TREMATODE LARVAE IN *LYMNAEA RUBIGINOSA* AND THEIR DEFINITIVE HOST IN IRRIGATED RICE FIELDS IN WEST JAVA

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(Received by the editor 5 January 1996)

ABSTRAK

ESTUNINGSIH, S.E. dan D.B. COPEMAN. 1996. Larva-larva trematoda pada siput *Lymnaea rubiginosa* dan inang definitifnya di persawahan irigasi di Jawa Barat. *Jurnal Ilmu Ternak dan Veteriner* 1 (3): 200-205.

Penelitian ini bertujuan untuk mengidentifikasi trematoda yang menggunakan *Lymnaea rubiginosa* sebagai inang antara pertama. Penelitian dilakukan di persawahan irigasi di Kabupaten Bogor dan Surade, Jawa Barat. Siput *L. rubiginosa* dikoleksi dari sawah irigasi di Kabupaten Bogor sebanyak 3.253 dan dari daerah Surade sebanyak 2.875. Kemudian dilakukan identifikasi larva trematoda yang terdapat pada siput tersebut. Hasil penelitian menunjukkan bahwa dari Kabupaten Bogor ditemukan serkaria echinostoma, strigeid, *Trichobilharzia* sp. dan Xiphidioserkaria, sedangkan dari daerah Surade ditemukan Xiphidioserkaria, serkaria *Fasciola gigantica, Schistosoma* dan echinostoma. Larva echinostoma yang ditemukan pada siput *L. rubiginosa* dan cacing echinostoma dewasa pada itik dan ayam kampung yang dilepas di daerah persawahan di Kabupaten Bogor telah diidentifikasi keduanya termasuk jenis *Echinostoma revolutum.* Cacing echinostoma dewasa tidak ditemukan pada 24 ekor tikus, 11 ekor kadal dan 35 ekor katak yang ditangkap di persawahan Kabupaten Bogor. Kemungkinan sumber strigeid, *Trichobilharzia* dan Xiphidioserkaria diuraikan dalam diskusi.

Kata kunci: *Fasciola gigantica,* trematoda, larva, *Lymnaea rubiginosa*

ABSTRACT

ESTUNINGSIH, S.E. and D.B. COPEMAN. 1996. Trematode larvae in *Lymnaea rubiginosa* and their definitive host in irrigated rice fields in West Java. *Jurnal Ilmu Ternak dan Veteriner* 1(3): 200-205.

The aim of this study was to identify trematodes which utilise *Lymnaea rubiginosa* as the first intermediate host. This study was conducted in irrigated rice fields in Bogor regency and at Surade, West Java. A total of 3,253 *L. rubiginosa* were collected from irrigated rice fields from Bogor regency and 2,875 from Surade. The results showed that cercariae of echinostomes, strigeid, *Trichobilharzia* sp. and Xiphidiocercariae were found in snails from Bogor regency, whereas Xiphidiocercariae and cercariae of *Fasciola gigantica, Schistosoma* sp. and echinostomes were found in snails from Surade. The larval echinostomes found in *L. rubiginosa* and adult echinostomes in domestic ducks and village chickens which grazed harvested rice fields in Bogor regency were both identified as *Echinostoma revolutum.* The adult echinostomes were not found in the 24 rats, 11 lizards and 35 frogs caught in the vicinity of the Bogor regency. The possible sources of strigeids, *Trichobilharzia* and Xiphidiocercariae are discussed.

Key words: *Fasciola gigantica,* trematode, larvae, *Lymnaea rubiginosa*

INTRODUCTION

Lymnaea rubiginosa, the only known intermediate host of *Fasciola gigantica* in Indonesia, may also act as either first or second intermediate host for other trematodes. Cercariae of echinostomes and *Trichobilharzia* sp. were found in *L. rubiginosa* from Surabaya Municipality, East Java (SUWANTI and PUSPITAWATI, 1990). In addition, SUARSINI *et al.* 1990) reported that *L. rubiginosa* collected from rice fields around Malang, East Java, were infected with cercariae of echinostomes, gymnocephalus (*Fasciola* sp.), strigeids and *Trichobilharzia* sp. However, there is no published information concerning trematodes which utilise *L. rubiginosa* as the first intermediate host in West Java and the definitive hosts of these trematodes have not been determined.

A variety of animals had access to the rice fields surveyed in this study and thus had the opportunity to deposit faeces containing fluke eggs and perhaps to become infected with trematodes. Between crops, buffaloes, cattle, sheep, goats, chickens and, in some areas, large flocks of domestic ducks, had access to the stubble; draught cattle and buffaloes were the source of traction for ploughing and preparing fields for planting; and aquatic, amphibious and terrestrial animals such as fish, frogs, lizards, snakes and rats were also observed in the fields. Furthermore, animal faeces is commonly used as a fertiliser in rice fields. The aims of this study were to identify cercariae from *L. rubiginosa* collected from rice fields in West Java and to gather information on the trematodes of some of the animals with access to rice fields as a guide to the hosts of the trematodes found in *L. rubiginosa.*

MATERIALS AND METHODS

Location

Rice fields in five villages within about 10 km of Bogor were chosen as the sites for collecting snails and animals with access to the fields. Sites were at Margadjaja and Bubulak in the subdistrict of Ciomas, and at Cibanon, Tanah Baru and Cimahpar in the subdistrict of Kedung Halang. All of the rice fields in these areas are irrigated, at an altitute of about 200 m and about 40 km inland. Snails were also collected from irrigated rice fields at Surade subsdistrict, a coastal area about 120 km south-east from Bogor. Each site included more than four adjacent rice fields covering an area of a few hectares.

Collection of samples

L. rubiginosa were collected from rice fields within about 1.5 m of the peripheral embankment around each field for one hour from each site on each sampling occasion. The snails were collected every four weeks for four months from January 1990 until April 1990. Rats, frogs and lizards were also collected from the study areas, while ducks and chickens were bought from the farmers. After collection snails were transported to the laboratory at Balitvet, Bogor in a container with approximately 10 cm depth of water from the rice fields.

In May 1990, 884 *L. rubiginosa* from irrigated rice fields at Ciomas and 2,875 from irrigated rice fields at Surade were also examined in the laboratory.

Laboratory procedures

In the laboratory, snails collected each four weeks from sites around Bogor were washed and divided into three groups: large, with shell length more than 15 mm; medium, with shell length between 10 and 15 mm; and small, with shell length less than 10 mm. The shell was measured from the apex to the aperture. Each snail was then placed in water in an individual glass tube within 24 hours of collection. Cercariae emerging from each snail were identified using the key to cercariae in SCHELL (1970). They were studied alive and after staining with 1% neutral red.

Snails in the large samples collected during May from Ciomas and Surade were also washed and measured. All snails from Ciomas and 200 of the 2,875 *L. rubiginosa* from Surade were dissected individually. The remainder from Surade were grouped into size-class and crushed, about 15 at a time but separated from each other, between two glass plates to facilitate microscopic examination of each snail for the presence of trematode larvae. The types of cercariae present were recorded and the presence of metacercariae of *Cotylurus* sp. was also recorded.

The chi-square test was used for analysing the data utilising the software package STATISTIX (N.H. Analytical Software). The null hypothesis used was that prevalence of each trematode species is independent of the prevalence of the others.

The lungs, liver and gastrointestinal tract from all animals collected were examined with the aid of a dissecting microscope for the presence of adult trematodes. Additionally, in ducks, blood vessels of the nasal turbinates and the lumen of the bursa fabricius were examined. All trematodes found were collected and fixed in Berland's fixative, stained with Gower's carmine and mounted in Canada balsam. The procedures for staining which were followed were those described by SCHELL (1970). Flukes were identified using the keys to family and genus in SCHELL (1970). All lizards, frogs and rats, after removal of their internal organs, were sent to the Zoological Museum in Bogor for identification of the species.

RESULTS

A total of 2,369 specimens of *L. rubiginosa* were collected progressively from the five sites around Bogor. Cercariae belonging to four different groups were found (Table 1).

Of the 2,369 snails collected, 562 (23.8%) were infected with cercariae. All infections were with one species only and no snails <10 mm long were infected. Prevalence of infection with echinostome cercariae in snails 10-15 mm in length from Margadjaja, Bubulak and Cibanon was lower than in snails 15 mm ($P<0.05$). No Xiphidiocercariae and strigeid cercariae were found in snails from Bubulak and Cibanon, and from Tanah Baru and Cimahpar respectively. Furthermore, echinostome,

Table 1. Types of cercariae in *Lymnaea rubiginosa* from irrigated rice fields at 5 locations around Bogor

Field site	Size of snail in mm	Ech no.& (%)	Str no.& (%)	Trich no.& (%)	Xip no.& (%)	Uninf no.& (%)	Number of snails
Margadjaja		0	0	0	0	133	133 (100)
	10-15	50 (19.5)	18 (7)	14 (5.4)	3 (1.2)	172 (66.9)	257
	15	74 (32.7)	17 (7.6)	3 (1.3)	1 (0.4)	131 (58)	226
Bubulak		0	0	0	0	93	93 (100)
	10-15	43 (38.4)	2 (1.8)	5 (4.5)	0	62 (55.3)	112
	15	105 (54.7)	7 (3.6)	33 (17.2)	0 (24.5)	47	192
Cibanon		0	0	0	0	98	98 (100)
	10-15	39 (17.3)	16 (7.1)	8 (3.5)	0 (72.1)	163	226
	15	57 (49.1)	8 (6.9)	9 (7.8)	0 (36.2)	42	116
Tanah Baru		0	0	0	0	68	68 (100)
	10-15	0	0 (1.6)	2	0 (98.4)	124	126
	15	4 (1.5)	0 (1.1)	3 (2.8)	7 (94.6)	247	261
Cimahpar		0	0	0	0	88	88 (100)
	10-15	6 (4.2)	0 (2.8)	4 (0.7)	1 (92.3)	133	144
	15	12 (5.2)	0 (0.9)	2 (3.9)	9 (90)	206	229
Total		390	68	83	21	1807	2369
%		16.5	2.9	3.5	0.9	76.2	100

Ech = Echinostome cercariae
Str = Strigeid cercariae
Trich = *Trichobilharzia* sp.
Xip = Xiphidiocercariae
Uninf = Uninfected

strigeid and *Trichobilharzia* sp. cercariae were predominantly present in the snails from Margadjaja, Bubulak and Cibanon whereas few snails from Tanah Baru and Cimahpar were infected.

Trematode larvae found in the 884 specimens of *L. rubiginosa* collected in May from irrigated rice fields at Ciomas are listed in Table 2. Whereas prevalence of infection with cercariae of echinostomes and *Trichobilharzia* sp. was higher in snails >15 mm long than in smaller snails ($P<0.05$), the opposite was true for infection with metacercariae of *Cotylurus* sp. ($P<0.015$).

Of the 2,875 snails collected in May from Surade, 187 (6.5%) were infected with cercariae (Table 3). Prevalence of infection with *F. gigantica* cercariae in snails >20 mm in length was higher than in snails <20 mm in length ($P<0.05$). Likewise, prevalence of infection with echinostome cercariae and Xiphidiocercariae in snails >20 mm in length was higher than in snails <20 mm in length ($P<0.05$).

Eleven lizards (*Mabuya multifasciata*), 35 frogs (*Rana limnocharis*) and 24 rats (*Rattus argentiventer*) were collected from the rice fields, and five domestic

Table 2. Trematode larvae in *Lymnaea rubiginosa* from irrigated rice fields at Ciomas

Size of snails in mm	Ech no.& (%)	Str no.& (%)	Trich no.& (%)	Xip no.& (%)	Cot no.& (%)	Str+ Cot no. &(%)	Ech+ Cot no. & (%)	Uninf no.& (%)	Number of snails
	1 (1.7)	0	0	0 (26.7)	16	0	0 (71.6)	43	60
10-15	118 (17.6)	60 (8.9)	12 (1.8)	1 (0.1)	199 (29.7)	3 (0.5)	2 (0.3)	276 (24.1)	671
15	64 (41.8)	13 (8.5)	5 (3.3)	1 (0.7)	25 (16.3)	0	0 (29.4)	45	153
Total	183	73	17	2	240	3	2	364	884
%	20.7	8.3	2.0	0.2	27.1	0.3	0.2	41.2	100

Ech = Echinostome cercariae; Str = Strigeid cercariae
Trich = *Trichobilharzia* sp. cercariae; Xip = Xiphidiocercariae
Cot = *Cotylurus* sp metacercariae; Uninf = Uninfected

ducks and 20 village chickens from flocks with access to rice fields after harvest were bought from farmers.

Three of the 11 lizards harboured trematodes in the intestine which were identified as members of the genus *Brachycoelium*. Seven of the 35 frogs also harboured trematodes in the intestine. These were members of the genus *Pleurogenoides*. All five ducks and 14 of the 20 village chickens were infected with echinostomes which were identified as *Echinostoma revolutum*. Two of the five ducks and 3 of the 20 village chickens also harboured *Prostogonimus* sp. in their bursa fabricius but no flukes were found in blood vessels of the nasal turbinates of the ducks examined. Rats were not infected with any trematode.

DISCUSSION

There is unequivocal evidence in this study that infection of *L. rubiginosa* with one species of trematode makes it refractory to infection with a second species of trematode. It may be calculated from the data in Table 1 using chi-square test for heterogeneity or independence that dual infection with cercariae of echinostomes and strigeids, and echinostomes and *Trichobilharzia* sp. (in snails >10 mm in length from Margadjaja, Bubulak and Cibanon) may be expected in 22 and 23 snails respectively if there is no interaction between these cercariae. However, since no dual infections were observed, this result departs significantly from the expected ($P<0.001$) and thus demonstrates the existence of strong antagonism between cercariae of these species. Similarly, if there was no antagonism between species of trematode, an estimated 51 of the snails from Ciomas (Table 2) would be expected to have dual infection with echinostome cercariae and *Cotylurus* sp. metacercariae. However, only two snails were infected with both species, a result which differs significantly ($P<0.001$) from that predicted in the absence of antagonism. A similar result was obtained with strigeid cercariae and metacercariae of *Cotylurus* sp. (Table 2).

These data thus demonstrate both antagonism between cercariae of different trematodes in *L. rubiginosa* and antagonism between cercariae and metacercariae; at least between the cercariae of echinostomes or strigeids and the metacercariae of *Cotylurus* sp. However, while the latter antagonism was strong it was not absolute. In contrast, not one dual infection with different species of cercariae was observed in the 6,128 snails examined in this study, suggesting that when *L. rubiginosa* is infected with one species of trematode this precludes infection with any other species of trematode; alternatively, one species dominates and eliminates other trematodes. The latter situation was describe by LIE *et al.* (1965). They found that when *L. rubiginosa* which were shedding *Trichobilharzia* sp. cercariae were exposed to miracidia of *Echinostoma audyi*, the production of *Trichobilharzia* sp. cercariae decreased gradually and finally disappeared. In another study, BASCH (1970) noted that *Cotylurus lutzi* miracidia cannot develop in *Biomphalaria glabrata* which have been exposed to *Paramphistomum segregatum* miracidia. Furthermore, when these snails exposed to *P. segregatum*

Table 3. Trematode larvae in *Lymnaea rubiginosa* from irrigated rice fields at Surade

Size of snails in mm	Fasc no.& (%)	Ech no.& (%)	Xip no.& (%)	Schisto no.& (%)	Uninf no.& (%)	Number of snails
10-15	7 (0.5)	16 (1)	14 (0.9)	0	1496 (97.6)	1533
15.1-20	9 (0.7)	35 (2.7)	80 (6.2)	1 (0.1)	1159 (90.3)	1284
20	2 (3.4)	6 (10.3)	17 (29.3)	0	33 (57)	58
Total	18	57	111	1	2688	2875
%	0.6	2	3.9	0.03	93.5	100

Fasc = *Fasciola gigantica* cercariae
Ech = Echinostome cercariae
Xip = Xiphidiocercariae
Schisto = Schistosome cercariae
Uninf = Uninfected

miracidia were a few days later exposed to *C. lutzi* cercariae, *P. segregatum* developed normally but metacercariae of *C. lutzi* were eaten by rediae of *P. segregatum* and finally disappeared.

In seeking to explain the large observed differences in prevalence of infection of snails with trematodes between sites, there is circumstantial evidence that the presence of ducks may be important. The presence or absence of large flocks of domestic ducks that grazed rice fields after harvest was the major difference between farming practices at each site. Ducks were present at Margadjaja, Bubulak and Cibanon but there were no large flocks at Tanah Baru, Cimahpar and Surade. Furthermore, sites where ducks were present in large numbers had a significantly higher ($P<0.05$) prevalence of larval echinostomes, strigeids and *Trichobilharzia* sp. (all of which are parasites of ducks) in *L. rubiginosa* than occurred at the other sites. Thus, it seems likely that the main source of infection of *L. rubiginosa* collected from rice fields around Bogor with echinostome cercariae is the flocks of ducks that graze in the fields after harvest.

The absence of strigeids and *Trichobilharzia* sp. from the ducks examined was unexpected in view of the relatively common occurrence of their larvae in *L. rubiginosa*. Nevertheless, ducks are their only plausible definitive host as water birds other than domestic ducks are almost unknown in the rice fields around Bogor. Moreover, there was a positive relationship between the prevalence of echinostome larvae and those of strigeids and *Trichobilharzia* sp. in *L. rubiginosa*, further supporting a conclusion that ducks are the common definitive host of all three species.

There are several possibilities for the absence of strigeids and *Trichobilharzia* sp. from the ducks examined. For strigeids, their short life span in ducks of less than two weeks (SCHMIDT and ROBERTS, 1989) could account for their absence if the ducks had not grazed in a harvested rice field and thus not been exposed to infection for more than about two weeks prior to necropsy. This is a realistic possibility with the farming systems in operation in the study area. On the other hand the most likely explanation for failure to find infection with *Trichobilharzia* sp. in the ducks is inadequate technique. Only vessels of the nasal mucosa were examined, whereas the only species of *Trichobilharzia* described in ducks in Indonesia which uses *L. rubiginosa* as an intermediate host, *T. brevis*, occurs in mesenteric blood vessels (MARGONO, 1968).

A number of possible sources of the Xiphidiocercariae found in *L. rubiginosa* were identified. Members of the genera *Brachycoelium* found in lizards, *Prostogonimus* in ducks and village chickens, and *Pleurogenoides* in frogs all have cercariae of this type. However, it is not known if they are able to use *L. rubiginosa* as an intermediate host.

The higher prevalence of infection with larvae of *F. gigantica*, echinostomes and *Trichobilharzia* sp. and with Xiphidiocercariae in larger than smaller snails observed in this study has also been reported as an occurence by others. SOUSA (1983) mentioned that this phenomenon occurs probably because the largest snails are the oldest snails in the population and thus have the most chronic exposure to infective miracidia. A contributing factor may also be the greater size achieved by snails infected with trematode larvae (ROTHSCHILD, 1941), thus placing a higher proportion of infected snails in the large size groups than would otherwise occur.

Cercariae of *F. gigantica* were found in only 18 of 2,875 (0.6%) *L. rubiginosa* collected from Surade. A similar low rate of infection was reported by SUHARDONO *et al.* (1988) who observed that the infection rate of *F. gigantica* in *L. rubiginosa* was less than 1% at Surade even though the infection rate with *F. gigantica* in cattle in the area was about 90%. The reason for this is not clear but may reflect a situation where faecal contamination (and thus infection of snails) in rice fields is limited, being restricted to the period after the crop is harvested and during ploughing in preparation for replanting when the number of *L. rubiginosa* present in rice fields is low (WIDJAJANTI, 1989). It may also be a biological feature of *Fasciola* spp. as there are a number of reports with *F. hepatica* that very low snail infection rates were sufficient to produce major infection in mammalian hosts (BORAY *et al.*, 1969; MALONE *et al.*, 1984; KHALLAAYOUNE *et al.*, 1991).

Results from this study did not reveal any unequivocal explanation for the absence of infection with *F. gigantica* in the 3,253 *L. rubiginosa* collected from the five sites around Bogor. However, because of the observed antagonism between different species of trematode in *L. rubiginosa* which resulted in monoinfection it is reasonable to expect that the approximately 60% of *L. rubiginosa* infected with larval trematodes at Margadjaja, Bubulak and Cibanon and the 10% at Tanah Baru and Cimahpar would have been refractory to infection with *F. gigantica*. The rate of infection of snails with *F. gigantica* would thus be reduced accordingly by about 60% and 10% at these two groups of sites respectively. There must, nevertheless, also be other factors contributing to the absence of infection with *F. gigantica* in *L. rubiginosa* from Bogor that are outsite the limits of observations made in this study.

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