LANKESTERIA CULICIS A GREGARINE PARASITE OF *AEDES POLYNESIENSIS* IN WESTERN SAMOA

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ABSTRACT. Field studies in Western Samoa showed that almost 40% of *Aedes polynesiensis* larvae were parasitized by the gregarine *Lankesteria culicis*. Adult trophozoites invade epithelial cells lining the gut of larvae and gametocyst formation and maturation occur in the Malpighian tubules of the pupa. The infection appeared to be associated with permanent larval habitats viz, tree-holes and crab-holes. Two companion species *A. samoanus* and *A. aegypti* were also examined. None of the 946 *samoanus* collected were infected and only 1 out of the 325 *aegypti* examined contained the parasite.

Aedes polynesiensis is the principal vector of aperiodic filariasis in the South Pacific. The species occurs in the following groups of islands: Fiji, Samoa, Horne, Wallis, Ellice, Tokelau, Cooks, Austral, Marquesas, Tuamoto, Pitcairn and Society Islands. The mosquito utilizes a wide range of natural containers as larval habitats, which include leaf-axils, tree-holes and crab-holes, and because of the physical impossibility of reaching these sites, a satisfactory method of control by conventional means does not seem possible.

In recent years there has been an increasing interest in the use of biological agents against the vector. Several species of *Coelomomyces* have been isolated from the mosquito in Fiji. Investigations in W. Samoa have revealed a widespread occurrence of a gregarine parasite in *A. polynesiensis*. This paper describes the pathology of infection and the probable identity of the pathogen.

MATERIALS AND METHODS

Larval and pupal samples of *A. polynesiensis* and two companion species, *A.*

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samoanus and *A. aegypti*, were collected from typical *polynesiensis* habitats on Upolu and Savaii, the 2 main islands of the W. Samoan group. The specimens were sorted in a field laboratory in Apia and preserved in MacGregor's solution (MacGregor 1924). Accurate identification of pupae of the 3 species was difficult, and these were not included in the survey except those pupating after sorting and prior to fixation.

The mean average dimensions of the adult trophozoite and spore stage were determined from dissections of preserved larvae and pupae. Ten infected larvae were used to obtain the trophozoite stage and 10 pupae for the spore stage.

Selected specimens of larvae and pupae and adults were embedded in paraffin wax, sectioned and stained in Delafield's iron haematoxylin.

RESULTS

INFECTION RATES. A total of 4,402 larvae belonging to the 3 species were examined. Most of the infection was recorded in *polynesiensis*, i.e., 1,236 out of 3,131 (or 39.5 percent); none in 946 *samoanus* and only 1 out of 325 (or 0.3 percent) in *aegypti*.

Infection rates in *polynesiensis* according to habitat. Most of the collections in this series were from tree-holes, one of the main preferred sites for *polynesiensis* breeding. Thirty-eight out of 45 tree-holes samples (or 84.4 percent) contained gregarine infected *polynesiensis* larvae. Another favored habitat sampled was crab-holes. Only 8 of these were studied because of the difficulty of extracting specimens from the burrows and 5 (or 62.5 percent) contained infected larvae. Infected larvae were also collected in a coconut shell and a rockhole. The habitats sampled and the mean rates of infection in each type are given in Table 1.

With the exception of tires all the habitats listed are natural containers. The trees are bread-fruit (*Atocarpus incisa*) and Pacific chestnut (*Inocarpus edulis*), both of which are cultivated in the village for food. The former produces rot-holes and the latter forms deep folds in the main trunk which collect water and serve as larval habitats for *polynesiensis*.

Several species of crabs belonging to the genus *Cardisoma* dig burrows extending to 1 meter or more along the supralittoral zone. These crab-holes are also favored larval habitat for *polynesiensis*.

OBSERVATIONS ON CONTINUOUS INFECTION IN TREE-HOLE HABITAT. Between June and December 1973 2 tree-holes were sampled 4 times, at approximately 2-month intervals. High rates of gregarine infection were recorded in the larvae from these holes throughout this period (Table 2).

Since the average time required for *polynesiensis* from egg to adult stage would not exceed 7 days, these observations indicated that new infections were occurring in the habitats throughout this period.

HISTOPATHOLOGICAL STUDIES. Longitudinal sectioning of infected larvae revealed that the young trophozoite matured intracellularly in the epithelial cell of the mid-gut. Initially it appeared as

Table 1. Infection rates in *polynesiensis* according to habitats.

Habitat	No. of habitats sampled	Total No. specimens	Range of specimens in habitat [mean]	No. infected percentage	No. habitat with infection
Tree-hole	45	2369	2–111(52.6)	968/40.9	38
Crab-hole	8	608	21–188(76)	260/42.8	5
Coconut shell	1	39	39	4/10.3	1
Rock-hole	1	27	27	4/14.8	1
Tires	4	78	10–42 (19.5)	0 0	0

Table 2. Continuous infection rates in larvae of *A. polynesiensis* in 2 tree-holes in 1973.

	Date sampled	No. sampled	No. infected	%
Tree-hole 1	June 25	31	24	77.4
	August 20	30	18	60.0
	September 29	58	28	48.28
	December 7	100	75	75
Tree-hole 2	June 26	232	103	44.40
	August 22	29	10	34.5
	October 30	253	188	74.3
	December 20	113	43	38.7
Total		846	489	57.8

a spherical body with a well stained nucleus and granulated cytoplasm. Multiply infected cells were occasionally observed (Fig. 1). As the parasite increased in size it became elongated and prior to rupturing the host cell resembled the pear-shaped form of the adult trophozoite. Following the breakdown of the epithelial cell, the trophozoite appeared to be attached to the damaged cell by a structure resembling the epimerite. The attachment, however, appeared short-lived for numerous observations of unattached trophozoites were made. At maturity the trophozoite had a mean dimension of 65 x 35 μm with an occasional individual reaching 85 x 35 μm.

Although initial observations of the intact larvae indicated that the adult trophozoites were situated outside the gut region, longitudinal sectioning revealed that these were confined in the space between the epithelial wall and the peritrophic membrane.

In some hosts as many as 10 percent of the epithelial cells were infected by the parasite, but the effect on the larva appeared minimal or negligible. Field observations in Samoa also support this view, in that heavily infected larvae were capable of pupating and producing apparently healthy adults.

GAMETOCYST MATURATION. All further stages in the parasite's life-cycle were observed in the Malpighian tubules of the pupa, which seemed to suggest that adult trophozoite migrate from the midgut during or just prior to pupation. Gamete formation was not observed, but examination of longitudinal sections of the Malphigian tubules showed spherical gametocysts, measuring approximately 40 μm in diameter at maturity (Fig. 2). Spore formation was accompanied by an in-

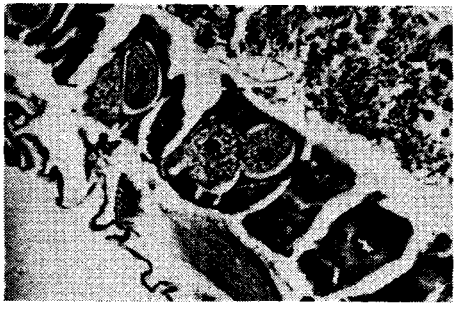

Fig. 1. Longitudinal sections of gut of *Ae. polynesiensis* larvae with developing trophozoites of *Lankesteria culicis* in the epithelial cell.

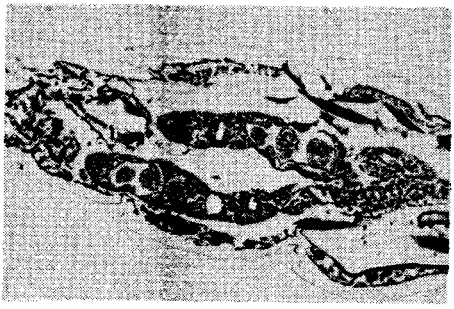

Fig. 2. Longitudinal section of pupa of *Ae. polynesiensis* showing maturing gametocysts in Malpighian tubules.

creased refractility of spores. The mean dimension of spores was 9.32 x 4.24 with a standard error of 0.0574±0.0447) (Fig. 3).

Fig. 3. Section of pupa showing mature spores.

DISCUSSION

On the basis of the life-cycle and adult morphology, the isolate described bears a close resemblance to *Lankesteria culicis* first described by Ross (1895) from *A. aegypti* in India. The parasite has been widely reported from this host in other parts of the world (Jenkins 1964). However, the published figures for the adult trophozoite size vary considerably in these reports. Ross recorded adults up to 200 μm in length. Wenyon (1926) gave an average figure of 50 μm for the species. Kudo (1950) and Steinhaus (1967) both accepted Wenyon's description of the life-cycle, yet Kudo in his classification of *L. culicis* stated that the trophozoites are 150–200 μm in length, although some specimens exceed this length. McCray et al. (1970), who studied the effects of the parasite on *A. aegypti* in the laboratory, quote the higher figure of 275–280 μm with some exceeding 300 μm. Despite these variations in the size of the adult trophozoite stage, spore dimension in these reports has remained consistently in the range 9–10 x 5.6 μm. Allowing for differing methods of fixation, it would appear that our figure of 9.32 x 4.2 μm (mean) for the Samoan isolate is fairly close to the range and therefore referable to *Lankesteria culicis*.

Elsewhere *A. aegypti* has been recorded as the main host of *L. culicis*. In W. Samoa the preferred host appears to be *A. polynesiensis*. This may indicate that permanent pools such as tree-holes and crab-holes utilized by *A. polynesiensis* are more favorable for its colonization. In these situations a concentration of spores could be built up to provide a reservoir which would give rise to infection in successive generations of larvae. This is indicated by the high successive rate of infection recorded in the 2 tree-holes (Table 2). *A. aegypti* in the South Pacific usually utilizes temporary or transient pools and is less likely to accumulate a concentration of spores. Occasionally *aegypti* shares permanent pools with *polynesiensis* and may become infected, but the effect of the parasite on either host appears minimal and therefore only perhaps marginally useful as a biocontrol agent. Although *A. samoanus* shares to some extent the same habitat as *polynesiensis*, the absence of infection in the former probably indicates the presence of a barrier.

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