Journal of the American Mosquito Control Association, 11(2):157–161, 1995

ARBOVIRUS SURVEILLANCE IN IOWA, USA, DURING THE FLOOD OF 1993[1]

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ABSTRACT. During the summer of 1993, mosquitoes were collected by dry ice-baited CDC light traps from July through September in 12 different cities in Iowa. In all, 169,907 mosquitoes belonging to 17 different species were collected. A total of 2,013 pools were processed for arbovirus isolation, from which 59 arbovirus isolates were obtained: 41 Flanders (FLA), 16 trivittatus (TVT), one Cache Valley (CV), and one Turlock (TUR). Supplementary sentinel chicken and human data are also included. In spite of the increase in larval habitats and elevated mosquito populations, there was not an increase in virus transmission.

INTRODUCTION

Since 1965, mosquito-borne arboviruses in Iowa have been studied through a cooperative effort between the Department of Entomology at Iowa State University and the Virology Division of the University Hygienic Laboratory at the University of Iowa (Dorsey et al. 1978). Several arboviruses cause encephalitis in equines and humans, including western equine encephalomyelitis (WEE), St. Louis encephalitis (SLE), and La Crosse (California) (LAC). Other arboviruses routinely isolated in Iowa include Flanders (FLA), trivittatus (TVT), Cache Valley (CV), and Turlock (TUR).

Known mosquito species that vector these arboviruses in Iowa are as follows (Andre et al. 1985): *Culex tarsalis* (Coq.) is the primary vector of SLE and WEE (Rowley et al. 1973; Wong et al. 1973, 1978; Dorsey et al. 1978). The *Culex pipiens* complex (*Culex pipiens* Linn., *Culex restuans* Theobald, and *Culex salinarius* Coq.) may contribute to the amplification of SLE and WEE viruses (Wong et al. 1978). In addition to being the major vector of TVT, *Aedes trivittatus* (Coq.) may also play an important role in the natural cycle of WEE virus (Green et al. 1980). La Crosse, the other arbovirus of public health significance in Iowa, has been isolated only from pools of *Aedes triseriatus* (Say) (Andre et al. 1985).

Unusually large amounts of precipitation in the summer of 1993 (Kunkel et al. 1994) contributed to greater than normal mosquito populations. It was anticipated that these above-normal mosquito populations would cause increased arbovirus activity in Iowa. This report outlines the results of the Iowa mosquito–arbovirus surveillance program for the summer of 1993 and includes sentinel chicken and human serum data.

MATERIALS AND METHODS

Mosquito collection: During the summer of 1993, mosquitoes were collected by dry ice-baited CDC light traps from July through September in 12 different cities in Iowa: Sioux City, Council Bluffs, Des Moines, Ames, Waterloo, Cedar Rapids, Iowa City, Burlington, Keokuk, Muscatine, Dubuque, and Davenport. Each city was trapped from one to 5 times. In each city, 4 CDC traps were set at 3 different sites, generally where such traps have been operated for several years, for a total of 12 traps per city. Site selection was originally based on availability of host animals, suitability of habitat for adult and larval mosquitoes, and accessibility (Rowley et al. 1973). The traps were set up between 1700 and 1900 h and taken down between 0600 and 0800 h the next morning. Standard techniques were used for collecting, processing, and handling the mosquitoes for subsequent virus isolation (Sudia and Chamberlain 1967).

Virus isolation: Mosquitoes were sorted and identified on entomological chill tables, then packaged into vials in pools of 4–100. Table 1 indicates the maximum number in each pool for each of the species collected. Maximum pool size varied, based on several factors such as number of a particular species collected, the relative importance of a known vector species, and frequency of isolation based on previous studies. For example, pools of 25 *Cx. tarsalis* and *Ae. triseriatus* were used because they are important vectors in Iowa, whereas some other species rarely, if ever, yield an isolate. *Aedes vexans* (Meigen) were discarded because of their abundance, and because, historically, arboviruses are rarely isolated from *Ae. vexans* in Iowa. *Orthopodomyia signifera* (Coq.) and *Psorophora ciliata* (Fabr.) were also discarded because they were rarely col-

[1] Journal Paper No. J-16070 of the Iowa Agriculture and Home Economics Experiment Station, Ames, Project 2053.

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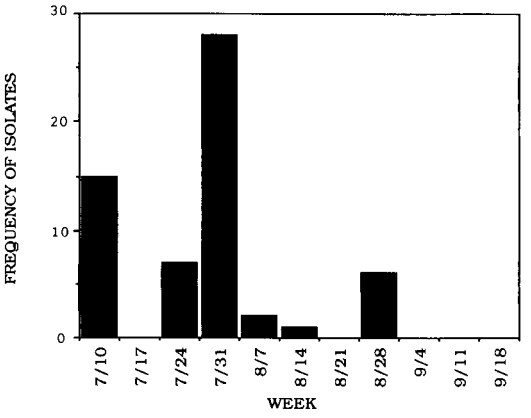

Fig. 1. Abundance of arbovirus isolations from Iowa mosquitoes per week, 1993.

lected. Vials were stored at −76°C until arbovirus isolation was performed by the University Hygienic Laboratory using suckling mice (Wong et al. 1971).

Sentinel chicken flocks: Sentinel chicken flocks (12 chickens/flock) were established in Sioux City, Des Moines, and Davenport. White Leghorn chickens were maintained in sentinel sheds similar to those described by Rainey et al. (1962). Chickens were bled from the wing vein before being established and every 1–2 wk thereafter by the health department of the county in which the flock was located. Approximately 1 ml of blood was collected and allowed to clot, then centrifuged at 1,000 rpm for 10 min. The resulting serum was transferred to a new tube and refrigerated until neutralization tests were conducted for antibodies against WEE and SLE antigens (Clarke and Casals 1958). Testing for LAC was not included because the vector of LAC, *Ae. triseriatus,* does not normally feed on chickens (W. A. Rowley, unpublished data).

Human surveillance: Clinically confirmed cases of encephalitis were monitored in humans. Human cases were verified as a routine procedure in the Virology Division of the University Hygienic Laboratory. Sera from suspected central nervous system disease cases from throughout Iowa were examined for a 4-fold rise in complement-fixing (CF), hemagglutination-inhibition (HI), and neutralizing (N) antibodies against WEE, SLE, and LAC prototype antigens (U.S. Public Health Service 1965, Lennette and Schmidt 1969). Sucrose–acetone-extracted infected mouse-brain antigens were used in the HI and CF tests. The microtiter system was employed for the N, HI, and CF tests. Baby hamster kidney (BHK-21) cells were used for the N test. All procedures have been described previously by Andre et al. (1985).

Table 1. Mosquito species collected, pooled, and viruses isolated in Iowa arbovirus surveillance, 1993. *Aedes vexans, Orthopodomyia signifera,* and *Psorophora ciliata* were discarded.

Species	No. collected	No. pooled	Maximum no./pool	No. of pools	Viruses isolated
Aedes atropalpus	6	6	25	1	
Ae. dorsalis	77	73	25	3	
Ae. nigromaculis	117	117	100	4	
Ae. sollicitans	13	13	100	2	
Ae. triseriatus	779	775	25	40	
Ae. trivittatus	19,255	19,254	50	393	1 FLA, 14 TVT
Ae. vexans	43,407	0	0	0	
Anopheles punctipennis	6,194	6,188	100	73	
An. quadrimaculatus	1,441	1,441	100	16	
Coquillettidia perturbans	773	769	100	11	
Culex pipiens complex[1]	80,710	80,710	100	817	15 FLA, 1 TVT, 1 CV
Cx. tarsalis	15,282	15,277	25	615	25 FLA, 1 TVT, 1 TUR
Culiseta inornata	281	274	100	10	
Orthopodomyia signifera	14	0	0	0	
Psorophora ciliata	4	0	0	0	
Ps. horrida	1,513	1,508	100	25	
Uranotaenia sapphirina	41	41	100	3	
Totals	169,907	126,446	—	2,013	59

[1] *Culex pipiens* complex mosquitoes include *Cx. pipiens, Cx. restuans,* and *Cx. salinarus.*

Table 2. Viruses isolated from mosquitoes collected in Iowa in 1993.

Date	Site	Mosquito	Pool size	Virus
July 14	Sioux City	*Cx. pipiens* complex[1]	100	1 FLA
July 14	Sioux City	*Cx. tarsalis*	25	4 FLA
July 16	Council Bluffs	*Cx. tarsalis*	25	3 FLA
July 16	Council Bluffs	*Ae. trivittatus*	50	1 FLA
July 16	Council Bluffs	*Cx. pipiens* complex[1]	100	4 FLA
July 16	Council Bluffs	*Ae. trivittatus*	50	2 TVT
July 27	Davenport	*Cx. pipiens* complex[1]	100	2 FLA
July 29	Waterloo	*Ae. trivittatus*	50	1 TVT
July 29	Ames	*Cx. tarsalis*	25	1 FLA
July 29	Ames	*Ae. trivittatus*	50	3 TVT
July 31	Council Bluffs	*Ae. trivittatus*	50	3 TVT
July 31	Council Bluffs	*Cx. pipiens* complex[1]	100	6 FLA
July 31	Council Bluffs	*Cx. pipiens* complex[1]	100	1 TVT
July 31	Council Bluffs	*Cx. tarsalis*	25	16 FLA
July 31	Council Bluffs	*Cx. tarsalis*	25	1 TVT
July 31	Council Bluffs	*Cx. tarsalis*	25	1 TUR
August 10	Davenport	*Cx. pipiens* complex[1]	100	1 FLA
August 11	Des Moines	*Cx. tarsalis*	25	1 FLA
August 20	Burlington	*Cx. pipiens* complex[1]	100	1 FLA
August 30	Davenport	*Cx. pipiens* complex[1]	100	1 CV
Sept. 2	Council Bluffs	*Ae. trivattatus*	50	5 TVT

[1] *Culex pipiens* complex mosquitoes include *Cx. pipiens, Cx. restuans,* and *Cx. salinarus.*

RESULTS

Mosquito collection: In all, 169,907 mosquitoes belonging to 17 species (Table 1) were collected, and 126,446 were pooled for virus isolation and identification. *Culex pipiens* complex mosquitoes were collected in all 12 cities; *Ae. triseriatus, Ae. trivittatus, Anopheles punctipennis* (Say), and *Cx. tarsalis* were trapped at all but one site.

Virus isolation: The 126,446 mosquitoes were combined into 2,013 pools to be processed for arbovirus isolation (Table 1). From the 2,013 pools, 59 arbovirus isolates were obtained: 69.5% FLA, 27.1% TVT, 1.7% CV, and 1.7% TUR. The date, site, mosquito, pool size, and virus for each isolate are listed in Table 2. Viral isolates were obtained from only 3 mosquito species: 45.8% from *Cx. tarsalis,* 28.8% from *Cx. pipiens* complex, and 25.4% from *Ae. trivittatus.*

Geographically, the western region of Iowa yielded the most virus isolates, with 48 of 59 or 81% of all virus isolations coming from Sioux City and Council Bluffs (Table 2). In addition, the western region also produced 68% of the total mosquitoes pooled for virus isolation, and among the 3 species from which virus isolates were obtained, it contained 85% of the total number of *Cx. tarsalis,* 67% of the total number of the *Cx. pipiens* complex mosquitoes, and 59% of the total number of *Ae. trivittatus* (Table 3). Chronologically, viral activity peaked from mid- to late July (Fig. 1).

Sentinel chicken flocks: During the summer of 1993, none of the 36 chickens seroconverted for WEE or SLE.

Human surveillance: Three LAC cases were confirmed by the Hygienic Laboratory in Iowa for the summer of 1993. All 3 cases were in male children, age 4–9 from Dubuque and Linn counties. No clinically confirmed cases of SLE, EEE, or WEE were reported.

DISCUSSION

Rainfall in Iowa during the summer of 1993 was excessive. From June 1 to August 31, 1993, most of Iowa received more than 600 mm of precipitation and 12 weather stations reported more than 900 mm. Normal precipitation for the period is approximately 300 mm (Kunkel et al. 1994; VanDyk et al., unpublished data). This excessive rainfall created standing water sites throughout the state, resulting in unusually large mosquito populations. Since 1966, the total number of mosquito specimens collected per year has ranged from 1,373 to 109,272, averaging 27,276 ± 25,977 (SD). The 1993 Arbovirus Surveillance Program trapped 169,907 mosquitoes;

Table 3. Distribution and abundance of mosquitoes pooled for virus isolation in Iowa arbovirus surveillance, 1993.

Region	City	Total no. mosquitoes	Total no. *Ae. trivittatus*	Total no. *Cx. pipiens* complex[1]	Total no. *Cx. tarsalis*
Western	Sioux City	11,867	408	7,040	4,117
	Council Bluffs	73,839	10,949	47,320	8,870
Central	Des Moines	7,637	1,431	5,017	485
	Ames	6,658	2,210	3,263	444
	Waterloo	10,069	2,968	5,192	787
Eastern	Cedar Rapids	2,466	949	1,310	52
	Iowa City	285	0	272	0
	Burlington/Keokuk/ Muscatine	1,988	80	1,370	20
	Dubuque	357	48	224	35
	Davenport	11,280	211	9,702	467
Total		126,446	19,254	80,710	15,277

[1] *Culex pipiens* complex mosquitoes include *Cx. pipiens, Cx. restuans,* and *Cx. salinarus.*

this number was significantly greater ($t = 5.5$, $P < 0.001$) than the 1966–92 mean.

From 1966 through 1992, the total number of viral isolates per year ranged from 0 to 133, with an average of 38.0 ± 33.9, and most isolates were FLA or TVT viruses. This compares with the 59 isolates for 1993, which were mostly FLA (Table 1). There is no significant difference between the number of isolates in 1993 as compared with 1966 through 1992 ($t = 0.60$, $P > 0.5$). Therefore, contrary to what might be expected, arbovirus activity in mosquitoes was not unusually large.

Sentinel chickens showed no evidence of arbovirus activity, which is consistent with past years. Since the inception of Iowa's surveillance program, 3 chickens in Council Bluffs seroconverted for WEE, one each in 1979 (Andre et al. 1985), 1985, and 1994 (W. A. Rowley, unpublished data).

From 1965 to 1992, a total of 171 laboratory-confirmed cases of human encephalitis have been reported by the University of Iowa Hygienic Laboratory. The number of cases ranged from 0 to 37 per year and averaged 6.0 ± 7.0. Comparing these data with 1993, which had 3 LAC cases, 1993 is consistent with past years for type of encephalitis cases and number of cases per year ($t = 0.44$, $P > 0.5$). Thus, the increased mosquito populations did not increase virus transmission.

ACKNOWLEDGMENTS

Special thanks to the University of Iowa's University Hygienic Laboratory for viral isolation and identification and Andrea Silvers and JB Silvers for mosquito pooling.

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