, as to the exact role that jackrabbits play in the summer transmission cycle of SLE virus in Butte County. The failure of experimentally infected subadult animals to develop significant levels of viremia suggested that they were dead-end hosts. However, Chamberlain *et al.*[3,4] found that up to 33% of *C. tarsalis* became infected with SLE virus after feeding on experimentally infected birds circulating levels of virus that were undetectable by intracerebral inoculation of weanling mice. Thus, further studies are needed to establish the threshold of infection of *C. tarsalis* with SLE virus before valid interpretations can be made between viremia levels in experimentally infected vertebrates and their potential role in the transmission cycle of SLE virus.

Jackrabbits were refractory to infection with TUR virus, another arbovirus transmitted by *C. tarsalis*.[11] The strain of TUR virus used in these experimental studies might not be representative of all field strains since a low prevalence of TUR viral antibody in jackrabbit sera was found in the present study and a strain of TUR virus has been isolated from the blood of a blacktail jackrabbit.[17] With available evidence, however, we must conclude that jackrabbits play little if any role in the transmission cycle of this virus.

Three *Culicoides* - transmitted viruses, BUT, LOK and MD, infected jackrabbits in the Sacramento Valley as they had been found to do earlier in Kern County.[11,18] However, in the present study the levels of activity of these viruses were quite low and no parallel studies were done to determine infection rates in *Culicoides*. Thus, little can be said about the seasonal dynamics of their transmission cycles in this area.

The technique used in this study to capture and recapture jackrabbits was netting at night from a moving vehicle. While this technique provided adequate sampling for the serologic studies, it failed to provide the type of quantitative data that are needed to calculate population densities. Further, the sampling was not random, as exemplified by the 2:1 ratio of adult females to adult males. In a previous study where 70 jackrabbits were shot at Llano Seco Rancho in 1969 and 1970, there was no significant deviation in either age group from the expected 1:1 sex ratio (authors' unpublished data). Thus, it was concluded that adult females were more easily captured by netting than were adult males.

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