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Serologic Evidence of Bunyamwera Group Arbovirus Infections in Wisconsin and Texas Deer*

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Abstract

Sera of 81 white-tailed deer from south Texas and 283 white-tailed deer from Wisconsin were tested for neutralizing and hemagglutination-inhibiting antibodies to arboviruses of the Bunyamwera group. Neutralizing antibodies were detected in 100% of the Texas deer sera and hemagglutination-inhibiting antibodies were detected in 61% of those sera collected in 1969 and 78% of those sera collected in 1963. The prevalence of both neutralizing and hemagglutination-inhibiting antibodies in Wisconsin deer sera varied from 72-100% and 42-79% respectively depending on the geographic area tested in the years 1963 and 1969.

Introduction

In Wisconsin the first isolations of arboviruses of the Bunyamwera group were made in 1964 and since that time numerous isolates have been made from mosquitoes from various locations in the state.^{1,2} In 1969, study of the Bunyamwera group of arboviruses in Wisconsin was stimulated by additional isolates from the blood of a horse³ and from the blood and brain of a caribou (*Rangifer tarandus*).⁴ Each animal was suffering an acute febrile illness at the time of virus isolation from its blood and each animal succumbed from its illness, although in neither case has the Bunyamwera group virus been documented as the etiologic agent. These two isolates are important

for two reasons: (1) they are among the first reported mammalian isolates of Bunyamwera group arboviruses in the United States, and (2) they were associated with fatal illnesses in these two animals, although not necessarily causally associated.

Members of the Bunyamwera group of viruses have been associated with human disease in other countries,^{5,6} but to date have only once been reported as associated with human disease in the United States.⁷ Serologic surveys have shown that Bunyamwera viruses do infect man and animals in the United States.^{8,9,10,11} In view of these reports and recent mammalian isolates, elucidation of the signifi-

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cance of Bunyamwera group arboviruses to animal and human health in the United States is indicated.

The white-tailed deer (*Odocoileus virginianus*) is one of the most widely distributed native mammalian species of North America. That, coupled with the fact that more than two million deer are harvested annually by hunters and serum

samples are readily available in many states, has led to the proposed use of deer as natural sentinels or indicators for the activity of various arboviruses.^{12,13}

The purpose of this paper is to attempt to elucidate the distribution and prevalence of Bunyamwera group virus infections in deer from Wisconsin and from a refuge in south Texas.

Materials and Methods

Wisconsin deer sera were collected at various deer registration stations in Wisconsin during the November hunting seasons of 1963 and 1969. Texas deer sera were from special collections during 1963 and 1969 at the Rob and Bessie Welder Wildlife Foundation in south Texas. All sera were heat inactivated at 56°C for 30 minutes before testing.

Not all Bunyamwera group arboviruses form hemagglutinating (HA) antigens. To date, HA activity has not been detected for the isolates from the horse and caribou or for prototype strain Cache Valley. Three strains of the Bunyamwera group of arboviruses were screened for use in the hemagglutination-inhibition (HI) tests: (1) Tensaw virus which was first isolated from *Anopheles crucians* mosquitoes in Alabama,¹⁰ (2) W-523-64 (Is. 523) which was isolated from *Aedes communis* mosquitoes in Wisconsin¹ and (3) W-933-64 (Is. 993) which was iso-

lated from *Aedes vexans* mosquitoes in Wisconsin.¹ Antigens were prepared by the sucrose-acetone extraction of infected mouse brain tissues.¹⁴

All sera to be tested in HI were acetone treated adsorbed with packed goose erythrocytes and tested by the microtechnique using 4 to 8 units of antigen per serum dilution.¹¹

Two strains of the Bunyamwera group of arboviruses were used in the neutralization (NEUT) tests: (1) W-523-64 (Is. 523) and (2) C-69-26, the isolate from the caribou in 1969. Sera were considered positive if they neutralized approximately 100 TCID₅₀ of the test virus in the metabolic inhibition test (MIT)¹⁵ using baby hamster kidney (BHK₂₁) cells, or approximately 100 LD₅₀ of the test virus in a neutralization test performed in one and two day old white mice using the intracerebral route of inoculation.¹⁴

Results

Since a number of local isolates as well as Tensaw virus were available for HI testing, a comparison of HI titers of deer sera was performed to choose the most suitable antigen to screen the sera. All sera positive to Is. 993 were positive to Is. 523. Tensaw virus was usually two-fold lower in activity than Is. 523 in both Wisconsin and Texas deer sera and much less sensitive to sera with low titers to Is. 523. Since Is. 523 seemed suitable and also because it has been used routinely in our laboratory it was chosen to test all deer sera.

A mammalian isolate (C-69-26) and a mosquito isolate (Is. 523) were compared for use in the NEUT test. Titers to Is.

523 were consistently higher than those to C-69-26. All sera which were positive on the HI test were positive to Is. 523 in NEUT tests whereas 2% of the HI positive sera were negative to C-69-26 in NEUT tests.

Results of the serologic study of Texas and Wisconsin deer sera are summarized in Table 1. All Texas deer sera from both 1963 and 1969 had NEUT activity. In 1963, 78% of the sera had HI activity and in 1969, 61% of the sera had HI activity to Is. 523. Tensaw virus was also used to screen the 1969 Texas deer sera on the HI test and only 39% of the deer sera reacted with Tensaw virus.

Serologic activity on both the HI and

NEUT tests were similar in those sera collected from west central Wisconsin in 1963 and 1969 (Table 1). The highest prevalence was in sera from east central Wisconsin and the lowest in sera from southwestern Wisconsin.

TABLE 1. Summary of Bunyamwera Group Arbovirus Antibody Study of 81 Texas Deer and 283 Wisconsin Deer.

Year	Area	HI Test ^①		NEUT Test ^②	
		No. Positive No. Tested	% Positive	No. Positive No. Tested	% Positive
1963	S. Texas	29/37	78	37/37	100
1969	S. Texas	27/44	61	44/44	100
1963	W. Central Wisconsin ^③	18/27	67	24/27	89
1969	W. Central Wisconsin ^③	96/123	78	114/123	93
1969	E. Central Wisconsin ^④	47/59	79	59/59	100
1969	S.W. Wisconsin ^⑤	31/74	42	53/74	72
Totals		248/364	68	331/364	91

^① Is. 523 was used in the HI test.

^② Is. 523 and C-69-26 were used in the NEUT test.

^③ Sera from Tomah and Black River Falls.

^④ Sera from Waupaca.

^⑤ Sera from Viroqua, Chaseburg, Holmen and Bangor.

Discussion

The serologic results of this study suggest widespread infection of deer in Wisconsin and south Texas with Bunyamwera group arboviruses. Prevalence of antibodies was found to be exceptionally high in the Wisconsin and Texas deer sera tested. In fact, these are the most prevalent arboviruses in Wisconsin and south Texas deer that have been studied.

The Wisconsin mosquito isolate W-523-64 (Is. 523) seems to be quite reactive in deer sera. It is not known if the high titered activity of deer sera to Is. 523 is a specific reaction or a high titered cross reaction. The virus (or viruses) of the Bunyamwera group which infects deer in Wisconsin or Texas is unknown but data suggests that in both states it is more closely related to Is. 523 and Is. 993 than to the isolate from the caribou or Tensaw virus.

Neutralizing antibodies to the Bunyamwera group of arboviruses were found in 91% of the sera and hemagglutination-inhibiting antibodies were found in 68% of the sera. Similar differences in rates of these antibodies have been noted previously¹⁰ and may be explained three ways: (1) neutralizing antibodies may persist for a longer period of time than HI antibodies, (2) the HI test may be more specific than the NEUT test is for the Bunyamwera group of arboviruses, and/or (3) the NEUT test may be more sensitive than the HI test.

The importance of deer in the epizootiology of Bunyamwera group arboviruses in Wisconsin is unknown but to our knowledge they are the only native wild species tested found to have antibodies, although the search has been limited. To fully assess the role of deer in the epizootiology of Bunyamwera

group arboviruses appropriate experimental studies need to be initiated utilizing deer and different strains of virus to establish the clinical response of deer, the titer and duration of viremia, the titer and persistence of antibodies, and to demonstrate transmission to and from suitable vectors.

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