

ARTICLES

ARBOVIRUS-VECTOR STUDIES IN THE CENTRAL VALLEY OF CALIFORNIA, 1969

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California experienced its largest epidemic of encephalitis in 1952, when 375 human cases of western encephalitis (WE) and 45 cases of St. Louis encephalitis (SLE) were recorded (Longshore *et al.*, 1956). Epidemiologic studies of these diseases have been made continuously in California since 1937 (Reeves *et al.*, 1964). These studies and others (Hess *et al.*, 1963) were directed toward developing an understanding of the climatological and ecological factors which precede epidemics of WE and SLE. Historically, excessive precipitation, in the form of rainfall and snowpack in the Sierra Nevada Mountains, results in extensive flooding in the Central Valley of California; conditions favorable to a rapid increase in the *Culex tarsalis* Coq. population are therefore created. This species is considered the primary vector of WE and SLE in California (Hardy, 1970).

Environmental conditions during the winter of 1963 and the spring of 1969, as described by Reeves, 1970, were essentially similar to those preceding the 1952 epidemic (Halverson *et al.*, 1953); they were also similar to conditions in 1958 when a WE epidemic was thought to have been aborted (Reeves *et al.*, 1964).

Because these circumstances might lead to an epidemic similar to that in 1952, steps were taken to increase mosquito control activities and to intensify virus surveillance through the collection and testing of mosquito vectors. The University of California, School of Public Health, Berkeley, maintained arbovirus surveil-

lance (Reeves, 1970) in Kern County (Lyness, 1970) and in Butte and Glenn Counties (Nelson, 1970). Staff members and students from the University of California, Los Angeles, School of Public Health, studied Inyo and Imperial Counties (Dr. T. H. Work, 1969, University of California, Los Angeles, personal communication).

In June 1969, the Arbovirus Ecology Laboratory (AEL), Virology Section, Center for Disease Control (CDC), was requested to join the California State Department of Public Health (CSDPH) in a cooperative arbovirus-vector surveillance in 14 other counties of the Central Valley. This study was unique in that no previous study had been done which attempted to perform a unified virus-vector survey in an area larger than 18,000 square miles. The results of this cooperative study are the subject of this report.

METHODS. U. S. highway 99 roughly divides the Central Valley into two parts, east and west. Beginning at the southern end of the valley, two 2-man teams from the AEL, each with a station wagon containing mosquito collecting equipment, were assigned daily a 40- to 50-mile zone on either side of the highway. Within each zone, attempts were made to locate suitable rural and urban sites, both within and outside of mosquito-controlled districts, for the collection of adult mosquitoes. Each team used 20 CDC battery-operated light traps (Sudia and Chamberlain, 1962) supplemented by dry ice (Newhouse, *et al.*, 1966). The traps were placed in the field in five groups of four traps or four groups of five traps to obtain adequate coverage and samples from each site studied. Unproductive sites were not revisited, but new sites were

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selected. Collections were made at all productive sites on each subsequent trip. More than 80 sites were used in this study. After each night's operation, the traps were removed and new trapping sites were located in the next 40- to 50-mile zone. Thus, about eight nights of operation per month were required to obtain samples of mosquitoes for virus tests from approximately

a 400-mile length of the Central Valley, Figure 1.

Mosquito collections were made on June 4-10, July 1-9, August 17-22, and September 9-18, 1969. Standard CDC methods for collecting, field handling, and laboratory processing of specimens for virus isolation were used (Sudia and Chamberlain, 1967). Some of the specimens col-

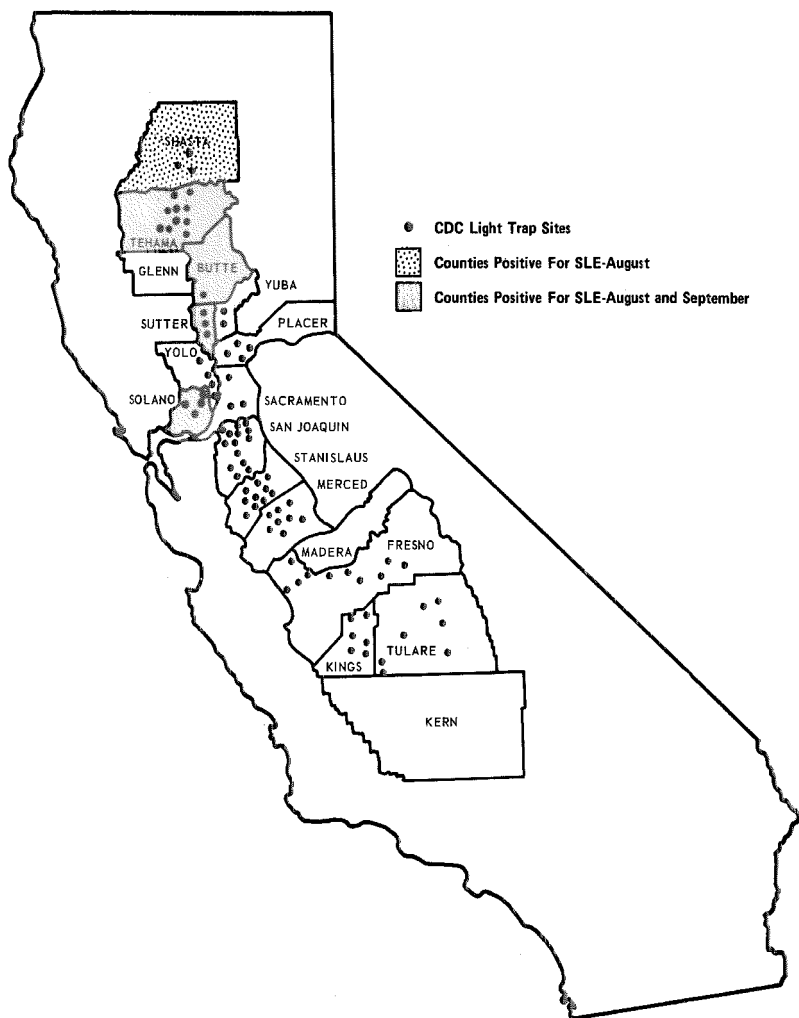


FIG. 1.—Locations of light trap sites, Central Valley, California

lected in June and July were sent to the Viral and Rickettsial Disease Laboratory (VRDL), CSDPH, Berkeley, for virus testing; the others were tested in the AEL facilities at CDC, Atlanta, Georgia.

CSDPH entomologists made additional CDC light trap collections in several of the counties, and a number of resting station collections were made in the vicinity of reported SLE cases. All of these specimens were sent to the VRDL in Berkeley, California, for testing.

At the AEL, the mosquitoes collected in June were tested by intra-cerebral inoculation of suckling mice (SM), 2 to 3 days old. Brains of mice that became sick or died were passed in additional litters of SM, and the brain harvest was used in CF tests for identification (Casey, 1965). Beginning in July, all mosquito pools were tested in duck embryo cell cultures (DEC) with agar overlay. The DEC cultures were inoculated with mosquito suspensions and observed for plaque formation daily for 10 days. Representative plaques from each isolate were passed in DEC fluid cultures. Viruses from these cultures were identified in DEC plaque reduction tests as described by Chappell, 1971.

Selected mosquito pools, previously tested in DEC were subsequently retested in SM in an attempt to isolate the viruses that do not plaque in DEC. These SM isolates were identified by CF tests as stated above.

In the VRDL, CSDPH, all mosquito suspensions were inoculated into SM. Brains harvested from ill mice were subpassaged into additional SM litters. Brain smears were prepared from first or second passage material, and the FA technique was used for rapid preliminary identification (Emmons, 1970 unpublished data). These identifications were confirmed by the neutralization tests in SM.

RESULTS. A summary of mosquito species collected monthly from June through September 1969 is given in Table 1. A total of 160,157 mosquitoes were collected in CDC light traps and tested in

3,487 pools; 1,404 of these pools, or slightly less than half, were *C. tarsalis* pools. Twenty-two mosquito species were represented. Tabulations of mosquitoes collected by county for the entire study are given in Table 2. These data indicate that *C. tarsalis* comprised 42 percent of the total mosquitoes collected. The lowest percentage occurred in Merced County (21%) and the highest (84%) in Kings County (Table 2).

Three other mosquito species were also abundant: *Aedes melanimon* Dyar—30,925, *Aedes vexans* (Meigen)—29,364, and *Anopheles freeborni* Aitken—15,088. The average number of mosquitoes captured in CDC light traps was 186. The averages for the individual counties ranged from a low of 43 mosquitoes per trap night in Stanislaus County to a high of 988 per trap night in the Sutter-Butte collections.

Supplementary resting station collections resulted in the capture and testing of an additional 1,693 mosquitoes, all *C. tarsalis*, with the exception of 214 *Culex peus* Speiser. These were tested in 37 pools (Table 3).

The viruses isolated during this study are presented in Table 4. Forty-eight virus isolations were made; 35 of these were SLE virus, 8 were Turlock virus, 4 were Hart Park-like, and 1 was Jamestown Canyon, a member of the California Group now considered identical to Jerry Slough virus. One pool of *C. tarsalis* from Tulare County collected in July was positive for both Turlock and Hart Park-like viruses. Western encephalitis virus was absent. An agent was isolated from *C. tarsalis* that produced small plaques unlike those of WE, SLE, or Turlock in DEC agar overlay. This agent did not produce symptoms in SM. Identification of this agent is continuing.

SLE virus was first isolated in August in the northern counties of the Sacramento Valley: Sutter-Butte, Tehama, and Shasta Counties. The numbers of SLE isolations were nearly equal for August (17) and September (18).

The SLE minimum field infection ratio

TABLE 1.

Summary of mosquito species collected monthly by CDC light trap from the central valley of California in 1969 and number of pools tested for arboviruses.

Mosquito Species	June	July	August	September	Total
<i>Aedes dorsalis</i>		2 (2)*	5 (-)**		7 (2)
<i>flavescens</i>			1 (1)		1 (1)
<i>inorepitis</i>	2 (1)	55 (2)			57 (3)
<i>melanimon</i>	183 (12)	9,010 (175)	5,539 (121)	16,193 (282)	30,925 (590)
<i>nigromaculis</i>	242 (11)	158 (9)	643 (28)	439 (19)	1,482 (67)
<i>sierrensis</i>	3 (3)	43 (9)		5 (3)	51 (15)
<i>sticticus</i>	3 (2)	8 (2)			11 (4)
<i>vexans</i>	430 (13)	16,743 (330)	4,457 (91)	7,734 (168)	29,364 (602)
<i>Anopheles</i>					
<i>franciscanus</i>	17 (4)	8 (5)	54 (9)	33 (9)	112 (27)
<i>freeborni</i>	229 (11)	2,591 (59)	8,441 (128)	3,827 (67)	15,088 (265)
<i>pseudopunctipennis</i>			3 (2)		3 (2)
<i>punctipennis</i>	66 (5)	515 (21)	142 (8)	42 (4)	765 (38)
<i>Culex</i>					
<i>erythrorhax</i>	113 (7)	1,556 (40)	137 (7)	1,695 (43)	3,501 (97)
<i>peus</i>	127 (10)	1,507 (52)	2,070 (28)	1,456 (45)	5,160 (135)
<i>pipiens complex</i>	113 (12)	631 (27)	255 (14)	2,629 (66)	3,628 (119)
<i>tarsalis</i>	4,848 (105)	26,923 (550)	15,251 (308)	20,849 (441)	67,871 (1,404)
<i>thriambus</i>			9 (-)		9 (-)
<i>Culiseta incidens</i>			8 (-)	7 (1)	15 (1)
<i>inornata</i>	578 (29)	111 (13)	16 (4)	61 (6)	766 (52)
<i>particeps</i>	206 (9)	767 (34)	10 (1)	9 (2)	992 (46)
<i>Mansonia</i>					
<i>perturbans</i>	17 (2)	306 (8)		11 (3)	334 (13)
<i>Orthopodomyia</i>					
<i>signifera</i>			3 (2)	12 (2)	15 (4)
Total	7,177 (236)	60,934 (1,338)	37,044 (752)	55,002 (1,161)	160,157 (3,487)

* Number of mosquitoes collected—(number of pools tested)

** Mosquitoes not tested.

(MFIR) ranged from 1:184 for *C. tarsalis* collected in Tehama County in September to 1:2871 in the same species from Sutter-Butte County in August (Table 5). Only one SLE virus isolation was made from *C. tarsalis* collected in resting stations. This isolate was obtained in September from Sutter County. The MFIR was 1:503, which is essentially the same (1:761) as that obtained from CDC light trap collections for the same time.

The MFIR for *C. peus* (1:257, Tehama, August and 1:100, Tehama, September) was equal to or lower than the rates for *C. tarsalis*.

MFIR were also calculated for each mosquito and virus based upon the totals of each for the month of isolation and for the entire 4-month period of the study, Table 6. The SLE MFIR for *C. tarsalis* for the month of isolation was 1:671, whereas the

season total MFIR was 1:2189. The seasonal rate for *C. peus* was 1:1290—higher than that of *C. tarsalis* but based on a much smaller sample.

The *A. melanimon*—Jamestown Canyon virus MFIR for the season was 1:30,925.

DISCUSSION. The major concern of health workers in California during the summer of 1969 was the potential for an outbreak of western encephalitis and/or St. Louis encephalitis. This study was initiated, in part, to locate areas of arbovirus activity so that control measures could be directed appropriately to prevent human illness. In our study and others (Reeves, 1970) (Lyness, 1970) (Nelson, 1970) more than adequate numbers of *Culex tarsalis* were collected and tested to detect the presence of WE virus in the vectors at levels of public health concern. Efforts were made to collect *C. tarsalis* in uncon-

TABLE 2. Summary of mosquito species collected by CDC light trap during arbovirus surveillance of 13 counties of the Central Valley of California, June-September 1969.

Mosquito Species	Kings	Tulare	Fresno	Merced	Stanislaus	San Joaquin	Solano	Sacramento	Yolo	Yuba-Placer	Sutter-Butte	Tehama	Shasta	Total Mosquitoes
<i>Aedes dorsalis</i>										1		6		7
<i>A. flavescens</i>												1		1
<i>A. inaeptus</i>				2				55						57
<i>A. melanmon</i>	141	372	1,839	13,760	9	36	2	48	21	859	13,529	307	2	30,925
<i>A. nigromaculis</i>	288	434	54	56	1		323		2	28	270	16	10	1,182
<i>A. sierrensis</i>		1	1	13		3		4		1		28		51
<i>A. sticticus</i>				3							1			11
<i>A. vexans</i>	1		5,607	1,909	55	83	1	2,216	48	1,907	12,414	5,077	46	29,361
<i>Anopheles franciscanus</i>														
<i>A. freeborni</i>		15	30	4	4		16	4				27	12	112
<i>A. pseudopunctipennis</i>	6	51	494	419	17	153	52	381	257	952	12,114	188	4	15,086
<i>A. punctipennis</i>	3	20	81	308	2	1		44		3	2	298	3	3
<i>Culex erythrothorax</i>														
<i>C. pus</i>		38	3	216	99	52	2	2,608		300	105	78		3,501
<i>C. pipiens</i>	8	513	710	231	52	216	12	229	357	162	324	2,273	73	5,160
<i>C. pipiens</i> complex	303	454	108	1,036	452	103		517	309	44	49	229	24	3,628
<i>C. tarsalis</i>	4,309	5,219	6,889	5,012	2,711	3,575	1,681	3,654	1,684	4,549	20,470	7,668	450	67,871
<i>C. thambus</i>													9	9
<i>Culiseta incidens</i>			7											
<i>C. inornata</i>	63	3	26	101	211	241	7	25		3	3	3	5	15
<i>C. parvipes</i>				926		2		42		2	4	9	7	766
<i>Mansonia perturbans</i>				31		1		302						992
<i>Orthopodomyia signifera</i>											1	13		334
<i>Orthopodomyia signifera</i>														
Total Mosquitoes	5,122	7,120	15,851	24,028	3,613	4,466	2,096	10,129	2,678	8,811	59,286	16,312	645	160,157
Total Trap Nights	71	76	158	107	84	84	10	49	9	24	60	124	6	6
Avg. Mosq./Trap Night	72	94	100	225	43	53	210	207	298	367	988	132	108	186
Avg. C. tarsalis/Trap Night	61	69	44	47	32	75	168	75	187	190	341	62	75	79
C. tarsalis % of Total Catch	84	73	44	21	75	80	80	36	63	52	35	47	70	42

TABLE 3.—Supplementary mosquito collections made from resting stations for arbovirus surveillance in four counties of the Central Valley of California July, September 1969

County	Species	July	September	Total
Placer	<i>C. tarsalis</i>	309 (7)*	292 (6)	601 (13)
	<i>C. peus</i>		214 (4)	214 (4)
Yuba	<i>C. tarsalis</i>	50 (1)	30 (1)	80 (2)
Colusa	<i>C. tarsalis</i>	50 (1)	75 (2)	125 (3)
Sutter	<i>C. tarsalis</i>	170 (4)	503 (11)	673 (15)
Total		579 (13)	1,114 (24)	1,693 (37)

* Number of mosquitoes collected—(number of pools tested).

trolled areas as well as in mosquito-controlled areas to determine if WE virus in sparsely inhabited natural areas could serve as a source for spread to more heavily inhabited rural and urban areas. A possibly oversimplified explanation for the absence of a WE epidemic is that the level of WE virus activity was extremely low. The low level is evidenced by the singular lack of WE virus in the large number of *C. tarsalis* tested in these studies. Our studies were designed to measure the presence of virus in the vector population of the Central Valley of California in 1969, not to explain its absence. For multifaceted consideration of possible mechanisms influenc-

ing presence or absence of virus, the reader is referred to Reeves, 1970.

The level and time of SLE virus activity in the Central Valley in 1969 were apparently not unusual for this area. In addition to the 35 SLE virus isolations reported here, Nelson, 1970, reported 12 isolations of SLE virus from nearly 15,000 *C. tarsalis* collected during the late summer in Glenn and Butte Counties. Apparently most of the SLE activity took place in the northern counties of the Sacramento Valley; no SLE virus isolations were reported from Kern County (Lyness, 1970). The SLE MFIR's for *C. tarsalis* in the northern counties studied were high

TABLE 4.—Arbovirus isolation from mosquitoes collected in counties of the Central Valley of California in 1969.

Month	County	Species	Arboviruses Isolated			
			SLE	Turlock	Hart Park-Like	Jamestown Canyon
June	Kings	<i>C. tarsalis</i>			2	
July	Tulare	<i>C. tarsalis</i>		4*	2*	
August	Tulare	<i>C. tarsalis</i>		1		
	Sutter-Butte	<i>A. melanimon</i>				1
	Sutter-Butte	<i>C. tarsalis</i>	3			
	Tehama	<i>C. tarsalis</i>	11	2		
	Tehama	<i>C. peus</i>	1			
	Shasta	<i>C. tarsalis</i>	2			
September	Solano	<i>C. tarsalis</i>	1			
	Sacramento	<i>C. tarsalis</i>		1		
	Sutter	<i>C. tarsalis</i>	5			
	Butte	<i>C. tarsalis</i>	1			
	Tehama	<i>C. tarsalis</i>	8			
	Tehama	<i>C. peus</i>	3			
Total			35	8	4	1

* 1 pool positive for both Turlock and Hart Park-like viruses.

TABLE 5.—Arbovirus minimum field infection ratios (MFIR) in mosquitoes for counties of the Central Valley of California, 1969, for months when isolations were made from listed species

Virus	Month	County	Mosquito Species	No. Mosquitoes Tested	No. Isolations	MFIR
SLE	August	Sutter-Butte	<i>C. tarsalis</i>	8,612	3	1:2,871
SLE	August	Tehama	<i>C. tarsalis</i>	3,873	11	1:352
SLE	August	Tehama	<i>C. peus</i>	257	1	1:257
SLE	August	Shasta	<i>C. tarsalis</i>	450	2	1:225
SLE	September	Solano	<i>C. tarsalis</i>	1,436	1	1:1,436
SLE	September	Sutter	<i>C. tarsalis</i>	503*	1	1:503
SLE	September	Butte	<i>C. tarsalis</i>	3,042	4	1:761
SLE	September	Tehama	<i>C. tarsalis</i>	1,400	1	1:1,400
SLE	September	Tehama	<i>C. tarsalis</i>	1,470	8	1:184
SLE	September	Tehama	<i>C. peus</i>	300	3	1:100
				Total	35	
Hart Park-like	June	Kings	<i>C. tarsalis</i>	2,072	2	1:1,036
Hart Park-like	July	Tulare	<i>C. tarsalis</i>	2,856	2	1:1,428
				Total	4	
Turlock	July	Tulare	<i>C. tarsalis</i>	2,856	4	1:714
Turlock	August	Tulare	<i>C. tarsalis</i>	616	2	1:308
Turlock	August	Tehama	<i>C. tarsalis</i>	3,873	1	1:3,873
Turlock	September	Sacramento	<i>C. tarsalis</i>	1,130	1	1:1,130
				Total	8	
Jamestown Canyon	August	Sutter-Butte	<i>A. melanimon</i>	3,750	1	1:3,750
				Total	1	

* Mosquitoes collected in resting stations; all others collected by CDC light trap.

enough to account for some local activity; they probably were not high enough, however, to achieve widespread transmission of this virus. Only 5 human cases of SLE were reported (Sacramento County, 2; Glenn County, 2 and Sutter County, 1), the lowest level of arbovirus encephalitis reported in California up to this time. An SLE isolate was obtained from resting station collections of *C. tarsalis* made during the investigation of the Sutter County

human case, which occurred in September.

At present, mosquito collections by California State Health Department personnel for arbovirus surveillance are made primarily from resting stations, sentinel chicken sheds, or American-New Jersey light traps. Our study points out the usefulness, if not superiority, of the CDC battery light trap for this purpose despite the modified desert conditions in California. *C. tarsalis*, which is considered the pri-

TABLE 6.—Arbovirus minimum field infection ratios in mosquitoes of the Central Valley of California, 1969

Virus	Mosquito Species	No. Isol.	MFIR/MI*	MFIR/T**
SLE	<i>C. tarsalis</i>	31	1:671	1:2,189
SLE	<i>C. peus</i>	4	1:139	1:1,290
Hart Park-like	<i>C. tarsalis</i>	4	1:1,232	1:16,967
Turlock	<i>C. tarsalis</i>	8	1:1,059	1:8,483
Jamestown C.	<i>A. melanimon</i>	1	1:3,750	1:30,925

* MFIR/MI—Minimum field infection rate per month of isolation.

** MFIR/T—Minimum field infection rate per season totals.

mary vector of SLE and WE in California, made up nearly half of the mosquitoes collected.

These traps should also prove useful in collecting various species of *Aedes* for the study of California Group viruses. In addition to the Jamestown Canyon virus isolation from *Aedes melanimon* reported here, 14 California Group viruses were isolated by these methods in the Owens Valley in 1969 (Dr. T. H. Work, 1970, Univ. of Calif., Los Angeles, personal communication).

Although favorable sites for the collection of *Anopheles freeborni*, the malaria vector in California, were not sought out in this study, 15,088 were taken, accounting for nearly 10 percent of the total catch. This fact suggests the usefulness of CDC light traps to study malaria as well as distribution of encephalitis virus.

SUMMARY. An outbreak of western encephalitis and/or St. Louis encephalitis appeared likely to occur in the Central Valley of California during the summer of 1969 because of heavy winter mountain snows and resulting high water tables. A cooperative arbovirus-vector surveillance study was conducted by the Arbovirus Ecology Laboratory, Center for Disease Control, and the California State Department of Public Health to detect any build-up of virus and to better direct control activities. Mosquitoes were collected from June through September in the Central Valley from King and Tulare Counties in the south to Shasta County in the north, an area of approximately 18,000 square miles. A total of 160,157 mosquitoes (42% *C. tarsalis*) were tested in 3,487 pools. No WE virus was isolated; however, 48 other virus isolations were made: SLE, 35; Turlock, 8; Hart Park-like, 4; and Jamestown Canyon, 1. Data are presented on mosquito catches by species, month, and county of capture. Minimum field infection rates were also calculated for those viruses which were isolated.

This study suggests that the CDC battery light trap may provide yet another

tool for arbovirus surveillance of mosquito vectors in the semi-arid conditions which occur in the Central Valley of California.

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A LIST OF OKLAHOMA MOSQUITOES

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An excellent survey by Rozeboom (1942) revealed 40 species of mosquitoes in Oklahoma. Roth (1945) added one additional state record. Griffith (1952) in a statewide study found 11 new state records which brought the total to 52. Carpenter and LaCasse (1955) list 54 Oklahoma species. Hill, *et al.*, (1958) recorded 55 species in the state.

Culex pipiens and *C. quinquefasciatus* are considered a complex in this paper and are recorded as one species. This work records three new state records, *Aedes spencerii*, *Ae. hendersoni* and *Uranotaenia lowii*. This brings the total of Oklahoma mosquitoes to 58.

Synonymy utilized here follows that of Stone, *et al.*, (1959) and Stone (1961).

The following list represents species collected by the authors as well as specimens identified at various museums, U. S. Army installations and state agencies.

Aedes aegypti (Linnaeus)
Aedes atlanticus Dyar and Knab
Aedes atropalpus (Coquillett)
Aedes canadensis canadensis (Theobald)

Aedes cinereus Meigen
Aedes dorsalis (Meigen)
Aedes dupreei (Coquillett)
Aedes fulvus-pallens Ross
Aedes hendersoni Cockerell
Aedes mitchellae (Dyar)
Aedes nigromaculis (Ludlow)
Aedes sollicitans (Walker)
Aedes spencerii (Theobald)
Aedes sticticus (Meigen)
Aedes taeniorhynchus (Wiedemann)
Aedes thelcter Dyar
Aedes tormentor Dyar and Knab
Aedes triseriatus (Say)
Aedes vexans (Meigen)
Aedes zoosophus Dyar and Knab
Anopheles barberi Coquillett
Anopheles crucians Wiedemann
Anopheles pseudopunctipennis franciscanus McCracken
Anopheles pseudopunctipennis pseudo-punctipennis Theobald
Anopheles punctipennis (Say)
Anopheles quadrimaculatus Say
Culex apicalis Adams
Culex erraticus (Dyar and Knab)
Culex nigripalpus Theobald
Culex peccator Dyar and Knab
Culex pipiens-quinquefasciatus Linnaeus

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