

THE DISTRIBUTION, ETHOLOGY AND CONTROL POTENTIAL OF THE *LANKESTERIA CULICIS* (ROSS)—*AEDES AEGYPTI* (L.) COMPLEX IN SOUTHERN UNITED STATES¹

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Since Ross and Smyth (1897) and Ross (1906) described *Lankesteria culicis* the occurrence of the protozoan in *Aedes* spp. has been reported in Africa, Asia, Europe and South America, and recently as a specific parasite of *Ae. aegypti* in North America (Barrett, 1968). The early reports are amply cited by Christophers (1960). The life cycle of the protozoan has been described by Wenyon (1911), Ray (1933) and Ganapati and Tate (1949). The taxonomic status of the species and additional biological and ethological data have been reported by Weiser (in Steinhaus, 1963) and presently by McCray *et al.* (1970).

Varied and contradictory statements have been made as to the pathogenicity of the protozoan to mosquitoes. Weiser (1963), in reviewing the gregarines inhabiting the insect gut, states that "*Lankesteria* does not cause pathology in the gut of the host or a destruction which could be characterized as representing pathogenicity, since all the cells destroyed are regenerated in excess by normal growth of the gut tissue." He states that most gut-inhabiting gregarines are harmless commensals.

Other authors, however, have reported larval mortality caused by *L. culicis*. Feng (1933) observed mortality in *Aedes koreicus* in China; Ganapati and Tate (1949) in *Aedes geniculatus* in England; and Hati and Ghosh (1963) in *Ae. aegypti* in India.

Barrett (1968) reports on larvae, pupae and adults of *Ae. aegypti* severely damaged by *L. culicis* under field conditions in Houston, Texas. Larvae infected with more than 250 trophozoites of *L. culicis* were almost always thin and stunted; dead or dying larvae were often seen. According to the same author, pupae and adults developing from larvae stunted by *L. culicis* were also stunted and their Malpighian tubes were severely damaged by the development of the gametocysts of the protozoan within the tubes. Gametocysts were generally rather equally distributed in the five Malpighian tubes of the pupa; from 8 to 25 gametocysts could destroy one-third of a single tube and in greater number could destroy the distal two-thirds of a tube. A damage of this magnitude occurring in all tubes generally caused death in the pupal stage.

In addition, preliminary evidence gathered at the Technical Development Laboratories, Center for Disease Control (CDC), Savannah, Georgia, indicated that DDT-resistant larvae heavily infected with *L. culicis* showed higher mortalities from DDT (McCray *et al.*, 1970).

The reported deleterious effect of the protozoan upon *Ae. aegypti* and the potential use of a biological control agent in the *Aedes aegypti* Eradication Program, CDC, justified a thorough evaluation of the protozoan. For this purpose investigations were made in 1968 by the operational and technical personnel of the *Aedes aegypti* Eradication Program and were aimed at gathering the following information.

1. Occurrence of the protozoan in the major areas of *Ae. aegypti* distribution in the continental United States.
2. The seasonal incidence of the protozoan in populations of *Ae. aegypti*.
3. The effect upon the host of the pro-

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- tozoan *per se* in association with other pathogens.
4. The feasibility of a controlled release of sporocysts, mass-produced under laboratory conditions, in both *Lankesteria*-free and *Lankesteria*-infected areas to permit workers to assess the establishment and dispersal of the protozoan and its effect upon the host population.
 5. The effect of *Lankesteria*-DDT complex upon both DDT-resistant and DDT-susceptible *Ae. aegypti*.
 6. The host range of *L. culicis* among mosquito species found associated with *Ae. aegypti* in field containers.
 7. Additional knowledge of the bio-nomics of the protozoan.

This paper describes the general procedures used in the surveys and correlates results obtained in specific studies. Details of the studies in Texas (Barrett *et al.*, 1971), in Louisiana (Hayes and Haverfield, 1971), and in Florida (Stapp and Casten, 1971) are given in separate papers.

SURVEILLANCE METHODOLOGY. Three teams making spring, summer and fall evaluations of *Ae. aegypti* and *L. culicis* during 1968 in Florida, Louisiana and Texas followed a standardized surveillance method. Supervisors selected urban centers that represented the main physiographic characteristics of the state, and designated a smaller satellite center, located within 20 miles of each urban center, to include smaller towns. With the exception of Houston, Texas, the centers and satellites were not scheduled to receive larvicide applications for *Ae. aegypti* eradication during the year. Twenty prime breeding sites (tire yards, car wrecking yards, garbage dumps, etc.) in each urban center and five or six sites in each satellite were selected and periodically inspected for the duration of the study.

During each seasonal cycle, the teams collected 20 to 30 juveniles in the 3rd or 4th larval instar or in the pupal stage from representative containers at each

prime breeding site. Samples of the live immature stages were gathered in vials according to site and type of container. Moribund individuals and those showing other signs of abnormality were also collected. Team members kept records on dead or moribund specimens, visible DDT residue, plant life, other mosquito species and the depth of the water in each container.

Within 48 hours of collection, juveniles were checked for the presence of *Lankesteria* and other known mosquito-inhabiting organisms. Each team supervisor was provided with a pictorial guide to many known mosquito pathogens. Team members first checked each specimen with a dissecting microscope for overall appearance, and then mounted it on a slide in a preservative stain [1:1 solution of glycerol and 20 percent formaldehyde with enough methylene blue to produce the desired intensity (McCray *et al.*, 1970)].

When the investigator applied a gentle pressure upon a coverglass overlaying the host specimen, the resulting rupture of the specimen permitted the detection and counting of the protozoan in the extruded digestive system of the juvenile. In other cases the gut of the specimen was teased out and then mounted in the staining media. Sample slides of abnormal and infected specimens were sent by the surveillance teams to the Technical Development Laboratories in Savannah, Georgia, for further study.

RESULTS AND DISCUSSION. The results of the seasonal surveys are depicted graphically in Figure 1 for six urban centers and their satellites in Texas: Corpus Christi (Sinton), San Antonio (Seguin), Houston (Cleveland), Temple (Cameron), Palestine (Rusk) and Nacogdoches (Lufkin); for six urban centers and their satellites in Louisiana: New Orleans (Covington), Baton Rouge (St. Francisville), Lake Charles (Orange, Texas), Alexandria (Colfax), Shreveport (Waskom, Texas), and Monroe (Vicksburg, Mississippi); and for six urban centers and their satellites in Florida: Jacksonville (Jacksonville Beach),

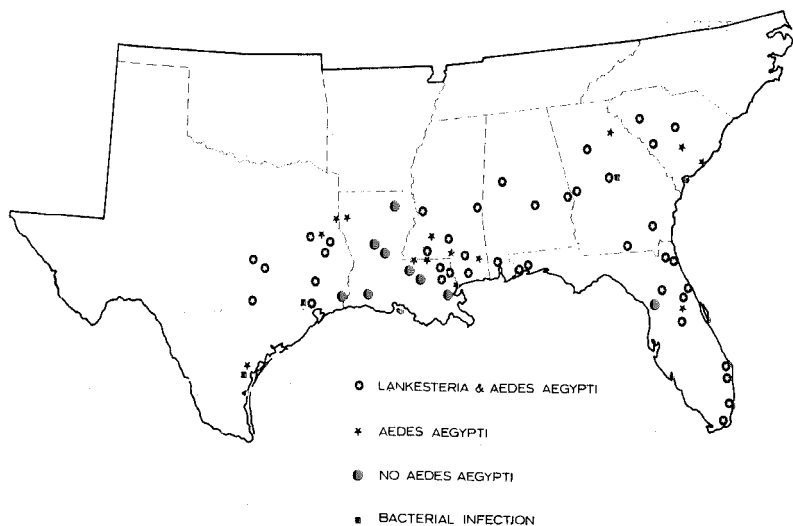


FIG. 1.—Occurrence of *Aedes aegypti* and *Lankesteria culicis* in selected sites in southern United States in a 1968 survey.

Daytona Beach (Deland), Orlando (Sanford), Ocala (Dunnellon), West Palm Beach (Boca Raton) and Miami (Homestead). In addition, the following urban centers were surveyed in the fall of 1968 to complete a general assessment of the geographical distribution of the parasite: Greenwood, Aiken, Columbia, Orangeburg and Charleston in South Carolina; Athens, Atlanta, Macon, Columbus, Valdosta and Waycross in Georgia; Tuscaloosa, Montgomery, Phenix City and Mobile in Alabama; Pensacola and Milton in Florida; and Meridian, Hazelhurst, Magee, Laurel, Hattiesburg, McComb, Woodville, Columbia, Poplarville, Lucedale and Wiggins in Mississippi.

L. culicis was found in all the known areas of distribution of *Ae. aegypti* in the continental United States. However, for reasons still unknown, the protozoan was not detected in a small number of urban centers with a history of recent *Ae. aegypti* reintroduction (i.e., according to Aedes aegypti Eradication Program records: Shreveport, Louisiana; Charleston

and Orangeburg, South Carolina; Corpus Christi, Texas). The protozoan was also absent from areas with a record of transient *Ae. aegypti* infestations, such as the southeastern area along the Louisiana-Mississippi border and along the northwest Louisiana-Texas border. It should also be noted that most of the surveyed areas of Louisiana did not yield *Ae. aegypti*. This confirmed a similar observation reported earlier by Hayes and Ritter (1966). However, the release of immature *Ae. aegypti* in field containers in one noninfested urban center (Monroe, Louisiana) resulted in their normal development to adulthood; neither *Lankesteria* nor any other pathogen was detected. Moreover, the release of sporocysts of *Lankesteria* in *Lankesteria*-free centers (Corpus Christi, Texas and Shreveport, Louisiana) led to the establishment of the organism in the local *Ae. aegypti* population and its dispersal to premises within a 900-ft. radius of the release site. Whether *Lankesteria* will continue to thrive and disperse within and beyond the release centers needs to be ascertained.

The ethology of *Ae. aegypti* and of the mosquito-protozoan complex in relation to ecological factors in areas where the two organisms do not have a history of permanence is not understood. For example, Louisiana is one nonendemic area that has no obvious barriers to invasion from surrounding endemic areas.

In a recent study of the temperature tolerance of *Lankesteria* under laboratory conditions, McCray *et al.* (1970) observed a marked loss of viability in free-living spores at water temperature above 90° F. During the field surveillance *Ae. aegypti* larvae infected with *Lankesteria* were recovered from field containers filled with water; the temperature of the water was sometimes as high as 102° F. It is conceivable that in endemic areas the temperature in a field container may reach the 90° to 100° range during the afternoon of summer days; at the same time we know that lower temperatures will occur in the same container. It is at these times that the larvae may ingest viable *Lankesteria* spores newly released by adults and thus perpetuate the infection in the container.

The same authors report spores remaining viable for 8 months under dry conditions in the laboratory. A prolonged period of drought may conceivably eliminate the two organisms from a given area, but we would expect the two organisms to be reintroduced from surrounding endemic areas when climatic conditions return to normal.

At the present time we have no plausible explanation for the gaps in the distributional pattern of the two organisms. We need to know, for example, why Pensacola, Florida, is heavily infested with *Lankesteria*-infected *Ae. aegypti* while New Orleans, Louisiana, is free of the two organisms; why Corpus Christi, Texas, is infested with *Lankesteria*-free *Ae. aegypti*, while the two organisms are found in most of the state north of Corpus Christi.

From the distributional surveillance in the spring, summer and fall periods of 1968, evidence was gathered which indi-

cates that the seasonal incidence of the protozoan follows the seasonal rise and fall of the population density of the host. Approximately 50 percent of the sites and containers inspected and 60 percent of the combined number of larvae and pupae collected were found infected with the protozoan. The number of trophozoites counted in the collected larvae ranged from 1 to 2,000; the highest frequency was within the 40-100 range. Gametocysts were not always found in all the Malpighian tubules of the pupae. As many as 300 gametocysts were observed in some specimens.

The surveys indicated that *L. culicis* is common in areas where the host is well established. Infected hosts were collected from all types of receptacles, including tree holes, and at water temperatures ranging from 70° to 102° F. No correlation was found between the incidence of the protozoan and water depth, sediment, detritus and vegetation present in the containers.

The data gathered in the surveillance of all the distributional areas yielded no convincing evidence of a deleterious effect of the protozoan upon the host populations in the field, even when larvae were infected with 500 or more trophozoites or when all the Malpighian tubes of pupae were heavily infected with gametocysts. Similar observations were made by McCray *et al.* (1970) in the laboratory; however, a slight reduction of the life span of the infected female was detected.

Observations made during the surveillance suggest that *L. culicis* is host specific. *Culex* spp. and *Aedes triseriatus*, often found associated with *Ae. aegypti*, were not infected with *L. culicis*; and in Texas, Louisiana and Florida, *Ae. triseriatus* was found infected with *L. barretti* Vavra and Levine, but no attempt was made to evaluate the protozoan as a control agent. A pseudomonas type bacterium, still unidentified, was found associated sporadically with *L. culicis* in larvae and pupae of *Ae. aegypti* collected in Houston, Texas, and Macon, Georgia. The same bacterium

was found not associated with the protozoan in Corpus Christi, Texas. The bacterium, with and without the protozoan, proved to be quite elusive as well as erratic in its pathogenicity under laboratory and field conditions, and also when released experimentally in Miami, Florida.

While the surveillance data were being gathered, a thorough study of the bionomics of the protozoan was conducted in Savannah, Georgia, at the Technical Development Laboratories (McCray *et al.*, 1970). It was at this time that sporocysts of *Lankesteria* were mass-produced for field release in endemic and nonendemic areas to enable investigators to study their effects upon the host populations. The data collected from the release experiments indicated that approximately 20,000 (in nonendemic Corpus Christi) to 36,000 (in endemic Miami) sporocysts per square foot of feeding area caused an erratic occurrence and temporary increase in the infection rate above the 40-100 trophozoite range found in naturally infected populations. Subsequent larval collections from test containers in release areas had a level of infection well within the natural range, indicating a rapid occurrence of nondeleterious symbiotic balance in the host-parasite complex.

Comparative measurements of infected and uninfected larvae and pupae on the release sites failed to indicate any stunting effect of the protozoan upon the host. In Corpus Christi, most of the adults (males and mated females) collected on the release site were infected with the protozoan.

Preliminary laboratory observations by McCray *et al.* (1970) indicated that larvae infected with *Lankesteria* had a lesser degree of resistance to DDT than did noninfected larvae. This finding led to a series of tests in Miami, Florida, where DDT-resistant populations had been found. In these tests, field containers received up to 36,000 sporocysts per square foot of feeding area and 200 parts per million of DDT. These tests indicated that the addition of sporocysts did not cause a persistent increase in the level of larval

infection nor a deleterious effect on the larval populations. A sporadic increase of mortality was observed in the test containers among larvae more heavily infected with *Lankesteria* and treated with DDT; at the same time, the increase in larval infection, above the field range, was only temporary. This finding indicates that a combination of DDT-*Lankesteria* has no practical application for control purposes.

One valuable observation made during the experiments concerns the larval survival in containers which received two treatments of 200 parts per million DDT at a 1-month interval. Water samples taken from treated containers 48 hours after treatment yielded less than 1 part per million DDT. Collections made from the treated containers within 28 days after treatment produced larvae and pupae in increasing numbers. In a second experiment in Florida, field containers were treated with a 2.5 percent suspension of DDT wettable powder using a hand sprayer, and following *Aedes aegypti* Eradication Program methodology. Rainfall during the 48-hour period after application amounted to 0.01 inch, and the temperature maxima ranged from 77° to 84° F. Water samples and sediment from the containers taken 48 hours after application and analyzed at the Savannah laboratory yielded less than 1 part per million DDT.

From these studies our investigation teams concluded that *L. culicis*, of common occurrence in the continental USA, may be successfully introduced into uninfected areas by means of sporocysts, and thereafter established and dispersed. However, laboratory and field tests failed to yield any evidence of a marked deleterious effect of the protozoan upon its host, *Ae. aegypti*.

SUMMARY. *L. culicis* was found in all areas in the continental United States where *Ae. aegypti* was known to exist except in a small number of urban centers that had a history of recent *Ae. aegypti* introduction. The protozoan follows the seasonal changes in host population densities, is host specific, and apparently has

little deleterious effect upon the host populations. Although temporary increases in field infection rates were obtained by artificial introductions of sporocysts, symbiotic balance in the host-parasite complex was rapidly reestablished.

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