HELICOSPORIDIUM SP. A NEW PARASITE OF MOSQUITOES[1]

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ABSTRACT. A *Helicosporidium* near *parasiticum* isolated from field-collected larvae of *Culex nigripalpus* infected larvae of 14 mosquito species in 6 genera and also 6 species of Diptera, Coleoptera, and Lepidoptera. The most susceptible mosquito hosts were *Anopheles albimanus*, *An. quadrimaculatus*, and *Cx. salinarius*. Because of differences in host specificity and pathology, and in spore morphology, the *Helicosporidium* of mosquitoes is apparently separate from both the *Helicosporidium* reported from a beetle and *H. parasiticum* as originally described.

The first record of a *Helicosporidium* in mosquitoes was that of Chapman et al. (1967) who reported an unknown species in the hemolymph of a field-collected larva of *Culex territans* in Louisiana. Kellen and Lindegren (1973) used inoculum of what they believed to be *Helicosporidium parasiticum* that was isolated from larvae and adults of *Carpophilus mutilatus* (Coleoptera:Nitidulidae) collected at College Station, Texas, to challenge insect species in the laboratory. They were successful in per os infection of 18 Coleoptera, Lepidoptera, and Acarina and of a mosquito (*Cx. pipiens quinquefasciatus*). Subsequently they maintained the parasite in the laboratory in *Paramyelois transitella* (Lepidoptera: Pyralidae). Also, Chapman (1974) was able to infect larvae of *Cx. territans* per os to this *Helicosporidium* (hereinafter called beetle *Helicosporidium*).

The present study was occasioned by two larvae of *Cx. nigripalpus* infected with what seemed to be *H. parasiticum* that were found in a collection from polluted water in a ditch adjacent to a slaughter house near Lake Charles, Louisiana. No larvae of other mosquitoes (*Cx. restuans, Cx. p. quinquefasciatus,* and *Culiseta inornata*) in the same collection were parasitized. To ascertain whether the strain of *H. parasiticum* from the mosquito (hereinafter called mosquito *Helicosporidium*) had a host range similar to that of the beetle *Helicosporidium*, studies of the susceptibility of non-mosquito hosts were conducted by Lindegren at the Stored Products Insects Research Laboratory in Fresno, California. Also, studies of mosquito host range and infectivity were made at the Gulf Coast Mosquito Research Laboratory in Lake Charles, Louisiana to determine the potential of the mosquito *Helicosporidium* as a biological control agent of mosquitoes.

MATERIALS AND METHODS

LABORATORY STUDIES. At Lake Charles, the two infected larvae were placed in 5 ml of distilled water in a 35x10-mm petri dish and their cuticles were ruptured to free the spores. Then approximately 200 first-instar larvae of *Cx. salinarius* from the laboratory colony were exposed in this pore suspension for 6 hours, transferred to enamel pans, and reared at 25° C in a liter of water on a diet of ground rabbit chow. The developing larvae were examined for infection daily until pupation with a dissecting microscope. Later, larger numbers of larvae of *Cx. salinarius* (or of other mosquito species) were exposed in 60x15-mm plastic petri dishes containing 10 ml of spore suspension. Two more tests were made with known numbers of spores, one with larvae exposed at various ages and the other with larvae exposed for various periods of time. The spore count in the suspensions was determined with a hemocytometer. At Fresno, fresh spores sus-

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pended in water were applied to the mouth parts of insects other than mosquitoes to determine host range; then after 20 days, fresh preparations of fat tissue of the treated insects were examined for spores and other parasite stages under phase contrast.

Spore morphology was studied from stained preparations obtained by rupturing the cuticle of infected larvae and allowing the hemolymph to air dry on glass microscope slides. The smear was then fixed for 1 min with absolute methanol, treated for 15 min with a 10% Giemsa solution in distilled water buffered at pH 7.41, and washed in tap water. Spore measurements were made with an image-splitting micrometer at 1000 X.

FIELD STUDIES. An old abandoned refrigerator body that had 2 to 3 inches of mud and organic debris in the bottom and held approximately 2 liters of water in which *Cx. territans* were breeding was treated with a suspension containing approximately 1×10^6 spores/ml of the mosquito *Helicosporidium*. Samples of larvae were collected before treatment, on 3 successive days after treatment, and 1 week and 2 weeks later. These larvae were examined immediately in the laboratory and then held and reared for further observations.

RESULTS

Infected mosquito larvae were first observed 4 days after exposure to the suspension of spores of the mosquito *Helicosporidium*. The maximum number of infected larvae was recovered 9 to 10 days after exposure. Mortality usually occurred during the fourth instar when the hemocoel became filled with spores, but some mortality also occurred in pupae and emerging adults that were heavily infected. The presence of spores in a few surviving adult mosquitoes suggested that mosquito *Helicosporidium* might be transmitted transovarially, but no infections were seen in 2060 larvae from 17 egg rafts of individual *Cx. salinarius* females exposed as third-instar larvae.

The results of exposing species of mosquitoes to mosquito *Helicosporidium* are summarized in Table 1. Fourteen of the 17 species challenged were susceptible to the pathogen. In the single exposure of *Aedes tormentor*, adverse rearing conditions caused complete larval mortality. Generally, the two *Anopheles* species appeared to be the most susceptible mosquito species; in two trials 89 and 93% of exposed *An. quadrimaculatus* were infected but in two others only 17 and 21% were infected. Also, several *Cx.* spp. appeared to be good hosts, but *Cx. p. quinquefasciatus* and *Cx. p. pipiens* were not. The comparison of infection in *An. quad-*

Table 1. Summary of results of exposing larvae of various mosquito species to mosquito *Helicosporidium* from *Culex nigripalpus*.[1]

Species exposed	No. survivors	Percent infection
Aedes		
aegypti	35	26
	478	4
epactius	1443	0.1
	152	16
taeniorhynchus	772	7
tormentor	0	..
Anopheles		
albimanus	1048	58
	855	35
quadrimaculatus	634	93
	1593	17
	800	89
	1726	21
Culex		
peccator	368	32
pipiens pipiens	981	0
pipiens quinquefasciatus	302	0
	425	0
salinarius	3039	37[2]
tarsalis	53	62
	728	4
territans	50	28
Culiseta		
inornata	75	12
	166	0
Psorophora		
ferox	115	27
horrida	30	4
varipes	116	4
Uranotaenia		
lowii	6	67

[1] Dosages were not checked quantitatively though they varied from 10^6 to 10^8 spores per ml.

[2] Average of 11 separate tests.

rimaculatus and *Cx. salinarius* exposed to known numbers of spores (Table 2) demonstrated that *An. quadrimaculatus* was about twice as susceptible as *Cx. salinarius*.

Table 2. Results of exposing larvae of two mosquito species to known dosages of mosquito *Helicosporidium* (500 larvae exposed in 5 ml of spore suspension for 4 hr).

No. spores/ml	No. larvae surviving	Percent infection
	Anopheles quadrimaculatus	
1.7×10^5	377	49
1.7×10^6	373	83
1.7×10^7	340	85
	Culex salinarius	
9.1×10^4	468	5
9.1×10^5	470	22
9.1×10^6	354	38

The results of the two other tests (Tables 3 and 4) indicated that when early-instar mosquito larvae were exposed to 10^7 spores per ml for longer than 4 hours, the mortality was so high that few infected larvae were recovered. For example, only 3% of the larvae that hatched in this suspension or were exposed to it as day-old larvae for 5 hours survived to the fourth instar (Table 3). Also (Table 4) survival was reduced more than 50% when day-old larvae were exposed for longer than 2 hours. These tests were not a direct indication of the effectiveness of the pathogen, but they do reflect the results of exposures at these conditions.

Table 3. Results of exposing various ages of larvae of *Culex salinarius* for 4 hr to mosquito *Helicosporidium* from *Cx. nigripalpus* (1000 larvae exposed to 8×10^7 spores per ml in a 10-ml spore suspension).

Larval age in days	No. larvae surviving	Percent infection
0[1]	31	71
1	30	87
2	691	49
3	964	33

[1]Allowed to hatch from egg rafts placed in spore suspension.

Table 4. Results of exposing 24-hour-old larvae of *Culex salinarius* to mosquito *Helicosporidium* from *Cx. nigripalpus* for various periods of time (200 larvae exposed to 2×10^7 spores per ml in a 10-ml spore suspension).

Exposure period (hr)	No. larvae surviving	Percent infection
1	151	56
2	96	48
4	88	21
8	17	65

The following non-mosquito insect species were established by laboratory tests as hosts of mosquito *Helicosporidium:* Diptera (*Culicoides* sp.), Coleoptera (*Carpophilus hemipterus, Lasioderma serricorne, Trogoderma variabile*), and Lepidoptera (*Galleria mellonella* and *Paramyelois transitella*).

When the artificial field site containing larvae of *Cx. territans* was treated with mosquito *Helicosporidium,* some small success was achieved. None of the larvae collected before treatment developed infections. However, of the 33 larvae collected 3 days after treatment, one developed a patent infection on the sixth day, and two more infected larvae were found the seventh day. None were found thereafter nor in collections made 1 week and 2 weeks after the treatment.

DISCUSSION

The external manifestations of larvae infected with the beetle *Helicosporidium* and the mosquito *Helicosporidium* differed in some important ways. Spores of the beetle *Helicosporidium* produced a localized infection in mosquito larvae, and a definite host melanization was evident; however, spores flowed in the hemolymph only when the infection was extremely heavy. The mosquito *Helicosporidium* produced a more general infection, no evidence of host melanization, and the spores flowed freely in the hemolymph as was typical of the infections observed by Keilin (1921) in *Dasyhelea.* Weiser (1970), who observed localized infections of *H. parasiticum* in *Hepialis*

pallens, attributed the differences in types of infection to the difference in the mode of invasion, chance invasion through cuticular wounds of terrestrial hosts versus massive invasions of the entire body of aquatic hosts. However, Keilin's (1921) suggestion that the primary route of infection by *H. parasiticum* was via ingestion of spores and penetration through the gut wall is supported by Kellen and Lindegren (1974) and by our studies with mosquitoes reported here.

Lindegren and Hoffman (1976) found that the spore and filament measurements of beetle and mosquito *Helicosporidium* were similar. However, the manifestations of *Helicosporidium* infections from the two hosts were clearly different in *Cx. salinarius.* Thus the difference in symptoms is not merely habitat- or host-oriented. Also, Kellen and Lindegren (1973) were able to infect *Cx. pipiens quinquefasciatus* with the beetle *Helicosporidium,* but the mosquito *Helicosporidium* has never produced infections in this species. Kellen and Lindegren (1974) reported the appearance of pellicles in their description of the life cycle of the beetle *Helicosporidium.* Giemsa-stained preparations of the mosquito *Helicosporidium* also revealed pellicles and the same general life cycle. The life cycles of the *Helicosporidium* from the two hosts are therefore similar, but differences in manifestation of infections and in infectivity to *Cx. p. quinquefasciatus* indicate that two *Helicosporidium* species are involved, both apparently separate from the *H. parasiticum* of Keilin.

Keilin (1921), Weiser (1970), and Kellen and Lindegren (1974) all discussed the biology and life cycle of *H. parasiticum* in detail but did not agree as to the tissues, source, and site of infection. Also Weiser (1970) proposed to transfer *H. parasiticum* from the Protozoa to the lower fungi. Kellen and Lindegren (1974), like Weiser, suggested removing the beetle *Helicosporidium* from the Protozoa, but they are not convinced it should be placed in the primitive Ascomycetes. In fact, Lindegren and Hoffman (1976) suggested that the beetle *Helicosporidium* is not an Ascomycete and that spore ultrastructure indicates an affinity to the Protozoa. We agree with many of the observations of the previous authors, since no new evidence was obtained to resolve the question of the taxonomic classification of *H. parasiticum.*

It is encouraging that the laboratory studies showed a wide host range of the mosquito *Helicosporidium* in mosquitoes and that it sometimes produced high levels of infection. However, the attractiveness of the pathogen as a biological agent is diminished by the high dosages required to produce substantial infections. Until the mosquito *Helicosporidium* has been tested for safety to mammals and non-target organisms, and has been thoroughly tested in the field, its value as a biological agent for mosquitoes can only be tentatively assessed.

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