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## Serologic Survey of a Deer Herd in California For Arbovirus Infections\*

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### ABSTRACT

Sera from 41 mule deer (*Odocoileus hemionus*) in Yosemite National Park, California, were tested for neutralizing antibody to 17 arboviruses which are known or suspected to occur in California. Antibody titers to California encephalitis, Trivittatus, Cache Valley, or Jerry Slough viruses were detected in 4 of the sera. Deer may be useful indicator hosts for the presence of arbovirus activity in a geographic area.

Although viral serologic surveys of deer in North America have not been extensive, there is some evidence that these animals are infected with certain of the arthropod-borne viruses (arboviruses) <sup>1-4, 6-8, 11-14</sup>. Serologic testing of deer, which are relatively long-lived, non-migrating animals and are hosts for ticks, mosquitoes, and other biting insects, should help to determine if arbovirus transmission has occurred in a geographic area. Population control (by shooting) of a portion of the mule deer herd (*Odocoileus hemionus*) in Yosemite National Park in central California during the fall and winter of 1965 provided an opportunity to obtain deer sera from an area which has not been extensively surveyed for arbovirus activity.

### METHODS

Deer sera were collected September 28 and 29, 1965, in cooperation with U.S. National Park Service personnel. Forty-one animals were bled just after they were shot, and blood samples were kept chilled until arrival in the laboratory. The sera were removed from the clots and stored at -70°C in a Revco refrigerator until all tests had been completed. Age of each deer was estimated from molar tooth wear. There were 8 male and 33 female deer. Seven were less than 1 year, 11 were 1-2 years, 7 were 3-5 years, and 16 were over 5 years of age.

The sera were tested for the presence of neutralizing antibody to the following 17 arboviruses which are known or suspected to occur in California (strain designation and mouse or cell-culture passage level indicated in parenthesis): California encephalitis (BFS 283, M11); Colorado tick fever (Florio, M 50); western encephalitis (A42, M18); St. Louis encephalitis (Ruis, M5); Turlock (MP-847-32, M3); Powassan (prototype, M6); Buttonwillow (A 7956, M12); Modoc (M544, M4); Cache Valley (Greeley 6V-633, M12 and M12 BHK<sub>21</sub>2; Kern Canyon (Borel, M6); Rio Bravo (M64, M4); Trivittatus (993, M11); epizootic hemorrhagic disease of deer (NJ-55, M24); bluetongue (8-25, M28); Hart Park (Ar 70, M 18); Jerry Slough (BFS-4474, M5 BHK<sub>21</sub>1); and vesicular stomatitis (NJ-Ogden, approximately 6 passages in embryonated eggs, 56 in chick embryo cell culture, and 1 in BHK<sub>21</sub> cells). Sera were heat-inactivated 56°C for 30 minutes, and were incubated for 1 hour at 37°C with equal volumes of a virus dilution that gave final serum-virus mixtures containing 50-150 LD<sub>50</sub> of virus. Mice 1-3 days old (1 litter of 6 mice per test) were inoculated intraperitoneally (i.p.) with 0.03 ml or intracerebrally (i.c.) with 0.02 ml volumes (for

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bluetongue and Hart Park viruses) and were observed for 14 days. Sera showing neutralization (survival of half or more of the mice) were then titrated in serial two-fold dilutions against approximately 100 LD<sub>50</sub> of virus and the antibody titer was calculated by the method of Reed and Muench<sup>9</sup>. Because of a shortage of mice, final titrations for vesicular stomatitis, Jerry Slough, and Cache Valley virus antibody were completed using BHK<sub>21</sub> hamster kidney cell cultures. Serial two-fold dilutions of heat-inactivated sera were incubated with equal volumes of virus (approximately 100 infective doses (TCID<sub>50</sub>) of virus per 0.1 ml) and 2 tube cultures were inoculated with 0.2 ml of each mixture, held in stationary racks at 37° C and observed for 7 days for cytopathic effects. The highest dilution of serum protecting at least one of the cultures was the titration endpoint.

#### RESULTS

There was no evidence of antibody to the following viruses in the sera tested: Colorado tick fever, western encephalitis, St. Louis encephalitis, Turlock, Powassan, Buttonwillow, Modoc, Kern Canyon, Rio Bravo, epizootic hemorrhagic disease of deer, bluetongue, Hart Park, and vesicular stomatitis. Serum No. 10 (3 yr old female) had a titer of 1:8 for California encephalitis virus, 1:8 for the closely related Trivittatus virus, and 1:32 for Cache Valley virus in the mouse tests. No serum remained for confirmatory tests in BHK<sub>21</sub> cell cultures, or for titration against Jerry Slough virus (closely related to California encephalitis and Trivittatus viruses).

Serum No. 7 (6 yr old female) had a titer of 1:16 in the mouse test and 1:4 in BHK<sub>21</sub> cells, against Cache Valley Virus.

Serum No. 16 (7 yr old female) had a titer of 1:8 and 1:16 against Jerry Slough virus on 2 separate titrations in BHK<sub>21</sub> cells.

Serum No. 35 (adult female) had a titer of 1:128 in mice and 1:32 in BHK<sub>21</sub> cells for Jerry Slough virus. The lower titers in cell cultures as compared with mice may be due to lower sensitivity of this system for the viruses tested.

#### DISCUSSION

Although few sera contained neutralizing antibody for the arboviruses tested, it is of interest that one had a low titer to California encephalitis and Trivittatus viruses (which are closely related by neutralization test as well as by other serologic tests). The serum had a higher titer to Cache Valley virus (Bunyamwera group). Two other sera contained Cache Valley virus antibody as well. California encephalitis virus has not been isolated in California since its initial discovery, but antibody surveys have indicated continued circulation of this or closely related viruses<sup>5</sup>. A virus or viruses related to Cache Valley virus also are known to occur in California, as are most of the other viruses included in this study<sup>10</sup>.

Deer sera from other areas in North America have shown antibody to California encephalitis virus<sup>2, 12</sup> and to a Bunyamwera group virus<sup>4</sup> by either neutralization or hemagglutination-inhibition tests. There is also evidence that deer can be infected with the viruses of western encephalomyelitis<sup>2, 3, 13</sup>, St. Louis encephalitis and Venezuelan encephalomyelitis<sup>2</sup>, vesicular stomatitis<sup>2, 6, 7</sup>, and epizootic hemorrhagic disease<sup>1, 8, 11, 14</sup>. Except for epizootic hemorrhagic disease virus, deer are probably not important hosts for the maintenance and spread of arboviruses, but can serve as indicators of past activity of the viruses.

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