LARVAL STAGES OF MEDICALLY IMPORTANT FLUKES (TREMATODA) FROM VIENTIANE PROVINCE, LAOS. PART II. CERCARIAE

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SUMMARY

The cercariae of five flukes, which are potential human parasites, *Opisthorchis viverrini* (family Opisthorchiidae), *Haplorchis taichui* (Heterophyidae), *Schistosoma spindale* (Schistosomatidae), *Fasciolopsis buski*, and *Fasciola gigantica* (Fasciolidae) were found in freshwater snails from Laos. The cercariae of the above

species, recorded in Laos for the first time, are illustrated and their morphology is briefly described. The snail *Helicorbis umbilicalis* represents a new intermediate host of *F. buski*. The occurrence of medically important snails in the Vientiane province will also be discussed.

RÉSUMÉ : Les stades larvaires de Distomes d'importance médicale dans la province de Vientiane, Laos. Deuxième partie : les cercaires.

Les cercaires de cinq espèces de Distomes (Trematoda) : *Opisthorchis viverrini* (Opisthorchiidae), *Haplorchis taichui* (Heterophyidae), *Schistosoma spindale* (Schistosomatidae), *Fasciolopsis buski* et *Fasciola gigantica* (Fasciolidae) ont été trouvées dans des Gastropodes du Laos. Ces cercaires trouvées au Laos pour la

première fois sont décrites et dessinées. La Gastropode *Helicorbis umbilicalis* représente un nouveau hôte premier intermédiaire pour *Fasciolopsis buski*. L'apparition des Gastropodes d'importance médicale dans la province de Vientiane est aussi mentionnée.

INTRODUCTION

During the study on opisthorchiasis carried out in Laos in 1989¹, snails and fish, potentially intermediate hosts of trematodes, were examined. The metacercariae found in fish have been described in the Part I (Scholz *et al.*, 1991). This paper gives the results of a survey of cercariae of some medically important trematodes which have been found in snails.

MATERIALS AND METHODS

A total of 6 520 water snails of 22 species and subspecies were examined for the presence of cercariae (Table I).

Snails were collected in the Vientiane province from the following localities (Table 1 shows the number of snails examined) : 1) Vientiane capital — drains collecting polluted water into Kua

Dinh swamp and Samgniekchinnaimo swamp; 2) Vientiane municipality — artificial fish ponds in Nong Teng; 3) Vientiane suburbs — rice fields and their irrigation systems in Khamavath, Dong Naxok, That Luang, Sikhay, Nong Sang Thoo, Kaoliao, Don Khoi, Bung Salakham drain; 4) Vientiane municipality (Chanthaboury district) — rice fields around Nong Ping; 5) Vientiane municipality — rice fields and their irrigation system near Thadeua; 6) Toulakhon district — rice fields around Ban Nanin near Ban Keun; 7) Keo-Oudom district — rice fields and their irrigation systems around the villages of Ban Phonmi, Phonsavath, and Naxon; 8) Keo-Oudom district — fish ponds near Ban Thinkeo; 9) Vientiane capital — the Mekong river; 10) Vientiane municipality — the Khu Khana river (30 km NW to Vientiane); 11) the Nam Ngum river in village of Ban Nanin and district centre Thinkeo; 12) Nam Ngum water reservoir (18 localities, in detail listed in a paper by Ditrich *et al.*, 1990). The number of snails does not reflect the actual occurrence within an individual species in a locality because bithyniid snails, which are potentially intermediate hosts of *Opisthorchis viverrini*, were predominately collected.

The snails were individually placed into test tubes. The shedding of the cercariae was stimulated by artificial light. All snails were dissected approximately 48 h after collection.

The cercariae were drawn alive and urea solution and neutral red were only used for highlighting the excretory system and penetration glands. The cercariae, fixed with a 4 % hot formaldehyde solution, were measured. Thirty specimens of each species were measured and the average and standard deviations were counted. All measurements are given in μm .

The cercariae used for scanning electron microscopical (SEM) study were fixed with a 4 % glutaraldehyde solution in cacodylate

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TABLE I. — Survey of snails examined.

Locality N°	1	2	3	4	5	6	7	8	9	10	11	12	Total
Viviparidae													
<i>Filopaludina</i>													
<i>martensi</i>	0	0	5	11	0	0	0	0	0	4	20	8	48
<i>Filopaludina</i>													
<i>polygrana</i>	0	0	11	0	0	0	0	0	0	0	0	10	21
<i>Idioma</i>													
<i>umbilicata</i>	0	0	32	0	0	0	0	0	0	0	0	0	32
<i>Trochotaila</i>													
<i>trochoides</i>	0	0	20	0	0	8	4	5	0	0	0	15	52
Ampullaridae													
<i>Pila</i>													
<i>ampullacea</i>	0	0	4	0	0	4	0	0	0	0	0	0	8
<i>Pila</i>													
<i>scutata</i>	0	0	10	0	0	5	6	0	0	0	0	0	21
<i>Pila</i>													
<i>polita</i>	0	0	22	0	8	0	0	0	0	0	0	0	30
Bithyniidae													
<i>Bithynia s.</i>													
<i>siamensis</i>	23	0	151	180	0	0	0	0	0	0	0	0	354
<i>Bithynia s.</i>													
<i>goniophthalus</i>	0	0	1811	0	48	0	47	0	0	0	0	2007	3913
<i>Bithynia s.</i>													
<i>laevis</i>	0	0	159	0	0	0	0	0	0	0	0	0	159
Hydrobiidae													
<i>nassa</i>	0	0	281	0	0	0	0	0	0	0	0	0	281
Thiaridae													
<i>Adamiella</i>													
<i>housei</i>	0	0	0	0	0	0	1	0	0	0	0	0	1
<i>Paracrostroma</i>													
<i>pseudosulcospira</i>	0	0	0	0	0	0	0	0	20	0	0	0	20
<i>Tarebia</i>													
<i>granifera</i>	0	0	0	0	0	0	0	0	0	0	212	100	312
<i>Thiara</i>													
<i>scabra</i>	0	0	0	0	0	0	0	0	0	0	62	0	62
<i>Melanoidea</i>													
<i>tuberculata</i>	0	0	17	18	0	0	3	0	21	10	0	0	69
Bucciniidae													
<i>Clea</i>													
<i>helena</i>	0	0	0	0	0	0	0	0	0	0	6	0	6
Lymnaeidae													
<i>Radix a.</i>													
<i>rubiginosa</i>	15	20	261	19	0	12	12	14	0	0	0	20	373
Planorbidae													
<i>Cantoceras</i>													
<i>jiraponi</i>	35	0	0	0	0	0	0	0	0	0	0	0	35
<i>Gyraulius</i>													
<i>convexisculus</i>	30	0	8	0	0	0	0	45	0	0	0	3	86
<i>Helicorbis</i>													
<i>umbilicalis</i>	40	0	122	0	0	0	0	0	0	0	0	0	162
<i>Indoplanorbis</i>													
<i>exustus</i>	0	40	334	12	28	6	12	8	0	0	0	35	475
Total	143	60	3248	240	84	35	85	72	41	14	300	2198	6520

buffer, dehydrated in alcohol series and acetone, dried in CO₂ by the critical-point method, gold-coated, and examined in a Tesla BS 300 electron microscope.

SURVEY OF CERCARIAE

Opisthorchiidae Braun, 1901.

1 — *Opisthorchis viverrini* (POIRIER, 1886) (Fig. 1A, B)

Description : Oculate, pleurolophocercous, pipe-form cercaria. Body length 178 ± 16 , body width 67 ± 11 . Tail length 390 ± 48 , tail width 25 ± 2 . Diameter of oral sucker 39 ± 3 . Pharynx length 9 ± 1 , width 7 ± 1 .

Body covered with minute spines; nine anterior rows with larger spines. Three transversal rows of preoral spines situated on dorsal lip of oral sucker. Ten long cilia visible on each lateral margin of body. Tail provided with wide dorsoventral fin-fold, dorsally beginning at first third of tail length and ventrally terminating in middle of tail. Tegument of tail apparently transversally striated on anterior third.

Oral sucker slightly subterminal, pharynx well visible, oesophagus and caeca not developed. Eyespots located anterolaterally to

pharynx. Central part of body occupied by penetration glands (at least 5 pairs); their ducts in two bundles on each side. Cystogenous glands mainly in lateral regions. Ventral sucker underdeveloped, spherical in shape, with diameter 38 ± 4 . Excretory vesicle thick-walled, cup-shaped, its diameter 53 ± 12 ; main collecting ducts divided into anterior and posterior branches at level of ventral sucker, flame cell formula $2 [(3 + 3) + (3 + 3 + 3)] = 30$. Brownish pigment scattered throughout lateral body fields.

Host: *Bithynia siamensis goniophthalus*, prevalence 0.6 % (number of snails examined—see Table 1).

Localities: 3-Khamsavath, Nong Sang Thoo; 12-Nam Ngum water reservoir (3 localities).

Comments: Cercariae under study well correspond to those described by Wykoff *et al.*, (1965).

Out of the potential host snails carrying *O. viverrini*, the cercariae of this fluke were only found in *Bithynia siamensis goniophthalus* in the present study, whilst *B. siamensis siamensis* (354 specimens examined) and *B. siamensis laevis* (159 spec.) were negative. However, Chanawong and Waikagul (1991) demonstrated that *B. s. goniophthalus* is 4–7 times less susceptible to *O. viverrini* infection than *B. s. siamensis* and *B. funiculata*. *B. s. goniophthalus* has been reported as the first intermediate host of this fluke in North Eastern Thailand (Brockelman *et al.*, 1985). *B. s. siamensis* in Central Thailand (Upatham and Sukhapanth, 1980), and a related species, *B. funiculata*, in Northern Thailand (Upatham, 1988).

Whilst *B. s. goniophthalus* was collected mainly from rice fields, *B. s. siamensis* and *B. s. laevis* were found in their irrigation systems (channels and drains). In the Nam Ngum water reservoir only one bythiniid snail, *B. s. goniophthalus*, was recorded. In our opinion, the above mentioned subspecies of *B. siamensis* should be considered as ecological forms of this snail species, similar to that suggested by Wykoff *et al.* (1965).

Snails of the genus *Bithynia* (subgenus *Digoniostoma*) have been reported as the exclusive first intermediate hosts of *O. viverrini* (SEAMEO-TROPED technical group 1986). Bisseru and Chong (1968) described a cercaria, designated as *O. viverrini*, from spontaneously and experimentally infected thiarid snail, *Melanoidea tuberculata*, in Malaysia. However, their species identification seems to be questionable. The tail of the cercaria, as represented by these authors, possesses a fin-fold beginning just in proximal part of the tail. This tail fin arrangement is more typical of heterophyid cercariae.

The occurrence of *O. viverrini* cercariae has been reported to exhibit a distinctly seasonal character (Wykoff *et al.*, 1965). It is assumed that this seasonal variation is caused by the lack of water in the rice fields during the dry season. Our observations in rice fields around the Vientiane capital support this assumption: *O. viverrini* cercariae were recorded in *Bithynia* snails from these fields as late as at the end of August and in September, while the snails collected there in June and at the beginning of July gave a negative result.

In contrast to our findings in rice fields, the ecology of *Bithynia* snails living around Nam Ngum water reservoir seems to be quite different because snails can be active throughout the whole year there. It is very probable that cercarial development in that locality does not have a seasonal character (Ditrich *et al.*, 1990).

FIGURE 1.

FIG. 1. — A : *Opisthorchis viverrini* (Poirier, 1886) — total view of cercaria. B : *O. viverrini* — lateral view of tail. C : *Haplorchis taichui* (Nishigori, 1924) — total view of cercaria. D : *H. taichui* — lateral view of tail.

FIGURE 1.

Heterophyidae Odhner, 1914.

2 — *Haplorchis taichui* (NISHIGORI, 1924) (Fig. 1C, D, Fig. 2)

Description: Oculate, pleurophocercous, spade-shaped cercaria with slightly curved tail. Body length 138 ± 18 , body width 56 ± 9 . Tail length 335 ± 52 , tail width 21 ± 2 . Diameter of oral sucker 37 ± 2 . Pharynx 7 ± 1 long and 4 ± 1 wide. Body surface covered with minute tegumental spines diminishing in size posteriorly. Dorsal lip of oral sucker bearing preoral spines arranged in three rows (Fig. 2A, B). At least 7 cilia located on both lateral margins of body; three anterior in front of eyespot, four posterior which are distinctly shorter, on hindbody. Tail with two lateral fin-folds in anterior third and dorsoventral fin-fold in other two posterior thirds (Fig. 2C). Oral opening subterminal, pharynx small, caeca rudimentary. Ventral sucker absent. Eyespots about half way between pharynx and oral sucker. Cystogenous glands with distinct nuclei in lateral fields of body, seven pairs of penetration glands in two longitudinal rows starting behind pharynx, reaching posteriorly to body extremity; their ducts in two bundles on each side. Flame cell formula not exactly determined (six pairs of flame cells observed); excretory vesicle round, its diameter 41 ± 9 ; tail excretory tubule bifurcating, opening on sides of tail at about one fifth of its length. Genital primordia situated in front of excretory vesicle.

Host: *Tarebia granifera*, prevalence 0.6 %.

Locality: 12) Nam Ngum water reservoir (1 locality).

Comments: The description of the cercaria corresponds with *H. taichui* from *Tarebia granifera* found in Hawaii (Martin, 1958), with the exception of the flame cell formula. While the above author described 7 pairs of flame cells, we observed only six. However, flame cells localized in the hindbody of the cercariae studied were not clearly visible and the presence of a seventh pair cannot be excluded.

In addition to the cercariae, other developmental stages (metacercariae, adults) were found in the same locality of the Nam Ngum water reservoir (Ditrich *et al.*, 1990). Human infection resulting from this trematode in the Vientiane province has also been confirmed (Giboda *et al.* 1991a).

The species *Haplorchis taichui* is a widely distributed parasite occurring in Hawaii, Iraq, India and Southeast Asia. Adults are localized in the intestine of fish-eating birds and mammals including man; metacercariae are found in freshwater fish (Pearson 1964, Pearson and Ow-Yang 1982). The cercariae of this species have hitherto been found in different species of thiarid snails (see Yamaguti, 1975).

FIG. 2. — *Haplorchis taichui* — SEM photomicrograph of cercaria. A : preoral spines, bar = 2 µm. B : oral sucker, bar = 5 µm. C : tail fin-fold, bar = 20 µm.

FIG. 3. — A : *Schistosoma spindale* Montgomery, 1906. B : *Fasciolopsis buski* (Lankester, 1857). C : *Fasciola gigantica* Cobbold, 1855.

Schistosomatidae Poche, 1907.

3 — *Schistosoma spindale* (MONTGOMERY, 1906) (Fig. 3A)

Description: Apharyngeal, brevifurcate cercariae. Body length 204 ± 16 , width 63 ± 9 . Tail stem length 287 ± 28 , width 29 ± 4 . Furca 111 ± 21 in length. Head organ pyriform in shape with strong muscular wall, its length 44 ± 5 , width on anterior end 32 ± 2 . Mouth on ventral side slightly behind anterior extremity. Oesophagus long, widened in its proximal part (10 ± 1), narrowing behind head organ, bifurcating into two short caeca at postequatorial level. Ventral sucker located at beginning of posterior fourth of body, its diameter 23 ± 3 . Five pairs of penetration glands located laterally to ventral sucker; their ducts opening at anterior body end near large apical papillae. Excretory vesicle bicornuate; flame cell formula $2 [2 + 2 + (1)] = 10$. Tail tubule bifurcating at distal end of tail stem, each branch opening at tip of furca. Genital primordia situated between ventral sucker and excretory vesicle.

Body, tail stem and furcae covered with minute tegumental spines.

Host: *Indoplanorbis exustus*, prevalence 0.2 %.

Locality: 6 (Ban Nanin).

Comments: The identification of the cercaria was not only based on its morphology but also the geographical distribution, the spectrum of snail intermediate hosts of *Schistosoma* species and findings of *S. spindale* eggs in the faeces from water buffaloes in the same locality were taken into consideration.

The planorbid snail, *Indoplanorbis exustus*, is a common intermediate host of this fluke in India, Sri Lanka, Indonesia, Malaysia, Thailand and Vietnam (Harinasuta *et al.*, 1965; Rollinson and Southgate, 1987).

Schistosoma spindale, a parasite of cattle, goats and buffaloes, can produce cercarial dermatitis in humans (Palmieri *et al.*, 1977). The inhabitants from the Vientiane province working in rice fields also suffer from dermatitis on their forearms. *Indoplanorbis exustus* snails are considered by these people as the causative agents of this itch.

Fasciolidae Railliet, 1895.

4 — *Fasciolopsis buski* (LANKESTER, 1857) (Fig. 3B)

Pigmentous, gymnocephalous cercaria. Body spinulate, tail glabrous. Body length 202 ± 17 , width 116 ± 19 . Tail length 426 ± 23 , width 27 ± 6 . Oral sucker diameter 38 ± 3 , ventral sucker diameter 44 ± 4 . Pharynx spherical with diameter 21 ± 3 . Oesophagus bifurcating in middle of body, caeca reaching to its posterior end. Ventral sucker postequatorial. Excretory vesicle bicornuate; vesicle and main collecting ducts containing refractile concretions; flame cell formula not determined. Inner structures not clearly visible because of number of dark cystogenous glands under tegument.

Host: *Helicorhis umbilicalis*, prevalence 1.9 %.

Locality: 3 (Bung Salakham)

Comments: The cercaria corresponds in its shape with that of the *Fasciolopsis buski* as described by Barlow (1925). Moreover, the

cercariae of *F. buski* have been found exclusively in planorbid snails, especially in members of the genera *Planorbis* and *Segmentina* (Yamaguti 1975, SEAMEO-TROPED technical group 1986). *Helicorhis umbilicalis* represents a new intermediate host for this medically important trematode. *F. buski* seems to be rather common in the study area because its eggs were found in 0.9-3.8 % of the inhabitants of the Vientiane province (Giboda *et al.*, 1991b).

5 — *Fasciola gigantica* (COBBOLD, 1855) (Fig. 3C)

Pigmentous gymnocephalous cercaria. Body surface covered with minute spines. Body length 343 ± 31 , width 276 ± 12 . Tail length 598 ± 32 , width 57 ± 4 . Oral sucker diameter 45 ± 6 , ventral sucker diameter 56 ± 5 . Spherical pharynx 19 ± 2 in diameter. Oesophagus bifurcating in front of ventral sucker, caeca reaching posterior end of body. Numerous cystogenous glands containing rod-like bodies beneath tegument, making entire body opaque. Excretory vesicle elongate, main collecting ducts filled with refractile excretory granules; flame cell formula not determined, tail tubule bifurcating in first fourth of tail length.

Host: *Radix auricularia rubiginosa*, prevalence 0.5 %.

Locality: 4 (Nong Ping) and 6 (Ban Nanin)

Comments: The cercaria corresponds in its morphology with those described by Alicata (1938). The findings of *F. gigantica* eggs from stool samples of cattle and water buffaloes in localities 3, 6 and 8 support species identification.

The snail *Radix auricularia rubiginosa* (syn.: *Lymnaea rubiginosa*) is a common intermediate host of *F. gigantica* in Thailand (Dissamarn, 1955), Malaysia (Palmieri *et al.*, 1977) and Indonesia (SEAMEO-TROPED technical group 1986). In addition to the cercariae, the sporocysts and rediae of *F. gigantica* were seen in dissected snails. Our observations therefore support the opinion of Sullivan *et al.* (1976) about the presence of the sporocyst in the life cycle of *F. gigantica*. Ogambo-Ongoma and Goodman (1976) on the other hand reported the development without the mother sporocyst stage.

DISCUSSION

The examination of snails from the Vientiane province revealed the presence of the cercariae of five medically important flukes. From a medical point of view, the common occurrence of the *Opisthorchis viverrini* cercariae is the most significant. This fact accords with the data obtained during previous studies which revealed a relatively high prevalence of the *O. viverrini* infection in humans (Sornmani *et al.*, 1977, Giboda *et al.*, 1991a,b). The frequent occurrence of the first intermediate host of this fluke in the Vientiane province is also important from epidemiological point of view.

The study on fish-borne trematodosis carried out in Laos (Giboda *et al.*, 1991a) revealed a simultaneous occurrence of heterophyid flukes and *O. viverrini* in both intermediate and definitive hosts. The combined task of distinguishing

the eggs in human stool and determining the metacercariae in fish is rather difficult and time consuming. On the other hand, the identification of cercariae — based on their morphology and the range of snail intermediate hosts — can serve as a quick way of proving of the presence of *O. viverrini* in an area studied.

Out of 4 species of haplorchine flukes recorded in the Vientiane province (Ditrich *et al.*, 1990, Scholz *et al.*, 1991), only the cercariae of *Haplorchis taichui* were found. However, a relatively frequent occurrence of potential intermediate hosts (*Melanoides tuberculata*, *Thiara scabra*, *Tarebia granifera*) indicates that additional haplorchine cercariae may be found after a more extensive examination.

The occurrence of *Fasciolopsis buski* and *Fasciola gigantica* in rice fields and their irrigation systems is also of interest from an epidemiological point of view. Many species of plants growing there are eaten raw and people can easily acquire both parasites by ingestion of their adolescents.

All species of cercariae have been recorded in snails from Laos for the first time. However, adult trematodes of *Opisthorchis viverrini*, *Haplorchis taichui* and *Fasciolopsis buski* have already been reported in humans (Segal *et al.*, 1968, Sornmani *et al.*, 1974, Giboda *et al.*, 1991a).

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