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Experimental Completion of the Life Cycle of the Lung Fluke, *Paragonimus westermani*, in the Laboratory

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The lung fluke, *Paragonimus westermani*, is one of the most important parasites of man in some of East Asian countries. Its life cycle has been elucidated. Yokogawa (1964, 1965) has reviewed previous work in this field. Attempts to infect either of the first or second intermediate host with the fluke have been made with successful results (Komiya *et al.*, 1961; Yokogawa, 1953). However, none have been able to complete the life cycle experimentally.

This paper reports the establishment of the entire life cycle in the laboratory.

Materials and Methods

Parasite and hosts

Metacercariae of *P. westermani* (triploid form†) were obtained from naturally infected *Eriocheir japonicus* caught on Tsushima Islands, Kyushu, Japan, in May 1978.

As the first intermediate host, young snails (2 to 9 mm in shell width) of *Semisulcospira libertina* were used. They were collected in the Metoba River at Asama, Matsumoto City, Nagano Prefecture, Japan, in August 1979. Most probably they were free from *Paragonimus* larvae of natural infection, because no *Paragonimus* larvae were detected in about 400 snails collected at the same locality in August and September 1979. They were reared in an aquarium at 25 C according to the method of Shimazu (1976).

As the second intermediate host, crabs of *Geothelphusa dehaani* and crayfish of *Procambarus clarki* were used. The crabs were collected at Midori, Iiyama City, Nagano Prefecture, in December 1979. They were considered to be free from *Paragonimus* metacercariae of natural infection, because none of about 800 crabs caught at the same locality during 1976 to 1980 were infected. The crayfish consisted of commercially supplied and laboratory-raised ones. These crabs and crayfish were kept all together at 25 C in another aquarium with various shelters to prevent them from eating one another, and given ground commercial food for mice, slightly salted dried fish and boiled lettuce twice per week.

As the final host, adult dogs were used. Fecal examinations prior to use showed that they were uninfected with *Paragonimus* parasites.

Experimental infection

Two dogs were given *per os* 30 and 40 of the above-mentioned metacercariae, respectively. Eleven months later, their feces containing numerous *Paragonimus* eggs

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† =*P. pulmonalis* (Baelz, 1880) Miyazaki, 1978.

were collected, dissolved in dechlorinated tap water, and filtrated through a metal sieve (mesh size, 0.210 mm). The eggs were obtained by repeated washing and sedimentation of the filtrate in dechlorinated tap water. They were incubated in water 15 mm deep in a 14-cm diameter Petri dish at 25 C in a dark condition. The water of the dish was not changed throughout incubation.

Fully-embryonated eggs at week 7 to 13 post-incubation were exposed to light at once, and mass hatching of miracidia was brought about in about 30 minutes. Not only the newly hatched miracidia but also unhatched eggs were thrown into the aquarium containing about 700 snails the day after the snails were collected. The number of miracidia per snail was not recorded. The snails were crushed for *Paragonimus* larvae at irregular intervals from 13 to 28 weeks after exposure.

Experimental infections of crabs and crayfish with mature cercariae, which were obtained 28 weeks after miracidial exposure, were made as follows. (1) Group A: Two crabs (25 mm in carapace width) were fed with fragments of the digestive gland of two infected snails each; two commercially supplied crayfish (38 mm in carapace length) were fed with those of four infected snails each. The fragments harbored a large number of mature cercariae. (2) Group B: Ten crabs (15 to 24 mm in carapace width) and ten laboratory-raised crayfish (13 to 24 mm in carapace length) were exposed directly to cercariae dissected out of infected snails. They were left sitting in water of 5 mm depth containing a large number of fully-grown cercariae in glass containers for 17 hours. At the beginning of the exposure, most of the cercariae were still within their rediae, but some were liberated. At the end, many were found free and still living. About 8 weeks after infection, these host animals were first individually examined organ by organ for *Paragonimus* metacercariae. Examined organs of each animal species were then combined according to the experimental group, minced, and filtrated through a single layer of gauze. The sediment of the filtrate was washed by several changes of physiological saline, and examined for metacercariae.

Table 1 Results of experimental infection of *Semisulcospira libertina* with miracidia of *Paragonimus westermani* (triploid form) at 25 C

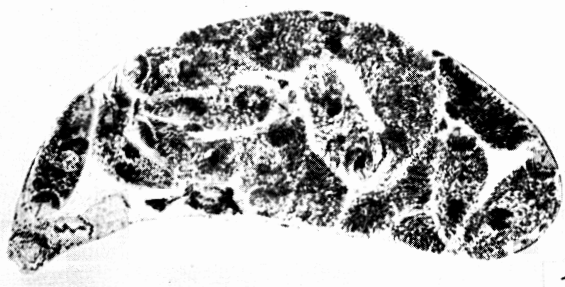
Weeks after infection	No. of snails examined	No. of snails infected with cercariae	% incidence
13	59	1	1.7
21	50	6	12.0
27	60	4	6.7
28	389	30	7.7
Total	558	41	7.3

Experimentally recovered metacercariae were fed to a dog, which was autopsied for adult flukes 13 weeks later.

Results

Results of experimental infection of the snails with the miracidia are summarized in Table 1. All of the infected snails harbored fully-formed cercariae. Figure 1 shows these mature cercariae in a redia at week 13. There were observed some sporocysts still living even at week 28. The larval stages obtained will be described elsewhere.

Until the examination about 8 weeks after infection, one crab and two crayfish in Group A and six crabs and nine crayfish in Group B survived. Out of them, one crab in Group B harbored six encysted metacercariae: three in the liver, and one each on the surface (?) of the pericardium, in the leg muscles and in the gills. The rest were all negative when being examined individually. However, an additional encysted metacercaria was recovered from the



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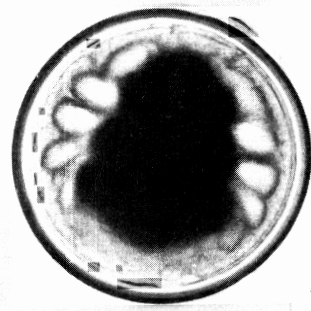

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Fig. 1 Mature cercariae in a redia (0.91 by 0.35 mm) of *Paragonimus westermani* (triploid form) experimentally obtained from *Semisulcospira libertina* 13 weeks after miracidial infection at 25 C, flattened, stained with alum carmine, mounted in balsam.
Fig. 2 Mature metacercaria of *P. westermani* (triploid form) experimentally obtained from *Geothelphusa dehaani* about 8 weeks after exposure to free cercariae at 25 C, living, 0.36 by 0.35 mm in inner cyst size.

sediment of the crabs in Group B. All of these seven metacercariae, but one found in the gills, were evidently mature, measuring 0.31 to 0.36 mm in diameter of inner cyst and 5–14 μm thick in inner cyst wall (Fig. 2). The exception, 0.22 by 0.37 mm in inner cyst size, seemed still immature, being enveloped in a thin inner cyst less than 5 μm thick.

The six well-developed metacercariae mentioned above were fed to a dog. On autopsy 13 weeks later, five worms were recovered: two each from two worm-cysts in the lung and one from the surface of the lung. Out of them, two found in one worm-cyst were gravid, but the others were immature. They proved to be morphologically identical with 22-month-old adult parent flukes which were obtained from the dog fed with the 40 original metacercariae: They were *P. westermani* (triploid form).

* As the source of their material, Komiya *et al.* and Yokogawa used naturally infected *E. japonicus* and *S. libertina,* respectively, both collected in Kannami Village, Shizuoka Prefecture. It is very likely from this that their parasites were of the triploid form of *P. westermani.*

Discussion

Mature cercariae were obtained at 25 C by 13 weeks after miracidial exposure in the present study. Komiya *et al.* (1961) found fully-grown cercariae about 25 C as early as 9 weeks after experimental exposure of *S. libertina* to recently hatched miracidia of *P. westermani**. However, they did not try to infect crab hosts with the cercariae they obtained.

Yokogawa (1953) succeeded in obtaining immature or mature metacercariae from *Potamon dehaani* (= *G. dehaani*), *E. japonicus* and *Cambarus clarkii* [sic] (= *Procambarus clarki*) by feeding them with the digestive gland of *S. libertina* naturally infected with *P. westermani** cercariae. In the present study, a direct contact of *G. dehaani* and *Pr. clarki* with free cercariae resulted in recovery of mature metacercariae from one or two crabs, but feeding with the digestive gland of infected snails failed to obtain metacercariae from both crabs and crayfish. This suggests that cercariae can penetrate crab hosts not only through

the mouth (Yokogawa, 1953) but also through the skin, although it cannot necessarily be denied the possibility that the crab or crabs may have acquired the infection by eating free cercariae or rediae containing cercariae. The failure of the feeding experiments may possibly be due to the fact that the crabs and crayfish used were too few as compared with those used by Yokogawa (1953). Concerning the mode of infection of the second intermediate host with cercariae, further experimental studies are needed.

In any case, this is the first report on a successful experiment in which the life cycle of *P. westermani* (triploid form) has been completed, indicating that it is practicable to maintain the fluke in the laboratory. This will facilitate further work on paragonimiasis and *Paragonimus* itself.

Summary

The life cycle of *P. westermani* (triploid form) was completed for the first time in the laboratory, beginning with the metacercarial stage of natural infection. Mature cercariae were obtained from *S. libertina* at 25 C by 13 weeks after miracidial exposure. A direct contact of *G. dehaani* with free cercariae obtained from experimentally infected snails resulted in recovery of well-developed metacercariae at 25 C about 8 weeks later. Fully gravid worms were recovered from a worm-cyst in the lung of a dog 13 weeks after administration of metacercariae experimentally obtained. Route of infection of *P. westermani* (triploid form) cercariae to the second intermediate host was briefly discussed.

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肺吸虫 *Paragonimus westermani* の生活史の実験室内再現について

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肺吸虫 *Paragonimus westermani* の生活史が解明されてから久しいが，その全生活史を実験的に回した報告はまだない．本研究では，メタセルカリア期より始めて，全生活史を実験室内で初めて再現することができた．

対馬産モクズガニ *Eriocheir japonicus* よりえた自然寄生のメタセルカリア（三倍体型）を犬に感染させた．この犬の糞便内虫卵を培養してえたミラシジウムをカワニナ *Semisulcospira libertina* に感染させたところ，セルカリアは 25 C で13週目にすでに成熟していた．実験的にえたセルカリアを遊離状態でサワガニ *Geothelphusa dehaani* に接触させて，25 C で約8週後に成熟メタセルカリアをえた．このメタセルカリアを犬に経口投与して，13週後に成虫を回収した．

この肺吸虫セルカリアの第2中間宿主への感染経路について少しく考察を試みた．

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