[Jpn. J. Parasitol., Vol. 36, No. 2, 94–99, April, 1987]

Food Preference of *Rattus rattus* for Some Terrestrial Molluscs and Experimental Infection of *R. rattus* with *Angiostrongylus cantonensis*

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(Received for publication; July 7, 1986)

Abstract

The feeding ability and preference of wild roof rat, *Rattus rattus* for four species of terrestrial molluscs were examined to clarify the importance of the molluscs in maintaining *Angiostrongylus cantonensis* in nature. Experimental infection of *R. rattus* with the parasite was also carried out.

R. rattus preferred the small molluscs, *Fruticicola despecta, Limax marginatus* and young *Achatina fulica* to adult *A. fulica* and *Laevicaulis alte.* The rats could not eat *A. fulica* more than 6 cm in shell length and starved to death. In experimental infection with *A. cantonensis,* overall recovery rate of worms was 65.9%. *R. rattus* harboring more than 90 worms died on day 27 or 28, during the period of worm migration from the brain to the pulmonary arteries. The parasite did not be matured and eggs were not found in the rats.

These facts indicate that small molluscan intermediate hosts, whose prevalence and intensity of infection with *A. cantonensis* are low, play a more important role in the transmission of the parasite in nature than heavily infected large molluscan intermediate hosts such as *A. fulica* or *L. alte.*

Key words: *Angiostrongylus cantonensis, Rattus rattus,* terrestrial molluscs, food preference, experimental infection

Introduction

It is well known that many species of terrestrial and fresh water molluscs can serve as intermediate hosts for *Angiostrongylus cantonensis* (Alicata and Jindrak, 1970; Otsuru, 1977). Among them, the giant African snail, *Achatina fulica,* and black garden slug, *Laevicaulis alte,* are widely spread and seem to be suitable intermediate hosts because of their high prevalence and intensity of infection with *A. cantonensis.* The molluscs are recognized as an important source for human angiostrongyliasis, because they harbor many *A. cantonensis* larvae and have many chances to contact with human (Alicata and Jindrak, 1970; Sato and Otsuru, 1983). However, the importance of the molluscs in maintaining the life cycle of the parasite is doubtful. The ingestion of heavily infected molluscs may have a lethal effect on rats in nature (Wallace and Rosen, 1969).

In the present study, the feeding ability and preference of wild roof rat, *Rattus rattus,* for four species of terrestrial molluscs were examined to clarify the role of the molluscs in the life cycles of *A. cantonensis* in nature. Experimental infection of *R. rattus* with the parasite was also carried out.

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This study was supported by a Grant-in-Aid for Scientific Research from the Japanese Ministry of Education, Science and Culture (No.57570161). The publication of this paper was supported by Kodama Memorial Fund for Medical Science Research.

Materials and Methods

Ability of R. rattus *to eat* A. fulica

R. rattus and *A. fulica* captured on Amami-oshima Island were used for the experiment. Four adult *R. rattus* (♂2, ♀2; 85–120 g in body weight) were separated by sex and kept in two wire cages at room temperature (25–30°C). Fifteen *A. fulica* were offered with water to each cage as food. Three snails, five, four and three were 2.0–2.9 cm, 3.0–4.9 cm, 5.0–5.9 cm and longer than 6 cm in shell length, respectively. Then ability of the rats to eat *A. fulica* was observed.

Preference of R. rattus *for terrestrial molluscs*

Nine *R. rattus* (♂3, ♀6; 53–130 g in body weight) were captured on Yoron Island to examine their food preference. Four species of terrestrial molluscs, *A. fulica, Fruticicola despecta, L. alte* and *Limax marginatus,* were collected on Yoron Island, Okinoerabu Island and Kagoshima City. These four species were recognized as a natural intermediate host for *A. cantonensis* in the Nansei Islands, Japan (Otsuru, 1977). The rats were individually kept in wire cages at room temperature (25–30°C), and after fasting for 48 hours each rat was given three molluscan species according to following three combinations (Table 1); experiment I: two *A. fulica* (6 cm in shell length), two *F. despecta* (2 cm in shell width) and two *L. alte* (4–5 cm in length), Experiment II: five *F. despecta* (2 cm), five *L. alte* (4–5 cm) and five *L. marginatus* (3 cm in length), and Experiment III: five *A. fulica* (3 cm), five *F. despecta* (2 cm) and five *L. marginatus* (3 cm). Additionally, 50 *F. despecta* (2 cm) were given in the same manner (Experiment IV), and five *A. fulica* (2 cm) and five *F. despecta* (2 cm) were offered with commercial food pellets (Experiment V). The number of molluscs eaten was recorded 20 hours later. After each experiment the rats were individually fed commercial food pellets for 3–7 days (depending on the nature of the experiment) by which time it was judged that the rats would be free from the effects of that experiment and ready for the next.

Experimental infection of R. rattus *with* A. cantonensis

Eight males and five females of 6-week-old *R. rattus* (44–82 g in body weight) taken from three pregnant females captured on Yoron Island, were orally injected with third-stage larvae of *A. cantonensis* using a stomach tube. The larvae used were isolated from experimentally infected *Biomphalaria glabrata,* and 20, 50, 100 and 200 larvae per rat were given to 4, 4, 2 and 3 rats, respectively. The rats were dissected to detect adult and larval worms on the day when rats died, or on the 53rd day after infection.

Results

Ability of R. rattus *to eat* A. fulica

In one cage three *A. fulica* shorter than 3 cm were eaten within two days and four snails 5.0–5.9 cm in shell length were eaten by day 5. However, the rats were unable to eat snails longer than 6 cm. Exactly the same results were obtained from the other cage. The rats died from starvation in one cage (male) on the seventh and eighth days and in the other cage (female) on eighth and ninth days.

Preference of R. rattus *for terrestrial molluscs*

The number of molluscs eaten by each *R. rattus* is shown in Table 1. Though one *A. fulica* (6 cm) was eaten partially and several *L. alte* were eaten, the rats preferred small species, *F. despecta* and *L. marginatus,* to *A. fulica* and *L. alte* (Exp. I and II). Young *A. fulica* (3 cm) were much preferred to *L. alte* and less to the small species (Exp. II and III). Four out of five rats ate more than 40 *F. despecta* within 20 hours (Exp. IV). Under the presence of commercial food pellets, the rats ate *F. despecta* and young *A. fulica* (2 cm) equally in Experiment V. There were no differences in the preferences between male and female rats, and between rats infected with *A. cantonensis* and uninfected rats.

Table 1 Number of four molluscan species, *Achatina fulica, Fruticicola despecta, Laevicaulis alte* and *Limax marginatus*, eaten by *Rattus rattus*

Species of mollusc (Size)	No. of molluscs given per rat	No. of molluscs eaten									
		Rat no. 1‡	2	3	4‡	5	6	7	8	9	Total*
		Sex ♂	♀	♀	♀	♂	♀	♀	♀	♂	
Experiment I :											
A. fulica (6 cm)	2	0	0	0	0	0	0	0	0	1†	1/18
F. despecta (2 cm)	2	2	2	1	1	1	1	0	1	2	11/18
L. alte (4-5 cm)	2	0	0	2	2	0	1	0	0	1	6/18
Experiment II :											
F. despecta (2 cm)	5	5	3	3	4	4	4	—	—	—	23/30
L. alte (4-5 cm)	5	0	1	2	0	0	0	—	—	—	3/30
L. marginatus (3 cm)	5	4	3	3	3	5	5	—	—	—	23/30
Experiment III :											
A. fulica (3 cm)	5	2	3	3	1	4	—	0	3	—	16/35
F. despecta (2 cm)	5	4	5	4	4	5	—	1	5	—	28/35
L. marginatus (3 cm)	5	5	5	5	5	5	—	5	5	—	35/35
Experiment IV :											
F. despecta (2 cm)	50	47	49	50	49	24	—	—	—	—	219/250
Experiment V : (With commercial food pellets)											
A. fulica (2 cm)	5	3	0	1	4	0	—	0	0	—	8/35
F. despecta (2 cm)	5	0	2	2	1	2	—	0	1	—	8/35

*: No. eaten/no. given †: partially eaten ‡: Infected with *Angiostrongylus cantonensis*

Table 2 Number of *Angiostrongylus cantonensis* recovered from experimentally infected *Rattus rattus*

Rat no. (Sex)	No. of larvae given	Days after injection	No. of worms recovered from				Egg-laying in pulmonary arteries
			Brain	Heart	Pulmonary arteries	Total (%)†	
1 (♂)	20	53	0	0	3	3 (15)	Yes
2 (♂)	20	53	0	0	18	18 (90)	Yes
3 (♀)	20	53	0	0	19	19 (95)	Yes
4 (♀)	20	53	0	0	17	17 (85)	Yes
5 (♂)	50	53	0	0	27	27 (54)	Yes
6 (♂)	50	53	1	0	36	37 (74)	Yes
7 (♀)	50	53	0	0	32	32 (64)	Yes
8 (♀)	50	53	0	0	21	21 (42)	Yes
9 (♂)	100	27*	36	43	13	91 (91)	No
10 (♀)	100	53	0	2	53	55 (55)	Yes
11 (♂)	200	27*	93	27	7	127 (64)	No
12 (♂)	200	28*	61	34	55	140 (70)	No
13 (♂)	200	28*	47	44	34	125 (63)	No

*: The day when rat died †: Percent recovery

Experimental infection of R. rattus *with* A. cantonensis

All *R. rattus* harbored *A. cantonensis* and overall recovery rate of worms was 65.9%. The number of worms recovered from each rat is shown in Table 2. The recovery rate in eight rats injected with 20 or 50 larvae (44–82 g in body weight) 15–95% on the 53rd day after infection. The worms were found in the pulmonary arteries except one male worm in the brain of rat No. 6 receiving 50 larvae. In these eight rats, maturation of worms and egg-laying were observed and first-stage larvae were detected in their feces. Among five rats given 100 or 200 larvae, only one female rat (No. 10) receiving 100 larvae was alive at the end of experiment (53 days after infection). It harbored 55 worms in its heart and pulmonary arteries and first-stage larvae were also observed in the feces. Four male rats (48–82 g in body weight) died on days 27 or 28, harboring more than 90 worms in the brain, heart and pulmonary arteries. Worms in these rats did not mature or lay eggs. Paralysis and heavy hemorrhage in the surface of brain were observed in the rats, while there were no macroscopical changes in their lungs.

Discussion

Since Nishimura *et al.* (1964) first found *A. cantonensis* in *R. norvegicus* on Iriomote Island, many epidemiological studies on the parasite have been carried out in the Nansei Islands, Japan (Otsuru, 1977). *A. fulica* and *L. alte* seemed to be suitable intermediate hosts because of their high prevalence and intensity of infection with *A. cantonensis,* and *A. fulica* was suspected as the source of infection in nine cases of human angiostrongyliasis and *L. alte* in two out of 16 cases in the Nansei Islands (Sato and Otsuru, 1983). As final host, *R. rattus* is dominant in the field and is suspected of playing a major role in prevalence of the parasite in some areas of the islands (Otsuru, 1977; Sato *et al.*, 1980). However, considering the abundance of *A. fulica* and a large number of the infective larvae in the snails, the number of worms detected from wild rats was too small (Noda *et al.,* 1987). Wallace and Rosen (1969) pointed out that lethal heavy infections commonly occurred in rats in nature, or that other rats possibly escape death by 1) feeding on molluscan species that were less heavily infected, 2) feeding on young individuals of heavily infected species, or 3) feeding on small portions of adults of heavily infected species.

In the present experiments, *R. rattus* could not eat *A. fulica* of more than 6 cm in shell length, and starved to death. *R. rattus* preferred small molluscs, *F. despecta, L. marginatus* and young *A. fulica,* to adult *A. fulica* or *L. alte.* Lim (1966) experimentally offered Malaysian molluscs to five *Rattus* species as food and reported that *Macrochlamys resplendens* (land snail) was most favored, with *Microparmarion malayanus* (slug) next in preference, and that the heavily shelled *A. fulica* was occasionally eaten but never completely eaten.

The worm burden in small molluscan species including *F. despecta* and *L. marginatus* was lower than that in *A. fulica* or *L. alte* (Wallace and Rosen, 1969; Hori *et al.,* 1973b; Noda *et al.,* 1982). The difference in the number of larvae among snail species may be due to behavior and life span of each species. Although young *A. fulica* are also susceptible to the first-stage larvae of *A. cantonensis* (Hori and Yamaguchi, 1982), the prevalence and intensity of infection with the parasite in young *A. fulica* were low, and then markedly increased in the snail more than 5 cm in shell length (Wallace and Rosen, 1969; Hori *et al.,* 1973b; Noda *et al.,* 1987). Matayoshi *et al.* (1980) investigated the growth of *A. fulica* on Yoron Island an Amami-oshima Island. They suspected that the smaller number of worms in young *A. fulica* was due to few opportunities for contact with the first-stage larvae, because it took only 3–4 months to grow up from young (2–3 cm) to adult (5–7 cm) in the summer. Further, Noda *et al.* (1985) reported that the prevalence of infection with *A. cantonensis* in *A. fulica* released in the endemic area was lower than that in the native *A. fulica* even 160 days after release. Therefore they concluded that *A. fulica* rarely contacted with the first-stage larvae of *A. cantonensis*.

These facts suggest that rats prefer small molluscs including young *A. fulica,* which harbor a small number of the larvae, and that they cannot break, in nature, solid shells of *A. fulica* longer than 6 cm in which the prevalence and intensity of infection with *A. cantonensis* are high.

In experimental infection, 65.9% of injected *A. cantonensis* were recovered from *R. rattus,* and the recovery rate was the same as in laboratory rats (Hori *et al.,* 1973a; Sato *et al.,* 1980). Lim *et al.* (1965) reported that laboratory white rats and laboratory-bred *R. rattus jarak* receiving 600 *A. cantonensis* third-stage larvae each, died after about 20 and 24 days, respectively. On the other hand, Nishimura (1966) reported that laboratory rats infected with 100 to 700 third-stage larvae died mostly in the period of from 27 to 40 days after infection and the death was considered mostly due to heart failure caused by adult worms stenosing or obstructing the pulmonary arteries. In the present study, *R. rattus* harboring more than 90 worms died on days 27 or 28, at the migration of the worms from the brain to the pulmonary arteries. As all dead rats had heavy hemorrhage in the brain and paralysis, hemorrhage or disorder in the central nerve system may be one of the cause of death. The parasite in such hosts did not mature sexually.

Although heavily infected molluscs such as *A. fulica* and *L. alte* are a possible source of human angiostrongyliasis, the importance of the large species in the transmission of the parasite to rats in nature is not clear. The facts mentioned above indicate that small-sized molluscan intermediate hosts, whose prevalence and intensity of infection with *A. cantonensis* were low, play a more important role in the transmission of the parasite in nature than large-sized molluscs. There is the possibility that *A. cantonensis* will be highly prevalent in rats and native molluscs in any areas where the large molluscs are not introduced. In fact, *A. cantonensis* infection is highly endemic in rats and *L. marginatus* and *Deroceras varians* (slugs) in harbor side areas of Tokyo (Hori *et al.,* 1972, 1973a). More attentions should be paid on small native molluscs to study plevalence of *A. cantonensis* in future.

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