FIELD STUDIES ON *LANKESTERIA CULICIS* AND *AEDES AEGYPTI* IN FLORIDA[1]

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As part of the general survey in the southeastern United States, following a uniform protocol (Gentile *et al.* 1971), the distributional pattern of the gregarine, *Lankesteria culicis,* in the yellow fever mosquito *Aedes aegypti,* was studied in Florida. Preliminary laboratory results had indicated that *Lankesteria*-infected mosquito larvae of a DDT-resistant strain were more susceptible to DDT than non-infected larvae (McCray *et al.,* 1970). To determine the validity of these observations under actual field conditions, studies of the effect of DDT larvicide applications on resistant larvae, heavily vs. lightly infected with *L. culicis,* were conducted in the Liberty City area of Miami during July, September and October 1968. Previous determinations (Flynn and Schoof, 1965) had indicated a degree of DDT resistance in the *Ae. aegypti* strain found in this field area.

METHODS AND MATERIALS. The distributional surveillance for *L. culicis* was conducted from field offices located in Jacksonville and Miami with teams composed of experienced state inspectors of the Aedes aegypti Eradication Program (AAEP). Six urban centers and six satellite communities were surveyed in three cycles during the 1968 season. The centers and satellites (in parentheses) selected were Jacksonville (Jacksonville Beach), Daytona Beach (De Land), Orlando (Sanford), Ocala (Dunnellon), Miami (Homestead), and West Palm Beach (Boca Raton).

In addition, 16 other Florida cities were inspected once: Apopka, Clermont, Fellsmere, Fernandina Beach, Fort Pierce, Gainesville, Green Cove Springs, Kissimmee, Leesburg, Palatka, St. Augustine, Starke, Stuart, Wabasso, Winter Park and Yulee. While making the *L. culicis* trophozoite counts, investigators recorded several other interesting biological organisms in the *Ae. aegypti* larvae collected.

The test site chosen for the DDT evaluations was in the central portion of Liberty City, the area supporting the DDT-resistant *Ae. aegypti.* The location had many types of containers showing active breeding. This site was divided into four areas, and in each, 10 containers breeding *Ae. aegypti* were marked with numbers. The four groups of containers were treated in the following manner:

Group 1. First-instar *Ae. aegypti* larvae and *L. culicis* sporocysts were added. *Lankesteria* trophozoite counts were subsequently made on the fourth-instar larvae.

Group 2. First-instar *Ae. aegypti* larvae and *L. culicis* sporocysts were added. A DDT treatment was made when the larvae reached third instar, and *Lankesteria* counts were made on surviving fourth-instar larvae.

Group 3. Only first-instar larvae were added; no *Lankesteria* sporocysts were added. Fourth-instar larvae were examined for trophozoites.

Group 4. Only first-instar larvae were added. DDT treatment was made when larvae reached third instar. Surviving fourth-instar larvae were examined for trophozoites.

The water volume of each container was measured and marked on the container with paint. For containers in groups 1

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and 2, the bottom feeding area for larvae was also measured. *Lankesteria* sporocysts furnished by the Biology Section, Technical Development Laboratories, Savannah, Georgia, were introduced into containers of groups 1 and 2 at the rate of 8,000 sporocysts per square foot of feeding area. The first-instar larvae were obtained from eggs collected in ovitraps placed at or near the test site.

Suspensions prepared with a 75 percent water-dispersible DDT powder were used for the insecticidal treatment of containers in groups 2 and 4. To insure accurate DDT concentrations, the water level in each container was adjusted to the originally marked level and maintained to the nearest liter throughout the experiment.

DISCUSSION AND RESULTS. The percent of sites positive for *Ae. aegypti* (Table 1) ranged from 0 to 100 percent, and those positive for *L. culicis* showed the same range. Since the detection of *L. culicis* was dependent upon the presence of *Ae. aegypti,* the sites positive for the parasite could not exceed the value for the mosquito host. Although the sampling areas were limited, the locations below 28° N. latitude, namely, West Palm Beach, Boca Raton, Miami and Homestead, showed higher positivity for both *Ae. aegypti* and *L. culicis* than did the more northerly localities. In those containers positive for *L. culicis,* from 32 to 86 percent of the *Ae. aegypti* were parasitized.

Results from the three cycles of inspection (Table 2) indicate seasonal increases of 39 to 71 percent positivity for *Ae. aegypti* and 30 to 47 percent for *L. culicis* from the first cycle through the third cycle. The degree of *L. culicis* infestation varied from 1 to over 800 parasites per *Ae. aegypti.* The mean number was somewhat lower during the second cycle and the mean for the entire study was 57 parasites per *Ae. aegypti.*

The first cycle of inspection in the northern and central counties of Florida

TABLE 2.—Cyclical variation in *Aedes aegypti* and *Lankesteria culicis* distribution and mean levels of infestation.

	Survey Cycles			Entire Survey
	1	2	3	
% Sites positive for *Ae. aeg.*	39	63	71	
% Sites positive for *L. c.*	30	40	47	
Mean level of *L. c.* infestation	66	44	63	57

TABLE 1.—General distribution of *Lankesteria culicis* in *Aedes aegypti* in Florida cities.

Cities	Sites			% *Ae. aeg.* infected with *L. c.* in positive containers
	Total	Percent Positive		
		Ae. aegypti	*L. culicis*	
Jacksonville	23	49	30	60
Jacksonville Beach	6	17	0	44
Daytona Beach	17	31	10	32
De Land	7	55	11	68
Orlando	18	56	21	41
Sanford	5	20	7	50
Ocala	15	32	30	61
Dunnellon	8	0	0	..
Miami	20	95	70	77
Homestead	4	100	83	83
West Palm Beach	20	98	86	71
Boca Raton	4	100	100	86

was conducted during dry conditions, but the other cycles occurred during very wet periods. *Ae. aegypti* increased to a greater degree with the wet conditions than did *L. culicis*. In the four southern cities with higher parasite infection rates of *Ae. aegypti* weather conditions were more uniformly wet.

At intervals between the regular cycles 16 cities were inspected once. No *Ae. aegypti* were detected at Apopka, Fellsmere, Ft. Pierce, Gainesville, Kissimmee, Wabasso or Yulee, Florida. No *L. culicis* were found in *Ae. aegypti* at Clermont, Green Cove Springs, Leesburg, St. Augustine, Starke, Stuart or Winter Park, Florida. The presence of *L. culicis* was confirmed only at Fernandina Beach and Palatka, Florida.

While making the *L. culicis* trophozoite counts, investigators noted several other interesting biological organisms in the *Ae. aegypti* samples: Jacksonville, red cells in the intestine of specimens which appeared sickly; in Jacksonville Beach, a nematode around the Malpighian tubules; in St. Augustine, algal strands attached to the exoskeleton; in Clermont, fungal hyphae throughout the body cavity; and in Milton, feathery growths just under the exoskeleton layers.

One month before the DDT-*Lankesteria* field studies, *Ae. aegypti* larvae from the DDT-treated Liberty City section of Miami and larvae from the southern part of Dade County which had never been under DDT treatment by AAEP were tested for resistance by the WHO test for mosquito larvae (Anon., 1960). Mean mortalities from five replicates with concentrations of 0.1, 0.5, and 2.5 p.p.m. DDT and of controls were as follows: Liberty City larvae 1.1, 14.6, 42.9 and 1.1 percent and South Dade larvae 19.8, 77.4, 100 and 1.1 percent.

In the first DDT-*Lankesteria* test (Table 3), the addition of 8,000 sporocysts per square foot of larval feeding area in containers of groups 1 and 2 produced marked increases in the average number of *Lankesteria* trophozoites per larva when compared with values for specimens from group 3 and 4 containers. A uniformly high infestation rate was not obtained, however, in group 1 and 2 larvae because an appreciable percentage of the population had less than 10 trophozoites per larva. The addition of 2 p.p.m. DDT to group 2 containers apparently reduced mainly the proportion of the population having the higher rates of trophozoites as shown by the cumulative percents of popu-

TABLE 3.—Relative parasitism of *Aedes aegypti* larvae in containers with and without the addition of 8,000 *Lankesteria* sporocysts per sq. ft. and 2 ppm DDT treatment. Field test 1, Miami, Florida.

Group	1		2		3		4	
Lankesteria added	7/26/68		7/26/68		..		..	
No. sporocysts per sq. ft.	8,000		8,000		..		..	
Aedes aegypti added	7/26/68		7/26/68		7/26/68		7/26/68	
DDT added			2 ppm				2 ppm	
			7/29/68				7/29/68	
Survivors collected	7/31/68		7/31/68		7/31/68		7/31/68	
No. *Lankesteria* per larva	No. Larvae	Cum. %	No. Larvae	Cum. %	No. Larvae	Cum. %	No. Larvae	Cum. %
0	9	7.2	13	12.4	53	48.2	44	34.0
1–9	19	22.5	42	52.4	30	75.5	35	61.1
10–49	42	56.4	18	69.5	20	93.7	36	89.0
50–99	13	66.9	11	80.0	6	99.1	9	96.0
100–249	19	72.2	14	93.3	0	99.1	5	100
250+	22	100	7	100	1	100	0	
Total	124		105		110		129	
Average No. *Lankesteria* per larva	144.6		45.4		12.0		18.2	

TABLE 4.—Relative parasitism of *Aedes aegypti* larvae in containers with and without the addition of 8,000 *Lankesteria* sporocysts per sq. ft. and 200 ppm DDT treatment. Field test 2, Miami, Florida.

Group	1		2		3		4	
Lankesteria added	9/13/68		9/13/68		..		..	
No. sporocysts per sq. ft.	8,000		8,000		..		..	
Aedes aegypti added	9/13/68		9/13/68		9/13/68		9/13/68	
DDT added			200 ppm				200 ppm	
	..		9/16/68		..		9/16/68	
Survivors collected	9/18/68		9/18/68		9/18/68		9/18/68	
No. *Lankesteria* per larva	No. Larvae	Cum. %	No. Larvae	Cum. %	No. Larvae	Cum. %	No. Larvae	Cum. %
0	11	12.9	7	58.3	48	39.0	0	0
1–9	18	34.1	4	91.6	39	70.7	1	25
10–49	35	75.3	1	100.0	33	97.5	3	100
50–99	10	87.1	0		3	100.0	0	
100–249	9	97.7	0		0		0	
250+	2	100.0	0		0		0	
Total	85		12		123		4	
Average No. *Lankesteria* per larva	19.8		3.7		9.3		19.8	

lations in groups 1 and 2. The larvicidal effect of 2 p.p.m. DDT was not of sufficient magnitude either in group 2 or group 4 to be of economic significance.

In the second test (Table 4), although the *Lankesteria* sporocysts were added to containers in groups 1 and 2 at the same rate used in the first test, the mean levels of parasites/larva were considerably lower and essentially equal to the levels obtained in containers without additional *Lankesteria* (groups 3 and 4). The effects of 200 p.p.m. DDT were marked, however, since the surviving larvae had infestation rates of less than 50 trophozoites/larva, whereas 25 percent of the group 1 larvae had higher infestation rates.

In the third test (Table 5), the intro-

TABLE 5.—Relative parasitism of *Aedes aegypti* larvae in containers with and without the addition of 24,000 *Lankesteria* sporocysts per sq. ft. and 200 ppm DDT treatment. Field test 3, Miami, Florida

Group	1		2		3		4	
Lankesteria added	10/11/68		10/11/68		..		..	
No. sporocysts per sq. ft.	24,000		24,000		..		..	
Aedes aegypti added	10/18/68		10/18/68		10/18/68		10/18/68	
DDT added	..		200 ppm		..		200 ppm	
			10/22/68				10/22/68	
Survivors collected	10/23/68		10/23/68		10/23/68		10/23/68	
No. *Lankesteria* per larva	No. Larvae	Cum. %	No. Larvae	Cum. %	No. Larvae	Cum. %	No. Larvae	Cum. %
0	18	13.7	1	4.8	8	33.3	3	15
1–9	32	38.2	2	14.3	7	62.5	7	50
10–49	36	65.6	5	38.1	5	83.3	6	80
50–99	18	79.4	3	52.4	3	95.8	4	100
100–249	11	87.8	4	71.4	1	100	0	
250+	16	100.0	6	100.0	0			
Total	131		21		24		20	
Average No. *Lankesteria* per larva	56.9		216		23.1		21.6	

duction of increased numbers of *Lankesteria* sporocysts (24,000 per sq. ft. of feeding area) produced somewhat higher mean infestation levels than did the 8,000 per square foot additions. The increased levels, however, were not proportional to the threefold increased additions. Further, about 50 percent of the larvae from groups 1 and 2 had fewer than 50 trophozoites/larva. This finding indicates that the increased numbers of added sporocysts did not influence the proportion of larvae with relatively low levels of infestation. In this test the sporocysts were added 1 week before the *Ae. aegypti* larvae, and mortality values were determined at 24 hours rather than at 48 hours.

SUMMARY. During all three cycles of inspection, the parasite *L. culicis* was commonly found throughout the Florida cities. Infestation with *L. culicis* in *Ae. aegypti* larvae was not high generally; mean values were 66, 44 and 63 trophozoites per positive larva for cycles 1 to 3, and heavily infected larvae with 200 to 800 trophozoites did not appear to be adversely affected to any extent by the parasites.

Levels of *L. culicis* infection in *Ae. aegypti* larvae can be increased by introducing parasite sporocysts into potential breeding containers. The levels of infection reached by the individual larva, however, are quite variable.

Although heavy infections of *L. culicis* in *Ae. aegypti* larvae of a DDT-resistant strain rendered the larvae more susceptible to DDT, the increased mortality is of questionable economic value in field use.

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