DISTRIBUTION AND DENSITY OF *AEDES AEGYPTI* (L.) AND *LANKESTERIA CULICIS* (ROSS) IN LOUISIANA AND ADJOINING AREAS[1]

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In an effort to determine the effects of *Lankesteria culicis* and other pathogens upon *Aedes aegypti,* the Aedes aegypti Eradication Program (AAEP) assigned three teams in 1968 to conduct simultaneous surveys and special studies on *L. culicis* within that portion of the continental United States in which *Ae. aegypti* is found or may be expected to occur. An additional aim was to explain the absence of *Ae. aegypti* from large geographic areas in the close proximity of other areas heavily infested by the species (Tinker and Hayes, 1959; Hayes and Ritter, 1966; Barrett, 1968). The results of surveys and studies made in an area extending from Temple, Texas, to Pensacola, Florida, are discussed in this paper.

Gregarines, in general, and *L. culicis,* in particular, have long been considered by many to have little effectiveness as agents in the control of mosquitoes. Barrett (1968), however, reported observations of some damage or death to *Ae. aegypti* larvae, pupae, and adults heavily infected with *L. culicis.* Gentile *et al.* (1971), in the first paper of this series, review the status of *L. culicis* as a parasite of *Ae. aegypti.* The possibility also exists that parasitism by *L. culicis,* while not lethal to a majority of the mosquito population, might act to potentiate the deleterious effects of other parasites or of insecticides or to render the infected individual less capable of coping with competitors or with adverse environmental factors such as severe climate. The apparent specificity of *L. culicis* for *Ae. aegypti* and the ability recently developed by AAEP to propagate and disseminate the parasite would enhance its addition to the armamentarium of the AAEP.

METHODS. Details of methods employed are given by Gentile in the first paper of this series. Ten urban centers were selected, each with a satellite community, in four states with Louisiana as a hub. Within Louisiana the six urban centers (and satellites) selected were New Orleans (Covington), Baton Rouge (St. Francisville), Lake Charles (Orange, Texas), Alexandria (Colfax), Shreveport (Waskom, Texas), and Monroe (Vicksburg, Mississippi). The centers were selected to afford general geographic representation of the State with physiographic characteristics of potential *Ae. aegypti* areas. Similar criteria were applied in selecting urban centers in other states. Of the urban sites and satellite communities within Louisiana, only Covington was known to have *Ae. aegypti* at the outset of the study. Vicksburg, Mississippi, and Waskom, Texas (satellites to Monroe and Shreveport, respectively) also were known to harbor the species. Urban centers and satellites in other states were known or believed to be infested with *Ae. aegypti.* These included Temple (Cameron, Texas), Palestine (Rusk, Texas), Nacogdoches (Lufkin, Texas), and Pensacola (Milton, Florida).

In addition to the seasonal surveillance for *L. culicis,* two special studies were conducted. One study was designed to determine whether an area from which *Ae.*

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aegypti had spontaneously disappeared contained elements in the environment inimical to the species. The other study was designed to evaluate the technique for introducing *L. culicis* into an environment positive for *Ae. aegypti,* but negative for the parasite, and to elucidate aspects of the parasite's establishment and spread, and, ultimately, its effect upon *Ae. aegypti.*

Two sites were selected for the first special study at Monroe, Louisiana, where light infestations of *Ae. aegypti* occurred during the 1950's and the last positives were found in 1959. On each site, 10 naturally-occurring containers of various size and type wre examined to verify the presence of adequate water and the absence of *Ae. aegypti.* Numerous inspections at other sites in the city, as well as the findings from an on-going program utilizing oviposition traps, further confirmed this negativity. Three control containers, 1-gallon wide-mouth glass jars, one-third filled with tap water, were added to each site. Fifty *Ae. aegypti* larvae, newly-hatched in the laboratory, were added to each container. Observations were made daily on their development. At the commencement of pupation all *Ae. aegypti* which could be captured were harvested, and the area was thoroughly treated with insecticide. A sample of the larvae was examined for pathogens and another sample was submitted to the Technical Development Laboratories (TDL) of the Center for Disease Control at Savannah, Georgia, for rearing and observation on such factors as longevity and vigor.

The second special study was inaugurated in Shreveport, Louisiana, and Waskom, Texas, where *L. culicis* was introduced. One site, selected in each city, had at least 20 containers (assorted, in Shreveport, all automobile tires in Waskom) with a high degree of permanence and in which *Ae. aegypti* were produced. The containers were marked for identification, and sporocysts of *L. culicis* were added to 10 containers on each site. In Shreveport the addition was made at the rate of approximately 8,000 sporocysts per square foot of feeding area available to the larvae. In Waskom 30,000 sporocysts were added to each tire so treated.

RESULTS. In the first cycle of the regular pathogen surveillance, *Ae. aegypti* was found for the first time since the mid-1950's in Shreveport. The species is known to have existed continuously in heavy infestation in Waskom, Texas, 21 miles to the west. The completed pathogen surveillance disclosed that Shreveport and Waskom were negative for *L. culicis.*

Table 1 presents the cumulative results of three cycles of surveillance of *L. culicis.* Shreveport and Covington were the only communities within Louisiana in which *Ae. aegypti* was found. Orange, Texas, on the Texas-Louisiana border was the only community outside of Louisiana in which *Ae. aegypti* was not found. *L. culicis* was found in all communities that had *Ae. aegypti* except Shreveport and its satellite community, Waskom, Texas, and Rusk, Texas, where *Ae. aegypti* was rare. In general, *L. culicis* was found more universally distributed and in greater numbers where *Ae. aegypti* populations were greatest. The parasite was most often absent or scarce in areas recently reinvaded by *Ae. aegypti* following an absence of the species. Exceptions were noted, however. In Temple, Texas, the westernmost of the communities surveyed, *Ae. aegypti* was abundant, but *L. culicis* was scarce. Waskom, Texas, with a large, long-established population of *Ae. aegypti* was without *L. culicis.* Generally, at least half of the *Ae. aegypti* larvae exposed to *L. culicis* became infected.

The number of sites and containers positive for *Ae. aegypti* and *L. culicis* were progressively greater throughout the season (Table 2). The percentage of *aegypti*-positive sites and of containers positive for *L. culicis* was also progressively greater in successive cycles of surveillance.

The number of *L. culicis*-infected larvae increased with subsequent cycles of inspection (Table 3); however, the average number of *L. culicis* per infected larva (as well as the greatest number) and the high-

TABLE 1.—Cumulative results of three cycles of surveillance for *Lankesteria culicis* in selected communities.

Community	Sites positive		Containers positive		*L. culicis* range per infected larva	Avg. No. *L. culicis* per infected larva	Percent *A.a.* infected when associated with *L.c.*
	A.a.	*L.c.*	*A.a.*	*L.c.*			
New Orleans, La.	0	0	0	0	0	0	0
Covington, La.	4	1	8	1	6	6	33
Baton Rouge, La.	0	0	0	0	0	0	0
St. Francisville, La.	0	0	0	0	0	0	0
Lake Charles, La.	0	0	0	0	0	0	0
Orange, Texas	0	0	0	0	0	0	0
Alexandria, La.	0	0	0	0	0	0	0
Colfax, La.	0	0	0	0	0	0	0
Shreveport, La.	5	0	9	0	0	0	0
Waskom, Texas	16	0	35	0	0	0	0
Monroe, La.	0	0	0	0	0	0	0
Vicksburg, Miss.	9	5	22	10	2–407	67.9	25
Temple, Texas	28	1	42	2	4–67	25.0	67
Cameron, Texas	15	10	44	17	1–615	41.2	58
Palestine, Texas	52	38	74	63	1–947	101.9	76
Rusk, Texas	4	0	4	0	0	0	0
Nacogdoches, Texas	36	18	55	25	1–476	65.2	60
Lufkin, Texas	16	11	29	22	1–415	64.2	70
Pensacola, Florida	60	42	125	69	1–456	50.6	58
Milton, Florida	15	14	40	23	1–248	42.9	59

est frequency of infection occurred during surveillance cycle II (mid-season for *Ae. aegypti*). With the conditions of this survey and in the geographic areas covered, the peak season for magnitude and frequency of *L. culicis* infections did not coincide with the peak season for *Ae. aegypti*.

Approximately 17,000 specimens of *Ae. aegypti* were examined microscopically in the course of the pathogen surveillance and special studies. No marked correlation was observed between sick, moribund, and dead larvae and parasitism by *L. culicis*. Other pathogens constituted only an occasional find. Larvae with more than 300 trophozoites and pupae with more than 50 gametocysts were encountered frequently without observable ill effects. Sick or moribund larvae were found with and without *L. culicis* about equally. In contrast to findings reported by Barrett (1968), wherein gametocysts were generally rather equally distributed in the five Malpighian tubules of pupae, gametocysts, at least in light infections of 15 or less, were limited more often to one or two of the tubules. With striking frequency, pupae devoid of *L. culicis* were observed from containers in which most of the larvae had significant numbers of trophozoites.

TABLE 2.—Summary of data relative to *Ae. aegypti*-positive sites and containers.

Cycle	Sites positive			Containers positive		
	A.a.	*L.c.*	Percentage	*A.a.*	*L.c.*	Percentage
I	80	34	43	143	49	38
II	87	44	50	155	73	47
III	93	62	67	179	100	55
Total	260	140	54	477	222	47

TABLE 3.—Summary of data on numbers of *L. culicis* infected larvae and the magnitude and frequency of infection.

Cycle	No. *Ae. aegypti* larvae positive for *L. culicis*	Total number *L. culicis*	Average number *L. culicis* per infected larva	% *Ae. aegypti* infected when associated with *L. culicis*
I	172	8,794	51.1	56
II	337	26,544	78.3	63
III	465	32,682	70.3	60
Total	974	68,020	69.7	60

Newly hatched *Ae. aegypti* larvae introduced into natural water in containers found in place and into control containers on two sites in a special study at Monroe, Louisiana (from which the species had spontaneously disappeared) developed normally through the larval stages. Subsequent rearing of specimens submitted to the laboratory at TDL resulted in large healthy pupae and adults. Examination of approximately 100 larvae revealed only two which were positive for *L. culicis*. These positives consisted of one very lightly infected larva from naturally-occurring containers on each site. The possibility of introduction of the parasite cannot be discounted since egg strips taken from a newly established laboratory colony in an *L. culicis*-infested area (Meridian, Mississippi) were used to obtain larvae.

L. culicis introduced onto sites in Shreveport and Waskom (communities where the parasite was not found) survived and spread. This special study was commenced late in the season in order to take advantage of the results of the regular pathogen surveillance in these communities. Accordingly, little time, under optimum breeding conditions for *Ae. aegypti,* was available to observe the results of the study. Some infected larvae were collected from containers into which the parasite had been added as few as 5 days before. Spread of *L. culicis* to some nearby containers was manifested in about 2 weeks. Unfortunately, insufficient time within the season remained to watch for subsequent spread both within and outside the perimeter of the study sites.

DISCUSSION. As far as reaching our goal of explaining by pathogen surveillance the diminution of *Ae. aegypti* infestations within one season in large areas like most of Louisiana and adjacent southeast Texas, the program did not succeed. However, a number of corollary benefits did accrue. In conjunction with statewide larval sampling and oviposition trap operations, information on the distribution of *Ae. aegypti* was updated and generally confirmed. The widespread distribution and abundance of *L. culicis* were established, and methodology for producing and distributing the parasite was developed and evaluated. Since *L. culicis* is apparently specific for *Ae. aegypti,* and future study may develop techniques for utilizing it to potentiate other factors, biological, chemical, or environmental, the above information may have much value.

Although evidence was insufficient to link *L. culicis* with significant mortality of *Ae. aegypti,* considerably more work will be required to rear and observe infected specimens through several generations to determine absence of subsequent deleterious effects. A colony presently maintained in the laboratory is apparently thriving, although infected with *L. culicis*. The survey disclosed that, in general, the heaviest and most widespread distribution of *L. culicis* was coincident with flourishing populations of *Ae. aegypti,* possibly a reflection that where the host is abundant the parasite thrives. In such areas, certainly, there is no indication that a serious limiting effect upon the *Ae. aegypti* population exists. On the other hand, some factor did operate to practically eliminate

once prodigious *Ae. aegypti* populations from Louisiana, and *L. culicis* might have figured in an adjunctive role.

The special study in Monroe, in which *Ae. aegypti* larvae developed normally after being introduced into sites from which they had spontaneously disappeared, suggests that an agent (agents) which may have figured in the demise of the mosquito was obligatory, and had, in turn, disappeared. *L. culicis* would fit this picture since it is apparently specific for *Ae. aegypti*.

SUMMARY. A survey was conducted during 1968 on the distribution, density and effects upon *Ae. aegypti* of *L. culicis* in an area extending from Temple, Texas, to Pensacola, Florida. The heaviest and most widespread distribution of *L. culicis* was coincident with flourishing populations of *Ae. aegypti*. There was no indication that the parasite presently exerts a serious limiting effect upon *Ae. aegypti* populations observed. However, *L. culicis* might act to potentiate the deleterious effects of other parasites, or of insecticides, or to render the infected individual less able to cope with adverse environmental factors.

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WET FILTER PAPER AS AN OVIPOSITION SUBSTRATE FOR MOSQUITOES THAT LAY EGG-RAFTS

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INTRODUCTION. It is general practice, when rearing many mosquito species that lay their eggs singly, to provide moist paper towels or filter paper as oviposition substrata (Gerberg, 1970). This technique probably arose from observations that in nature these species usually oviposit on moist debris near the edges of their breeding pools.

In contrast, mosquitoes that lay their eggs in groups to form "egg-rafts" are generally considered to select free water surfaces for oviposition (Wallis, 1954). This assumption had been widely held for mosquitoes of the genus *Culex* (e.g. Carpenter and La Casse, 1955; King *et al.*, 1960) until Mattingly (1970) recognized this belief as incorrect and listed the following exceptions: *C. abominator* Dyar and Knab on the upper surface of *Lemna* fronds (Coad, 1913); *C. chrysonotum* Dyar and Knab on grass or sedge about one inch above the water surface (Arnett, 1948); *C. territans* Walker from above the water line to as much as 6–8 inches above it (Knab, 1904); *C. fergusoni* Taylor and *C. douglasi* Dobrotworsky on moist filter paper 2–5 and 1–3 inches respectively above the water line (Dobrotworsky, 1956); and *C. hayashii* Yamoda and *C. infantulus* Ed-