[Jpn. J. Parasitol., Vol. 38, No. 5, 290—295, October, 1989]

Migration and Emergence of *Eurytrema pancreaticum* Daughter Sporocysts from Host Land Snails (Trematoda, Dicrocoeliidae)

TOMOYUKI KOZUTSUMI AND HIROSHI ITAGAKI

(Accepted for publication; August 16, 1989)

Abstract

Dicrocoeliids utilize pulmonate land snails as the first intermediate host, so that their sporocysts or cercariae emerging from host snails are different in morphology and ecology from those of the species that utilize aquatic snails. In snail hosts, *Bradybaena similaris, Eurytrema pancreaticum* daughter sporocysts were produced by mothers in the sinus near the posterior part of the stomach and migrated through the rectal or the lateral sinus to reach the mantle near the pneumostome. They emerged to the exterior by penetrating the mantle on either side of the pneumostome: those migrating through the rectal sinus emerged on the right side and those through the lateral sinus on the left side of the pneumostome. No daughter sporocysts emerged through the pneumostome.

Key words: *Eurytrema pancreaticum,* daughter sporocyst, emergence, migration, snail host, *Bradybaena similaris*

Introduction

All dicrocoeliid species, as far as known, require the first and the second intermediate hosts to complete their life cycle even when they are parasitic in hervivorous final hosts.

In Japan 2 *Eurytrema* species occur: *E. pancreaticum* and *E. coelomaticum*. The main first intermediate hosts of these 2 species are pulmonate land snail, *Bradybaena similaris* and *Acusta despecta sieboldiana,* which are widely distributed in Japan.

The development of *E. pancreaticum* in the first intermediate host has been studied by some authors, but no precise reports have dealt with the intramolluscan migration and emergence of daughter sporocysts from host snails.

Materials and Methods

Materials:

Uterine eggs were dissected out of mature flukes obtained from a cow slaughtered in Tokyo and from artificially infected goats and rabbits in the laboratory. Eggs were then preserved at 5°C within 6 months before inoculation to host snails.

Inoculation of snails with eggs and maintenance of infected snails:

After kept for 3 or 4 days without food, snails, *Bradybaena similaris,* were fed with a small piece, about 5 × 5 mm in size, of Standen's food (Standen, 1951), on which 4 or 5 mature eggs were pipetted with a small amount of water. Twenty five inoculated snails each were kept at 26°C in plastic containers, 25 cm × 15 cm and 5 cm in height, with a thin layer of moistened pebbles on the bottom. The containers were cleaned and snails were supplied with lettuce every 2 or 3 days.

Macro- and microscopic observation of infected snails:

About 100 days after inoculation when daughter sporocysts began to emerge from snails, infected snails were individually transferred to small plastic cases with a lid. Some of the infected

Department of Parasitology, Azabu University School of Veterinary Medicine, Fuchinobe, Sagamihara, Kanagawa 229, Japan
小堤知行　板垣　博（麻布大学獣医学部寄生虫学教室）

snails that began to shed daughter sporocysts were dissected to observe daughter sporocysts macroscopically, and some were used for microscopic observation. For light microscopy snails were fixed in 5% formalin after killed by immersing them in the water and sections were stained with hematoxylin and eosin. For scanning electron microscopy, snails were fixed in 5% formalin, refixed in 3% $K_2Cr_2O_7$ solution, dehydrated in ethanol, dried at critical point and deposited with gold or carbon by vacuum evaporation.

Results

Migration of daughter sporocysts

The main migration routes of *E. pancreaticum* larvae in snail hosts are illustrated in Fig. 1 schematically. The development and migration of the larval stages are given in detail as under.

A great number of mother sporocysts were parasitic on the outer wall of the posterior most part of stomach where the digestive tract turns anteriorly. Histologically, the proliferated connective tissue closely surrounded sporocysts, some of which were degenerate (Fig. 2).

Soon after shed by mothers, daughter sporocysts were found near mothers in the subrenal and the visceral sinuses near the stomach, but never in the parenchyma of the digestive gland (midgut gland), stomach, intestine, and albumen gland nor in the cephalopedal sinus. Then daughter sporocysts migrated toward the mantle collar through the rectal or the lateral sinus: those parasitic in the sinus dorsal to the stomach (the visceral sinus) migrated through the rectal sinus (the right arm of afferent branchial vessel) (Fig. 3), whereas those in the sinus around the posterior most part of the esophagus (the subrenal sinus) migrated through the lateral sinus (the left arm of the afferent branchial vessel) (Fig. 4). In heavily infected snails, daughter sporocysts were in masses in the subrenal and the lateral sinuses, while they were arranged in a single file in the narrow rectal sinus. After reaching the sinus near the mantle collar, daughter sporocysts penetrated the mantle to the exterior, but no daughter sporocysts were found in the respiratory cavity (mantle cavity) nor in the efferent branchial vessels (pulmonary vessel).

Most daughter sporocysts migrating through the rectal or the lateral sinus were subspindle-shaped but they varied in shape, this indicating

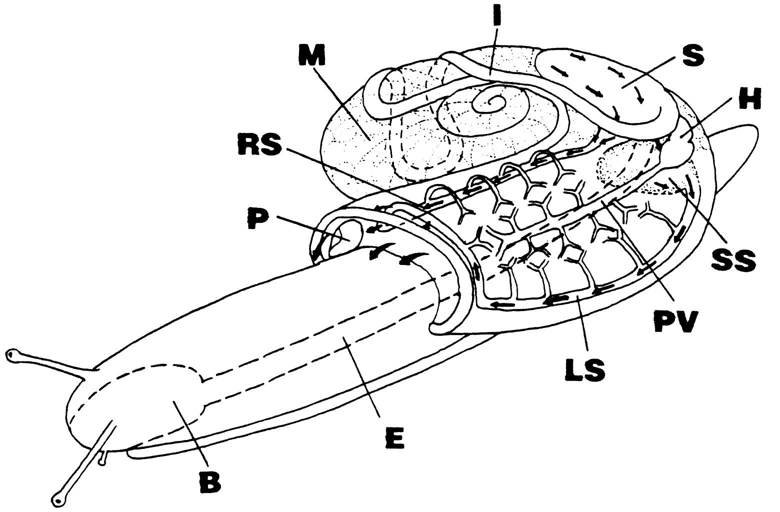
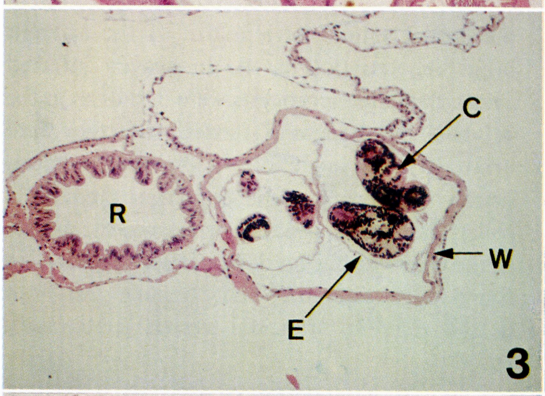


Fig. 1. Migration and emergence routes (arrows) of daughter sporocysts from visceral mass to the exterior. Kidney is removed. B: buccal mass, E: esophagus, H: heart, LS: lateral sinus, P: pneumostome, PV: efferent branchial vessel (pulmonary vessel), RS: rectal sinus, S: stomach, SS: subrenal sinus.


s
s
2

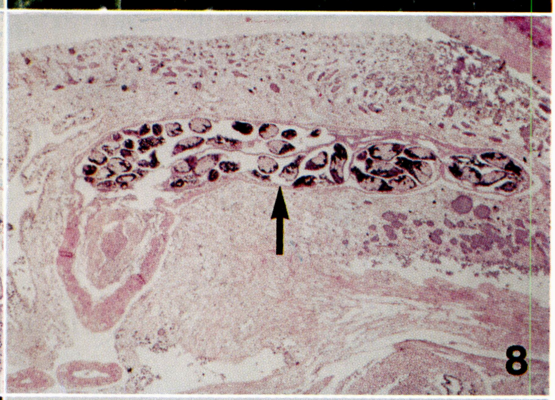

C
R
W
E
3

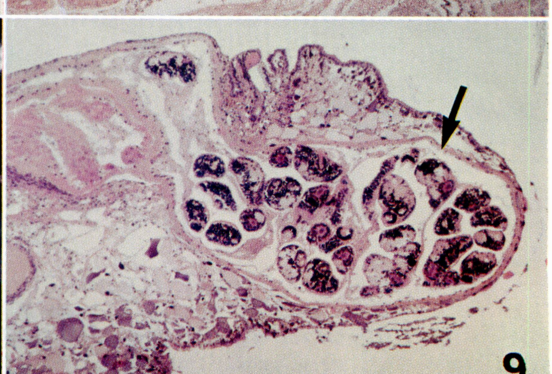

E
E
4


R
L
5


6


7


8


9

their active vermicular locomotion during migration.

The daughter sporocysts migrating through the lateral sinus were greater in number than those through the rectal sinus since the former sinus is thicker than the latter.

Emergence of daughter sporocysts

Daughter sporocysts began to emerge from snails about 30 min after the snails began to crawl more vigorously. Just before daughter sporocysts emerged, their anterior end appeared on the mantle on both lateral sides of the pneumostome (respiratory orifice) (Figs. 5, 6, 7). Dissection of infected snails revealed that the sporocysts migrating through the lateral sinus emerged from the mantle on the left side of the pneumostome, whereas those through the rectal sinus on the right side (Fig. 5). Histological examinations revealed that emergent daughter sporocysts were spindle-shaped, while those migrating in the sinus were elongate fusiform (Figs. 8, 9).

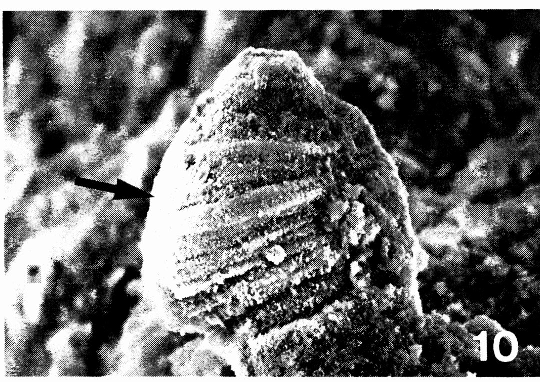
Emergent daughter sporocysts were left behind the creeping snail after deposited on its "neck" for a while (Figs. 6, 7).

Sporocysts were nearly transparent and stream-lined during emergence from snails. Just after emerging from snails, sporocysts became slenderer in shape and opaque nearly white in color, and continued to expand and contract at regular intervals. After emerged from snails, daughter sporocysts took the characteristic spindle-shape with the proboscis-like anterior, the elliptical middle and the thinner tail-like posterior parts, since the endocyst moved posteriorly to the middle portion of daughter sporocysts. It took 5 to 10 min for daughter sporocysts to complete their emergence from snails.

Light- and electron-microscopies revealed that migrating and emergent daughter sporocysts had the annular structures and the verrucose formations on the surface of the anterior and the posterior portions, respectively (Figs. 10, 11).

Discussion

Dicrocoeliid eggs hatch in the alimentary canal of suitable land snails after ingested by them. The



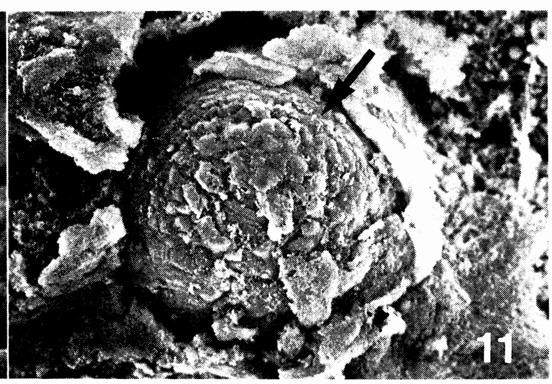


Fig. 10. Anterior end of daughter sporocyst (arrow) emerging from mantle collar (SEM).
Fig. 11. Anterior end of daughter sporocyst (arrow) perforating mantle collar (SEM).

Fig. 2. Mother sporocysts (arrow) around stomach. S: stomach.
Fig. 3. Daughter sporocyst in rectal sinus, including cercariae. C: cercaria, E: endocyst, R: rectum, W: sporocyst wall.
Fig. 4. Daughter sporocyst (arrow) in lateral sinus. E: esophagus.
Fig. 5. Daughter sporocysts (arrow) emerging from a snail at mantle collar on both sides of pneumostome. R: right side, L: left side.
Fig. 6. Daughter sporocysts (arrow) emerging from a snail.
Fig. 7. Daughter sporocysts (arrow) emerging from a snail.
Fig. 8. Daughter sporocyst (arrow) migrating in mantle collar.
Fig. 9. Daughter sporocyst (arrow) just before emergence to the exterior.

sites for ingested eggs to hatch can be located by the habitats of mother sporocysts, since miracidia penetrate the alimentary canal wall soon after they hatch, where they develop into mother sporocysts on its outer surface. Tang (1950) reported that most mother sporocysts of *Eurytrema pancreaticum* are parasitic in both the posterior portion of the stomach and the follicles of the digestive gland. In the present study, however, mother sporocysts were located on the stomach wall in the posterior portion but never in the follicles of the digestive gland.

Dicrocoeliid mother sporocysts, then, produce daughters. Daughter sporocysts or cercariae, shed by them, migrate actively or are passively carried by venous blood flow through the sinuses toward the mantle collar. Pulmonate land snails have the open vascular system. In *Helix* the blood is brought back from the visceral hump and head-foot toward the mantle collar through the right and the left arms of the afferent branchial vessels respectively (Bullough, 1958). The venous blood flow is the same in both *Helix* and *Bradybaena,* although there exists the difference in anatomical terminology of snails between previous authors.

The habitats of dicrocoeliid cercariae or daughter sporocysts in snails have been reported by many authors: the mantle cavity wall, the softer dorsal tissue of body and mantle collar (Denton, 1944), the veins in the pulmonary cavity wall (Denton, 1944, 1945), the site near the "origin" of the thick, muscular mantle edge (Krull and Mapes, 1952a), the mantle cavity (Neuhaus, 1936; Denton, 1944, 1945), the hemocoel in the digestive gland, albumen gland and kidney wall, and the connective tissue of the pneumostome and along the intestine (Denton, 1945; Krull and Mapes, 1952a). These habitats suggest that dicrocoeliid species migrate through the hemocoel (sinus), although some of the habitats were described without histological details. Dicrocoeliid daughter sporocysts or cercariae that finally reach the sinus near the mantle collar through the lateral or the rectal sinus escape to the exterior by perforating the tissue near mantle collar or through the pneumostome according to the species.

The emergent site of larvae from host snails to the exterior is different in different dicrocoeliid species. Daughter sporocysts of *E. procyonis* (= *C. procyonis*) and *D. petiolatum* (= *Z. petiolatus*), and the "slime balls" of *B. americanum* are shed through the pneumostome (Denton, 1944, 1945; Timon-David, 1960), but in the case of *D. dendriticum* the results are divergent by the authors (Neuhaus, 1936; Krull and Mapes, 1952a). The divergence in the emergent sites will arise from the difficulty in accurate location of the site. In the present study, *E. pancreaticum* daughter sporocysts emerged from snails by penetrating the mantle on both sides of the pneumostome but never through the orifice, although Tang (1950) observed daughter sporocysts in the "anterior parts of respiratory chamber".

Dicrocoeliid larvae are different in ecology from those utilizing aquatic snails as intermediate hosts. The larvae emerging from land snails have some characteristic structures against desiccation and are morphologically divided into two types. The first type is active, long-tailed cercariae enveloped by the mucous membranes of parasite and/or host origin, so called the "slime balls", whereas the second type is daughter sporocysts which include many sluggish, mature micro-cercous cercariae and have no birth pore nor canal.

Daughter sporocysts of the first type release active mature cercariae during their migration through the host sinuses after shed from mothers, whereas *E. pancreaticum* daughter sporocysts, in this study, were released from mothers in the visceral and subrenal sinuses, but they released no cercariae. *Dicrocoelium* and *Brachylecithum* larvae belong to the first type, while those of *Eurytrema, Concinnum,* and *Dicrocoelioides,* partially a synonym of *Zonorchis,* to the second type (Denton, 1944, 1945; Timon-Davis, 1960).

Emerging daughter sporocysts of *E. pancreaticum* took the characteristic spindle-shape as described by Tang (1950), and the verrucose formations were located on the thinner posterior portion and its transitional site to the thicker middle portion of daughter sporocysts, although Zdárská *et al.* (1978) reported that the formations are situated on the anterior proboscis-like portion and its transitional site.

References

1) Bullough, W. S. (1958): Practical Invertebrate Anatomy. 2nd ed., Macmillan, London, 365–366.
2) Denton, J. F. (1944): Studies on the life history of *Eurytrema procyonis* Denton, 1942. J. Parasitol., 30, 277–286.
3) Denton, J. F. (1945): Studies on the life history of *Brachylecithum americanum* n.sp., a liver fluke of passerine birds. J. Parasitol., 31, 131–141.
4) Krull, W. H. and Mapes, C. R. (1952a): Studies on the biology of *Dicrocoelium dendriticum,* including its relation to the intermediate host, *Cionella lubrica.* 3. Observations on the slimeballs of *Dicrocoelium dendriticum*. Cornell Vet., 42, 253–276.
5) Krull, W. H. and Mapes, C. R. (1952b): Studies on the biology of *Dicrocoelium dendriticum,* including its relation to the intermediate host, *Cionella lubrica.* Infection experiments involving definitive hosts. Cornell Vet., 42, 277–285.
6) Neuhaus, W. (1936): Untersuchungen über Bau und Entwicklung der Lanzettegel-cercariae (*Cercaria vitrina*) und Kalrstellung des Infektionsvorganges beim Endwirt. Z. Parasitenkd., 8, 431–473.
7) Standen, O. D. (1951): Some observations upon the maintenance of *Australorbis glabratus* in the laboratory. Ann. Trop. Med. Parasitol., 45, 80–83.
8) Tang, C. C. (1950): Studies on the life history of *Eurytrema pancreaticum* Janson, 1889. J. Parasitol., 36, 559–573.
9) Timon-Davis, J. (1960): Recherches expérimentales sur le cycle évolutif de *Dicrocoelioides petiolatum* (Railliet, 1900) (Dicrocoeliidae). Ann. Parasitol. Hum. Comp., 35, 251–267.
10) Zdárská, Z., Panin, V. Ya. and Nesterenko, L. G. (1978): Morphology, histochemistry and ultrahistochemistry of special verrucose formations in daughter sporocysts of *Eurytrema pancreaticum*. Folia Parasitol. (Praha), 25, 41–47.