SUSCEPTIBILITY OF *AEDES AEGYPTI* AND *ANOPHELES QUADRIMACULATUS* LARVAE TO INFECTION WITH THE CERCARIAE OF *PLAGIORCHIS NOBLEI* (TREMATODA: PLAGIORCHIIDAE)

R. A. WEBBER,[1] M. E. RAU[2] AND D. J. LEWIS[3]

ABSTRACT. The impact of interspecific behavioral differences on the relative susceptibility of third instar *Aedes aegypti* and *Anopheles quadrimaculatus* larvae to infection with cercariae of *Plagiorchis noblei* was determined. When permitted to move freely in a column of water, larvae of *Ae. aegypti* were significantly more susceptible to infection with the parasite than were *An. quadrimaculatus* larvae. This difference is ascribed to the significantly greater activity of *Ae. aegypti* larvae in the water column. Since cercariae are suspended in the column, particularly near the bottom, contact with larvae of *Ae. aegypti* may be enhanced, whereas contact with *An. quadrimaculatus* larvae, which tend to remain near the surface, may be reduced. Interspecific differences other than behavior are not thought to play a major role, since immobilized larvae of the two species did not differ significantly in their susceptibility to this parasite.

INTRODUCTION

Recent studies suggest that the entomophilic larvae of some digenetic trematodes may be effective in controlling mosquito populations. Thus, Rao et al. (1985) have documented the destruction of *Culex* larvae by cercariae, and have suggested the possible use of these parasites as agents for the biological control of mosquitoes.

Plagiorchis noblei Park is a parasite of the intestinal tract of birds and mammals. Eggs passed into water with the feces of the definitive host may be ingested by lymnaeid snails. The miracidium escapes from the egg, penetrates the tissues of the snail and transforms into a sporocyst. Polyembryony gives rise to massive numbers of cercariae which emerge from the snail host and penetrate the cuticle of a wide range of aquatic insect larvae. In the tissues of the insect host, cercariae encyst to form metacercariae. After three days, such metacercariae are infective to the definitive host which acquires the infection by ingesting infected insect larvae (Blankespoor 1977).

Laboratory studies have shown that *Aedes aegypti* Linn.) larvae infected with metacercariae of *P. noblei* are unable to complete their development to the pupal and adult stages (Dempster et al. 1986). Furthermore, infected larvae are less active and spend a greater amount of time near the surface of the water. Such behavioral changes may render them more susceptible to predation by surface-feeding birds or mammals (Webber et al 1986a). Little is known about the susceptibility of other mosquito species to this parasite. Our study assesses the relative susceptibility of larvae of *Ae. aegypti* and *Anopheles quadrimaculatus* Say to infection by cercariae of *P. noblei*, and attempts to gain some insight into the factors that determine susceptibility to this parasite.

[1] Imperial College, London SW7 2BB, England.
[2] Institute of Parasitology of McGill University, Canada H9X 1CO.
[3] Department of Entomology of McGill University, Canada H9X 1CO.

MATERIALS AND METHODS

Eggs of *Aedes aegypti* were obtained from Concordia University (Montreal, Quebec). *Anopheles quadrimaculatus* eggs were acquired from the University of Notre Dame, Notre Dame, Indiana and the U.S. Department of Agriculture, ARS Laboratory in Gainesville, Florida. Larvae were hatched in plastic rearing trays (58 x 48 x 7 cm) and maintained at room temperature (20–22°C) on a 16L, 8D photoperiod and a food source consisting of tropical fish food (TetraMin,® Tetra Co.). *Plagiorchis noblei* Park cercariae were obtained from naturally infected lymnaeid snails (*Stagnicola elodes* (Say)) as described by Webber et al. (1986b).

Five thousand freshly emerged cercariae of *P. noblei* were added to the surface of a 13.2 L (35 x 21 x 22.5 cm) aquarium containing 12 liters of aerated tap water (20°C) and 2 g of tropical fish food. Ninety third-instar *Ae. aegypti* and *An. quadrimaculatus* larvae (a composite of three replicates) were then introduced at the surface of the aquarium and allowed to disperse. Since cercariae of *P. noblei* emerge from the snail host at dusk (Webber et al. 1986b), mosquito larvae were exposed to cercariae for 12 hours in the dark to simulate natural conditions. Mosquito larvae were subsequently removed from the aquarium, rinsed in a gentle flow of aerated tap water (20°C) and transferred to a plastic container (15.5 x 9.5 cm) for one hour. Individual larvae were subsequently crushed between two microscope slides and examined under a compound microscope to determine the prevalence and intensity of infection.

Larvae of *Ae. aegypti* and *An. quadrimaculatus* differ markedly in their locomotory behavior in the water column. In order to determine whether differences in susceptibility to cercarial penetration exist in the absence of these behavioral components, larvae of both species were immobilized during their exposure to cercariae. Sixty third-instar larvae of both *Ae. aegypti* and *An. quadrimaculatus* (a composite of three replicates) were randomly distributed along the bottom of an aquarium filled to a depth of 0.5 cm of aerated tap water (20°C) and gently covered with a nylon mesh so as to render them immobile. One thousand freshly emerged cercariae were then added, and left for one hour. Larvae were subsequently removed from the aquarium, rinsed in aerated tap water (20°C) and transferred to a plastic container for one hour. The larvae were then crushed to determine the level of infection.

The behavior of individual *Ae. aegypti* and *An. quadrimaculatus* larvae both in the presence and absence of the cercariae was analyzed through the use of 10 minute videotapes: Forty third-instar *Ae. aegypti* and *An. quadrimaculatus* larvae were introduced into individual 30 ml, clear plastic observation chambers containing 12 ml of aerated tap water (20°C) and 0.003 g of tropical fish food. The chambers were placed in a 20°C incubator for one hour and then removed individually in order to videotape each larva for 10 minutes. Immediately prior to videotaping, 15 freshly emerged *P. noblei* cercariae were introduced into each of 40 chambers in order to expose 20 larvae of each species to the parasites. The remaining 20 larvae of each species were not exposed and served as controls. All exposed larvae were subsequently crushed to ensure that no cercarial penetration had occurred. Only larvae free of metacercariae were included for analysis. Four aspects of the behavior of *Ae. aegypti* and *An. quadrimaculatus* larvae were recorded: 1) the number of wriggling movements; 2) the time (seconds) spent suspended from the surface of the water; 3) the time (seconds) spent in the bottom half of the observation chamber; and 4) the number of looping or grooming movements. Data were analyzed using analysis of variance (ANOVA) with the exception of prevalence data which were analysed using Fisher's exact test (Sokal and Rohlf 1981).

RESULTS AND DISCUSSION

In the absence of a behavioral component, i.e., when rendered immobile, *Ae. aegypti* and *An. quadrimaculatus* did not differ significantly in their susceptibility to attack and penetration by *P. noblei* cercariae (ANOVA, $P > 0.05$) (Table 1). However, when such larvae were permitted to behave normally in the water column, larvae of *Ae. aegypti* were significantly more susceptible to infection with cercariae than were larvae of *An. quadrimaculatus*. Thus, all *Ae. aegypti* larvae (mean intensity 19.1 ± 1.5), but only 38% of the *An. quadrimaculatus* larvae were infected (mean intensity 1.9 ± 0.28, $P < 0.01$). This suggests that the observed interspecific differences in susceptibility to infection with *P. noblei* have a behavioral basis. Non-behavioral factors are not likely to be of major importance. The latter may include structural differences such as cuticle thickness, setal length, etc.

Table 1. Prevalence and mean intensity (±SE) of infection with *Plagiorchis noblei* metacercariae in immobile and mobile *Aedes aegypti* and *Anopheles quadrimaculatus* larvae.

	Prevalence (%)		Mean intensity (±SE)	
Motility	*Aedes*	*Anopheles*	*Aedes*	*Anopheles*
Immobile	100	97	7.9 ± 1.0	7.3 ± 0.79
Mobile	100	38**	19.1 ± 1.5	1.9 ± 0.28**

** Significant at the 0.01 level.

In the absence of cercariae, *Ae. aegypti* larvae were more active, spent less time suspended from the surface of the water and more time in the lower half of the observation chamber, and exhibited a greater number of looping movements than did *An. quadrimaculatus* larvae ($P < 0.01$) (Table 2). In the presence of cercariae, these differences remained unchanged. However, the number of looping movements performed by *Ae. aegypti* larvae increased significantly from 1.5 ± 0.42 to 34.7 ± 11.6 ($P < 0.01$) as did the number of wriggling movements performed by larvae of *An. quadrimaculatus* (from 0.4 ± 0.35 to 13.3 ± 4.9, $P < 0.05$).

Kavelaars (1965)[4] has shown that the reduced activity of *Culex pipiens* (Linn.) larvae renders them less susceptible than *Ae. aegypti* larvae to infection with *P. noblei* cercariae. In a similar manner, the activity of *Ae. aegypti* larvae may make them more susceptible than *An. quadrimaculatus* larvae. Furthermore, the surface feeding behavior of the anopheline larvae would seem to reduce contact with cercariae, since cercariae generally sink towards the bottom soon after emerging from the snail host (Bock 1984). Likewise, the reduced susceptibility of anopheline larvae to infection with *Bacillus thuringiensis* H-14 is a result of the feeding behavior

[4] Kavelaars, J. 1965. Host-parasite relationships between cercariae of *Plagiorchis noblei* Park and *P. peterborensis sp. N.*, and mosquito larvae. M.Sc. thesis. University of Western Ontario, London, Canada.

Table 2. Behavior of *Aedes aegypti* and *Anopheles quadrimaculatus* larvae in the absence of *Plagiorchis noblei* cercariae (mean/10 min. ± SE).

Activity	*Aedes*	*Anopheles*
No. wriggling movements	981.5 ± 139.2	0.4 ± 0.3**
Time at surface (sec)	200.4 ± 15.9	599.6 ± 0.01**
Time at bottom (sec)	289.5 ± 14.1	0.0 ± 0.0**
No. looping movements	1.5 ± 0.42	0.0 ± 0.0**

** Significant at the 0.01 level.

of the larvae and the rapid settling of the *B. thuringiensis* crystalline inclusions (Standaert 1981).

In response to attacking cercariae, *Ae. aegypti* larvae conduct looping movements, presumably in an attempt to detach the parasites. However, as observed by Rees (1952) with chironomid larvae, the effectiveness of this maneuver is limited since the larvae cannot reach all of their body surface with their mouthparts. In contrast, the anopheline larvae attempt to dislodge attacking cercariae with sudden, vigorous wriggling movements. Whereas such an increase in activity may conceivably enhance infection by increasing the frequency of host-parasite contact, this may be more than offset in that the anopheline larvae remain close to the surface of the water at a distance from the settling *P. noblei* cercariae.

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