

## PLAGIORCHIS NOBLEI IN AEDES AEGYPTI: CERCARIAL AGE AND INFECTIVITY

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*Plagiorchis noblei* (Park) is an entomophilic digenetic trematode. The adult stage of this parasite is found in the intestinal tract of a variety of birds and small mammals. Eggs are passed with the host feces and are ingested by the molluscan first intermediate host, *Stagnicola elodes*. Eggs hatch in the snail intestine and the miracidia penetrate the hepato-pancreas where they multiply asexually, producing thousands of cercariae each day.

Upon their release from the molluscan first intermediate host, cercariae undergo a brief, free-swimming existence. Contact with the second intermediate host, generally aquatic insect larvae, occurs within this period. The cercariae penetrate the cuticle of the insect host with the aid of a stylet and histolytic enzymes, and transform into metacercariae. The definitive host acquires the infection by ingesting insects harboring metacercariae. Kennedy (1975) states that the frequency of host-parasite encounters may ultimately depend on the distribution and mobility of the target organism. Contact between free-swimming *P. noblei* cercariae and larvae of *Aedes aegypti* (Linn.) is primarily a function of host size and distance traveled (Dempster and Rau 1987). Very little is known about how the infectivity of cercariae to the second intermediate host changes over the course of their free-swimming existence. Several laboratory studies report that the cercariae of various *Plagiorchis* sp. have a life span of no more than 24 hours (Bock 1984, Kavelaars and Bourns 1968, Velasquez 1964). Macy (1960), however, states that at room temperature, *P. vespertilionis parorchis* cercariae may live as long as 36 hours, and can still infect *Culex* larvae after 26 hours. The objective of the present study is to determine the relationship between the age of *Plagiorchis noblei* cercariae and their infectivity to *Aedes aegypti* larvae.

### MATERIALS AND METHODS

A glass aquarium (48 × 48 × 26 cm) was filled to a depth of 3.5 cm with aerated tap water and maintained at 20°C. The bottom of the aquarium was divided into 36 numbered, 8 × 8 cm squares. Approximately 84,000 freshly emerged *P. noblei* cercariae were randomly suspended in the water (10 cercariae/cc). Ten nylon mesh

enclosures, 7.5 cm diam. × 5.0 cm height, were lowered to the bottom of the aquarium. The open top of the enclosure projected above water level. The location of the 10 enclosures was selected at random from the 36 squares. The mesh (0.15 cm<sup>2</sup>) prevented the escape of mosquito larvae from a given test area but did not hinder cercarial movement.<sup>1</sup> Concentrations of cercariae were equal within and outside the enclosures. One *Ae. aegypti* larva was introduced into each enclosure and remained there for 20 minutes. The enclosures were then gently raised to retrieve the larvae. Mesh enclosures were rinsed and gently lowered to new, randomly selected locations within the aquarium. The enclosures remained undisturbed in position for at least 30 minutes before the next 10 mosquito larvae were introduced. This procedure was repeated at 2-hour intervals over a period of 30 hours. The above experimental protocol simulates conditions under which cercariae are allowed to age in intermittent contact with the second intermediate host, as is most likely to occur in the field.

Upon their removal from the aquarium, all mosquito larvae were first dipped into a large volume of aerated tap water to avoid the transfer of loose and loosely adhering cercariae. Larvae were then transferred to individual styrofoam cups (7.5 cm diam. × 4.5 cm height), filled to a depth of 3.5 cm with aerated tap water, provided with food *ad libitum* and stored at 21°C. Three days after exposure to cercariae larvae were crushed under a coverslip and examined microscopically (×40). The numbers of metacercariae were recorded.

The first 10 hours of data are comprised of two pooled replicates (n = 20) with homogeneous variances. A single classification ANOVA on the number of metacercariae recovered from mosquito larvae confirmed a random distribution of cercariae throughout the aquarium (*F*-statistic = 1.2601). Prevalence of infection (%) and mean abundances ( $\bar{x} \pm SE$ ) are reported.

To determine the temporal distributions of

<sup>1</sup> Webber, R. A. 1987. Behavioral aspects of the host-parasite association between *Aedes aegypti* (Linn.) larvae and *Plagiorchis noblei* Park, 1936. M.Sc. Thesis, McGill University, Montreal, Canada.

mosquito targets within the water column, ten 4th instar larvae were observed individually in a 30-ml clear plastic chamber (7.3 cm width  $\times$  3.0 cm depth  $\times$  12.0 cm height) filled to a depth of 3.5 cm with aerated tap water (20°C) (Webber et al. 1987). The amount of time larvae spent within 3.5 mm of the bottom of the flask was recorded over a 5-minute period.

## RESULTS

Within one-half hour of emergence, cercariae of *P. noblei* were highly infective to the experimental second intermediate host. Thus, 4th instar *Ae. aegypti* larvae acquired a mean of  $8.35 \pm 1.28$  metacercariae. By 2 hours after emergence the numbers of metacercariae acquired had declined precipitously to only  $1.95 \pm 0.71$ . The subsequent decline in the level of acquisition was more gradual over the next 6 hours. By 10 hours post-emergence, acquisition had been reduced to a mean abundance of  $0.20 \pm 0.18$  metacercariae and was maintained close to this level for the next 20 hours (Fig. 1). Prevalence of infection followed a similar pattern (Fig. 1).

Within 2 hours of their introduction into the aquarium, cercariae had settled to the bottom of the chamber. Some cercariae were crawling along the substrate, while others remained in suspension within approximately 3 mm of the bottom. *Aedes aegypti* larvae spent less than 3% of their time within 3.5 mm of the bottom of the chamber ( $8.0 \pm 2.52$  seconds out of 300 seconds).

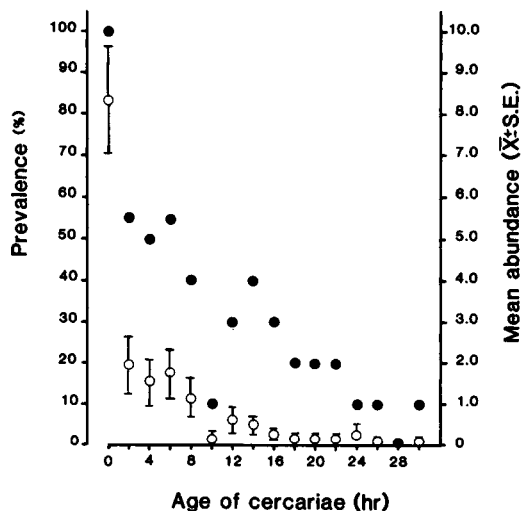


Fig. 1. The influence of cercarial age on the prevalence (%) and abundance ( $\bar{x} \pm SE$ ) of *Plagiorchis noblei* metacercariae in 4th instar *Aedes aegypti* larvae. Closed and open circles indicate prevalence and abundance, respectively.

The remainder of this time was spent in the water column.

## DISCUSSION

The major challenge facing each larval stage of a parasite's life-cycle is the location and infection of a suitable host. Ecological links between the intermediate and definitive host often enhance the chances of location of such hosts. Thus, the behavior of the parasite and the host before and after infection may determine the probability of their contact (Kennedy 1975).

When the mosquito larvae were first introduced into the aquarium, cercariae were randomly distributed in the water column and encounters between the host and parasite were independent of the spatial distributions of the larvae. The subsequent precipitous decline in the acquisition of the parasite by mosquito larvae may be due to the settling of the cercariae to the bottom of the aquarium. The bottom 3.5 mm of the chamber contain 10% of the total volume of water but after 2 hours contain virtually 100% of the cercariae. Since the bottom is visited only 3% of the time (rather than 10%) by the mosquito larvae, parasite acquisition may be reduced by a factor of three. This approximates the observed decline from slightly more than 8 metacercariae to almost two. The pattern of decline in parasite acquisition is characteristic of infectivity and survivorship curves (Evans and Gordon 1983). Large numbers of cercariae are released by the snail intermediate host at dusk over 24-hour intervals (Webber et al. 1986). Maximum transmission of cercariae occurs within the first few hours after their release into the aquatic environment but persists for up to 30 hours.

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