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SEROLOGICAL EVIDENCE OF CALIFORNIA SEROGROUP VIRUS ACTIVITY IN OREGON

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ABSTRACT: We wished to demonstrate evidence of the presence of California serogroup viruses in Oregon and to test for the presence of certain other arboviruses in large ungulates. Blood samples from black-tailed deer (*Odocoileus hemionus columbianus*), mule deer (*O. hemionus hemionus*), and Roosevelt elk (*Cervus elaphus roosevelti*) from nine counties in Oregon were tested by serum-dilution plaque reduction neutralization for antibody to California serogroup viruses, including snowshoe hare, California encephalitis, and Jamestown Canyon, as well as to Cache Valley (Bunyamwera serogroup) and Klamath, an ungrouped rhabdovirus. Of 132 samples tested, 60 (46%) were found to be seropositive at a dilution of $\geq 1{:}10$ for at least one of the five different arboviruses. Forty (30%) samples contained antibody to more than one arbovirus, and 15 samples (11%) contained antibody to all five. Of these 15, 14 were from 75 black-tailed deer sera collected in Lincoln County, Oregon. Seropositivity rates for black-tailed deer ranged from 23% to 35%, with all five arboviruses represented. Positive reactions for all five arboviruses were represented among mule deer sera at rates from 5% to 29%. Elk sera were found to be positive for four of the viruses (none for Klamath virus). Although Cache Valley and Klamath viruses have been reported from Oregon, these data represent the first evidence of a California serogroup virus in the state.

Key words: Arboviruses, Oregon, California serogroup viruses, ungulates, mule deer, white-tailed deer, Roosevelt elk.

INTRODUCTION

Mosquito-borne virus diseases have been known to occur in Oregon since at least the first decades of this century. However, of these diseases, only western equine encephalomyelitis (WEE), a disease that affects horses and humans, and St. Louis encephalitis (SLE), a human disease, have been reported (Oregon State Board of Health, 1969; Oregon State Department of Agriculture, 1969). WEE virus is classified in the family Togaviridae. SLE virus is in the family Flaviviridae. In Oregon these viruses are transmitted by the mosquito *Culex tarsalis* to vertebrate hosts primarily in irrigated areas of the central and eastern parts of the state. Various passerine birds serve as reservoir hosts.

Since the 1960's, in other areas of North America and especially in the midwestern United States, mosquito-borne viruses belonging to the California serogroup (family Bunyaviridae, genus *Bunyavirus*) have assumed increasing importance as causes of human illness (Calisher and Thompson, 1983). For these viruses, mammals serve as reservoir hosts, and mosquito vectors typically are species in the genera *Aedes* and *Culiseta* (LeDuc, 1979; Turell and LeDuc, 1983). In North America, several California serogroup viruses have been associated with human illness: California encephalitis (CE), Jamestown Canyon (JC), snowshoe hare (SSH), LaCrosse (LAC), and trivittatus (TVT) (Grimstad et al., 1982, 1986a). The importance of these viruses as causes of disease in domestic animals and wildlife is unknown, although evidence of

infection of deer, elk, and other large mammals is widespread (Emmons, 1968; Watts et al., 1982; Zarnke et al., 1983; Grimstad et al., 1986a, b).

There have been no confirmed human infections due to a California serogroup virus reported in Oregon, nor has evidence been presented which would indicate the presence of any of these viruses in the state. There has been evidence of California serogroup virus activity in other Pacific Coast states. California has had California encephalitis as well as isolations of California serogroup viruses from mosquitoes (Sudia et al., 1971). In Alaska, there have been isolations of California serogroup viruses from mosquitoes as well as serologic evidence of human infections (Calisher, 1983; Zarnke et al., 1983). There is also evidence of California serogroup virus activity in western Canada (Artsob, 1983). Because of the presence of virus in other western North American areas, we felt that some members of this serogroup may occur undetected in Oregon, and may have some importance from the standpoint of public health and wildlife disease management. We knew that the occurrence of LAC virus, the most important member of this group from the public health standpoint, was unlikely in Oregon. It is vectored by *Aedes triseriatus*, a treehole breeding mosquito which does not occur in Oregon. However, JC and SSH have been isolated in Alaska (Zarnke et al., 1983) and western Canada (Artsob, 1983), and CE and JC are known to occur in California (Reeves et al., 1983). CE and JC antibodies were detected in mule deer sera collected in Yosemite National Park (Emmons, 1968).

Until recently, the potential public health significance of JC virus has not been fully appreciated (Deibel et al., 1983). However, recent studies have shown that JC virus is a more important cause of human disease than was believed previously, and that human infection with this virus may be associated with areas having large white-tailed deer populations (Grimstad et al., 1982, 1986a).

In 1985, we conducted a limited survey of mule deer, black-tailed deer, and elk from various areas of Oregon for neutralizing antibody to certain California serogroup viruses in an attempt to obtain evidence of virus activity. We also tested these animals for Cache Valley and Klamath viral antibody. This report describes the results of these tests on 132 samples.

MATERIALS AND METHODS

Blood samples were collected from black-tailed deer (*Odocoileus hemionus columbianus*), mule deer (*O. hemionus hemionus*), and Roosevelt elk (*Cervus elaphus roosevelti*). Samples were obtained by wildlife biologists of the Oregon Department of Fish and Wildlife from carcasses of freshly killed animals at hunting check stations and from live-captured anesthetized animals. All animals were from areas of medium to high elevation (300–1,000 m). Blood was collected using Vacutainer® tubes, and placed in household refrigerators within a few hours of collection. After 1–7 days whole blood specimens were shipped on wet ice to the Department of Microbiology, Oregon State University (OSU), where they were centrifuged and the serum was removed by aspiration and frozen. A group of blood samples from Douglas County was frozen before shipment to OSU without separation of serum (as whole blood). After processing at OSU, all samples were shipped frozen on dry ice (−70 C) to the Division of Vector-Borne Viral Diseases, Centers for Disease Control, Fort Collins, Colorado, for serological screening tests.

Viruses used in serologic tests were prototype strains maintained at the Centers for Disease Control, Fort Collins, Colorado: snowshoe hare (Original), California encephalitis (BFS-283), Jamestown Canyon (61V-2235), Cache Valley (6V-633), and Klamath (M-1056). These viruses were included because they have been isolated from arthropods or mammals in Oregon (Klamath) or because their known geographic distributions suggested the probability of their presence in Oregon.

Deer and elk sera were heat-inactivated at 56 C for 30 min, and tested for antibody by the serum dilution-plaque reduction neutralization test (Lindsey et al., 1976). Testing was done using Vero cell cultures grown in 6-well plastic plates. Sera were diluted 1:5 in medium 199 containing Earle's balanced salt solution with

TABLE 1. Seropositive sera for five different arboviruses from ungulates collected from Oregon.

County	Arbovirus					Total tested
	SSH*	CE	JC	CV	KLA	
Crook	—	—	2	—	—	6
Deschutes	1	1	3	—	1	8
Douglas	2	2	7	4	—	11
Jackson	1	1	3	4	—	11
Lane	—	—	1	1	—	1
Lincoln	22	21	27	20	18	75
Sherman	—	—	—	—	—	2
Wallowa	—	—	—	—	—	1
Wasco	1	1	1	1	1	10
Unrecorded	1	3	3	4	1	7
Totals	28	29	47	34	21	132

* Abbreviations for viruses: SSH, snowshoe hare; CE, California encephalitis; JC, Jamestown Canyon; CV, Cache Valley; KLA, Klamath.

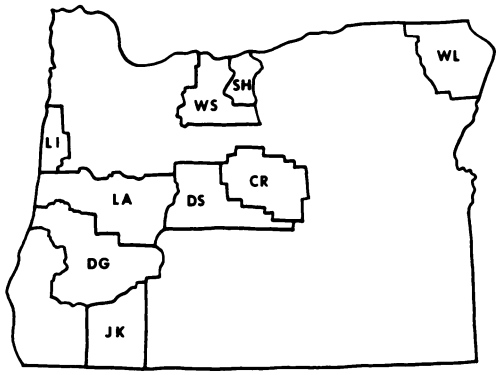

FIGURE 1. Counties in Oregon from which deer and elk sera tested for California serogroup viruses were collected. The following are the codes for counties: CR, Crook; DG, Douglas; DS, Deschutes; JK, Jackson; LA, Lane; LI, Lincoln; SH, Sherman; WL, Wallowa; WS, Wasco.

0.05 M Tris, pH 7.6, 1% bovine albumin, 0.35 g/liter sodium bicarbonate, and 100 IU penicillin, 100 μg streptomycin, and 1 μg fungizone per ml. When mixed with an equal 0.1 ml of virus suspension containing a pretitrated 200 plaque-forming units, the sera were considered diluted 1:10. After incubation of each serum-virus mixture at 4 C overnight, 0.1 ml was inoculated onto a single well containing Vero cells. The mixture was allowed to adsorb to the cells at 37 C for 45 min, and the cells were overlayed with 3 ml maintenance medium containing 1% agar (agarose). The cultures were then incubated in the presence of 5% CO_2 for 3 days at 37 C, a second agar overlay (2.5 ml) with 1:20,000 neutral red was added, and the cells were incubated an additional 24 hr at 37 C. When plaque counts were reduced 90% or more as compared with negative control sera (serum samples from deer previously shown not to have neutralizing antibody to these viruses), test sera were considered positive (it was assumed they contained antibody). Positive sera were tested for determination of endpoint (titer) by serial two-fold dilution in Medium 199. Titers are expressed as the highest dilution of serum that reduced the plaque count by 90%, as compared with a negative control serum sample.

RESULTS

Evidence of antibody to snowshoe hare (SSH), California encephalitis (CE), Jamestown Canyon (JC), Cache Valley (CV), and Klamath (KLA) was detected among 132 deer and elk sera collected in nine Oregon counties. Of the samples tested, 60 (46%) were positive in the initial screening (serum dilution of 1:10) against at least one arbovirus. Forty samples (30%) were positive for more than one arbovirus, and 15 samples (11%) were positive for all five viruses.

Seropositive samples were detected from seven counties (Table 1, Fig. 1). The highest prevalence of seropositives was from Douglas County, where eight of 11 samples had antibody to JC virus. The greatest number of samples came from Lincoln County. Antibody to all five viruses were seen in these samples, with prevalences ranging from 24% (KLA) to 36% (JC). Of the 15 samples with antibody to all five viruses, 14 were from a group of 75 from Lincoln County. The samples were collected from black-tailed deer at a hunting check station representing deer from a large area of the Siuslaw National Forest between the Siuslaw and Siletz Rivers. Among the 15 samples showing antibodies to all five arboviruses, five had titers to CV virus two or more dilutions higher than to any other virus. Among the other ten, it was not possible to distinguish any one virus on the basis of titer.

High prevalences of seropositives were

TABLE 2. Seropositive sera for five different arboviruses collected from deer and elk in Oregon.

Mammal host	Arbovirus					Total tested
	SSH[a]	CE	JC	CV	KLA	
Mule deer	2 (9.1)[b]	2 (9.5)	6 (28.6)	1 (4.8)	2 (9.5)	21
Black-tailed deer	22 (28.6)	21 (27.3)	27 (35.1)	20 (26.0)	18 (23.4)	77
Elk	4 (16.0)	4 (16.0)	12 (48.0)	10 (40.0)	—	25
Unrecorded	—	2	2	3	1	9

[a] Abbreviations for viruses: SSH, snowshoe hare; CE, California encephalitis; JC, Jamestown Canyon; CV, Cache Valley; KLA, Klamath.
[b] Percent positive.

seen in both black-tailed deer and elk (Table 2). The percentage of positive black-tailed deer samples was roughly equal for each of the five viruses. Among elk samples, the percentage of seropositives was highest for JC and CV. Four elk samples each had antibody to SSH and CE viruses, but none had antibody to KLA virus. Mule deer serum samples showed relatively low rates for all viruses except JC virus (29%). Titers for most positive sera ranged from 20 to 40. The highest titers were seen in the group of samples which tested positive for all five viruses. These titers ranged from 160 to 640.

DISCUSSION

The data presented in this paper represent the first presumptive evidence of California serogroup virus activity in Oregon. Because of the limited number of sera tested and the few counties sampled in Oregon, few conclusions can be reached regarding the geographic distribution of virus activity within the state. It seems unlikely that the 15 seropositive samples to all five viruses represent multiple infections, although such a possibility cannot be definitely ruled out. Cross-reactivity seems unlikely between KLA (a rhabdovirus) and the other four (bunyaviruses), but it is possible among the latter four viruses (especially among the three California serogroup viruses). Of the California serogroup viruses tested, SSH and CE are the most closely related (both are antigenic variants of California encephalitis virus). However, 13 of the 132 sera tested had antibody to one, but not the other of these two viruses. This confirms the specificity of the test used. Unfortunately, we lack data on the ages of the black-tailed deer collected in Lincoln County. A possible explanation for samples showing antibody at high titers for all agents is that they represent previous infections to CV and KLA viruses, and to repeated infections with a California serogroup virus (probably JC). Such repeated infections can produce an anamnestic reaction resulting in production of antibody less specific than those encountered in animals infected with only one California serogroup virus (Dr. Paul Grimstad, pers. comm.). This explanation would be consistent with the high titers observed in these instances.

It is premature to speculate on the ecology of these viruses in Oregon, other than to note that many of the mosquito species from which California serogroup agents have been isolated elsewhere (Turell and LeDuc, 1983) are present in Oregon, including the counties from which the samples were taken (Gjullin and Eddy, 1972). Both SSH and JC have been isolated repeatedly from various snow pool *Aedes* spp. in North America (Turell and LeDuc, 1983) and many of the same species are

common in Oregon, especially at higher elevations. However, *Culiseta inornata* has been regarded as the principal vector of both viruses (Turell and LeDuc, 1983) and this species is common at medium to high elevations throughout the state (Yates, 1953).

Cache Valley virus, a bunyavirus classified in the Bunyamwera serogroup, has been isolated previously from Oregon, from pools of *Culiseta inornata* (Calisher et al., 1986). KLA virus was first isolated from Oregon from the tissues of a mountain vole (Zarnke et al., 1983), but nothing is known about its arthropod vectors.

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