

ARTICLES

PRECIPITIN TESTS OF BLOOD-FED MOSQUITOES COLLECTED DURING THE VEE SURVEILLANCE SURVEY IN THE SOUTHERN UNITED STATES IN 1971¹

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INTRODUCTION. In the course of mosquito surveillance surveys by U.S. Army personnel during the 1971 epidemic of Venezuelan equine encephalitis in the southwestern U.S., over 500,000 adult female mosquitoes were collected, identified and pooled for virus isolation (Eldridge *et al.*, 1972). To provide more information on the vector link in this epidemic cycle it was considered desirable to obtain host affiliation data especially on those mosquito species implicated as primary vectors. Some 700 mosquitoes in the surveillance collection had taken recent blood meals and were segregated and tested for host identification. This paper presents the results of these tests.

MATERIALS AND METHODS. Mosquitoes were collected with CDC miniature light traps supplemented with dry ice. Collections were frozen in the field and air-shipped on dry ice for initial processing at the Fifth U.S. Army Medical Laboratory, Ft. Sam Houston, Texas. Identification and pooling was accomplished over wet ice. The abdomens of mosquitoes which showed evidence of recent blood meals were removed and placed in gelatin capsules. The remainder of the bodies of the mosquitoes were pooled with non-engorged specimens for virus isolation attempts. The abdomens were then refrozen and held at -40° C. until tested.

The preparation and testing of blood

meals followed the modified precipitin method described by Tempelis and Lofy (1963). Initial screening against mammal, bird, and reptile was accomplished using broadly reacting antisera produced in rabbits.² The positive reactors were then tested against specific antisera produced in roosters. The production of antisera was closely monitored by titration and only those displaying a homologous titer of 1:10,000 were used. The flocculation test described by Tempelis and Reeves (1962) was used to determine titers of antisera. Precipitin tests were conducted in capillary tubes with readings recorded at 3 hours and then overnight. Controls and dilutions to attempt elimination of deer-bovine-goat crossings were as described by LeDuc *et al.* (1972).

RESULTS AND DISCUSSION. A total of 700 blood-engorged mosquitoes comprising 28 species were tested (Table 1). Ninety-four percent of all mosquitoes tested were positive against mammal screening antisera. A wide variety of specific antisera reactions were obtained, reflecting the diversity of collection areas ranging from arid West Texas to the salt marshes of Louisiana (Table 2). Few mosquito species fed both on horses and man. Significant numbers of horse feedings were evident in *Psorophora cyaneescens* (62%), *P. confinnis* (28%), *Aedes*

¹ From the Department of Entomology, Walter Reed Army Institute of Research, Washington, D.C. 20012. The opinions or assertions contained herein are the private views of the author and are not to be construed as official or as reflecting the views of the Department of the Army or the Department of Defense.

² In conducting the research described in this report, the investigators adhered to the "Guide for Laboratory Animal Facilities and Care," as promulgated by the Committee on the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Academy of Sciences—National Research Council.

nigromaculis (29%), and *A. vexans* (13%). These also showed human involvement with feeding ratios of horses to humans of 17:1, 19:1, 20:1, and 15:1 respectively. Two of these species (*P. cyanescens* and *P. confinnis*) were previously implicated with VEE transmission in South Texas (Sudia and Newhouse, 1971). The predominance of large animal feeding by *Psorophora* has been reported by other workers. Whitehead (1951) in Arkansas found that cattle (69%), pigs (16%), horses (14%), and humans (1%) constituted the major hosts for *P. confinnis*. Schaefer and Steelman (1969) found that *P. confinnis* fed primarily on cattle (95%) in the gulf coast area of Louisiana.

Avian feeding represented 3.3 percent of the total blood engorged mosquitoes.

The majority of bird feeding (Table 3) was attributed to *Culex tarsalis* (26%), *C. quinquefasciatus* (22%), and *C. (Melanoconion) spp.* (22%). Greatest avian host preference was shown by *C. quinquefasciatus* (56%), *C. tarsalis* (23%), and *C. (Melanoconion) spp.* (16%). Several other workers have reported a high percentage of bird feeding by *C. tarsalis* (Anderson *et al.*, 1967; Tempelis *et al.*, 1967; Gunstream *et al.*, 1971) and *C. quinquefasciatus* (Tempelis and Reeves, 1964).

The mosquitoes attracted to reptiles comprised 1.1 percent of the total. All reptile positive mosquitoes reacted to turtle specific antiserum. *P. confinnis* made up 50 percent of the total positive reptile feeders. No previous reptilian host record

TABLE 1.—Results of tests of mosquito blood meals with screening antisera.

Mosquito species	Number tested	Mammal positive	Bird positive	Reptile positive	Double feeding	Negative
<i>Aedes atlanticus-tormentor</i>	18	14	2	2
<i>A. atropalpus</i>	1	1
<i>A. dorsalis</i>	1	1
<i>A. dupreei</i>	2	2
<i>A. fulvus pallens</i>	1	1
<i>A. nigromaculis</i>	66	66	4	..
<i>A. sollicitans</i>	10	9	..	1
<i>A. sticticus</i>	2	2	2	..
<i>A. taeniorhynchus</i>	3	3
<i>A. thelcter</i>	9	9
<i>A. vexans</i>	111	111	1	1
<i>A. spp.</i>	3	3	1	..
<i>Anopheles crucians</i> complex	2	2
<i>An. pseudopunctipennis</i>	1	1
<i>An. punctipennis</i>	1	1
<i>An. quadrimaculatus</i>	3	3	1	..
<i>Culex coronator</i>	7	7
<i>C. quinquefasciatus</i>	9	4	5	..	1	..
<i>C. salinarius</i>	137	132	2	3
<i>C. tarsalis</i>	26	20	6	..	2	..
<i>C. (Culex) spp.</i>	31	29	2	..	1	..
<i>C. (Melanoconion) spp.</i>	31	26	5
<i>Culiseta melanura</i>	1	..	1
<i>Mansonia perturbans</i>	1	1
<i>Psorophora ciliata</i>	3	3
<i>P. confinnis</i>	72	67	..	4	..	1
<i>P. cyanescens</i>	114	108	4	6
<i>P. discolor</i>	1	1
<i>P. ferox</i>	3	2	1
<i>P. signipennis</i>	13	13
<i>P. spp.</i>	4	4
Unidentified	13	13
Total	700	659	23	8	17	10

TABLE 2.—Identification of mosquito blood meals positive for mammal antisera.

Mosquito species	Number tested ¹												
		Bovine	Cat	Deer	Dog	Goat-sheep	Horse	Human	Opossum	Pig	Rabbit	Raccoon	Deer-goat-bovine
<i>Aedes atlanticus-tormentor</i>	14	I	..	I	I	3	I	..	6	..	I
<i>A. atropalpus</i>	I	I
<i>A. dorsalis</i>	I	I
<i>A. dupreei</i>	2	I	..	I
<i>A. fulvus pallens</i>	I	I
<i>A. nigromaculis</i>	70	2	..	6	..	17	20	I	..	I	9	..	14
<i>A. sollicitans</i>	9	I	4	I	I	..	2
<i>A. sticticus</i>	4	2	2
<i>A. taeniorhynchus</i>	3	2	I
<i>A. thelcter</i>	9	I	7	..	I
<i>A. vexans</i>	112	6	I	6	..	38	15	I	..	2	7	..	36
<i>A. spp.</i>	4	I	I	..	I	I
<i>Anopheles crucians</i> complex	2	I	I
<i>An. pseudopunctipennis</i>	I	I
<i>An. punctipennis</i>	I	I
<i>An. quadrimaculatus</i>	4	I	I	2
<i>Culex coronator</i>	7	7
<i>C. quinquefasciatus</i>	5	I	I	I	..	I	I
<i>C. salinarius</i>	132	4	..	30	5	32	I	..	3	I	12	I	43
<i>C. tarsalis</i>	22	4	I	5	2	..	I	I	2	..	6
<i>C. (Culex) spp.</i>	30	4	..	2	..	13	2	I	..	8
<i>C. (Melanoconion) spp.</i>	26 ²	3	..	2	..	3	I	5	..	11
<i>Mansonia perturbans</i>	I	I
<i>Psorophora ciliata</i>	3	I	2
<i>P. confinnis</i>	67	3	..	2	..	29	19	I	7	..	6
<i>P. cyanescens</i>	112	I	..	6	..	9	69	4	I	2	13	..	7
<i>P. discolor</i>	I	I
<i>P. ferox</i>	2	I	I
<i>P. signipennis</i>	13	I	4	I	6	..	I
<i>P. spp.</i>	4	4
Unidentified	13	I	..	9	2	..	I
Total	676	30	I	6I	15	162	135	13	9	16	88	2	143

¹ Total includes double feeds.² Includes one unidentified blood meal.

TABLE 3.—Identification of mosquito blood meals positive for bird antisera.

Mosquito species	Number tested	Anseri-formes	Ciconii-formes	Columbi-formes	Galli-formes	Grui-formes	Passeri-formes
<i>Aedes atlanticus-tormentor</i>	2 ¹	I	..
<i>Culex quinquefasciatus</i>	5	2	..	I	2
<i>C. salinarius</i>	2	I	I
<i>C. tarsalis</i>	6	I	2	..	I	..	2
<i>C. (Culex) spp.</i>	2 ¹	I
<i>C. (Melanoconion) spp.</i>	5	..	I	..	I	..	3
<i>Culiseta melanura</i>	I	I
Totals	23	4	3	I	3	I	9

¹ Includes one unidentified blood meal (each).

for this species was noted. *C. salinarius* was previously found to feed on turtles by Hayes (1961). Murphey *et al.* (1967) reported limited feeding on turtles by *Aedes sollicitans* and were collected from tethered turtles by Crans and Rockel (1968).

Fourteen multiple feedings were noted involving seven species. All except *A. sticticus* and *Anopheles quadrimaculatus* were previously reported to possess such feeding behavior by Edman and Downe (1964).

SUMMARY. Seven hundred engorged specimens of 28 species were collected from four southern-southwestern states during VEE surveillance in July-August 1971. The blood meals were identified by precipitin tests. Little host specificity could be demonstrated for any species tested. Ninety-four percent of the specimens contained mammalian blood with 3 percent positive for avian hosts and 1 percent indicating reptilian affiliation. Evaluation of host preference for known VEE vectors indicates that *P. confinnis* and *P. cyanoescens* are of considerable epidemiological importance by showing horse to human feeding ratios of 19:1 and 17:1 respectively.

ACKNOWLEDGMENTS. The authors thank the survey team members who worked diligently and capably under very difficult circumstances. MAJ John F. Reinert, MAJ Reginal Richardson, CPT Edward S. Saugstad, CPT Bruce Harrison and one of the authors (BFE) identified the mosquitoes.

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