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## Serologic Survey for Arbovirus Activity in Deer Sera from Nine Counties in New York State\*

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### Abstract

Sera from 352 deer from nine New York State counties were tested for neutralizing and hemagglutination-inhibiting antibodies to six arboviruses representing four groups. Antibody titers to California encephalitis and Cache Valley viruses were detected in varying frequency in the 9 counties with foci for the Bunyamwera group in Seneca, Dutchess, and Erie counties. Neutralizing antibodies to western equine encephalomyelitis were noted in sera collected in 1959-61 from Albany and Seneca counties while a focus of group B arbovirus activity, most probably due to Powassan virus, was found in Shelter Island, Suffolk County. Our experience indicates the usefulness of deer as natural indicators of activity of certain arboviruses.

### Introduction

Antibody surveys in sera of wild animals have been used by many workers to detect evidence of arbovirus activity.<sup>5,8</sup> Serologic surveys of deer as suggested by Emmons may be particularly useful in locating foci of recent or past arbovirus activity since these animals seldom roam great distances, are relatively long-lived, and are easily accessible to biting or sucking arthropods.<sup>3</sup> Deer sera have also been investigated by Trainer in Wisconsin to detect activity of California encephalitis (CE) virus and reported by Thompson.<sup>7</sup> We surveyed deer sera taken from nine counties in New York State over a period of 10 years at post-season hunts. The results of this study are the subject of this report.

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### Materials and Methods

**Sera.** In collaboration with the New York State Conservation Department, 337 blood samples were collected from deer killed in post-season hunts and 15 samples from live-captured animals in Hamilton County. After removal from the clot, the serum was stored frozen at  $-20^{\circ}\text{C}$  until examined. Ages of the animals were determined by replacement and wear of the teeth.

**Viruses.** The same strains of eastern and western equine encephalomyelitis (EEE, WEE), from group A, and Powassan (POW) and St. Louis encephalitis (SLE), within group B, were employed as antigen in both hemagglutination-inhibition (HI) and neutralization (N) tests. For the Bunyamwera (BUN) group, the Maguari (MAG) strain was used for HI and the Cache Valley strain (CV) for N tests; the CE complex was represented by the prototype BFS283 and the New York strain 65-8569 for HI and N tests, respectively. History of the viruses was recently described.<sup>8,9,11</sup>

Preparation of the sucrose-acetone extracted antigens and hyperimmune sera, technics of the HI and N tests, and interpretations of the results have been published.<sup>8,9,11,12</sup>

**HI tests.** All sera, except the samples collected in Seneca County in 1959-60, were screened for group A and B antibodies by the microtechnic; HI studies for the BUN group were included for sera after 1964 and for the CE group, for sera after 1965. The sera were acetone-treated and then adsorbed with packed goose erythrocytes.

**N tests.** The Seneca County sera collected in 1959-60 and those from Albany in 1959-61 were screened for both group A and B neutralizing antibodies. Thereafter N tests were carried out only for A and B antibodies on sera which had HI reactions. All sera were examined for BUN and CE neutralizing antibodies. Nylar strain mice, one or two days old, were used for the *in vivo* tests. Undiluted serum, not inactivated, was mixed with an equal volume of test virus diluted to contain approximately 100 LD<sub>50</sub>. The intraperitoneal route of inoculation was used for the A and B groups; the intracerebral route for the BUN and CE groups.

### Results

While WEE neutralizing antibodies were noted infrequently among the Albany County and Seneca County deer sera collected in 1959-61, no group A arbovirus reaction was observed by HI (Table 1). Reproducible reactions by HI to POW and SLE viruses were occasionally detected in sera from deer in the upstate areas. We failed, however, to demonstrate neutralizing antibodies to these viruses.

In Suffolk County, the incidence of HI reactions was significantly higher: 40% of the deer reacted with POW antigen, with titers ranging from 10 to 40. Five of these sera also cross reacted with SLE antigen. Sufficient material for N tests remained from 10 sera, 8 of which had reacted with POW and 2 with SLE. The presence of neutralizing antibodies for POW and SLE viruses confirmed the *in vitro* results.

Widespread activity was suggested for the arboviruses of the BUN and CE groups but there was considerable variation in the incidence of antibody for each group. Antibody to BUN was found in 3 to 7% of deer in Cayuga, Delaware, Hamilton, and Suffolk counties, whereas antibody to CE virus was detected in 15 to 62%. In contrast, deer in Erie County showed a significantly higher antibody incidence to BUN than to the CE group.

Antibody for groups B, BUN, and CE was detected in all age groups; the lowest incidence was in animals under 6 months of age (Table 2). In this group, the presence of maternal antibody cannot be ruled out. The highest incidence of antibody was found by HI and N tests with BUN strains in the 7 months to 1½ years group. Neutralizing antibody to CE virus was more frequently noted than HI reactions.

TABLE 1. *Antibody survey in 352 deer from nine New York State counties*

Per cent reacting sera													
County	Years collected	No. sera	Hemagglutination-inhibition						Neutralization				
			Group A		Group B		BUN	CE	Group A		BUN—Cache Valley		CE
			EEE & WEE	POW	SLE	MAG	BFS283	EEE	WEE	BUN	Valley	NY 65-8569	
Albany	1959-61	10	0	0	0	10	nt	nt	0	10	20	nt	nt
Cayuga	1969	16	0	0	0	0	6	0	nt	nt	6	62	62
Delaware	1965	30	0	0	0	0	3	0	nt	nt	3	20	20
Dutchess	1967	63	0	0	0	0	16	6	nt	nt	34	22	22
Erie	1965	25	0	0	0	0	36	0	nt	nt	60	17	17
Hamilton	1969	15	0	0	0	0	0	0	nt	nt	7	33	33
St. Lawrence	1964	14	0	0	0	7	0	nt	nt	nt	nt	36	36
Seneca	1959-60	67	nt	nt	nt	nt	nt	nt	0	3	nt	nt	nt
	1968	50	0	0	0	0	56	16	nt	nt	62	48	48
	1969	28	0	4	0	0	14	21	nt	nt	34	25	25
Suffolk	1969	34	0	40	15	3	0	0	nt	nt	3	15	15
BUN	=	Bunyamwere group											
CE	=	California encephalitis group											
EEE	=	eastern equine encephalomyelitis											
WEE	=	western equine encephalomyelitis											
MAG	=	Maguari virus											
POW	=	Powassan virus											
SLE	=	St. Louis encephalitis virus											
nt	=	not tested											

BUN = Bunyamwera group  
 CE = California encephalitis group  
 EEE = eastern equine encephalomyelitis  
 WEE = western equine encephalomyelitis  
 MAG = Maguari virus  
 POW = Powassan virus  
 SLE = St. Louis encephalitis virus  
 nt = not tested

TABLE 2. Incidence of antibody to arboviruses in 216 deer of different ages from six New York State counties

TABLE 2. Incidence of antibody to arboviruses in 216 deer of different ages from six New York State counties									
Age	No. of sera	Per cent reacting sera					Neutralization		
		Hemagglutination-inhibition							
		Group B		BUN		CE			
		POW	SLE	Maguari	BFS283	BUN	CE	Cache Valley	NY 65-8569
0-6 mos.	64	3	1	9	0		12	14	
7 mos. - 1½ yrs.	41	5	2	39	17		62	51	
1 yr. 7 mos. - 2½ yrs.	52	8	4	21	12		30	35	
2 yrs. 7 mos. - 3½ yrs.	22	14	0	18	9		25	60	
3 yrs. 7 mos. +	37	11	5	19	8		35	31	

BUN = Bunyamwera group  
CE = California encephalitis group  
POW = Powassan virus  
SLE = St. Louis encephalitis virus

Bunyamwera group reactions were more frequent than CE reactions by the *in vitro* method. All HI reactions were confirmed by detection of neutralizing antibodies with the following exceptions: one serum did not neutralize the BUN strain and 3 sera failed to neutralize the CE virus strain. Thirty-eight deer demonstrated both CV and CE neutralizing antibodies.

### Discussion

The antibody findings suggest that deer may be reservoirs or at least important links in the infectious cycles of a number of arboviruses. The variation in incidence of antibody in the geographical areas studied indicates that factors such as differences in the arthropod populations, climatic and ecologic conditions may play an important role in distribution and spread of arboviruses. Foci of POW arbovirus activity appear to be on Shelter Island in Suffolk County. The island, 11½ square miles in area, is rural and not connected to land by either sandspits or bridges.<sup>1</sup> It has many large estates, and its ecology is different from that of nearby Long Island. There is an overpopulation of deer. Shelter Island was studied extensively in the late 1940's because of the severity of the Rocky Mountain spotted fever infection in this particular area. Before setting up a tick eradication program, Collins, Nardy, and Glasgow<sup>2</sup> reported the prevalence of seven species of ticks: *Dermacentor variabilis* (Say), *Haemaphysalis leporis-palustris* Packard, *Ixodes cookei* Packard, *I. dentatus* Neumann, *I. marxi* Banks, *I. muris* Bishopp & Smith, and *I. scapularis* Say. *I. muris* was the most prevalent species associated with mammals, and infested predominantly the following animals: white-footed meadow mouse, shrew, gray squirrel, house rat, eastern skunk, chipmunk, and Virginia deer. Powassan virus has been isolated from several species of ticks, *I. marxi*, *D. andersoni*, and *I. cookei*, as well as from blood and tissues of woodchucks and red squirrels and from the brain of a fox.<sup>4,9,10</sup> The deer of Shelter Island may possibly act as yet another host for POW virus.

Serologic data demonstrate considerable activity of viruses of the BUN group in Seneca, Erie, and Dutchess counties while differences in CE group activity from county to county are not so pronounced.

Primary infections with BUN or CE group agents seem to occur in animals 7 months to 1½ years as suggested by increased antibody findings in this age group. Decreasing incidence of serologic reactions to the BUN group by HI and N in older animals may indicate limited persistence of antibody. These observations could explain less frequent antibody findings in Seneca County in 1969 as compared with those in 1968. These data would also suggest that a booster of response resulting from natural reinfection did not occur during this period and that BUN group activity in the same area occurred in 1968 or before.

California encephalitis virus neutralizing antibody was detected more often than HI antibody, independent of the geographical location or the age group. This finding may point to longer persistence of N antibody. It may, however, also reflect differences in the test strains or a lower sensitivity of the HI test (antigens are known to be difficult to prepare from CE group strains). Thus, the importance of employing 2 different serologic procedures in surveys is emphasized.

Our data suggest that deer are an excellent natural indicator for activity of POW, BUN group, CE group and possibly other members of the group A and B arboviruses. Studies in consecutive years in the same area and also of deer of different age groups may aid in determining the time of the occurrence of arbovirus infections in the deer population.

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