

## ISOLATION OF CALIFORNIA GROUP ARBOVIRUSES FROM PENNSYLVANIA MOSQUITOES—1971, 1972

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**ABSTRACT.** During 1971 and 1972, 58,163 mosquitoes from eight locations within the Commonwealth of Pennsylvania were processed for virus isolation. Twenty-six virus isolations were made from 756 pools of mosquitoes which represented 6 of the 8 locations. Seventeen of the isolates were identified as belonging to the California group of arboviruses. Nine of these were isolated from *Aedes trivittatus* and were identified as Trivittatus subtype. Four isolated from *Culex salinarius*, one from *C. pipiens* and one from *Culiseta melanura* were identified as South

River (NJO-94F) subtype. A Keystone subtype was isolated from a pool of *Anopheles punctipennis* while one isolate from *Aedes trivittatus* was not subtyped. In addition, Flanders virus was isolated from a pool of *Culiseta melanura*. The other eight isolates were not identified. This report includes 2 new host records for South River (NJO-94F) subtype, a new host record for Keystone subtype, and new geographical records for Trivittatus, South River (NJO-94F) and Keystone.

The growing importance of California group arboviruses as etiologic agents of human disease in North American has been well documented (Sudia *et al.*, 1971; Henderson and Coleman, 1971). Over the past several years serologic surveys demonstrated the presence of California virus in Pennsylvania (Pidcoe, Wills, and Satz, unpublished).

In 1971, sentinel rabbits were employed to assess the distribution of this arbovirus throughout Pennsylvania (Wills and Pidcoe, 1972). As a result of demonstrating antibody to California virus in serum samples from some of the sentinel rabbits, 8 locations were selected to collect mosquitoes for the purpose of virus isolation and identification. This study was stimulated by the reports of a large number of cases of California encephalitis in Ohio (Master-son *et al.*, 1971) and of the isolation of California group viruses from New York and New Jersey (Whitney *et al.*, 1969). Pennsylvania had only three confirmed cases of California encephalitis reported through 1970 (CDC Encephalitis Summary, 1972). An additional case was con-

firmed in 1972. Every year many cases of "encephalitis-etiology unknown" are reported in Pennsylvania, and it is suspected that some are California virus infections.

### MATERIALS AND METHODS

Mosquito collections were made using CDC light traps with a modification of the electrical system (Carroll and Wills, 1973) and dry ice as an attractant. Mosquitoes were killed in dry ice, placed in shell vials with a rubber stopper, taped shut, marked and stored in dry ice. At the end of each week they were sent to the Pennsylvania Department of Health Laboratories in Philadelphia where they were stored at  $-70^{\circ}\text{C}$ . Using a chill table, the mosquitoes were sorted to species and placed in pools of 100. Pools were processed using the CDC method of Sudia and Chamberlain (1967) and injected intracerebrally into suckling mice (SM). When neurological symptoms occurred in the mice, a second passage was made in SM as well as an attempt at isolation in duck embryo tissue culture (DETC). Mice demonstrating neurological symptoms through two passages of brain homogenate were considered tentatively positive for arbovirus activity. Viral identification of positive mouse brain material was performed by inoculating isolates in primary DETC. Isolates demonstrating plaques

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were tentatively identified by the plaque reduction neutralization test (PRNT) using CDC reference antisera to California, eastern equine encephalitis, western equine encephalitis and St. Louis encephalitis viruses.

## RESULTS AND DISCUSSION

Of the 8 locations chosen for mosquito collections, viruses were isolated from 6 (Table 1). These locations were Shamokin Dam, Snyder County; Tinicum Marsh, Philadelphia County; Camp Speers, Pike County; State College, Centre County; Raccoon Creek State Park, Beaver County; and Pulaski Township, Lawrence County.

At the other 2 locations 8,157 mosquitoes were collected, but all pools were negative. One provided good mosquito yields, while the other site was relatively unproductive.

Table 2 summarizes the mosquito species collected monthly in 1971 and 1972 and the number of pools tested for arboviruses.

Table 3 summarizes the mosquito species collected in 6 counties and the number of virus isolations.

From the mosquitoes collected in 1971 and 1972, a total of 26 viruses were iso-

lated in SM. Of the 26, 18 were tentatively identified by PRNT as California virus; 7 were lost due to a freezer breakdown; and one could not be identified. The 19 isolates were forwarded to Dr. Charles Calisher of the Arbovirus Reference Unit, Center for Disease Control, where 17 of the 18 tentatively identified as California viruses were confirmed as California virus by the complement-fixation test. The remaining one was not viable. The 17 viruses were further subtyped by CDC and the results are as follows:

Trivittatus (TV)	9
South River (NJO-94F)	6
Keystone (Key)	1
Subtype not determined	1

The SM isolate not identified in our laboratory was identified by the CDC as Flanders virus.

ISOLATIONS. The California group is divided into three complexes: Trivittatus, Melao and California. Isolations of members of the Melao complex were not found during this study.

CALIFORNIA COMPLEX. The California complex is represented by two subtypes in Pennsylvania: South River (NJO-94F) and Keystone.

TABLE 1. California group virus isolated from Pennsylvania mosquitoes, 1971 & 1972

Month	County	Species	Arbovirus isolated		
			Trivittatus	South River	Keystone
June	Snyder	<i>Aedes trivittatus</i>	1	..	..
July					
August	Snyder	<i>Aedes trivittatus</i>	1	..	..
	Beaver	<i>Aedes trivittatus</i>	4	..	..
		<i>Culex salinarius</i>	..	4	..
	Philadelphia	<i>Aedes trivittatus</i>	1	..	..
	Pike	<i>Culiseta melanura</i>	..	1	..
	Lawrence	<i>Culex pipiens</i>	..	1	..
	Centre	<i>Anopheles punctipennis</i>	..	..	1
September	Snyder	<i>Aedes trivittatus</i>	2	..	..
			9	6	1

TABLE 2. Mosquito species collected monthly in 1971 &amp; 1972 from six locations in Pennsylvania and the number of pools tested for arboviruses.

	Mosquitoes					
	June	July	August	September	October	
<i>Aedes canadensis</i>	1,078 (12)	4,748 (50)	250 (10)	2 (1)	...	6,078 (73)
<i>Aedes trivittatus</i>	486 (5)	749 (10)	3,539 (44)	2,829 (29)	15,583 (154)	23,186 (242)
<i>Aedes vexans</i>	...	510 (11)	1,895 (29)	384 (5)	1,066 (16)	3,855 (61)
<i>Aedes triseriatus</i>	...	...	...	9 (1)	...	33 (2)
<i>Culex pipiens</i>	337 (4)	1,538 (17)	24 (1)	52 (1)	...	2,134 (28)
<i>Culex salinarius</i>	...	1,385 (16)	11,655 (110)	18 (1)	...	13,058 (136)
<i>Anopheles punctipennis</i>	...	...	245 (15)	107 (2)	...	352 (17)
<i>Culiseta melanura</i>	...	...	386 (5)	...	...	386 (5)
<i>Protophthora ferox</i>	...	5 (3)	1 (1)	32 (1)	26 (3)	64 (8)
<i>Coquillettidia perturbans</i>	...	477 (10)	342 (11)	...	...	819 (21)
Dark Legged <i>Aedes</i>	...	...	41 (2)	...	...	41 (2)
Totals	1,901 (21)	9,412 (117)	18,585 (243)	3,433 (41)	16,675 (173)	50,006 (595)

(Number of pools tested for virus)

TABLE 3. Summary of mosquito species collected in six counties, 1971-1972, in Pennsylvania and number of virus isolations.

	Snyder County		Pike County		Philadelphia County		Beaver County		Lawrence County		Centre County		Total
	County		County		County		County		County		County		
<i>Aedes canadensis</i>	...	5991	...	14	...	1	...	60	...	12	...	6,078	
<i>Aedes trivittatus</i>	20,975 (4)	1,238 (1)	...	6	...	836 (4)	...	106	...	25	...	23,186 (9)	
<i>Aedes vexans</i>	1,367	481	...	811	...	106	...	584	...	506	...	3,855	
<i>Aedes triseriatus</i>	...	...	...	...	...	...	...	...	...	...	...	33	
<i>Culex pipiens</i>	52	1,936	...	...	...	83	...	63 (1)	...	...	...	2,134 (1)	
<i>Culex salinarius</i>	...	1,144	...	11,853 (4)	...	...	...	...	...	61	...	13,058 (4)	
<i>Anopheles punctipennis</i>	16	38	...	23	...	26	...	52	...	197 (1)	...	352 (1)	
<i>Culiseta melanura</i>	...	386 (1)	...	...	...	...	...	...	...	...	...	386 (1)	
<i>Protophthora ferox</i>	58	5	...	...	...	...	...	...	...	...	...	64	
<i>Coquillettidia perturbans</i>	...	561	...	257	...	...	...	...	...	...	...	819	
Dark Legged <i>Aedes</i>	...	...	...	...	...	...	...	41	...	...	...	41	
Total	22,468 (4)	11,780 (2)	...	12,964 (4)	...	1,952 (4)	...	906 (1)	...	836 (1)	...	50,006 (16)	

(Number of virus isolations)

South River (NJO-94F) is as yet an uncatalogued virus; however, it is believed by some workers to be a bona fide subtype (Henderson and Coleman, 1971; and Sudia *et al.*, 1971). This subtype has been isolated only 3 times from New Jersey and is poorly understood. The 6 isolations from Pennsylvania mosquitoes provide new information about the geographical distribution (Figure 1). New mosquito host records for this subtype are *Culex pipiens*, and *C. salinarius*. The ecology of the 3 geographical areas of the State is different. Tinicum Marsh is near the highly urbanized area of Philadelphia; Pulaski Township in Lawrence County in the western part of Pennsylvania is semi-rural;

while Pike County in the Pocono Mountains is a sylvan area. The common denominator is the avian population. We feel there is a strong possibility that this subtype of the California complex is an avian virus, although much more work is needed to substantiate this hypothesis.

The Keystone subtype isolated from a pool of *Anopheles punctipennis* mosquitoes in Central Pennsylvania (Figure 2), represents a new mosquito host. Prior to this isolation, this subtype was known to occur only as far north as Maryland.

TRIVITTATUS COMPLEX. This complex is represented by only one subtype, Trivittatus. Although as many as 15 species of mosquitoes have been found to be infected

Figure 1

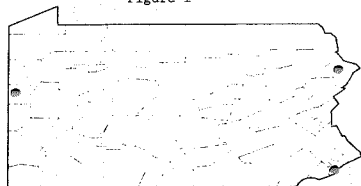
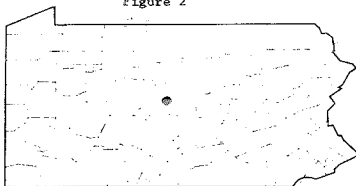
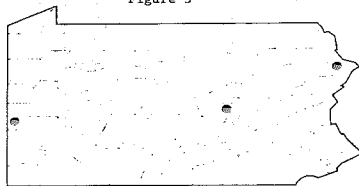
SOUTH RIVER  
(NJO-94F)

Figure 2



KEYSTONE

Figure 3



TRIVITTATUS

Figure 4

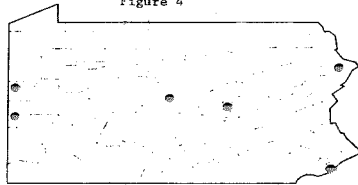
Distribution of California Group Viruses  
in Pennsylvania

FIG. 1. Map of Pennsylvania showing isolations of South River (NJO-94F) subtype, California group virus in Lawrence, Pike and Philadelphia counties.

FIG. 2. Map of Pennsylvania showing isolations of Keystone subtype, California group virus in Centre County.

FIG. 3. Map of Pennsylvania showing isolations of Trivittatus subtype, California group virus in Beaver, Snyder and Pike counties.

FIG. 4. Map of Pennsylvania showing the distribution of all California group virus isolates.

with this subtype, the only isolations in Pennsylvania have been from *Aedes trivittatus*. This virus has been isolated from 3 separate counties in Pennsylvania (Figure 3) representing new geographical records.

#### STUDY LOCATIONS

**SHAMOKIN DAM, SNYDER COUNTY.** Shamokin Dam is a small community directly north of Selinsgrove on U.S. Route 11 approximately 40 miles north of Harrisburg. The collection site was on the historic Erie Canal that at one time ran from Erie to Philadelphia. The canal is still present in about one-fourth of its original length of over 400 miles and produces hordes of mosquitoes where it still holds water. Summer homes face the Susquehanna River and are within about 100 yards from the heavily wooded breeding sites. In 1971 over 22,000 mosquitoes were collected from this area in August, September and October. In June, 1972 additional collections were made from this area but, due to mosquito control pressure, collections were meager. On June 21 tropical storm Agnes completely devastated the area including the mosquito fauna. Four of the virus isolates from pools of *Aedes trivittatus* were identified by CDC as the subtype *Trivittatus* of California virus.

**CAMP SPEERS, PIKE COUNTY.** Camp Speers is a YMCA camp approximately 40 miles north of Stroudsburg in the center of the Pocono Mountains. The camp is used by several hundred children throughout the summer from the middle of June until the end of August. Sentinel rabbits in three different locations in Pike County showed neutralizing antibodies to California virus (Wills and Pidcoe, 1972). Camp Speers was chosen for mosquito collections because of its close proximity to a large swamp. Collections were made in August, 1971 with 903 specimens sorted into 7 pools. In 1972 collections were made in June, July and August, and 11,780 mosquitoes were separated into 141 pools. Flanders virus was isolated

from a pool of 105 specimens of *Culiseta melanura* collected in 1971. Two isolations from 1972 collections were made from pools of *Culiseta melanura* and *Aedes trivittatus*. The isolate from *Culiseta melanura* was identified as California virus, subtype South River (NJO-94F).

**TINICUM WILDLIFE PRESERVE, PHILADELPHIA COUNTY.** Tinicum Wildlife Preserve is located in southwestern Philadelphia County approximately 3 miles from the Philadelphia International Airport. It abounds in many species of migrating birds as well as resident species such as pheasant. The land area is small and peninsular-like with a few trees. Collections were made in July and August, 1972. *Culex salinarius* represented about 90% of the 12,964 mosquitoes collected and sorted into 151 pools. Four isolations of South River (NJO-94F) subtype of California virus were made from *Culex salinarius*. All occurred in collections made August 8 and 9, 1972 (Table 1). These isolations represent a new mosquito host record for South River (NJO-94F) subtype.

**RACCOON CREEK STATE PARK, BEAVER COUNTY.** The actual collections from this area were made from the Western Pennsylvania Wildflower Reserve adjacent to Raccoon Creek State Park about 40 miles from Pittsburgh. This area consists of a mixed hardwood and pine stand of forests. Nature trails lead from the caretaker's residence to a small ravine where a small stream flows, surrounded by marshy areas. Although collections here were small due to extremely heavy rain, we were able to isolate 4 viruses from 9 pools of *Aedes trivittatus* collected in August, 1972.

**PULASKI TOWNSHIP, LAWRENCE COUNTY.** The site of this collection was adjacent to the residence of a 6-year-old girl who had California virus encephalitis in July of 1972. The area was semi-rural and consisted of a hardwood forest with a junkyard of discarded cars and tires approximately 100 yards behind the house. Mosquitoes were also being produced in untreated effluent from an over-flowing septic system. This area is only one-half

mile from the Ohio state line. Collections were made on August 24 and 25. One isolation of South River (NJO-94F) was made from a pool of 31 *Culex pipiens*. This again is a new host record for this subtype of California virus.

STATE COLLEGE, CENTRE COUNTY. The site of these collections was an experimental area at Pennsylvania State University that uses treated sewage effluent for spray irrigation. Traps were located in a stand of hardwood trees that abounds in a wide variety of birds and mammals. Keystone virus was isolated from a pool of *Anopheles punctipennis*. This is a new mosquito host record and a new geographical record for this subtype.

### CONCLUSION

From these studies we found that California group arboviruses are widely distributed throughout the Commonwealth of Pennsylvania (Figure 4). At least 2 of the 3 complexes of this group were identified. A puzzling aspect of this study is the overwhelming evidence of the presence of these viruses and the small number of acute cases reported. Due to the loss of 7 isolates, minimum field infection rates (MFIR) are not established in this study. They would, however, be extremely high due to the large number of isolations from the relatively small number of mosquitoes processed for virus isolation.

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