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## SEROLOGIC EVIDENCE OF INFECTION OF WHITE-TAILED DEER IN TEXAS WITH THREE CALIFORNIA GROUP ARBOVIRUSES, (JAMESTOWN CANYON, SAN ANGELO, AND KEYSTONE)

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**Abstract:** Sera collected from 187 white-tailed deer (*Odocoileus virginianus*) from southern Texas in 1963, 1970, 1971, and 1972 were tested for the ability to neutralize several California group arboviruses. Jamestown Canyon virus was specifically neutralized by 99 of the 187 sera (53%). San Angelo and Keystone viruses were specifically neutralized by four and one sera respectively. Serologic evidence of infection of deer with California encephalitis or LaCrosse virus was not detected. Results of limited inoculation studies indicate that white-tailed deer are probably incidental, dead-end hosts for San Angelo and Keystone viruses. White-tailed deer are sensitive indicators of Jamestown Canyon virus and may be an important vertebrate host for this virus.

### INTRODUCTION

Recent studies have indicated the relative importance of California group arboviruses and the growing need to distinguish between these closely related viruses and their antibodies.<sup>2</sup> It seems possible that each of the viruses of the California group indigenous to North America is maintained in a distinct hematophagous arthropod-mammal cycle in nature.

Four California group viruses occur in Wisconsin,<sup>8</sup> and white-tailed deer may be an important vertebrate host for one of them — Jamestown Canyon (JC) virus. Briefly, studies with deer have demonstrated four main points. First, JC virus replicates to relatively high titer when subcutaneously inoculated in deer.<sup>5</sup> Second, it is possible to differentiate antibodies elaborated by deer after infection with a single California group arbovirus.<sup>5</sup> Third, JC virus commonly infects deer in Wisconsin.<sup>6</sup> Fourth, JC virus has been isolated from the blood of a naturally infected deer.<sup>3</sup>

Previous serologic surveys performed on Texas deer with Snowshoe Hare

virus<sup>1,10</sup> have indicated exposure of deer to California group viruses, but no comparative testing was done to determine the responsible virus. Four California group viruses have been isolated from mosquitoes in Texas—JC, San Angelo (SA), Keystone (KEY), and California encephalitis (CE) virus. This study was initiated to serologically determine which of those viruses infect deer in southern Texas.

### MATERIALS AND METHODS

#### Deer:

Blood samples from white-tailed deer (*Odocoileus virginianus*) were collected during special hunts at the Rob and Besie Welder Wildlife Foundation near Sinton, Texas in 1963, 1970, 1971 and 1972. The sera were harvested according to standard procedures,<sup>5</sup> heat inactivated at 56C for 30 minutes, and stored at -25C until tested. Ages of the deer were determined by personnel of the Welder Wildlife Foundation according to tooth replacement and wear.<sup>9</sup>

#### Viruses and Neutralization Tests:

Prototype strains of JC, LaCrosse (LAC), KEY, CE, and SA viruses in 10% suckling mouse brain suspensions were used in the neutralization tests. JC and LAC viruses were obtained from Dr. W. H. Thompson, University of Wisconsin, and KEY, CE, and SA viruses were obtained from the Center for Disease Control. All sera were tested at an initial 1:10 dilution in a constant virus-varying serum dilution technique of a tissue culture neutralization test.<sup>7</sup> The test utilized BHK<sub>21</sub> cells (obtained from Dr. T. M. Yuill, University of Wisconsin) and was performed in microtiter tissue culture plates. A sample was considered positive if it neutralized at least 30 x 30 tissue culture 50% infective doses) of one or more of the viruses used. If the positive serum neutralized only one virus, or if it neutralized a given virus to a 4-fold higher titer, it was considered to specifically neutralize that virus. If the serum neutralized more than one virus in high titer, it was placed in an "undetermined" category.

#### Inoculation Studies:

Prototype strains of KEY and SA viruses in doses of  $10^{4.0}$  and  $10^{4.5}$  suckling mouse 50% intracerebral lethal doses (SMICLD<sub>50</sub>) were each subcutaneously inoculated into one yearling white-tailed deer. The deer were repeat-

edly sampled for viremia and antibody data as described previously.<sup>5</sup> These deer were raised at the University of Wisconsin's Charman Research Center in Madison. They were bled prior to inoculation and no detectable neutralizing antibodies were found at a 1:2 serum dilution against several California group arboviruses mentioned above.

#### RESULTS

Results of the serologic survey are presented in Table 1. Fifty-three percent (99 of 187) of the deer sera specifically neutralized JC virus. Titers of those sera ranged from 1:10 to 1:160 with a geometric mean titer of 1:22. Eighty nine of those 99 sera neutralized only JC virus. Ten of the 99 JC specific sera also neutralized KEY virus but reacted in a 4 to 8-fold higher titer to JC virus. Only four samples specifically neutralized SA virus and only one KEY virus. Seven of the twelve "undetermined" sera neutralized both JC and KEY viruses; all seven reacted in 2-fold higher titer to JC virus. Three samples neutralized JC, KEY and SA viruses and two samples neutralized all five viruses in high titer.

All samples which neutralized more than one virus and a sample of those sera neutralizing only JC virus were tested against CE virus. Only two sera (see above) neutralized 30 TCID<sub>50</sub> of CE virus.

TABLE 1. Neutralization of California Group Arboviruses by Texas Deer Sera.<sup>①</sup>

Year	Number Tested	JC	Number Showing Specific Neutralization <sup>②</sup> of			Und <sup>③</sup>
			(%)	SA	KEY	
1963	47	16	(34)	3	1	0
1970	69	37	(54)	0	0	5
1971	41	23	(56)	0	0	5
1972	30	23	(77)	1	0	2
Totals	187	99	(53)	4	1	12

① All sera were tested for their ability to neutralize at least 30 TCID<sub>50</sub> of Jamestown Canyon (JC), San Angelo (SA), Keystone (KEY) and LaCrosse (LAC) viruses. Selected sera were tested against California encephalitis (CE) virus.

② See text for criteria of specific neutralization.

③ Undetermined. See text for explanation.

TABLE 2. Age Specific Neutralizing Antibody Rates of Texas Deer<sup>[1]</sup> to California Group Arboviruses.

Age (months)	Number Tested	Percent Positive [2]
0-5	15	60
6-12	10	40
13-24	25	64
25-36	21	76
37-48	18	78
49-60	15	73
61-127	24	71

[1] White-tailed deer sampled at the Wedder Wildlife Refuge in 1970, 1971, and 1972.

[2] All samples which neutralized JC virus to highest titer.

Samples with available age data from 1970, 1971, and 1972 were analyzed for age-specific neutralizing antibody rates to JC virus (Table 2). The majority of samples were collected from deer over 25 months of age, ranging to 127 months. The antibody rates were lowest in the fawns aged 6-12 months.

KEY virus was subcutaneously inoculated into one white-tailed deer. Viremia was detected in trace amounts on post-inoculation (PI) days 2 and 3. Serum from that deer titered 1:64 at 21 and 90 days PI against 30 TCID<sub>50</sub> of KEY virus.

Viremia was not detected in the deer subcutaneously inoculated with SA virus. Serum from that deer also titered 1:64 at 21 and 90 days PI against 30 TCID<sub>50</sub> of SA virus. Sera collected 21 days PI from the deer inoculated with KEY and SA viruses were comparatively tested against 30-100 TCID<sub>50</sub> of JC, LAC, KEY and SA viruses. A 1:4 dilution of those sera neutralized only the homologous virus.

#### DISCUSSION

The results presented here and those published elsewhere<sup>8,9,6</sup> indicate that white-tailed deer are sensitive indicators

for JC virus. They are commonly infected and may be important amplifier or reservoir hosts for JC virus.

The results of the inoculation study, although based on admittedly small numbers, suggest that deer are probably incidental, dead-end hosts for KEY and SA viruses, but would serve as sensitive indicators for these two viruses in nature. The low prevalence of antibodies to KEY and SA in the sera of deer from southern Texas, reflects either on the sensitivity of deer as indicators for these viruses in nature or the relative absence of these viruses from the area. To our knowledge, only SA virus has been isolated from Southern Texas (D. Sudia, Center for Disease Control, personal communication).

The lower rates of antibody in fawns 6-12 months of age agrees with studies of white-tailed deer in Wisconsin,<sup>4,6</sup> where maternal antibody to JC virus is prevalent.

The prevalence of antibodies neutralizing JC virus suggests yearly enzootic transmission of JC virus to deer. This further supports the claim that white-tailed deer may be important vertebrate hosts for JC virus.

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