ARBOVIRUS SURVEILLANCE IN CONNECTICUT IV. BUNYAMWERA GROUP[1]

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ABSTRACT. One strain of a Cache Valley-like virus (Bunyamwera serogroup) was recovered from 204,753 female mosquitoes representing 36 species collected and tested in Connecticut from 1969 through 1980. This isolate, identified by complement-fixation and suckling mouse neutralization tests, was from a pool of 22 *Aedes triseriatus* collected in light traps during September 1979. Virus was not detected in 431 adult male or 8,317 immature *Ae. triseriatus* from the same area. Neutralizing antibody against this virus was demonstrated in 7 of 49 deer sera from Connecticut, but not in a small number of sera from cottontails, chipmunks, and squirrels from the same area.

This isolate extends the recognized geographic range of the Bunyamwera group into New England and it is the first Cache Valley-like strain reported from *Ae. triseriatus*.

INTRODUCTION

Cache Valley virus was originally isolated from a pool of 50 *Culiseta inornata* (Williston) collected in the Cache Valley of northern Utah (Holden and Hess 1959). Since that time, viruses of the Cache Valley complex, namely Maguari in South America (Shope and Whitman *in* Berge 1975) and Cache Valley (including Tlacotalpan virus) in North America (Hess and Holden *in* Berge 1975, Bishop and Shope 1979), have been reported from mosquitoes in Argentina, Colombia, Brazil, Guyana, French Guiana, Trinidad, Jamaica, Mexico, United States including Virginia (Buescher et al. 1970), New York (Srihongse et al. 1980), Kentucky and Illinois (Kokernot et al. 1969), Indiana (Chamberlain *in* Berge 1975), Iowa (Wong et al. 1970, 1978), North Dakota (Eklund *in* Berge 1975), and Wisconsin (Anslow et al. 1969), and in Manitoba (Sekla et al. 1980) and Saskatchewan (Burton et al. 1973) Canada.

Cache Valley-like virus has been reported from at least 15 species of mosquitoes in 5 genera in the United States and Canada. Nearly three-quarters of these isolates were from 5 species: *Cs. inornata* (23%) (Holden and Hess, 1959, Burton et al. 1973, Eklund *in* Berge 1975, Wong et al. 1978, Sekla et al. 1980), *Anopheles quadrimaculatus* Say (21%) (Kokernot et al. 1969, Wong et al. 1970), *Aedes vexans* (Meigen) (11%) (Anslow et al. 1969, Wong et al. 1970, Srihongse et al. 1980), *Ae. sollicitans* (Walker) (11%) (Buescher et al. 1970, Srihongse et al. 1980), and *Ae. taeniorhynchus* (Wiedemann) (8%) (Buescher et al. 1970). Occasional isolates were reported from the *An. bradleyi* group, *An. punctipennis* (Say), *An. walkeri* Theobald, *Ae. cinereus* Meigen, *Ae. communis* group, *Ae. nigromaculis* (Ludlow), *Ae. stimulans* group, *Ae. trivittatus* (Coquillett), *Coquillettidia perturbans* (Walker), *Psorophora columbiae* (Dyar and Knab), and *Ps. ferox* (Von Humboldt) (Anslow et al. 1969, Kokernot et al. 1969, Buescher et al. 1970, Chamberlain *in* Berge 1975, Wong et al. 1978, Srihongse et al. 1980).

The isolation and identification of a Cache Valley-like virus strain, the first from *Ae. triseriatus* and the first from New

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England, is described in the present paper.

MATERIALS AND METHODS

The methods and procedures employed in collecting and processing arthropods, in virus isolation and identification, and in antibody detection were described in detail in the first paper of this series (Main et al. 1979). Virus isolations were attempted in suckling mice by intracerebral inoculation and identified by complement-fixation and constant serum varying virus neutralization tests in suckling mice. Neutralization tests on native mammals were done by incubating undiluted serum with an equal volume of a virus suspension containing approximately 100 SMic LD_{50}/0.02 ml and assayed in suckling mice by the intracerebral route of inoculation. These sera were collected from white-tailed deer *(Odocoileus virginianus)* shot during the 1979 hunting season in Connecticut or from live-trapped eastern cottontails *(Sylvilagus floridanus)*, gray squirrels *(Sciurus carolinensis)*, and eastern chipmunks *(Tamias striatus)* in southern Connecticut.

RESULTS

A Bunyamwera group virus was recovered from a single pool of 22 nonblooded adult female *Ae. triseriatus* collected between 10–14 September 1979 in light traps in North Madison (New Haven Co.) Connecticut. This was the only Bunyamwera group isolate from 204,753 adult female mosquitoes representing 36 species tested in Connecticut since 1969 (Table 1). Virus was not recovered from 431 adult male or 8,317 immature *Ae. triseriatus* collected in the same area from 1974 through 1979.

This strain, Ar-560-79, was identified as a Cache Valley-like virus by complement-fixation (Table 2) and suckling mouse neutralization (Table 3) tests.

Neutralizing antibody was not detected in sera from 16 cottontail rabbits, 16 chipmunks, or 16 gray squirrels collected in south-central Connecticut. However, sera from 7 of 49 (14.3%) deer did neu-

Table 1. Adult female mosquitoes from Connecticut, for the years 1979 and 1980 and the totals for 1969 through 1980, tested in suckling mice for arboviral infections.

Species	Year		
	1979	1980	1969–80
Anopheles punctipennis	261	6	4035
Anopheles quadrimaculatus	12	0	774
Anopheles spp.	33	0	136[a]
Aedes communis group	2804	419	9372[b]
Aedes sollicitans	72	0	1628
Aedes stimulans group[c]	1646	52	8193
Aedes triseriatus group[d]	438	13	4532
Aedes trivittatus	6	0	672
Aedes spp.	4853	246	52185[e]
Psorophora spp.	0	0	244[f]
Coquillettidia perturbans	1905	374	30628
Culiseta spp.	2900	53	24811[g]
Culex spp.	677	29	25116[h]
Uranotaenia sapphirina	175	87	3184
Orthopodomyia signifera	0	0	4
Totals	17729	1304	204753

[a] *Anopheles* spp. includes 3 *barberi*, 23 *crucians*, 107 *walkeri* and 3 unidentified *Anopheles*.

[b] *Aedes communis* group includes 9104 *abserratus*, 248 *communis*, 9 *intrudens*, 1 *provocans*, and 10 *sticticus*.

[c] *Aedes stimulans* group includes *excrucians, fitchii*, and *stimulans*.

[d] *Aedes triseriatus* group includes *triseriatus* and probably *hendersoni*.

[e] *Aedes* spp. includes 1,185 *aurifer*, 39,581 *canadensis*, 10,887 *cantator*, 459 *thibaulti*, and 73 unidentified *Aedes*.

[f] *Psorophora* spp. includes 1 *ciliata* and 243 *ferox*.

[g] *Culiseta* spp. includes 5 *inornata*, 19,189 *melanura*, 5,615 *morsitans*, and 2 *sylvestris minnesotae*.

[h] *Culex* spp. includes 3,473 *pipiens*, 7,819 *restuans*, 5,302 *salinarius*, 1,111 *territans*, and 7,411 unidentified *Culex*.

Table 2. Results of complement-fixation tests comparing Ar-560-79 with the prototype strain of Cache Valley virus (CV-633).

	Ascitic fluid		
Antigen	Ar-560-79	Cache Valley	Normal
Ar-560-79	32/16*	16/16	0
Cache Valley	64/128	16/256	0
Normal	0	0	0

* Reciprocal of serum titer/reciprocal of antigen titer; 0 = < 4.

tralize Ar-509-79 virus. There was a slightly greater proportion of seropositive bucks (18.5%) than does (9.1%); however, this difference was not statistically significant and was probably the result of longer exposure of the bucks to infected mosquitoes.

DISCUSSION

The recovery of a Cache Valley-like virus from *Ae. triseriatus* in Connecticut extends the recognized geographic range of the Bunyamwera serogroup into New England. However, the identification of this isolate must remain tentative until it has been compared more closely with other isolates of Cache Valley virus from the East and Midwest.

This is the first report of a Bunyamwera group virus from *Ae. triseriatus*. This species feeds readily on humans and other mammals and is an important vector of California group viruses in the East and Midwest (Sudia et al. 1971), but it is not known if it will transmit Bunyamwera group viruses. Saliba and co-workers (1973) were unable to infect colonized *Ae. triseriatus* with either of two strains of Cache Valley-like virus from Wisconsin. However, these experiments were done with low titered, high passage mouse brain seed virus with which they were unable to infect other mosquito species including *An. quadrimaculatus*, the most efficient transmitter tested in their studies.

Other species of mosquitoes from which Cache Valley-like viruses have been isolated repeatedly include *Cs. inornata*, *An. quadrimaculatus*, *Ae. vexans*, *Ae. sollicitans*, and *Ae. taeniorhynchus*. *Cs. inornata* and *Ae. taeniorhynchus* are not abundant in New England, and, although both species occur in Connecticut, few specimens were tested in the present study (Table 1). A total of 28,987 *Ae. vexans* and 1,628 *Ae. sollicitans* was examined without any Bunyamwera group isolates. *Aedes vexans* was shown to be a relatively ineffective transmitter of Cache Valley virus in the laboratory (Saliba et al. 1973) and minimum field infection rates were greater than 1:4000 and 1:135,000 in Wisconsin (Anslow et al. 1969) and New York (Srihongse et al. 1980), respectively. *Aedes sollicitans*, as well as *Ae. taeniorhynchus*, were readily infected and transmitted Cache Valley (Yuill and Thompson 1970), but low field infection rates of approximately 1:20,000 were reported in Virginia (Buescher et al. 1970) and New York (Srihongse et al. 1980). Except in 1972, when more than 600 were tested, very few *An. quadrimaculatus* from Con-

Table 3. Results of neutralization tests comparing Ar-560-79 with the prototype strain of Cache Valley virus.

	Ascitic fluid				
	Ar–560–79		Cache Valley		Normal
Virus	LLD_{50}	LNI	LLD_{50}	LNI	LLD_{50}
Ar–560–79	2.1	1.5	2.3	1.3	3.6
Cache Valley	2.5	2.0	3.8	0.7	4.5

LLD_{50} = Log_{10} of the suckling mouse, intracerebral, LD_{50}
LNI = Log_{10} of the neutralizing index.

necticut have been examined. This species was shown to be an efficient transmitter in the laboratory (Saliba et al. 1973) and high infection rates of 1:400 were reported in Illinois (Kokernot et al. 1969). More extensive surveillance of this species may give a better indication of the prevalence of Bunyamwera group viruses in New England.

Viruses of the Bunyamwera serogroup have been associated with human disease in several parts of the world (Berge 1975) including Bunyamwera and Ilesha in Africa, Calovo in Europe and Guaroa and Wyeomyia in Central and South America. Although neutralizing antibody to Cache Valley virus was reported in human serosurveys in New York (Whitney et al. 1968), Virginia and Maryland (Buescher et al. 1970), the seropositives were not associated with disease. A case of encephalitis associated with Tensaw virus was reported from Indiana in 1964 (McGowan et al. 1973). Cache Valley, but not Tensaw, has been isolated from mosquitoes in Indiana and neighboring states (Berge 1975). Tensaw virus is another Bunyamwera group virus reported from southeastern United States but not recognized outside of Florida, Georgia, and Alabama (Karabatsos *in* Berge 1975). The diagnositic criteria, the tests and antigens employed, and the travel history of the patient were not reported in this case. Cache Valley should be included in the battery of antigens used in diagnostic laboratories.

The detection of neutralizing antibody to Cache Valley virus in white-tailed deer in Connecticut is consistent with similar serosurveys of deer in New York (Whitney et al. 1969), Virginia (Buescher et al. 1970), Wisconsin (Issel et al. 1970, Hoff et al. 1970), North Dakota (Hoff et al. 1973), and Texas (Issel et al. 1970). High antibody rates have also been reported in other species of wild and domestic ungulates (Whitney 1965, Buescher et al. 1970, Yuill et al. 1970, Hoff et al. 1970, 1973) with little or no antibody detected in small or medium-sized animals from some of the same areas (Whitney et al. 1968, Buescher et al. 1970). Strains of Cache Valley-like virus were isolated from a caribou and a horse in Wisconsin (Hoff et al. 1970).

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Literature Cited

Anslow, R. O., W. H. Thompson, P. H. Thompson, G. R. DeFoliart, O. Papadopoulos and R. P. Hanson. 1969. Isolation of Bunyamwera-group viruses from Wisconsin mosquitoes. Am. J. Trop. Med. Hyg. 18:599–608.

Berge, T. O. (Editor). 1975. International catalogue of arboviruses including certain other viruses of vertebrates. USDHEW Publ. No. (CDC) 75-8301. 789 pp.

Bishop, D. H. L. and R. E. Shope. 1979. Bunyaviridae. Chap. 1. Comprehensive Virology. Fraenkel-Conrat, H. and R. R. Wagner (Ed.) 14:1–156.

Buescher, E. L., R. J. Byrne, G. C. Clarke, D. J. Gould, P. K. Russell, F. G. Scheider and T. M. Yuill. 1970. Cache Valley virus in the Del Mar Va Peninsula. I. Virologic and serologic evidence of infection. Am. J. Trop. Med. Hyg. 19:493–502.

Burton, A. N., J. McLintock and D. B. Francy. 1973. Isolation of St. Louis encephalitis and Cache Valley viruses from Saskatchewan mosquitoes. Can. J. Publ. Hlth. 64:368–73.

Hoff, G. L., C. J. Issel, D. O. Trainer and S. H. Richards. 1973. Arbovirus serology in North Dakota mule and white-tailed deer. J. Wildlife Dis. 9:291–95.

Hoff, G. L., J. Spalatin, D. D. Trainer and R. P. Hanson. 1970. Isolation of a Bunyamwera

group arbovirus from a naturally infected caribou. J. Wildlife Dis. 6:483–87.

Holden, P. and A. D. Hess. 1959. Cache Valley virus, a previously undescribed mosquito-borne agent. Science 130:1137–88.

Issel, C. J., G. L. Hoff, D. O. Trainer and W. H. Thompson. 1970. Serologic evidence of Bunyamwera group abrovirus infections in Wisconsin and Texas deer. J. Wildlife Dis. 6:479–82.

Kokernot, R. H., J. Hayes, C. H. Tempelis, D. H. M. Chan, K. R. Boyd and R. J. Anderson. 1969. Arbovirus studies in the Ohio-Mississippi basin, 1964–1967. IV. Cache Valley virus. Am. J. Trop. Med. Hyg. 18:768–73.

McGowan, J. E., J. A. Bryan and M. B. Gregg. 1973. Surveillance of arboviral encephalitis in the United States, 1955–1971. Amer. J. Epid. 97:199–207.

Main, A. J., A. L. Smith, R. C. Wallis and J. Elston. 1979. Arbovirus surveillance in Connecticut. I. Group A viruses. Mosq. News 39:544–51.

Saliba, E. K., G. R. DeFoliart, T. M. Yuill and R. P. Hanson. 1973. Laboratory transmission of Wisconsin isolates of a Cache Valley-like virus by mosquitoes. J. Med. Entomol. 10:470–76.

Sekla, L. H., W. Stackiw and R. A. Brust. 1980. Arbovirus isolations from mosquitoes in Manitoba. Mosq. News 40:377–82.

Srihongse, S., J. P. Woodall, M. A. Grayson, R. Deibel, T. F. Bast, C. D. Morris, E. M. Bosler, J. L. Benach, J. J. Howard and J. Berlin. 1980. Arboviruses in New York State: surveillance in arthropods and nonhuman vertebrates 1972–1977. Mosq. News 40:269–76.

Sudia, W. D., V. F. Newhouse, C. H. Calisher and R. W. Chamberlain. 1971. California group arboviruses: isolations from mosquitoes in North America. Mosq. News 31:576–600.

Whitney, E. 1965. Arthropod-borne viruses in New York State: Serologic evidence of Group A, B, and Bunyamwera viruses in dairy herds. Am. J. Vet. Res. 26:914–19.

Whitney, E., H. Jamnback, R. G. Means and T. H. Watthews. 1968. Arthropodborne-virus survey in St. Lawrence County, New York: Arbovirus reactivity in serum from amphibians, reptiles, birds and mammals. Am. J. Trop. Med. Hyg. 17:645–650.

Whitney, E., A. P. Roz, G. A. Rayner and R. Deibel. 1969. Serologic survey for arbovirus activity in deer sera from nine counties in New York State. Bull. Wildlife Dis. Assoc. 5:392–397.

Wong, Y. W., D. C. Dorsey, M. J. Humphreys and W. J. Hausler. 1970. Arbovirus encephalitis surveillance in Iowa. Health Lab. Sci. 7:117–123.

Wong, Y. W., W. A. Rowley, D. C. Dorsey and W. J. Hausler. 1978. Surveillance of arbovirus activity in Iowa during 1972–1975. Mosq. News 38:245–251.

Yuill, T. M., W. S. Gochenour, F. R. Lucas, M. J. Collins and E. L. Buescher. 1970 Cache Valley virus in the Del Mar Va. Peninsula. III. Serologic evidence for natural infection of dairy cattle. Am. J. Trop. Med. Hyg. 19:506–12.

Yuill, T. M. and P. H. Thompson. 1970. Cache Valley virus in the Del Mar Va Peninsula. IV. Biological transmission of the virus by *Aedes sollicitans* and *Aedes taeniorhynchus*. Am. J. Trop. Med. Hyg. 19:513–19.