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AEDES ALBOPICTUS AND OTHER CONTAINER-INHABITING MOSQUITOES IN THE UNITED STATES: RESULTS OF AN EIGHT-CITY SURVEY[1]

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ABSTRACT. Extensive surveys were conducted in 1987 in Baytown, TX; Lafayette, Shreveport and Baton Rouge, LA; Memphis, TN; Kansas City, MO; Evansville, IN; and Jacksonville, FL. The program objective was to determine the intensity of *Aedes albopictus* infestations, to evaluate the degree to which *Ae. albopictus* had spread into residential areas, to document habitat selection and to obtain background information for possible suppression or eradication projects. This report describes the survey methods and presents a preliminary analysis of the data. Larvae, pupae and adult mosquitoes were collected from container habitats in a randomized selection of urban premises as well as at and around sites known to be at high risk for introduction of *Ae. albopictus*. Adult or larval mosquitoes were collected from 24.4% of 5,728 premises inspected, and there were an average of 3.27 positive containers per positive premise. Several known disease vectors, especially *Culex pipiens* (*s.l.*), were frequently found in urban container habitats. The large numbers of specimens collected during the surveys and the detailed information available for each collection make this a useful database for comparison in future studies.

INTRODUCTION

Since its discovery in Houston, Texas, in 1985 (Sprenger and Wuithiranyagool 1986) *Aedes albopictus* (Skuse) has rapidly spread throughout the eastern United States (Moore et al. 1988). Concern has been expressed by public health officials regarding the potential involvement of this species as a vector of viruses of public health importance in the U.S. All evidence indicates this species is sufficiently adaptable to colonize a wide variety of container habitats throughout the eastern and midwestern United States (Nawrocki and Hawley 1987, Hawley 1988), possibly replacing indigenous container-breeding mosquito species in the process (e.g., Sprenger and Wuithiranyagool 1986, Hawley 1988; J. E. Freier, personal communication). In addition to assessing the possible vector role of this exotic invader, an introduction of this sort presents an opportunity for a wide variety of observations on altered ecologic relationships, particularly with relation to interspecific competition, niche partitioning and predator-prey interactions (Diamond 1986).

In 1987, we conducted extensive surveys in 8 cities to determine the intensity of *Ae. albopictus* infestations, to evaluate the degree to which *Ae. albopictus* had spread into residential areas, to document habitat selection and to obtain background information for possible suppression or eradication projects. We describe here our study methods and provide a preliminary analysis of data collected in those surveys.

MATERIALS AND METHODS

Survey teams: Six teams were formed, each consisting of one team leader and 3 to 5 inspectors. All team leaders had previous experience in the biological sciences, and most had had previous experience in mosquito biology or surveillance. Team leaders attended a 3-day training session in New Orleans, Louisiana, in which they were familiarized with project objectives, survey methods and team supervision. Survey team members, including team leaders, were given 2 to 3 days of training in methods of collecting and specimen handling, data recording, specimen processing and public relations. Special emphasis was placed on record keeping and on preventing the accidental transport of larvae or adults from one site to another in contaminated equipment.

Selection of study areas: Eight cities were chosen for the project: Baytown, TX; Lafayette, Shreveport and Baton Rouge, LA: Memphis, TN; Kansas City, MO; Evansville, IN; and Jacksonville, FL. These cities were known to have *Ae. albopictus* infestations (Moore et al. 1988) and were believed to have differing intensities of infestation based on preliminary surveys by CDC or by state and local agencies. Therefore, we expected these cities to provide a range of

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infestation intensity and problems for control or eradication of *Ae. albopictus*. Surveys were conducted between July 22 and August 13, 1987. Since there were only 6 teams, the surveys were not simultaneous in all cities; Jacksonville and Shreveport were surveyed after completion of the Kansas City and Lafayette surveys, respectively. Random surveys were not conducted in the latter 2 cities due to lack of time. Close coordination was maintained between the inspection teams and state and local health/vector control agencies. Several of those agencies supplied additional personnel to assist with the inspections, greatly facilitating the surveys.

Selection of sites within study areas: Both random and nonrandom sampling methods were used in this study. Nonrandom surveys included premises at highest risk of infestation and, where positive premises were found, areas surrounding those foci.

"High risk" premises: Sites at highest risk of infestation included truck and large equipment tire dealers, tire retreaders, used tire stockpiles, salvage yards and illegal dumps. Commercial sites were located by consulting the appropriate categories of the telephone directory in each city. Additional sites were frequently identified in conversations with employees at premises described above. Tire dumps, both legal and illegal, were usually located by consulting local health department or mosquito control staff.

Perifocal areas: When one or more *Ae. albopictus* adults were found at a high risk site, a 1-mi. grid was constructed with the infested premise at the grid center. The team then drove through the delimited area, identifying and inspecting all premises with potential breeding sites.

Random surveys: A random cluster sampling technique was used to sample residential premises. Each of the study cities was located inside Standard Metropolitan Statistical Areas (SMSAs) as designated by the Bureau of the Census. Census tracts were chosen randomly on the basis of probability proportional to size (estimated number of premises, data from the 1980 census). Thus, census tracts with a large number of premises had (theoretically) a higher chance of being sampled. Before choosing census tracts, all those containing fewer than 640 premises were combined with an adjacent tract. A minimum of 160 residential premises were inspected in each selected tract. The number of census tracts chosen for a city varied with the size of the city. Within the chosen census tracts, 40 random numbers were used to pinpoint the block location of the target premises. The randomly selected target premise and the three nearest premises on that block formed the cluster of premises to be inspected.

Specimen collection and handling: Larval collecting equipment consisted of large soup ladles, white enamel pans, tea strainers, kitchen basters and small pipettes. Adult collecting equipment consisted of battery-powered aspirators. Prior to leaving each site, team members carefully cleaned and inspected all equipment to ensure that specimens were not transferred between sites.

Water-holding containers at or near ground level on each premise were inspected. Samples of up to 100 larvae and pupae were removed from containers by pouring or by dipping with a tea strainer or ladle. Specimens were concentrated into a small volume of water and placed in labeled plastic bags. The bags were placed in insulated coolers for later processing. Specimens from individual containers were to be kept separate to permit subsequent analysis of species associations.

Adult mosquitoes were collected with an aspirator as they flew around the inspectors or as they attempted to bite. Additional specimens were flushed by kicking or moving tires and other containers. Specimens were transferred to labeled holding cages and placed in insulated coolers for later processing.

Larvae were killed in hot water (Belkin et al. 1965) and preserved in 70% alcohol in labeled vials. Whenever possible, pupae were held for adult emergence in holding cages and then processed as adults. When this was impossible, pupae were treated in the same manner as larvae. Adults were immobilized with triethylamine and placed between protective tissue plugs in labeled vials. All specimens from a given site were placed in a single bag that was labeled with the site identification. This was done to reduce the likelihood of mixing specimens during later laboratory identification of the specimens. Once or twice each week, specimens were packed with associated data forms, and sent via express mail to the Division of Vector-Borne Viral Diseases (DVBVD), CDC, Fort Collins, Colorado. In addition to location, date and time of inspection, data forms recorded the type of inspection (i.e., high risk, perifocal, random), characteristics of the site and estimated total containers. Containers were listed by type, amount of shade, water volume and whether they contained larvae or pupae. Collections of adults were also recorded. All specimens were identified microscopically at DVBVD.

RESULTS

A total of 5,728 premises were inspected in the 8 cities, of which 1,399 (24.4%) were positive for one or more mosquito species (Table 1). There were 11,929 water-filled containers inspected, of which 4,574 (38.3%) were positive, for an overall average of 3.27 positive containers per positive premise. Positive premises had an average of 7.07, 2.73 and 0.37 containers in high risk, perifocal and random surveys, respectively. A total of 11,700 larvae (40.50 larvae/premise) were collected at high risk premises; 9,403 larvae (41.98 larvae/premise) were collected at perifocal sites; and 23,064 larvae (4.42 larvae/premise) came from the random surveys. The largest number of specimens (14,950) was collected in Baton Rouge, LA, and the smallest from Evansville, IN (1,607). There were 3,581 adults collected during the surveys. Nearly half of the adults (1,729 or 48.3%) were collected from high-risk sites.

Tables 2, 3 and 4 show the Breteau Indices (BI, the number of positive containers per 100 premises) for random, high risk and perifocal surveys, respectively. The number of species found in surveys in the 8 cities varied from 8 in Shreveport and Jacksonville (which lacked random surveys) to 13 in Baton Rouge and Memphis. The *Culex pipiens* Linn. complex was common in all cities, with BIs ranging from 11 to 24 in random, 27 to 392 in high risk and 26 to 170 in perifocal surveys. *Culex salinarius* Coq. was common (BI = 0 to 67) at high risk premises,

Table 1. Numbers of premises, positive premises (larvae or adults) and containers found in surveys of 8 U.S. cities, July–August, 1987. Data for an additional 329 specimens were excluded for lack of indication of survey type.

City	High-risk			Perifocal			Random			Total		
	Premises			Premises			Premises			Premises		
	Total	Pos.	Pos. containers	Total	Pos.	Pos. containers	Total	Pos.	Pos. containers	Total	Pos.	Pos. containers
Baytown	12	11	1,063	8	8	107	398	123	276	418	142	1,446
Lafayette	16	16	125	18	16	45	779	260	438	813	292	608
Shreveport	38	29	160	27	20	88	ND*	ND	ND	65	49	248
Baton Rouge	16	12	130	67	65	238	953	288	714	1,036	365	1,082
Memphis	94	51	222	ND	ND	ND	1,093	181	275	1,187	232	497
Kansas City	22	18	129	26	24	26	1,271	93	127	1,319	135	282
Evansville	11	11	89	5	5	15	721	52	89	737	68	193
Jacksonville	80	46	125	73	70	93	ND	ND	ND	153	116	218
Totals	289	194	2,041	224	208	612	5,215	997	1,919	5,728	1,399	4,572

* ND = not done.

Table 2. Breteau Index (number of positive containers per 100 premises) for collections of immatures of different species in 6 of 8 survey cities, July–August, 1987. Random surveys of urban areas. (Random surveys were not conducted in Shreveport or Jacksonville.)

Species	Baytown	Lafayette	Baton Rouge	Memphis	Kansas City	Evansville
Ae. aegypti	0.00	34.15	34.31	4.85	0.00	0.00
Ae. albopictus	32.16	25.16	1.78	0.26	0.16	0.55
Ae. atropalpus	0.00	0.00	0.00	0.00	3.46	0.00
Ae. hendersoni	0.00	0.13	0.10	0.09	0.00	0.00
Ae. triseriatus	0.00	5.65	5.35	0.37	3.70	2.08
Ae. species	0.25	0.13	0.52	0.00	0.08	0.14
Cx. nigripalpus	0.00	0.13	0.10	0.18	0.00	0.00
Cx. pipiens (s.l.)	9.05	14.63	19.62	2.84	3.86	4.99
Cx. restuans	0.00	0.00	0.21	0.27	0.47	2.50
Cx. salinarius	0.75	0.13	0.10	0.00	0.00	0.28
Cx. territans	0.00	0.00	0.63	0.09	0.00	0.14
Cx. species	0.00	0.13	0.00	0.00	0.08	0.00
Anopheles spp.	0.00	0.00	0.00	0.18	0.08	0.42
Culiseta spp.	0.00	0.26	0.00	0.00	0.00	0.14
Orthopodomyia spp.	0.00	0.26	0.31	0.09	0.24	0.69
Psorophora spp.	0.00	0.00	0.10	0.00	0.00	0.00
Toxorhynchites spp.	0.00	0.13	0.10	0.27	0.08	0.55
Premises with containers	321	589	773	179	320	315
Containers with water	884	1,407	2,744	277	334	411

Table 3. Breteau Index (number of positive containers per 100 premises) for collections of immatures of different species at 8 survey cities, July–August, 1987. High risk surveys.

Species	Baytown	Lafayette	Shreveport	Baton Rouge	Memphis	Kansas City	Evansville	Jacksonville
Ae. aegypti	0.00	312.50	176.32	86.67	105.32	0.00	0.00	42.50
Ae. albopictus	1,083.33	462.50	150.00	20.00	42.55	36.36	0.00	6.25
Ae. atropalpus	0.00	0.00	0.00	0.00	1.06	95.45	18.18	0.00
Ae. hendersoni	0.00	12.50	0.00	0.00	0.00	0.00	0.00	0.00
Ae. triseriatus	16.67	18.75	23.68	0.00	9.57	63.64	100.00	7.50
Ae. species	8.33	0.00	2.63	0.00	1.06	4.55	9.09	0.00
Cx. nigripalpus	8.33	0.00	0.00	13.33	0.00	0.00	9.09	3.75
Cx. pipiens (*s.l.*)	391.67	237.50	176.32	100.00	93.62	68.18	100.00	17.50
Cx. restuans	8.33	0.00	0.00	0.00	18.09	59.09	36.36	0.00
Cx. salinarius	66.67	18.75	0.00	20.00	3.19	13.64	9.09	1.25
Cx. territans	8.33	0.00	0.00	6.67	2.13	0.00	0.00	0.00
Cx. species	0.00	0.00	0.00	0.00	6.38	0.00	0.00	0.00
Anopheles spp.	41.67	0.00	0.00	0.00	1.12	9.09	9.09	0.00
Orthopodomyia spp.	25.00	37.50	10.53	6.67	21.28	13.64	18.18	2.50
Psorophora spp.	8.33	0.00	2.63	0.00	0.00	0.00	0.00	0.00
Toxorhynchites spp.	41.67	0.00	5.26	0.00	13.83	0.00	0.00	2.50
Premises with containers	12	16	27	15	94	22	11	51
Containers with water	1,551	268	438	559	1,562	189	87	367

Table 4. Breteau Index (number of positive containers per 100 premises) for collections of immatures of different species in 7 of 8 survey cities, July–August, 1987. Perifocal surveys. (Perifocal surveys not conducted in Memphis.)

Species	Baytown	Lafayette	Shreveport	Baton Rouge	Kansas City	Evansville	Jacksonville
Ae. aegypti	0.00	172.22	144.44	110.45	0.00	0.00	78.08
Ae. albopictus	362.50	138.89	92.59	111.94	38.46	120.00	31.51
Ae. atropalpus	0.00	0.00	0.00	0.00	23.08	0.00	0.00
Ae. epactius	0.00	0.00	0.00	0.00	3.85	0.00	0.00
Ae. hendersoni	0.00	0.00	0.00	2.99	0.00	0.00	0.00
Ae. triseriatus	0.00	5.56	0.00	22.39	19.23	160.00	4.11
Cx. nigripalpus	0.00	0.00	3.70	0.00	0.00	0.00	1.37
Cx. pipiens (*s.l.*)	125.00	44.44	170.37	94.03	19.23	100.00	23.29
Cx. restuans	0.00	5.56	0.00	4.48	7.69	20.00	0.00
Cx. salinarius	0.00	0.00	0.00	5.97	0.00	0.00	1.37
Cx. territans	0.00	0.00	0.00	4.48	0.00	0.00	0.00
Cx. species	0.00	0.00	0.00	2.99	0.00	0.00	0.00
Anopheles spp.	0.00	0.00	0.00	0.00	0.00	20.00	0.00
Culiseta spp.	0.00	0.00	0.00	2.99	0.00	0.00	0.00
Orthopodomyia spp.	12.50	0.00	0.00	1.49	7.69	40.00	0.00
Toxorhynchites spp.	0.00	0.00	0.00	1.49	3.85	0.00	4.11
Premises with containers	8	18	27	65	19	5	58
Containers with water	135	77	150	589	34	15	106

but was rare in perifocal and random surveys.

Table 5 shows the abundance of adult mosquitoes in all cities except Memphis. Data for adults from Memphis could not be properly assigned by survey type or other characteristics, and therefore were deleted from the analysis. In general, adult collections paralleled larval collections, but anthropophagic and day-biting species (*Aedes*, *Psorophora*) were somewhat more commonly collected than ornithophagic or nocturnal species (e.g., the *Culex* species). As with the larval collections, adult mosquitoes were more common at high risk and perifocal premises than at random survey premises (Table 6). *Aedes aegypti* (Linn.) and *Ae. albopictus* were the most common species collected as adults.

Table 5. Percent of premises positive for adults of different species, all survey types combined, in 7 U.S. cities (Memphis adults excluded—see text), July–August, 1987.

Species	Baytown	Lafayette	Shreveport	Baton Rouge	Kansas City	Evansville	Jacksonville	Total
Ae. aegypti	0.2	10.0	35.4	10.2	0.0	0.0	36.6	4.7
Ae. albopictus	25.8	8.7	21.5	3.1	1.4	0.8	14.4	4.7
Ae. atropalpus	0.0	0.0	0.0	0.0	0.2	0.0	0.0	0.1
Ae. triseriatus	0.2	0.7	0.0	0.7	0.4	1.6	0.0	0.5
Ae. species	8.6	2.1	12.3	2.7	0.4	0.9	7.2	2.0
Cx. pipiens (*s.l.*)	0.7	0.6	6.2	1.0	0.0	0.0	2.0	0.4
Cx. salinarius	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Cx. species	4.5	3.7	3.1	4.2	0.0	0.7	2.0	1.8
Anopheles spp.	0.7	0.4	0.0	0.3	0.0	0.3	0.0	0.2
Orthopodomyia spp.	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Psorophora spp.	0.7	0.4	1.5	4.0	0.0	0.0	2.0	0.9

Table 6. Percent of premises positive for adults of different species by survey type, all cities combined, in 7 U.S. cities (Memphis adults excluded—see text), July–August, 1987.

Species	Random	High risk	Perifocal	Total
Ae. aegypti	2.6	23.5	27.2	4.7
Ae. albopictus	3.0	12.8	34.8	4.7
Ae. atropalpus	0.0	0.0	1.3	0.1
Ae. triseriatus	0.2	2.8	5.4	0.5
Ae. species	1.1	9.0	12.5	2.0
Cx. pipiens (*s.l.*)	0.3	3.1	0.9	0.4
Cx. salinarius	0.0	0.0	0.0	0.0
Cx. species	1.2	5.2	10.7	1.8
Anopheles spp.	0.2	0.7	0.0	0.2
Orthopodomyia spp.	0.0	0.3	0.0	0.0
Psorophora spp.	0.7	2.4	3.1	0.9

DISCUSSION

In this study there were many opportunities for errors in the data. Since each city was served by a different team, the possibility exists that some of the apparent differences between cities were due to procedural differences or to the level of experience of the teams (e.g., the diligence with which the teams removed larvae from each container). We attempted to minimize these factors by thorough pre-survey training and by balancing the composition of the teams with respect to experience. Since samples from individual containers were not exhaustive (i.e., samples were limited to ≤ 100 larvae), the total number of larvae in certain habitats was underestimated. It is also possible that some species were present but not collected. Similarly, since it was frequently impossible to sample all containers at high risk sites, estimates of the Breteau Index for those sites are minimum estimates.

We built a certain amount of redundancy into the field data forms to prevent losses due to inadequate information (e.g., from inability to link field records to specimens, etc.). Even so, we were unable to use certain records for portions of the analysis (e.g., records for adults collected in Memphis, and those lacking data on container type or amount or shade). Finally, it is difficult to estimate the accuracy of the actual numbers of, for example, total containers on a given premise. Those numbers undoubtedly became less accurate as the total increased, simply because many containers within large concentrations were hidden from view (e.g., large tire piles). Although we recognize the foregoing potential difficulties, we feel the data collected in this study still represent the most complete estimates available to date.

The large difference in frequency of positive containers between the different survey types probably reflects both real differences and differences in sampling. High risk premises were not randomly selected and were the most likely sites to have large numbers of positive containers. Thus, those sites may provide an estimate of the upper limit of the frequency of positive containers (recognizing that these are minimal estimates of the BI for each premise, as indicated above) within the 8 cities. The frequency of positive containers in the random surveys (0.37) was somewhat higher than the frequency of *Ae. aegypti*-infested containers (0.26) at urban premises in Puerto Rico studied by Moore et al. (1978). The random surveys give the most statistically accurate estimate of infestation rates in the surveyed cities.

The mosquito fauna of urban container habitats is quite diverse. Several of the more commonly encountered species are known vectors of human disease (*Ae. triseriatus* (Say), *Ae. aegypti*, *Ae. albopictus*, *Cx. pipiens* and *Cx. nigripalpus* Theobald). *Culex pipiens* (*s.l.*), the major vector of St. Louis encephalitis in the eastern U.S.

(Mitchell et al. 1980), was particularly abundant in all of the random surveys. We wish to emphasize the importance of containers as a larval habitat for this important vector species. Our observations should serve as a reminder that conditions still exist for outbreaks of St. Louis encephalitis.

The general decline in abundance of *Ae. albopictus* with increasing distance from Houston supports but does not prove the hypothesis that other infestations are due to spread from a single introduction into the Houston area. *Aedes albopictus* may have been established in Memphis as early as 1983 (Reiter and Darsie 1984). The north-south distribution of the survey cities clearly shows the change in abundance of regional species such as *Ae. aegypti* and *Ae. triseriatus*. The surveys show that *Ae. atropalpus* has continued the southward and westward spread reported by others (Restifo and Lanzaro 1980, Nawrocki and Craig 1989). The collection of *Cx. nigripalpus* in Evansville apparently represents a new state record for that species. Since *Cx. nigripalpus* is found just south of Evansville in Kentucky (Darsie and Ward 1981, p. 262), this new record is not surprising.

The data collected during the 1987 surveys produced a large body of detailed data on *Ae. albopictus* and other container-breeding mosquitoes in urban environments. The data should provide a good baseline for future studies that examine changes in species composition and habitat related to the movement and spread of *Ae. albopictus*. We could find no evidence from the data collected in 1987 to suggest that *Ae. albopictus* may be unable to invade any container habitat, or that it will be locally restricted in its distribution.

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