IMPACT OF THE ENTOMOPHILIC DIGENEAN *PLAGIORCHIS NOBLEI* (TREMATODA: PLAGIORCHIIDAE) ON THE SURVIVAL OF *AEDES PROVOCANS* UNDER FIELD CONDITIONS[1]

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ABSTRACT. Under field conditions, exposure of *Aedes provocans* to *Plagiorchis noblei* cercariae reduced the number of pupae produced to about one-third of control values. Larvae bearing more than 2 metacercariae rarely survived, and pupal mortality was almost 3 times higher among exposed individuals than controls. Adult females exposed to cercariae as pre-imagos experienced a 32% reduction in longevity. The life span of males was not affected. The potential of entomophilic digeneans as biological agents in the control of mosquitoes is discussed.

INTRODUCTION

The use of helminth parasites in the control of insect pests of medical and veterinary importance has been limited almost exclusively to nematodes, specifically mermithids (Hominick and Tingley 1984). More recently, Rao et al. (1985) suggested that the cercariae of certain entomophilic digeneans may be of value in the control of mosquito larvae. The cercariae of *Plagiorchis noblei* Park, one such digenean, are produced asexually by sporocysts in the hepatopancreas of the molluscan first intermediate host, *Stagnicola elodes* (Say), for at least one or 2 years (Blankespoor 1977). Cercariae are released by the thousands into the aquatic environment each day at dusk (Webber et al. 1986). The stylet-bearing cercariae (xiphidiocercariae) can penetrate a number of aquatic insect species where they encyst to form metacercariae. The infection is transmitted to the vertebrate definitive host by ingestion of the insect (Blankespoor 1977). In the laboratory, daily exposure of *Aedes aegypti* (Linn.) larvae causes significant host mortality (Dempster and Rau 1991). This study examines whether the deployment of snails shedding cercariae of *P. noblei* can reduce the production of pupae and adults of the snow pool mosquito *Ae. provocans* (Walker) under controlled field conditions.

MATERIALS AND METHODS

Circular enclosures (45 cm diam × 51 cm deep) were constructed in a temporary, water-filled, woodland ditch in the Morgan Arboretum, Macdonald College, McGill University, Quebec, Canada. They were set up in early April when newly hatched larvae of *Ae. provocans* first appeared. Enclosures consisted of rigid, brown plastic tubes, open at both ends, whose serrated lower edges were twisted firmly through a layer of leaf litter into the soft clay subsoil, and staked into place. Water levels within the enclosures were in equilibrium with the ditch, ranging from 21 to 28 cm at the beginning of the study. Mosquito larvae and cercariae were unable to leave or enter the enclosures. A total of 22 enclosures were established. The number of larvae in each enclosure was estimated over the first 7 consecutive days following its placement. Aggregates of larvae were scooped from the surface of undisturbed enclosures and counted. The highest counts were considered to be the best estimate of the total larval population at the beginning of the study. Adjacent enclosures, less than 20 cm apart, were paired: one received a field-collected specimen of *S. elodes* shedding approximately 1,000 cercariae of *P. noblei* per day (as determined in the laboratory over the preceding 7-day period), while the other received an uninfected snail (control). During the following 3 wk enclosures were monitored daily for water temperature, water depth, the presence of other species (insects, molluscs and crustaceans), enclosure integrity, viability of the snail and the production of mosquito pupae. Pupae were removed from the enclosures, counted and transferred to the laboratory where they were reared individually in 100-ml styrofoam cups containing 40 ml of aerated tap water. The deaths of pupae and adults during emergence were recorded and pooled as pupal mortality.

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Successfully emerged adults were sexed and identified to species, and all but 10 ml of water was drained from the cups. Adults were provided with 10% sucrose solution *ad lib* via a cotton plug inserted through the clear plastic lid of the cup. The viability of adult mosquitoes was monitored daily. Dead larvae and pupae were crushed gently under a coverslip and examined for metacercariae of *P. noblei* under a compound microscope (×10). Prevalence and intensity of infection were recorded. Adult mosquitoes, because of the heavy sclerotization and melanization of their tissues, received only a cursory examination and no attempt was made to quantify infection. Water temperatures and volumes within the enclosures, the numbers of larvae before the introduction of the parasite, the numbers of pupae produced, intensities of infection and the longevity of adult *Ae. provocans* are given as means ± SE. Where the frequency distributions of data did not conform with the assumptions of normality after a series of standard transformations (Sokal and Rohlf 1981), nonparametric tests were employed.

RESULTS

Larvae of *Ae. provocans* were first seen during the second week of April; pupation commenced about 3 wk later. Pupa production extended over a 2-wk period ending during the third week of May. Mean water temperatures changed little during the course of the study, but remained near 10.9 ± 2.6°C. The mean volume of water per enclosure declined from a high of 36.5 ± 1.4 liters at the beginning of the study, to 28.3 ± 1.3 liters when third instar larvae first appeared and finally to 21.3 ± 1.4 liters when fourth instar larvae and pupae were most abundant. All enclosures remained intact, and no snails died during the course of the study.

Data from 3 of the 11 pairs of enclosures were considered unusable when a second species, *Ae. canadensis* (Theobald), appeared in large numbers among the emerging adults. This cast some doubt on the composition of the pretreatment population of first instar larvae in these enclosures, making any assessment of the impact of infection on pupa production unreliable.

Before the introduction of snails shedding *P. noblei* cercariae, the mean numbers of first instar larvae in the remaining 8 pairs of field enclosures were 41.9 ± 22.9 and 41.0 ± 22.6, and were not significantly different ($P > 0.50$, Kruskal-Wallis test, $H = 0.23$). Enclosures containing infected snails produced a mean of 22.6 ± 5.6 pupae, whereas the corresponding controls produced 63.4 ± 13.0. These means were statistically significantly different ($P < 0.01$, Kruskal-Wallis test, $H = 6.90$). Nineteen dead larvae, primarily fourth and a few third instars, were found in the enclosures containing *P. noblei*-infected snails; all dead larvae were infected. The mean intensity of infection was 7.7 ± 2.4 metacercariae (median = 4.0). Only 3 dead larvae were recovered from control enclosures; none was infected.

Pupal mortality was 8.3% in the treated group ($n = 169$) and 3.1% in the controls ($n = 421$). Differences in the proportion of dead pupae were statistically significant ($P < 0.01$, chi-square test). Eleven dead pupae or incompletely transformed adults obtained from enclosures containing *P. noblei*-infected snails were examined for metacercariae: all but one were infected (mean intensity 2.0 ± 0.5 metacercariae).

The sex ratios of 79 males to 73 females among the treated group, and 187 males to 213 females among the controls did not differ significantly from the expected ratio of 1:1, nor did they differ significantly from each other ($P > 0.50$, chi-square test).

The longevity of adult females from treated groups was 15.7 ± 1.3 days ($n = 72$), whereas controls lived 23.2 ± 1.1 days ($n = 137$). These means were significantly different at the 0.001 level (Kruskal-Wallis test, $H = 21.71$). The mean longevity of adult males was independent of treatment. Treated males lived 9.5 ± 0.5 days ($n = 77$); controls lived 9.3 ± 0.3 days ($n = 128$) ($P > 0.50$, Kruskal-Wallis test, $H = 0.21$). Both males and females harbored infections.

DISCUSSION

Exposure of *Ae. provocans* in the field to cercariae of *P. noblei* for most of their aquatic life reduced the number of pupae to about one-third of control values. The data indicated that mosquito larvae died when infection intensities exceeded 2 metacercariae (median 4). Few larvae bearing more than 2 metacercariae managed to reach the pupal stage and pupal mortality was significantly higher among exposed individuals than among controls. Furthermore, adult female *Ae. provocans*, which had been exposed to cercariae as pre-imagos, experienced a 32% reduction in longevity. The longevity of exposed males was not impaired. The basis for this difference remains obscure. The combined effects of increased pre-imago mortality and reduced adult longevity may amount to a 77% reduction in the population of female *Ae. provocans* exposed to infection with *P. noblei*. This reduction may be

somewhat of an underestimate. In our study pupae were removed from further infection when they were transferred to the laboratory. However, the underestimate may be small. Dempster and Rau (1987) have shown that the susceptibility of *Ae. aegypti* pupae to infection with *P. noblei* is relatively low when compared with third and fourth instar larvae.

Environmental factors may have influenced parasite transmission. Since the volume of water in the enclosures declined by more than 40% by the time the most susceptible stages, third and fourth instar larvae, had reached peak abundance, the concentration of cercariae and consequently the level of transmission may have increased substantially. The relatively low May temperatures may have reduced the impact of the parasite on host survival. Low environmental temperatures do not appear to affect the activity of *P. noblei* cercariae; they readily penetrate mosquito larvae at 4°C. Furthermore, although low temperatures increase the developmental time of mosquitoes and thus the period of exposure to cercariae, parasite acquisition and host mortality remain essentially unaffected (Dempster and Rau 1991), presumably due to a compensatory reduction of larval activity at such temperatures. Larval activity is a major factor in parasite acquisition; the greater the activity, the greater the prevalence and intensity of infection (Dempster and Rau 1991). However, low temperatures do impair the release of cercariae by the snail host, and hence transmission. At 10°C, cercarial release is approximately 25% of 25°C peak values (M. E. Rau, unpublished data).

The data suggest that *P. noblei* has some potential as a biological agent in the control of mosquitoes. The sustained release of cercariae produced asexually by the snail intermediate host, for a time, frees the *P. noblei* from density dependent constraints that have traditionally hampered conventional biological control agents. To cycle successfully through a target species, the control agent must not be too pathogenic lest it drive itself to extinction (Anderson 1982). This trade-off between pathogenicity and the ability to recycle has led to treatment by inundative release with no attempt to recycle. Agents are used like biological insecticides and require repeated, frequent application (Hamon 1981). Entomophilic digeneans may provide both high pathogenicity and daily, inundative releases of cercariae for one or 2 seasons, once infected snails have been introduced or indigenous small populations have been infected with parasite eggs. Since the cercariae of entomophilic digeneans are essentially density independent in their action, they may be easily integrated with other agents such as chemical insecticides, *Bacillus thuringiensis* var. *israelensis* or predatory fish (Webber et al. 1987).

The genus *Plagiorchis*, of which *P. noblei* is a typical representative, is cosmopolitan in its distribution. *Plagiorchis noblei* exhibits a low degree of intermediate and definitive host specificity. A wide range of lymnaeid snails, insects, passerine birds and micromammals may serve its life cycle (Blankespoor 1977). Nevertheless, the fine-grained distribution of this parasite is heterogeneous in space and time. Presumably, few habitats consistently provide the prerequisites for the completion of the life cycle. The introduction of infected snails or the infection of indigenous snail populations with parasite eggs may allow an expansion of this digenean into environments that may be unsuitable for the completion of the life cycle but may sustain cercarial release for extended periods of time (without the need to recycle).

The impact of such introductions on nontarget species needs further study. Preliminary findings suggest that although organisms such as damselfly and dragonfly naiads will acquire the infection, they are not adversely affected at levels of exposure that invariably kill mosquito larvae. Furthermore, natural environments where virtually all adult snails are shedding large numbers of *P. noblei* cercariae support an abundance of chironomid larvae and odonate naiads. Such differential pathogenicity may enhance the usefulness of entomophilic digeneans as agents in the biological control of mosquito larvae.

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