THE INFLUENCE OF EXPERIMENTAL

PLAGIORCHIS NOBLEI (TREMATODA: PLAGIORCHIIDAE)

INFECTIONS ON THE SURVIVAL AND DEVELOPMENT

OF

AEDES AEGYPTI

by

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A thesis submitted to the faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of Master of Science.

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ABSTRACT

Experimental infections with Plagiorchis noblei metacercariae impaired the survival and development of Aedes aegypti larvae. The digenean interfered with pupation and the emergence of adults from the pupal case. The survival of infected Ae. aegypti appeared to be governed by the stage of development of the pre-imago at the time of parasite acquisition and by the location of metacercariae in the body of the insect host. Acquisition of the parasite by Ae. aegypti larvae increased significantly with each instar but declined precipitously upon pupation. Freshly emerged P. noblei cercariae were highly infective to 4th instar mosquito larvae, but their infectivity declined sharply within 2 hours and then more gradually over the next 28 hours. Daily exposure of Ae. aegypti pre-imagos to small numbers of P. noblei cercariae, throughout the course of their development, resulted in dose-dependent host mortality. Both the prevalence and intensity of infection were independent of environmental temperatures.
L'infection, en laboratoire, des larves d'Aedes aegypti à l'aide de Plagiorchis noblei a démontré que ce parasite affecte leur survie et leur développement. Le trematode interfère au niveau de la nymphose et de l'émergence des adultes. La survie d'Aedes aegypti semble reliée au stade préimaginal infecté et à la localisation des métacercaires dans le corps de l'insecte. L'acquisition du parasite par les larves d'Aedes aegypti augmente de façon significative avec chaque stade du développement mais diminue rapidement après la nymphose. Les jeunes cercaires de Plagiorchis noblei sont hautement infectueux pour les moustiques du 4ème stade larvaire. Leur capacité infectieuse diminue rapidement durant les 2 premières heures de leur vie aquatique et puis plus lentement durant les 28 heures qui suivent. L'exposition journalière des stades préimaginaux d'Aedes aegypti aux cercaires de P. noblei a démontré que le degré de mortalité chez l'hôte s'accroît en fonction de la dose appliquée. La température ambiante n'affecte pas la prévalence et l'intensité de l'infection.
SUGGESTED SHORT TITLE:

Plagiorchis infections in mosquitoes
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Finally, I am most grateful to my family and Mrs. Joan Buffey for their encouragement and support, and to Gary Desmarais, a very special person, for his love and infinite patience.
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GENERAL INTRODUCTION AND LITERATURE REVIEW

A vast array of entomophilic parasites and parasitoids are known to affect the survival and development of their hosts. This includes protozoans, microsporidians, cestodes, nematodes and braconid wasps. A variety of developmental anomalies occur and can be summarized as follows (modified after Beckage, 1985):

The parasite
(a) hasjuvenilizing effects on host morphological features.
(b) interferes with emergence of the adult host.
(c) causes a partial or complete cessation of egg maturation in female hosts.
(d) prematurely ends diapause (suspended development) of the host larva.
(e) causes the host larva to stop feeding prematurely and undergo precocious metamorphosis.
(f) induces the host to molt to supernumerary larval instars.
(g) extends the final instar of the host but suppresses its pupation.

To date though, very little research has been done on the effects of larval trematodes on the insect host. Incidental infections with entomophilic digenetic trematodes have been found in a variety of mosquito
species, particularly in the genus Anopheles (Hall 1929, Timon-David 1958). Many authors have reported successful experimental infections of mosquito larvae with stylet bearing cercariae (xiphidiocercariae) (McMullen 1937, Macy 1960, Dolfus et al. 1960). More recently, laboratory studies by Rao et al. (1985) have shown that xiphidiocercariae of the genus Prosthogonimus can kill Culex quinquefasciatus larvae. Work by Webber et al. (1987) suggests that Aedes aegypti larvae infected with Plagiorchis noblei (Trematoda: Plagiorchiidae) metacercariae exhibit behavioural modifications that may render the infected larvae more susceptible to being preyed upon by the definitive host.

Monoecious adult flukes of the genus Plagiorchis occupy the small intestine of a variety of vertebrates, including birds and small mammals. Parasite eggs are passed with the faeces of the definitive host and are ingested by lymnaeid snails, such as Stagnicola elodes, which serve as the first intermediate host. The eggs hatch and the embyros (miracidia) penetrate through the gut wall into the hepato-pancreatic tissues of the snail where they transform into mother sporocysts. Mother sporocysts give rise to numerous daughter sporocysts asexually. The daughter sporocysts, in turn, produce large numbers of cercariae. After approximately 40 days,
xiphidiocercariae emerge from the snail each evening at dusk in response to decreasing light intensity (Webber et al., 1986), and penetrate the cuticle of a wide variety of insect larvae. Metacercariae are formed in the tissues of the insect larvae, and require three days to become infective. The infection is transmitted to the vertebrate definitive host by ingestion of the insect (Blankespoor, 1977). This life-cycle has been maintained in our laboratory with gerbils serving as the definitive hosts.

The mosquito *Aedes aegypti* is a vector of diseases such as yellow fever and dengue and thus, of great medical importance (Harwood & James, 1979). It is also known as the "white mouse of entomology" and for these reasons was chosen to serve as the second intermediate, experimental host of *P. noblei*. The eggs of *Ae. aegypti* are deposited singly by the adult female on the edge of temporary bodies of water such as puddles, tree holes, or flower pot plates. Flooding stimulates the 1st instar larvae to hatch from the eggs (Christophers, 1960). Larvae undergo three molts during which they grow and change physiologically. Approximately 24 hours are required for each molt. However, water temperature can influence the duration of the aquatic stages of this insect. The fourth instar larva molts to a non-feeding pupal stage, which lasts only 2-3
days. The pupa transforms into the adult mosquito. After mating, the adult female must take a blood meal in order to allow the developing eggs to mature. This life-cycle has been maintained in our laboratory.

The overall objectives of the present study are to determine some of the factors that govern the acquisition of Plagiorchis noblei infections by the mosquito Aedes aegypti, and to document the effects of these infections on the survival and development of the host.

In chapter 1, fourth instar mosquito larvae are infected with a single pulse of cercariae in order to determine the effects of the parasite on the survival and development of the intermediate host. Chapter 2 discusses how a single pulse of infection affects the survival and development of each of the four larval instars and the pupal stage of the mosquito host. Chapter 3 determines the susceptibility of each pre-imago stage to infection with P. noblei cercariae, and speculates as to what factors may influence this susceptibility and the distribution of the parasite within the intermediate host’s body. Chapter 4 describes the infectivity pattern of cercariae over time, and Chapter 5 simulates field conditions by exposing the host to daily doses of the parasite throughout the course of its development at various temperatures.
Literature cited


Plagiorchiidae), and observations on the effects of light on the emergence of the cercariae. J. Parasitol. 46: 337-345.


STATEMENT OF AUTHORSHIP

"While the inclusion of manuscripts co-authored by the Candidate and others is not prohibited by McGill, the Candidate is warned to make an explicit statement on who contributed to such work and to what extent, and supervisors and others will have to bear witness to the accuracy of such claims before the Oral Committee. It should also be noted that the task of the External Examiner is made much more difficult in such cases, and it is in the Candidate's interest to make authorship responsibilities perfectly clear."¹

All experiments, analyses of data, and writing of manuscripts were carried out by the Candidate. Each manuscript (chapter) of the thesis is written in accordance with the guidelines set by the journal of submission.

CHAPTER 1

The effects of *Flagiorchis noblei* metacercariae on the development and survival of fourth instar *Aedes aegypti* larvae in the laboratory.*

by

Shiona J. Dempster, Richard A. Webber,

Manfred E. Rau and David J. Lewis

ABSTRACT

*Plagiorchis noblei* infections impair the survival and development of fourth instar *Aedes aegypti* larvae. Mortality during the larval and pupal stages reached 92%, and 60% of the emerging adults were malformed. The metacercariae interfere with pupation and the emergence of adults. Larvae and pupae that fail to transform to the next developmental stage within the normal time characteristically persist for extended periods, but invariably die without transforming. Whereas 82% of the control larvae gave rise to functional adults, only 4% of infected larvae managed to do so. Such effects may facilitate the transmission of the parasite.
INTRODUCTION

A wide variety of entomophilic parasites and parasitoids are known to affect the development of their hosts. Thus, the protozoan *Nosema albarea* has been shown to retard the development of its mosquito host *Anopheles stephensi* (Haq et al., 1981). Lengthened instar duration has been documented for lepidopterans infected with parasitoids (Lewis, 1970; Jones and Lewis, 1971; Jones et al., 1982). In contrast, the braconid parasitoid *Chelonus* sp. initiates precocious pupation in its larval host, *Trichoplusia ni* (Noctuidae), but also blocks its completion (Jones et al., 1981). Furthermore, laboratory studies (Rao et al., 1985) have shown that xiphidiocercariae of the genus *Prosthogonimus* can kill *Culex quinquefasciatus* larvae. Preliminary sentinel studies suggest that *Aedes aegypti* larvae can acquire significant numbers of *Plagiorchis noblei* metacercariae in the field. The present study assesses the effect of *P. noblei* metacercariae on the development and survival of their experimental second intermediate hosts, fourth instar larvae of *A. aegypti*, under laboratory conditions. Aquatic insect larvae acquire the infection when they come into contact with the xiphidiocercariae which are released by the lymnaeid snail host in response to decreasing light intensity (Webber et al., 1986). Metacercariae are formed in the
tissues of the insect larvae. The infection is transmitted to the vertebrate definitive host by the ingestion of the insect. Adult worms inhabit the small intestine and produce eggs that are passed with the feces. The eggs are ingested by the snail first intermediate host, the miracidia emerge and penetrate the tissues of the snail to initiate the intra-molluscan phase of the life cycle. This life cycle is maintained in our laboratory and the parasite has been identified as P. noblei on the basis of adult and egg morphometrics (Blankespoor, 1974).
MATERIALS AND METHODS

Two aquaria (35 X 19.5 X 20 cm) were filled to a depth of 20 cm with 13 L of aerated tapwater (20°C). Seventy-eight fourth instar larvae of A. aegypti were introduced into one aquarium, seventy-three into the other. Pre-soaked tropical fish food (Tetramin, Tetra Co.) (0.2 g dry weight) was provided as a source of food for the developing larvae. Freshly emerged cercariae of P. noblei from field collected Stagnicola elodes were added to the first aquarium giving an estimated density of 0.40 ± 0.17 cercariae/ml; the other contained no cercariae and served as a control. The mosquito larvae remained in the aquaria overnight for 12 hr, after which they were transferred to individual styrofoam cups containing 85 ml of aerated tap water (20°C) and 0.003 g of fish food. Cups containing individual larvae were kept in a 20°C incubator for the duration of the experiment and examined at 12 hr intervals to determine the numbers of living and dead larvae and pupae, as well as emerged adults of A. aegypti. Larvae and pupae that died, and adults that emerged within any one 12 hr period, were crushed and examined for metacercariae under a compound microscope (X 40) to determine the intensity of infection. Observations were carried out until all larvae had died or transformed into adults (35 days). Data were analysed using analysis of variance and Fisher’s test (Sokal and
RESULTS

All but one of the 78 mosquito larvae that were exposed to cercariae of *P. noblei* acquired the infection (mean intensity of infection ± standard error), 7.8 ± 6.6 metacercariae). Data on the survival and development of control and infected mosquito larvae are presented in Figure 1. Two mosquito larvae died during the 12hr exposure period. In contrast, none of the controls died during the concomitant period of sham exposure. The first deaths among the controls coincided with the advent of pupation, approximately 2.5 days after the end of sham exposure. However, very few of the control larvae died during the course of the experiment (7%); most pupated successfully. In contrast, in the infected group a significantly greater proportion of larvae died (60%, *P*<0.01). The mean times of pupation were statistically indistinguishable for the infected and control groups (5.3 and 4.7 days post-exposure respectively, *P*>0.05). Pupation in both groups was complete by 7 days post-exposure. However, although 15% of the infected larvae were still alive at this time, and indeed some persisted for as long as 35 days post-infection, all eventually died without pupating. Concomitantly, on day 7 only 4% of control larvae persisted. These differences were
Figure 1. The effects of *Plagiorchis* noblei metacercariae on the survival and development of *Aedes aegypti* from the fourth instar larva to the adult. The lines enclose cumulative percentages of the various developmental stages at various days post-exposure. (Curves incorporate all data points.)

A: Survival and development of sham-exposed larvae.

B: Survival and development of infected larvae.
significant at the 0.05 level. In both groups pupae began to die six to seven days after exposure. The mean times of adult emergence were statistically indistinguishable for the infected and the control group (9.7 and 8.8 days respectively, \( P>0.05 \)). No adults emerged later than days 10 to 11. Although some infected pupae persisted as long as day 13 post-exposure, these did not give rise to adults. Among the controls only 12\% (n=66) of the pupae failed to transform into adults; the corresponding figure for the infected group was 77\% (n=30). These differences are significant at the 0.0001 level. Furthermore, 60\% of the infected adults were incapable of flight due to the malformation or complete absence of wings and/or legs. None of the control adults were noticeably deformed. These differences are significant at the 0.01 level. Overall, 82\% of the 73 control larvae gave rise to presumably functional adult mosquitoes. This is in sharp contrast with 4\% of 78 larvae in the infected group (\( P<0.0001 \)).
DISCUSSION

_Plagiorchis noblei_ infections seriously impair the survival and development of fourth instar _A. aegypti_ larvae. Mortality during the larval and pupal stages is high, and many of the emerging adults are malformed. The data suggest that in a significant portion of individuals, infection interferes with pupation. Consequently, larval stages often persist well beyond the normal time of pupation, and invariably die without pupating. Similar effects have been documented for _Romanomermis culicivorax_ infections in _Aedes vexans_ (Galloway and Brust, 1985). Infection may also interfere with emergence of the adults. Again, some pupae persist beyond the normal time of adult emergence. This extension is not as pronounced as in larvae, presumably because pupae have a finite energy supply whereas larvae continue to feed. As with larvae, pupae that fail to metamorphose within the normal time invariably fail to metamorphose at all and die as pupae. The malformations observed among infected adults were severe, and presumably few, if any, such adults would survive and reproduce. Whereas the parasite may conceivably impair the competitive fitness of the insect host, it may well enhance its own. Heavily infected larvae are generally found near the surface of the water where they are more susceptible to predation by avian or mammalian definitive
hosts (Webber et al., 1986). The persistence of the larval and pupal stages, as well as the emergence of malformed adults, may further facilitate transmission.

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The authors would like to thank Professor R. T. Cronin of Concordia University for his generosity in supplying Aedes aegypti eggs. The technical assistance of Kathy Keller is gratefully acknowledged. Research at the Institute of Parasitology is supported by the Natural Sciences and Engineering Research Council of Canada and the Fonds pour la Formation de Chercheurs et Aide à la Recherche (FCAR).
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host-parasite relationship between *Heliothis* *zea* and *Microplitis croceipes*. Journal of Insect Physiology 17: 921–927.


CONNECTING STATEMENT

Chapter 1 established that a single exposure to freshly emerged cercariae of Plagiorchis noblei severely impairs the survival and development of 4th instar Aedes aegypti larvae. The data suggest that metacercariae found in the insect's body interfere with the process of pupation and the emergence of adults. Furthermore, a significant proportion of emerging adults was grossly malformed and largely incapacitated. The question arose whether all developmental stages of Ae. aegypti are similarly affected by P. noblei infections and whether the location of the metacercariae in the body of these experimental second intermediate hosts influences their survival. Chapter 2 addresses these questions.
CHAPTER 2

The effects of single exposures of Aedes aegypti larvae and pupae to Plagiorchis noblei cercariae in the laboratory*

by

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and

Manfred E. Rau

*for submission to Journal of Parasitology
ABSTRACT

The mortality of *Aedes aegypti* pre-imagos harboring metacercariae of *Plagiorchis noblei* is governed by the stage of development of the host at the time of parasite acquisition and the location of the parasite in the insect body. First and 2nd instar larvae generally succumbed to cercarial invasion, regardless of site. Invasion of the head and thorax of 3rd and 4th instar larvae was generally lethal, or gave rise to non-functional adults. However, older instars frequently survived abdominal infections. Pupae showed considerable tolerance to cephalic, thoracic and abdominal infections, and generally emerged as adults. Many such infected adults, however, were non-functional.
INTRODUCTION

Recent laboratory studies (Rao et al., 1985) revealed that the entomophilic xiphidiocercariae of the genus Prosthogonimus are capable of killing Culex quinquefasciatus larvae. Similarly Dempster et al. (1986) have established that exposure to cercariae of Plagiorchis noblei severely impairs the development and survival of 4th instar larvae of Aedes aegypti which may serve as experimental second intermediate hosts. The cercariae of this digenean parasite are released by the molluscan first intermediate host into the aquatic environment. After a brief, free-swimming existence cercariae penetrate into the tissues of aquatic insects where they encyst to form metacercariae. When infected insects are ingested by the vertebrate definitive host, the metacercariae excyst and develop into adult flukes in the small intestine. Parasite eggs are passed with the host feces and are ingested by the lymnaeid snail Stagnicola elodes (Blankespoor, 1974).

The present study examines the effects of a single exposure to P. noblei cercariae on the survival of the four larval instars and the pupa of Ae. aegypti. Both the stage of development of the insect host at the time of exposure, and the position of metacercariae in the body of the experimental host are considered.
MATERIALS AND METHODS

*Aedes aegypti* larvae and pupae were exposed once, individually to varying doses\(^1\) of *P. noblei* cercariae in 3.0 ± 0.1 ml of aerated tap water (21°C) for 30 minutes. Forty-two larvae of each of the 1st, 3rd, and 4th instars, 23 second instar larvae and 37 pupae were infected with the parasite, while 6 sham exposed larvae from each instar and the pupal stage served as controls. Larvae and pupae were maintained individually in styrofoam cups (7.5 cm diameter x 4.6 cm height) containing 80 ml of aerated tapwater at 21°C. Food (Tetramin, Tetra Co.) was provided *ad libitum*. The development and survival of the mosquito host were charted at 12 hour intervals thereafter. Dead larvae and pupae, as well as emerged adult mosquitoes, were crushed and examined under a coverslip at X 100 magnification.

Adults with broken or missing appendages as well as adults unable to free themselves from the pupal case were considered to be non-functional. It was presumed that few, if any, such adults would survive and reproduce. For each instar, the percent mortality of infected individuals (regardless of site of infection) as well as the corresponding intensities of infection, were determined.\(^1\) Pupae were exposed to high doses of the parasite due to their low susceptibility to infection (Dempster & Rau, 1987).

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\(^{1}\)Pupae were exposed to high doses of the parasite due to their low susceptibility to infection (Dempster & Rau, 1987).
In addition, the significance of differences in the distribution of head, thoracic and abdominal infections between the above two groups (infected, dead and non-functional versus infected, live individuals) was determined using the R x C test (Sokal & Rohlf, 1981) which tests for independence in contingency tables by means of the G-test. Differences in the mean intensities of infection for each stage of development of the host among and between dead and surviving individuals were tested for significance using the Student's t-test.
RESULTS

The early developmental stages of *Aedes aegypti* infected with metacercariae of *Plagiorchis noblei*, namely 1st, 2nd, and 3rd instars, suffered significantly greater mortality than infected 4th instar larvae and pupae (88% versus 50% mortality respectively; *P* < 0.001, Table 1.).

The mean intensity of infection (regardless of site of infection) increased significantly (*P* < 0.05) from the first to the last developmental stage in both dead and living hosts (Table 1.). In addition, dead individuals were consistently and significantly more heavily infected than living individuals for any given stage except the first (*P* < 0.05, Table 1.). Although dead first instar larvae were more heavily infected than the living, this difference was not statistically significant.

Of the 42 first instar *Aedes aegypti* larvae exposed to *Plagiorchis noblei*, only 6 (14%) acquired the infection. Five of these infected larvae were found dead with metacercariae in the head and thoracic regions of the body. One larva, infected as a 1st instar in the abdomen, survived to the adult stage. This relationship between the site of infection and mortality however, was not statistically significant (*P* > 0.05).

Fourteen of the 23 second instar larvae (61%) exposed to *P. noblei* cercariae acquired the infection. Thirteen
TABLE 1: *Plagiorchis noblei* in *Aedes aegypti*: host mortality and intensity of infection.

<table>
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<tr>
<th>STAGE</th>
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<td>14</td>
<td>93 a</td>
<td>4.31±0.41 bc</td>
</tr>
<tr>
<td>3rd</td>
<td>25</td>
<td>80 a</td>
<td>3.35±0.52 b</td>
</tr>
<tr>
<td>4th</td>
<td>38</td>
<td>47 b</td>
<td>4.25±0.61 bc</td>
</tr>
<tr>
<td>Pupal</td>
<td>28</td>
<td>54 b</td>
<td>5.13±0.76 c</td>
</tr>
</tbody>
</table>

* values within column bearing the same letter are not significantly different at the 0.001 level.

** values within rows and columns bearing the same letter are not significantly different at the 0.05 level.
of these infected larvae were found dead with 6 individuals infected in the head and thorax and 7 infected in the abdomen. Only one infected larva reached the adult stage harboring a metacercaria in the abdomen. There was no significant relationship between the site of infection and host mortality (P>0.05).

Forty-two 3rd instar larvae were exposed to cercariae: 22 larvae (52%) became infected. Fifteen of these infected larvae carried metacercariae in the head and thorax: all of these died. Only 2 larvae died from abdominal infections. The remaining five larvae had only abdominal infections: all reached the adult stage. In contrast to the 1st and 2nd instars, the relationship between the site of infection and host mortality among 3rd instar larvae was statistically significant (P<0.05).

Of the 42 fourth instar Ae. aegypti larvae exposed to P. noblei, 36 (85%) acquired infections. Sixteen of these infected larvae were found dead with metacercariae in the head and thorax. Only 6 larvae with cephalic and thoracic infections survived to the adult stage. None of the larvae harboring abdominal infections died, and all emerged as adult mosquitoes. These differences in mortality are statistically significant at the 0.05 level.

Twenty-eight of 37 pupae (76%) exposed to cercariae acquired the infection. Fourteen of these were found dead
with cephalic and thoracic infections: only one was found
deading with an abdominal infection. Thirteen infected pupae
successfully emerged as adults. Six of these carried
metacercariae in the abdomen while 7 harbored infections
in the head and thorax. These differences were not
significant at the 0.05 level. Three of the fifteen dead
individuals succumbed as pupae: the remaining 12 reached
the adult stage but were grossly malformed, did not become
functional, and died at the surface of the water.
DISCUSSION

The effects of Plagiorchis noblei infections on the mortality of Aedes aegypti are governed by the stage of development of the pre-imaggo at the time of parasite acquisition and by the location of the metacercariae in the body of the host. In spite of the fact that the early stages of the host are less heavily infected with the parasite than the later stages, for both dead and living individuals, the early instars nevertheless suffered higher levels of mortality than later instars. The data on the outcome of infection (prevalence) alone indicates that the early instars are sensitive to infection: first and 2nd instar larvae appear to be the most sensitive. Perhaps because of the relatively small size of these instars, the presence of metacercariae in any body region (head, thorax, or abdomen) will cause death. Third and 4th instar larvae are somewhat more robust and seem to be able to cope better with the infections. As with 1st and 2nd instars, head and thoracic infections are lethal, or give rise to non-functional adults. However, 3rd and 4th instar larvae appear to be able to survive abdominal infections. This sensitivity to infections of the head and thorax may be associated with damage to nervous tissues and imaginal buds. Some pupae will survive infections of all body regions unless they are associated with gross morphological
malformations. It remains to be determined whether abdominal infections affect the longevity and reproductive success of surviving adults.
ACKNOWLEDGEMENTS

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LITERATURE CITED


CONNECTING STATEMENT

Chapter 2 suggested that not all developmental stages of *Aedes aegypti* are equally sensitive to infection with metacercariae of *Plagiorchis noblei*. Chapter 3 will investigate the susceptibility of the five aquatic stages of the mosquito host to infection with the parasite and will provide some insight into the factors that govern this susceptibility.
CHAPTER 3

Factors affecting the acquisition of *Plagiorchis nobelii* (Tremaotoda: Plagiorchiidae) metacercariae by larvae and pupae of *Aedes aegypti* in the laboratory*

by

Shiona J. Dempster
and
Manfred E. Rau

*Journal of the American Mosquito Control Association 3: 607-610.*
ABSTRACT: When exposed to concentrations of less than one Plagiorchis noblei cercariae per cc of water, the acquisition of metacercariae by Aedes aegypti larvae increased significantly with each successive instar but declined precipitously upon pupation. Thus, first instar larvae acquired no metacercariae, whereas 4th instars had a mean abundance of 6.15; pupae only acquired a mean of 0.62 parasites. Parasite acquisition was largely a function of host size and activity. Other factors, such as grooming and feeding behavior, may affect the success of cercarial penetration and the distribution of metacercariae in the body of the insect host.
INTRODUCTION

Natural infections with entomophilic digenetic trematodes have been found in a variety of mosquito species, particularly in the genus *Anopheles* (Hall 1929, Timon-David 1958). Furthermore, many authors have reported successful experimental infections of mosquito larvae with stylet-bearing cercariae (xiphidiocercariae) (McMullen 1937, Macy 1960, Dolfus et al. 1960). More recently, laboratory studies revealed that xiphidiocercariae of the genus *Prosthoqonimus* will kill *Culex quinquefasciatus* (Say) larvae (Rao et al., 1985). Similarly, *Aedes aegypti* (Linn.) larvae infected with *Plagiorchis noblei* Park metacercariae exhibit reduced survival and extensive developmental changes (Dempster et al. 1986), and behavioral modifications (Webber et al. 1987a). These changes may render the infected mosquitoes more susceptible to being preyed upon by the definitive host (Webber et al. 1987b).

When insect larvae infected with *P. noblei* are ingested by a vertebrate definitive host, the metacercariae excyst and develop into adult flukes in the intestine. Parasite eggs are passed with the feces of the definitive host and are ingested by lymnaeid snails which serve as the first intermediate host. Within the aquatic snail host, polyembryony gives rise to xiphidiocercariae and, after a
brief, free-swimming existence, actively penetrate aquatic insect larvae. Here they encyst to form metacercariae (Blankespoor 1977).

Kavelaars\(^1\) has shown that the acquisition of *Plagiorchis* metacercariae by *Ae. aegypti* larvae increases progressively from the 1st to 4th instar of development. Since the cercariae of *Plagiorchis* are relatively small and disperse slowly (Kavelaars and Bourns 1968), encounters between the parasite and the insect host may be primarily a function of host size and activity. Once contact has been made however, other factors, such as grooming behavior, may influence the success of cercarial penetration. Grooming behavior may also affect the distribution of the metacercariae within the body of the insect host. This study was conducted to gain some insight into the factor(s) that govern the acquisition of *P. noblei* metacercariae by larvae and pupae of *Ae. aegypti*.

MATERIALS AND METHODS

To determine acquisition of *P. noblei* metacercariae by the pre-imago stages of *Ae. aegypti*, 1st, 2nd, 3rd and 4th instar larvae \( n = 140, n = 240, n = 140, n = 190 \), respectively) and pupae \( n = 190 \) were placed in an aquarium \( 35 \times 19.5 \times 22 \) cm filled to a depth of 20 cm with 13 liters of aerated tap water \( 20^\circ \text{C} \). Presoaked tropical fish food (Tetramin, Tetra Co.) was provided as a food source for the larvae. The larvae and pupae were exposed jointly to \( 9,430 \pm 538 \) \( \bar{X} \pm \text{standard error} \) freshly emerged cercariae of *P. noblei* obtained from field-collected *Stagnicola elodes* according to the method of Webber et al. (1986). The cercariae exhibit no phototactic responses and remain in suspension for about 12 hrs (Bock 1984). After 30 minutes the larvae and pupae were removed from the aquarium and rinsed with aerated tap water to remove loosely adhering cercariae. Larvae, separated according to instar, and pupae were transferred to plastic containers \( 10.5 \text{ cm diam.} \times 9.0 \) cm filled with aerated tap water, provided with food and maintained at \( 20^\circ \text{C} \) for 3 days. The pre-imagos were then crushed and examined under a compound microscope (X40) to determine the prevalence (percent of larvae infected), mean intensity (mean number of parasites within infected larvae) and mean abundance (mean number of parasites within all larvae

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examined i.e. the product of mean intensity and prevalence) of infection. These terms were used in accordance with the definitions established by Margolis et al. (1982). The data set consisted of two replicates. Homogeneity of variances permitted pooling of replicates. Mean intensity and abundance of infection were compared using a t-test (McPair test): prevalence was compared using the Chi-square test (Sokal and Rohlf 1981).

To determine whether differences in parasite acquisition among the various instars are an inherent attribute of their level of development, or whether they are a function of body size, 4th instar larvae of widely divergent sizes were exposed jointly to cercariae of _P. noblei_. Small larvae were produced by rearing them under crowded conditions (1 larva/cc of water, n = 25) whereas larvae of normal size were reared at a density of 1 larva/3.4 cc (n = 24). Both groups were provided with food _ad libitum_. Once larvae attained the 4th instar, they were exposed jointly to 11,982 ± 493 cercariae in 13 liters of aerated tap-water for 30 minutes. Larvae were then removed from further exposure and maintained in containers as described previously.

Ten randomly selected, uninfected larvae from both the normal and crowded populations were killed in household bleach, transferred to a slide, and measured under a
microscope (10 X). Body length was measured from the anterior surface of the head to the posterior end of the last anal segment. Body width was measured at the first abdominal segment. The calculation of surface area (mm\(^2\)), modified after Kavelaars\(^1\) \(( l \times 2\pi r + 2\pi r^2 \) where \( l = \) length and \( r = \) radius), provided a measure of contact between the host and parasite. Three days after exposure infected larvae were crushed and the numbers of metacercariae were recorded. Data were analysed using analysis of variance (ANOVA). The relationship between infection level and surface area (mm\(^2\)) was assessed by linear regression (Sokal and Rohlf 1981).

To determine the distance travelled by the larvae, 10 larvae each of the 2nd and 4th instars were observed in a graduated cylinder (3.5 cm diam. x 25 cm) filled with aerated tap water to 20 cm and provided with food. Each larva was observed for 30 minutes and all vertical movements were measured. Larvae were then measured as described previously. As a mosquito larva travels through a suspension of cercariae in the water column, the predicted number of parasites encountered is contained within a volume of water enclosed by the length and width of the larva and the distance travelled. Second and 4th instar larvae from the same population \((n = 43\) and 37, respectively) were jointly exposed to a mean of 5,400 ± 301
cercariae in 13 liters of aerated tap water (0.42 cercariae /cc). The predicted number of cercariae encountered by 2nd and 4th instar larvae were calculated (volume x cercarial density) and compared with the actual number of metacercariae acquired. The prevalence and mean intensity and abundance of infection were then calculated for both instars.

To determine whether the distribution of metacercariae between the head, thorax, and abdomen of mosquito larvae is a function of the relative size of these body regions, ten randomly selected 2nd, 3rd, and 4th instar larvae were measured. Length and width of each region were recorded. Then 100 larvae of each instar were exposed jointly to 10,615 ± 476 cercariae of P. noblei for 30 minutes as described above and then transferred to separate containers. The number of metacercariae in each body region was determined 3 days later. A Chi-square test compared the observed partitioning of metacercariae between the head, thorax, and abdomen with the distribution of the parasite as predicted on the basis of size (length x width) of these regions. Such a comparison was carried out for each susceptible instar (2nd - 4th).

Since the distribution of metacercariae within the population of mosquito larvae was overdispersed, data were normalized by square-root transformation (Sokal and Rohlf
1981). Adjusted means and 95% confidence limits are reported. Distances travelled, lengths, and widths are given as $\bar{x} \pm$ standard error.
RESULTS

None of the 1st instar larvae of *Ae. aegypti* acquired *P. noblei* infections. Second, 3rd and 4th instar larvae acquired a mean abundance of 0.54, 2.71 and 6.15 metacercariae, respectively (Table 1). Successive instars bore significantly larger numbers of the parasite (*P<0.05*). Pupae acquired a mean abundance of only 0.62 metacercariae, which differed significantly from that of the 3rd and 4th instars (*P<0.05*).

Normal fourth instar larvae were significantly longer and wider (7.71 ± 0.10 mm and 0.89 ± 0.01 mm, respectively) than crowded fourth instar larvae (5.65 ± 0.11 mm and 0.63 ± 0.02 mm, respectively, *p<0.05*). Normal larvae contained significantly larger numbers of metacercariae than did stunted individuals (\( \bar{X} = 5.42; 95\% \text{ C.L.} = 3.72 - 7.45 \)) and \( \bar{X} = 1.10; 95\% \text{ C.L.} = 0.55 - 1.84 \), respectively). Regression of larva size (surface area) against level of infection was significant (*P<0.001*, \( F = 26.464 \), regression coefficient = 0.432, *y*-intercept = -3.907).

Fourth instar larvae travelled significantly greater distances in the water column than did 2nd instars (451.8 ± 22.8 cm and 212.7 ± 34.2 cm, respectively, *P<0.05*) and were significantly larger in size (body length \( \times \) width) than the latter (4.26 ± 0.26 mm\(^2\) and 0.69 ± 0.05 mm\(^2\), respectively, *P<0.05*). Thus, 2nd instar larvae travelled through 1.47cc,
Table 1: Prevalence, intensity and abundance of *Plagiorchis noblei* metacercariae found in the pre-imago stages of *Aedes aegypti*.

<table>
<thead>
<tr>
<th>Stage</th>
<th>n</th>
<th>Prevalence (%)</th>
<th>Intensity</th>
<th>Abundance</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st</td>
<td>140</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2nd</td>
<td>240</td>
<td>21.2</td>
<td>2.54</td>
<td>0.54</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(2.39, 2.71)</td>
<td>(0.46, 0.63)</td>
</tr>
<tr>
<td>3rd</td>
<td>140</td>
<td>73.6</td>
<td>4.24</td>
<td>2.71</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(3.79, 4.72)</td>
<td>(2.65, 2.72)</td>
</tr>
<tr>
<td>4th</td>
<td>190</td>
<td>93.2</td>
<td>6.91</td>
<td>6.15</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(6.30, 7.56)</td>
<td>(5.47, 6.81)</td>
</tr>
<tr>
<td>Pupa</td>
<td>190</td>
<td>26.3</td>
<td>2.65</td>
<td>0.62</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(2.45, 2.86)</td>
<td>(0.52, 0.74)</td>
</tr>
</tbody>
</table>

1) 95% confidence limits
Table 2: Observed and predicted distributions of *Plagiorchis noblei* metacercariae in *Aedes aegypti* larvae

<table>
<thead>
<tr>
<th>Instar</th>
<th>Body region</th>
<th>Area (mm²)</th>
<th>No. of metacercariae</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Observed</td>
<td>Predicted</td>
</tr>
<tr>
<td></td>
<td>H</td>
<td>0.43</td>
<td>11</td>
</tr>
<tr>
<td>2nd</td>
<td>Th</td>
<td>0.53</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>Ab</td>
<td>1.74</td>
<td>72</td>
</tr>
<tr>
<td>3rd</td>
<td>Th</td>
<td>1.03</td>
<td>114</td>
</tr>
<tr>
<td></td>
<td>Ab</td>
<td>3.67</td>
<td>117</td>
</tr>
<tr>
<td>4th</td>
<td>Th</td>
<td>1.44</td>
<td>147</td>
</tr>
<tr>
<td></td>
<td>Ab</td>
<td>4.94</td>
<td>350</td>
</tr>
</tbody>
</table>

1) H: Head, Th: Thorax, Ab: Abdomen
2) observed and predicted values are significantly different at the 0.05 level
whereas 4th instar larvae travelled through 19.30 cc. The predicted number of cercariae encountered by 2nd and 4th instar larvae, based on the above volumes and the density of cercariae (0.42/cc), was 0.61 and 8.11 cercariae, respectively. The actual number acquired (mean abundance) was 0.49 (95% C.L. = 0.32 - 0.68) for 2nd instar larvae and 8.12 (95% C.L. = 7.95 - 8.29) for 4th instars.

Although *Ae. aegypti* larvae grew significantly with each successive instar, the relative size (length x width) of the head, thorax and abdomen of 2nd, 3rd and 4th instar larvae did not differ significantly (P>0.05) (Table 2). These findings are consistent with those reported by Christophers (1960). For 2nd instars, the distribution of metacercariae among the three body regions conformed with their relative size (P<0.05) (Table 2). In contrast, 3rd and 4th instar larvae exhibited fewer metacercariae than predicted in the abdomen, and more than predicted in the head. Levels of infection in the thorax showed no consistent relationship with area (Table 2).
DISCUSSION

The various larval instars and pupae of Ae. aegypti differed in their susceptibility to infection with metacercariae of P. noblei. Thus, 1st instar larvae were never found to be infected. Thereafter susceptibility increased with each successive instar, confirming the findings of Kavelaars¹. Susceptibility declined abruptly upon pupation.

Exposure of 4th instar larvae of varying sizes suggests that susceptibility to infection is not an inherent attribute of the level of development. Rather, it is, at least in part, a function of the body size (surface area) of the host. Host size (surface area) alone accounted for ca. 38% of the difference in the observed acquisition of cercariae by 2nd and 4th instar larvae. However, size (body length x width) in conjunction with distance travelled accounted for ca. 80% of observed parasite acquisition. A comparison of the predicted number of cercariae encountered with the actual number of metacercariae acquired confirms this relationship. Fourth instar larvae theoretically encountered 8.11 cercariae and acquired 8.12 metacercariae. Similarly second instar larvae theoretically encountered 0.61 cercariae in their travels and acquired 0.49 metacercariae. Thus, virtually all cercariae encountered by the mosquito
larvae gain entrance. Other factors that may influence the level of infection include parasite-associated changes in host behavior, avoidance reactions, grooming responses, and feeding behavior. Webber et al. (1987a) have presented evidence that 3rd instar larvae become more active in the presence of cercariae. Whereas light infections cause a further increase in activity, heavy infections precipitate a decline. Since 2nd instars are only one sixth the size of 4th instar larvae, even light infections may conceivably cause a reduction in activity in the former, while comparable infections in the latter may result in an increase in activity. Both responses may contribute to the observed difference in infection between 2nd and 4th instar larvae. The relative size of cercariae and larvae of various instars may account for the absence of infection in 1st instar larvae and the low levels in 2nd instar individuals. Such small larvae actively avoid the relatively large cercariae.

Once cercariae have attached themselves to the cuticle of the larvae, grooming responses may dislodge penetrating parasites. However, as observed by Rees (1952) with chironomid larvae, the effectiveness of these manoeuvres is limited by the inability of the larva to reach its entire body with its mouthparts. An assessment of the distribution of *P. noblei* metacercariae between the head,
thorax, and abdomen in various instars suggests that grooming responses may be effective in 3rd and 4th stage larvae in reducing parasite penetration in the abdominal regions. Thus, the head of both 3rd and 4th instar larvae and the thorax of 3rd instar larvae harbor significantly more metacercariae than predicted on the basis of area (length x width). This may be attributable to the periodic feeding activity of larvae during which they propel themselves head forward along the bottom of the aquarium by means of their labial brushes. The relatively low susceptibility of pupae to *E. noblei* infection may reflect their low activity and thick cuticle (Christophers, 1960).
ACKNOWLEDGMENTS

The authors wish to express their gratitude to Professor R.T. Cronin of Concordia University, Montreal for his generosity in supplying Aedes aegypti eggs. Research at the Institute of Parasitology is funded by the Natural Sciences and Engineering Research Council of Canada and les Fonds pour la formation de chercheurs et aide à la recherche (FCAR).
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KAVELAARS, J. and T.K.R. BOURNS. 1968. Plagiorchis peterborensis sp. n. (Trematoda: Plagiorchiidae), a parasite of Lymnaea stagnalis appressa, reared in the


The preceding chapters indicated that Plagiorchis noblei infections can kill Aedes aegypti larvae and pupae. The most sensitive stages are the first and second instars; the least sensitive are the pupae. Nor are the various stages equally susceptible to infection. Although vulnerable to the effects of infection, 1st and to a lesser extent, 2nd instar larvae do not acquire the infection readily. Instead, 3rd and 4th instar larvae are the most susceptible stages, although they are somewhat less sensitive than the younger and smaller larvae. Pupae, however, are neither sensitive nor susceptible. In general, the larger the larva grows, the more likely it is to acquire the infection. Cercariae are released by the snail first intermediate host in large numbers for one or two hours at dusk each day. The cercariae do not feed and when their energy stores are depleted, they die. Presumably, they will lose their ability to penetrate the insect intermediate host some time before that. Chapter 4 deals with changes in the infectivity of cercariae over time.
CHAPTER 4

Plagiorchis noblei in Aedes aegypti: cercarial age and infectivity*.

by
Shiona J. Dempster
and
Manfred E. Rau

*Journal of the American Mosquito Control Association (accepted for publication).
ABSTRACT: Within one half hour after emergence from the first intermediate snail host, cercariae of Plagiorchis noblei were highly infective to 4th instar larvae of Aedes aegypti and parasite acquisition was maximal at 8.35 metacercariae. Two hours after cercarial emergence parasite acquisition declined to 1.95 metacercariae, and after ten hours acquisition reached a low of 0.20. A small proportion of cercariae retained their infectivity for up to 30 hours. The prevalence of infection followed a similar pattern. The precipitous decline in transmission is, at least in part, due to the settling of the cercariae and the non-random distribution of mosquito larvae in the water column. The subsequent, more gradual decline in parasite acquisition reflects the diminishing infectivity and survival of the cercariae over time.
INTRODUCTION

*Plagiorchis noblei* (Park, 1936) 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 hystolytic 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. It has previously been established that contact between free-swimming *P. noblei* cercariae and larvae of *Aedes aegypti* is primarily a function of host size and distance travelled (Dempster & 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 & Bourns 1968, and 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

An all-glass aquarium (48 x 48 x 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, 8cm x 8 cm squares. Approximately 84,000 freshly emerged P. noblei cercariae were randomly suspended in the water (estimated density of 10 cercariae/cc). Ten nylon mesh enclosures, 7.5 cm diameter x 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 cm²) prevented the escape of mosquito larvae from a given test area but did not hinder cercarial movement. 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


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mosquito larvae were introduced. This procedure was repeated at two 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 diameter x 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 (X 40). The numbers of metacercariae were recorded.

The first ten 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 (± standard error) are reported.

To determine the temporal distributions of mosquito targets within the water column, ten 4th instar larvae were
observed individually in a 30 ml clear plastic chamber (7.3 cm width x 3.0 cm depth x 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 five minute period.

To determine the temporal distribution of cercariae within the water column, 10 cercariae were added to each of 10 test tubes filled to a depth of 3.5 cm with aerated tap water (20°C). The number of cercariae found within the bottom 3.5 mm of the test tubes were recorded every 15 minutes until no cercariae remained above this mark.
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 ± 1.28 metacercariae. By two hours after emergence the numbers of metacercariae acquired had declined precipitously to only 1.95 ± 0.71. The subsequent decline in the level of acquisition was more gradual over the next six hours. By 10 hours post-emergence, acquisition had been reduced to a mean abundance of 0.20 ± 0.18 metacercariae and was maintained close to this level for the next 20 hours (Fig. 1). Prevalence of infection follows 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. Ae. aegypti larvae spent less than 3% of their time within 3.5 mm of the bottom of the chamber (8.0 ± 2.52 seconds out of a total of 300 seconds). The remainder of this time was spent in the water column.
Figure 1: The influence of cercarial age on the prevalence (%) and abundance ($\bar{x} \pm$ standard error) of Plagiorchis noblei metacercariae in 4th instar Aedes aegypti larvae. Closed and open circles indicate prevalence and abundance, respectively.
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 behaviour 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 3. This approximates the observed decline from slightly more than 8 metacercariae to almost 2. The pattern of decline in parasite acquisition is characteristic of infectivity and survivorship curves (Evans & 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.
ACKNOWLEDGMENTS

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Plagiorchis noblei (Trematoda: Plagiorchiidae) 

metacercariae on the behaviour of Aedes aegypti 

CONNECTING STATEMENT

Chapter 4 showed that the behaviour of both the parasite and the target host influence the probability of their contact. The settling of *Plagiorchis noblei* cercariae and the non-random distribution of *Aedes aegypti* mosquito larvae in the water column accounted, in part, for the severe decline in parasite acquisition. However, in a field situation, environmental factors can easily influence transmission of the parasite to the next host. Chapter 5 determines the effects of exposing *Ae. aegypti* larvae to various doses of cercariae at different temperatures. Both host mortality and parasite acquisition will be monitored.
CHAPTER 5

Plagiorchis noblei in Aedes aegypti: parasite acquisition and host mortality in trickle infections*

by

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and

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*for submission to Journal of the American Mosquito Control Association.
ABSTRACT

The prevalence and intensity of experimental infections of *Aedes aegypti* with the digenean *Plagiorchis noblei* increased significantly with the level of trickle exposure to cercariae. Daily exposure to doses of 16 cercariae yielded a mean infection intensity of 13.0 metacercariae; doses of 1 cercariae/day resulted in only 2.4 metacercariae per infected host. The prevalence of infection rose from 46% at an exposure of 1 cercariae/day to 99% at 16 cercariae/day. Host mortality rose concomitantly from 25% to 88%.
INTRODUCTION

Entomophilic digeneans may affect the survival and development of experimental mosquito intermediate hosts. Studies by Rao et al. (1985) have shown that xiphidiocercariae of the genus Prosthogonimus can kill Culex quinquefasciatus larvae. Similarly, Aedes aegypti larvae infected with Plagiorchis noblei cercariae experience reduced survival and extensive developmental abnormalities (Dempster et al., 1986). In both of the above studies mosquito larvae were exposed to the parasite only once and for a short time. However, in the field mosquito larvae may be exposed to the parasite for extended periods, conceivably from the time of hatching until ecdysis. The above time interval may vary greatly. Thus, at a temperature of 28°C, newly hatched 1st instar larvae of Ae. aegypti require only 6-8 days to complete their development to the adult stage. At lower temperatures, however, the rate of development is greatly reduced (Christophers, 1960). Consequently, the period of exposure to cercariae of P. noblei in a field situation may vary with temperature. Cercariae of P. noblei emerge in large numbers from the snail host each day at dusk (Webber et al., 1986). The present study examines the effect of long term trickle exposure to graded doses of P. noblei cercariae on the level of parasite acquisition as well as
host development and survival over a range of environmental temperatures. This range includes the minimum (21°C) and optimal (28°C) temperatures necessary for development of the host.

MATERIALS AND METHODS

Groups of 20 mosquito larvae were individually exposed to 0, 1, 2, 4, 8, or 16 cercariae/day at each of five temperatures (21°C, 23°C, 25°C, 27°C, and 29°C). First instar Aedes aegypti larvae were kept in individual styrofoam cups (7.5cm diameter x 4.5 cm height), filled with 80cc of aerated tap water, provided with food ad libitum, and maintained at the appropriate temperature (± 0.5°C) for the duration of the experiment. At 24 hour intervals, in the evening, counted numbers of P. noblei cercariae were introduced to each cup. The development and survival of the mosquito host were charted at 12 hour intervals thereafter. Dead larvae and pupae, as well as emerged adult mosquitoes, were crushed and examined under a coverslip at X 100 magnification. Adults with broken, missing, or atrophied appendages, as well as adults unable to free themselves from the pupal case were considered to be non-functional.

Since the distribution of metacercariae within the host population of mosquitoes was overdispersed, the data were normalized by square-root transformation (Sokal &
Rohlf, 1981). The effects of the two variables, environmental temperature and the level of daily exposure to the parasite, on the intensity of infection were analyzed using ANOVA and Duncan's Multiple Range Test (Sokal & Rohlf, 1981). The effects of these variables on the prevalence of infection and host mortality were analyzed using the Chi-square test (Sokal & Rohlf, 1981). A linear regression compared the mean time from hatching to adult emergence of the sham exposed group at each of the five experimental temperatures.
RESULTS

Temperatures below 29°C significantly decreased the rate of development of uninfected control Aedes aegypti. At 29°C the mean time of emergence was 7.1 days whereas at 21°C, 11.4 days were required (P < 0.001, F = 65.9711, regression coefficient = -0.7273, y-intercept = 31.9143). Temperature, however, had no statistically significant effect on either prevalence or intensity of infection (P = 0.294 and 0.499, respectively), or on host mortality (P = 0.246). In contrast, the prevalence of infection increased significantly with the level of daily exposure to cercariae (P<0.001). Thus, at the lowest dose of 1 cercariae/day the prevalence of infection was 46%; at the highest dose (16 cercariae/day) prevalence reached 99%. The intensity of infection manifested a similar, but somewhat less pronounced, relationship with the intensity of exposure (P<0.05, Table 1). Concomitantly, the host suffered 88% mortality when exposed to 16 cercariae/day but experienced only 25% mortality at levels of 1 cercariae/day: 11% of sham exposed larvae died. These differences are statistically significant (P<0.001).
Table 1: Parasite acquisition and host mortality following exposure of *Aedes aegypti* to daily doses of *Plagiorchis noblei* cercariae.

<table>
<thead>
<tr>
<th>Exposure no. of cercariae/day</th>
<th>Mortality (%) n=100</th>
<th>Prevalence (%) n=100</th>
<th>Mean intensity n=100</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>11</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>25</td>
<td>46</td>
<td>2.396</td>
</tr>
<tr>
<td>2</td>
<td>48</td>
<td>70</td>
<td>3.588</td>
</tr>
<tr>
<td>4</td>
<td>50</td>
<td>85</td>
<td>5.720</td>
</tr>
<tr>
<td>8</td>
<td>64</td>
<td>92</td>
<td>7.422</td>
</tr>
<tr>
<td>16</td>
<td>88</td>
<td>99</td>
<td>12.998</td>
</tr>
</tbody>
</table>

All dose effects are significant at the 0.05 level.
DISCUSSION

The effect of temperature on the rate of development of uninfected *Ae. aegypti* larvae confirms the work of Christophers (1960). Thus, the time required from the 1st instar to the adult stage is considerably shorter at higher than at lower temperatures. It would follow that maximal exposure to cercariae for quickly developing hosts might be relatively low when compared to slowly developing hosts. Thus, quickly developing *Ae. aegypti* might conceivably escape lethal infection. However, this is not the case. Host mortality and parasite acquisition, both prevalence and intensity, appear to be independent of environmental temperatures within the narrow range tested. This may be due to the fact that mosquito larvae are more active at high than at low temperatures (Christophers, 1960). The greater activity of the mosquito larvae, the greater is the frequency of encounters with cercariae (Dempster & Rau, 1987). Conceivably, an increase in the frequency of encounters may compensate for the reduced period of exposure. Although a concomitant effect of temperature on the activity of cercariae cannot be ruled out, its impact on the frequency of encounters would be relatively minor, since the distance travelled by cercariae is essentially insignificant when compared to that travelled by the mosquito larvae (Dempster & Rau, 1987).
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LITERATURE CITED


GENERAL CONCLUSIONS

The host-parasite association between the digenetic trematode *Plagiorchis noblei* and the mosquito *Aedes aegypti* was examined in order to determine some of the factors influencing parasite acquisition and to assess the effects of the parasite on the survival and development of this experimental second intermediate host.

*P. noblei* infections were found to impair the survival and development of fourth instar *A. aegypti* larvae. The metacercariae interfered with pupation and the emergence of adults. Larvae and pupae that failed to transform to the next developmental stage within the normal time characteristically persisted for extended periods, but invariably died without transforming. The majority of emerging adults were malformed.

The fate of *Ae. aegypti* larvae and pupae infected with *P. noblei* metacercariae was determined by the stage of development of the host at the time of parasite acquisition and by the location of the parasite in the insect body. Any infection in 1st and 2nd instar larvae almost invariably led to death. In 3rd and 4th instars head and thoracic infections were generally lethal or gave rise to non-functional adults. In contrast, abdominal infections appeared to have little, if any, effect on the survival of the older instar larvae. Pupae showed no significant
relationship between location of the parasite and host mortality.

The acquisition of metacercariae by *Ae. aegypti* larvae was shown to increase significantly with each successive instar but declined precipitously upon pupation. Parasite acquisition was largely a function of host size and activity. The relatively low susceptibility of pupae to *P. noblei* infections may reflect their low activity and thick cuticle. Other factors such as grooming and feeding behaviour, may affect the success of cercarial penetration and the distribution of metacercariae in the body of the insect host.

Within one half hour after emergence from the snail first intermediate host, cercariae of *P. noblei* were highly infective to 4th instar larvae. Two hours after emergence parasite acquisition declined by a factor of 4 and after ten hours, acquisition was almost zero. A small proportion of cercariae retained their infectivity for up to 30 hours. The precipitous decline in transmission is, at least in part, due to the settling of the cercariae and the non-random distribution of the mosquito larvae in the water. The subsequent, more gradual decline in parasite acquisition reflects the diminishing survival and infectivity of the cercariae over time.

An increase in the daily dose of *P. noblei* cercariae
gave rise to a significant increase in the mean intensity of infection in *Ae. aegypti* larvae and pupae that were found dead and adults that were non-functional. However, host mortality and parasite acquisition, both prevalence and intensity, appeared to be independent of environmental temperatures. This may be due to the fact that mosquito larvae are more active at high than low temperatures.

Thus, evidence presented in this study clearly indicates that digenetic trematodes, such as *Plagiorchis nobleti*, effect the survival and development of their insect hosts, and that they should be considered along with other natural enemies as potential agents in the biological control of mosquito larvae.