INGESTION OF *FASCIOLA GIGANTICA* METACERCARIAE BY THE INTERMEDIATE HOST SNAIL, *LYMNAEA OLLULA*, AND INFECTIVITY OF DISCHARGED METACERCARIAE

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Abstract. The rate of ingestion of *Fasciola* normal metacercariae (NMc) encysted on plants by *Lymnaea ollula* was examined, and the infectivity of the ingested metacercariae (IMc) in the feces of the host snail to mice was studied. As a result of ingestion by snails, the metacercarial outer cyst disappeared in about 50% of IMc in feces. There was no significant difference in the liver juvenile recovery at autopsy between mice inoculated with NMc and IMc kinds of metacercariae. Compared with NMc, the number of IMc could more easily be counted, because the separation of IMc from fecal contents under a microscope was not laborious.

INTRODUCTION

Fascioliasis due to *Fasciola* infection is not confined to ruminant, such as cattle. Instead, like several types of helminthiasis, it may be a zoonosis, *ie* an infection or disease naturally transmitted between man and other animals. It is well known that a principal source of *Fasciola* infection in most countries is grazing on green grass in pastures contaminated with *Fasciola* metacercariae (normal metacercariae, NMc). In Japan, most cattle are given a large quantity of the stems of harvested rice plants, *Qryza sativa*, year round. A rice field filled with water is a favorable habitant for the life of *Lymnaea ollula*, an intermediate host snail for *Fasciola gigantica* (Hashimoto *et al*, 1997), during early summer to early autumn. The feeding of rice plants contaminated with NMc of the fluke is a main source of *Fasciola* infection in domestic animals.

Examination of the behavior of the cercariae of *F. gigantica* under laboratory conditions demonstrated ingestion of NMc by the intermediate host snails and the presence of NMc in the feces of the snails.

Few studies have been carried out on the viability of *Lymnaea* snail-ingested *Fasciola* NMc (IMc) (Kendall and McCullough, 1951; Taylor and Parfitt, 1957; Yadav and Gupta, 1988). An attempt was made to determine the role of the IMc present in snail feces in *Fasciola* infection.

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MATERIALS AND METHODS

Snails infected with *F. gigantica*

The gallbladder was obtained from infected cattle at an abattoir in Hachiouji, Tokyo. The eggs of *F. gigantica* were separated from the bile by washing the eggs with fresh water. Intermediate host snails, *L. ollula*. which originated from Sagamiko, Kanagawa Prefecture, were utilized for the examinations. Infected snails were prepared in the laboratory according to the method described previously (Ueno and Yoshihara, 1974). A large number of cercariae that emerged from the snails 45 days after exposure to the miracidiae were examined.

Water pot used

The type of water pot used previously (Ueno and Yoshihara, 1974) was employed in the present examination. The pot with a stump of rice plant and the irrigation system are illustrated in Fig 1. For cercarial shedding, a cylindrical stainless cage, 7 cm in diameter and 25 cm in height, was also placed in the pot.

Transplantation of plants in pot

The plants used for encystment of cercariae

were rice plants, Japanese parsley and water lily. They were obtained from paddy fields and planted in pots.

Ingestion of NMc by *L. ollula*

A cylindrical cage containing 50 infected snails was placed in a pot containing a transplanted rice plant for 72 hours. Then, cercariae shedding was observed and the cercariae were observed to be encysted on the rice plant and the inside wall of the pot. The plant and snail cage were removed from the pot 72 hours later, and the number of NMc on the plant and the inside wall of the pot were counted. After that, the rice plant was transplanted again in the same position, and 40 non-infected snails were bred freely in the pot with the rice plant for 24 hours. After ingestion of the NMc by the snails, the number of NMc on the plant and the container were counted. When water lily were used instead of rice plants for encystment of cercariae, the evaluation of ingestion was carried out by the same procedure. In the case of Japanese parsley, drain 2 in Fig 1 was used in the examination.

Observation of IMc in snail feces

Almost all of the cercariae were encysted on the surface of plants and the inside wall of the pot and about half of them were ingested by snails. As a result, a large numbers of feces containing IMc fell to the bottom of the pot. Observation of the feces was performed macroscopically and microscopically.

Infectivity of IMc to mice

As shown in Table 2, ten male mice of *ddy* strain were divided into two groups, A and B. Each mouse of group A was inoculated orally with 40 NMc on a small piece of cabbage and each mouse of group B with 40 IMc. Fourteen days later, all the animals used were sacrificed under anesthesia, juvenile flukes were recovered from the livers by cutting and squeezing thin slices of the liver which were then left in warm saline for an hour before being squeezed again. Finally, all the flukes recovered were counted.

RESULTS

Rate of ingestion of NMc by *L. ollula*

Of 6,163 NMc on the inside wall of a pot, 3,216 NMc (51.9%) were ingested by the snails. Many of the NMc, half of the total, were found within the region from the surface of the water in

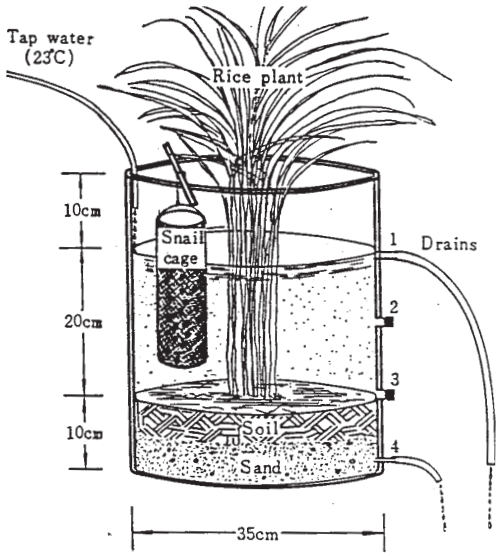

Fig 1–The water pot used in the present study.

Table 1
Ingestion of metacercariae encysted on plants in pot by *L. ollula.*

Plant used for encystment (Parts of plants encysted)	No. of metacercariae		Ingestion rate (%)
	NMc[a] (Before)	IMc[b] (After)	
Rice plant (stem)	64	34	46.5
Japanese parsley (leaf and stem)	80	41	50.6
Water lily (leaf and stem)	463	274	40.8

[a] Normal metacercariae; [b] Ingested metacercariae.

Table 2
Infectivity of ingested *Fasciola* metacercariae in snail feces to mouse.

Group	Mouse no.	No. of metacercariae inoculated		Worms recovered
		NMc[a]	IMc[b]	
A	1	40	0	7
	2	40	0	4
	3	40	0	9
	4	40	0	4
	5	40	0	5
B	6	0	40	6
	7	0	40	3
	8	0	40	14
	9	0	40	8
	10	0	40	11

[a]Normal metacercariae; [b]Ingested metacercariae.

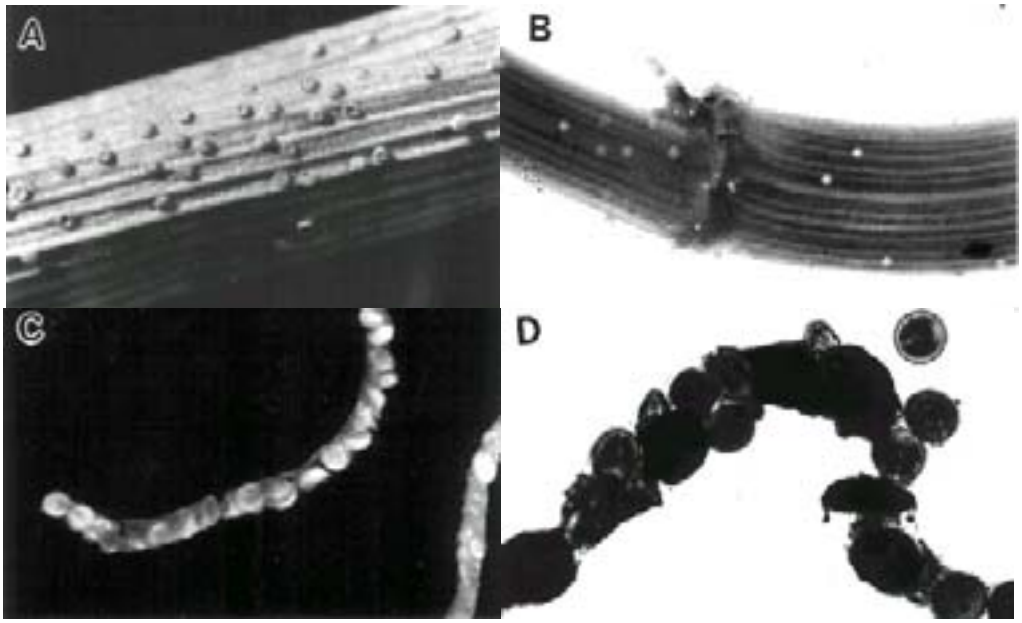

Fig 2–Metacercariae encysted on the stem of a rice plant (A) and of Japanese parsley (B). Macroscopical (C) and microscopical (D) observations of IMc in snail feces.

the pot to a level 1.6 cm below the surface of the water, and the ingestion rate was 74.4% in this area. As shown in Table 1, the rate of ingestion of NMc encysted on rice plants (Fig 2A) by *L. ollula* was 46.5%, while that on Japanese parsley (Fig 2B) was 50.6% and that on water lily was 40.8%.

Observation of IMc in snail feces

Macroscopically, IMc in the feces (Fig 2C) were white or cream colored. Microscopical findings showed that 45% of IMc in feces had lost their outer cyst (Fig 2D). Cercariae of the flukes could not be detected in the fecal samples.

Infectivity of IMc to mice

The number of juvenile flukes recovered from each group of mice is shown in Table 2. The average number of flukes obtained from the mice of group A was 5.8 and that from group B 8.4. There was no significant difference between the number of flukes collected from the mice of the two groups.

DISCUSSION

The ingestion by intermediate snail hosts, *Lymnaea* sp, has been demonstrated using *Fasciola* NMc encysted on the walls of glass vessels (Taylor and Parfitt, 1957) and the surfaces of polythene (=polyethylene) sheets (Yadav and Gupta, 1988). In the present study, the same phenomenon was observed for NMc encysted on the surface of three plants obtained from paddy fields. These plants are widely found in marshes and brooks in Japan.

No *Fasciola* cercariae were observed based on morphological criteria in microscopic observation of snail feces. In contrast, Campbell and Todd (1956) reported the presence of cercariae of *Fascioloides magna* in the feces of the intermediate host, *Stagnicola reflexa*. Kendall and McCullough (1951), who studied the relationships between *F. hepatica* and *L. truncatula*, and also described the same findings and mentioned that some of the cercariae might have penetrated the gut wall of the snails and have been expelled in the snail feces. It seems likely that the differences in the findings of the present examination and theirs may have been due to differences in the condition of the cercariae when they were encountered by the snails in the various studies. Almost all of the cercariae in the present examination had metamorphosed into NMc on the surface of the plants and the inside wall of the water pot before examination of ingestion. It is very unlikely that cercariae present on these surfaces were ingested by the snails in the present examination.

Concerning the infectivity of *F. magna* metacercariae in feces, Campbell and Todd (1956) described that since sheep were not susceptible to *F. magna*, the metacercaria in their feces could be considered indicative of the non-viability of the flukes. In order to avoid damage or destruction of the *F. gigantica* metacercariae, in another study, *L. natalensis* snails were placed in a cage consisting of a nylon net (Madsen and Monrad, 1981). Those reports suggested the harmful influence of ingestion by snails on the activity of NMc. In contrast, metacercariae of *F. hepatica* were recovered from the feces of the intermediate host, *L. truncatula* and shown to be infective to mammals (Kendall, 1965). It was reported that *F. hepatica* metacercariae found in the feces of *L. truncatula* snails are often infective to mice (Taylor and Parfitt, 1957). The results obtained in the present examination demonstrate that *F. gigantica* IMc in the feces of an intermediate snail host, *L. ollula*, are infective to mice.

One interesting finding of the present study was that examination and inoculation of mice with IMc is very easy. This may be due to the fact that IMc have lost their rough gelatinous outer cyst coat (Taylor and Parfitt, 1957). Therefore, the procedure using IMc may be useful for the inoculation of experimental animals with a precisely known number of metacercariae.

Contrary to our expectation, the rate of ingestion of NMc on the surfaces of plants was high. There are some reasons to doubt the significance of that result. Namely, the population density of the snails used for ingestion in the pot was higher than that in field conditions. Many kinds of snails, such as *Physa* sp that ingest NMc, live in paddy fields and small streams and on their banks.

Intermediate host snails play an important role in the dissemination of *Fasciola* infection in the natural environment (Yadav and Gupta, 1988). *Lymnaea* snails may be paratenic hosts in *Fasciola* infection. The results obtained here suggest that mammals, including humans, might be infected with *Fasciola* sp by drinking the water of small streams or banks contaminated with the IMc in epidemic areas.

Further studies should be performed to examine the infectivity of IMc in snail feces eaten by small fishes, small crabs, and other animals in fresh water under laboratory conditions.

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REFERENCES

Campbell WC, Todd AC. Emission of cercariae and metacercariae in snail feces. T*rans Am Micros Soc* 1956; 75: 241-3.

Hashimoto K, Watanobe T, Liu CX, *et al*. Mitochondrial DNA and nuclear DNA indicate that the Japanese *Fasciola* species is *F. gigantica*. *Parasitol Res* 1997; 83: 220-5.

Kendall BS. McCullough FS. The emergence of the cercariae of *Fasciola hepatica* from the snail *Lymnaea truncatula*. *J Helminthol*1951; 25: 77-92.

Kendall BS. Relationships between the species of *Fasciola* and their molluscan host: Advance Parasitology. Vol 3. Academic Press, 1965.

Madsen H, Monrad J. A method for laboratory maintenance of *Lymnaea natalensis* and for mass production of *Fasciola gigantica* metacercariae. *J Parasitol* 1981; 67: 735-7.

Taylor EL, Parfitt JW. Mouse test for the infectivity of metacercariae with particular reference to metacercariae of snail faeces. *Trans Am Micros Soc* 1957; 76: 327-8.

Ueno H, Yoshihara S. Vertical distribution of *Fasciola gigantica* metacercariae on stems of rice plant grown in a water pot. *Natl Inst Anim Health Q* 1974; 14: 54-60.

Yadav SC, Gupta SC. On the viability of *Fasciola gigantica* ingested by *Lymnaea auriculria*. *J Helminthol* 1988; 62: 303-4.