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Earthworms as a Transport Host of the Rat Cecal Worm, *Heterakis spumosa*

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Abstract

Larval nematodes obtained from earthworms *Pheretima hilgendorfi* in the field were fed to chickens, Japanese quails and rats. Adult worms were recovered from rats alone and were identified as *Heterakis spumosa*. To determine the role of earthworms as a transport host of the nematode, another species of earthworms, *Eisenia foetida*, were fed with embryonated eggs and examined for nematode larvae with time after ingestion. The number of larvae in *E. foetida* was almost the same from day 20 to 60 after infection. Larvae invaded into the epithelial tissue of digestive canal and were larger than those in embryonated eggs in length and width by 128 μm and 6 μm respectively on day 35 after infection. Larval nematode recovered from earthworms had higher infectivity to rats of advanced eggs than the embryonated eggs.

Key words: *Heterakis spumosa*, earthworms, rat, transport host

Introduction

The rat cecal worm, *Heterakis spumosa* Schneider, 1866, is worldwide in distribution and parasitizes rats in the cecum and colon. The nematode has the direct life cycle (Winfield, 1933; Smith, 1953), but is not known to utilize a transport host although another species of *Heterakis*, the chicken cecal worm *H. gallinarum*, is known to utilize earthworms as a transport host (Ackert, 1917; Lund *et al.*, 1966; Anderson, 1992).

Larval nematodes obtained from earthworms, *Pheretima hilgendorfi*, were identified and the role of earthworms as a transport host was determined using the earthworms, *Eisenia foetida*, by feeding them with embryonated eggs.

Materials and Methods

Earthworm: Two species of earthworms were used in the present experiment. Earthworms of *P. hilgendorfi*, collected in the field of Yokohama city harbored the larval nematodes, most likely belonging to the genus *Heterakis*. Earthworms, *E. foetida*, that used the experimental infection were collected in the campus of Azabu University, Kanagawa. Because this species of earthworm can be easily raised in the laboratory. Before the experiments earthworms were kept on wet filter paper for one day to take off free-living soil nematodes adhere to the body surface and to free the digestive canal of fecal contents. And 100 earthworms collected same place of the campus were examined for endoparasitic larval nematodes and confirmed they were free from such larvae.

Parasite: Larval nematodes obtained from earthworms, *P. hilgendorfi*, were fed to each of 5 male chickens, Japanese quails, and Wister rats of 15, and 28 days old, respectively. All animals used were killed by anesthetic after 37 days of ingestion and adult nematodes were recovered only from rats.

Embryonated egg: Eggs of the *H. spumosa* were obtained from the feces of the artificially infected rats by the flotation method with saturated NaCl solution on days 30 to 37 after infection. These eggs were incubated in 1% formalin at 25°C for 14 days to make embryonated.

Food for earthworm: Food for earthworms was

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prepared as follows: moistened pellets for rats were completely fermented, thoroughly screened and washed with tap water until they lose the odor. The resultant was dried and pulverized.

Experimental infection of earthworms with embryonated eggs: Ten earthworms, *E. foetida*, were kept at 25°C in each plastic container with a lid 16×12×4 cm in size, on a wet piece of filter paper on the bottom. They were allowed to feed on 250 mg of feed containing 3000 embryonated eggs for 3 to 5 days. After feeding earthworms were removed to and kept in another plastic container with clean wet peat moss on the bottom to about a half of the depth.

Habitat of larvae in earthworms: Ten earthworms each were examined on days 10, 20, 30, 40, 50 and 60 after the start of feeding by dissecting them under the stereoscopic microscope. Earthworms were divided into three parts of body, the anterior, middle, and posterior, and the parts were individually examined for larvae to determine the distribution of larvae in earthworm. Five days after the start of feeding ten infected earthworms were fixed in 70% ethanol, sectioned and stained with hematoxylin and eosin to confirm the habitat of larvae.

Development of larvae in earthworms: Larvae recovered from the infected earthworms by the needle under the stereoscopic microscope and fixed in 5% hot formalin were measured with an Abbe's drawing apparatus.

Infectivity of the larvae recovered from the earthworm to Wister rats: On days 35 after the feeding of the embryonated eggs, lively larvae in the earthworms recovered same way as mentioned above were stored in 0.4% NaCl solution. Within an hour, these 100 lively larvae were fed to five male Wister rats each of 4, 6 and 10 weeks olds, whereas control rats were given 100 embryonated eggs.

Statistical comparisons: Student's *t* test was used.

Results

Morphology of the nematode: Adult worms of both sexes had three lips with two papillae each and prominent cervical alae. Male worms were 8.3–11.2×0.3–0.5 mm in size and the esophagus including the bulb was 928.4 μm in average length. The caudal alae were prominent and the round preanal sucker, 115.0 μm in average diameter, was situated anterior to the cloacal opening. The papillae were comprised of 2 and 8 pairs of the adanal and the caudal ones respectively and a floriform structure with many minute processes was located between the preanal sucker and cloacal opening. Both spicules were equal in length. Female worms were 9.6–16.0×0.3–0.6 mm in size and the esophagus including the bulb had an average length of 993.0 μm. The vulva opened on the midline of body at an average distance of 6.6 mm from the anterior end of body and 2 and 3 characteristic processes were situated anterior and posterior to the vulva, respectively. Eggs were 63.0–78.0×46.0–54.0 μm in size (Table 1).

Experimental infection of earthworms with embryonated eggs:

1) *Passage of larval nematodes into earthworm feces*: For 5 days after feeding of earthworms with nematode eggs, all the feces passed by earthworms were examined every day and a total of 38 embryonated eggs (74.5%), 11 egg shells (21.6%), and 2 larvae (3.9%) were recovered.

2) *Habitat of larvae in earthworms*: On days 10, 20, 30, 40, 50 and 60 after feeding, 565, 166, 244, 177, 167 and 234 larvae were recovered from earthworms, respectively. Of all the 1553 larvae, 98.9% were recovered from the middle part of body (Table 2). Histological examination revealed that larvae invaded between the epithelial cells of intestine on day 5 after exposure (Figs. 1 and 2).

3) *Development of larvae in earthworms*: Larvae were 365.2–387.6×14.6–22.5 (370.2×18.8 on average) μm in size on day 35 after ingestion and were larger than those developed in eggs, which measured 231.2–274.2×11.3–15.1 μm with an average of 242.4×12.5 μm, in average length and width by 128 and 6 μm, respectively (Table 3).

4) *Infectivity of recovered larvae to rats*: Average recovery rates of adults worms were 12.4%, 10.2% and 8.0% from rats of 4, 6 and 10 week old respectively and no significant differences existed in the rate between different age groups of rat.

Table 1 Comparison of measurements of *H. spumosa* by different authors (mm)

	Yamaguti (1954)	Present author*
Male		
body length × width	9.3–10.2×0.4–0.45	8.30–11.20×0.33–0.46
oesophagus (including pharynx and bulb)	0.9 –0.97	0.89–0.97
nerve ring (from head end)	0.27 –0.28	0.25–0.30
cervical papillae (from head end)	0.45	0.43–0.56
excretory pore (from head end)	0.35 –0.41	0.36–0.41
tail length	0.32 –0.36	0.33–0.36
preanal sucker (diameter)	0.105–0.11	0.11–0.13
spicules length	0.34 –0.37	0.34–0.38
Female		
body length × width	9.5–15.0×0.35–0.5	9.60–16.00×0.43–0.57
oesophagus (including pharynx and bulb)	0.875–1.025	0.95–1.05
nerve ring (from head end)	0.27 –0.33	0.28–0.36
cervical papillae (from head end)	0.47	0.47–0.50
excretory pore (from head end)	0.33 –0.4	0.37–0.43
tail length	0.77 –0.95	0.77–1.00
vulva (from head end)	4.6 –6.7	5.20–7.20
egg (μm)	63–73×42–50	63.0–78.0×46.0–54.0 (67.9×49.4)†

* Twenty five male and 25 female worms were measured.
† Average values in parentheses (n=50)

Table 2 Number of larvae parasitic in earthworms, *E. foetida*, fed with embryonated eggs

Days after feeding	Portion of body of earthworm*			Total	No. of larvae per earthworm (±SE)
	anterior	middle	posterior		
10	1	558	6	565	56.5±26.7
20	1	165	0	166	16.6± 6.7
30	4	240	0	244	24.4±10.5
40	0	176	1	177	17.6± 4.2
50	1	166	0	167	16.7± 5.7
60	3	231	0	234	23.4±10.9

*The body of earthworms was divided into 3 parts with equal length.
Ten earthworms were used in each examination.

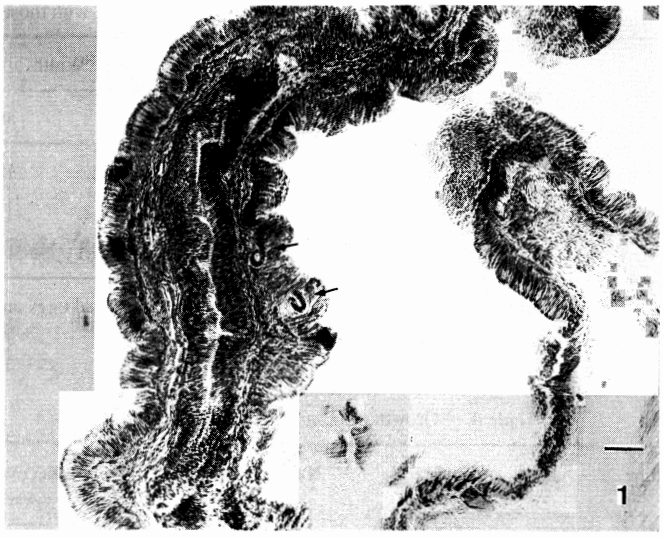

Fig. 1 *H. spumosa* larvae (arrows) in an earthworm, *E. foetida*, 5 days after feeding earthworms to embryonated eggs. Bar=100 μm.

Fig. 2 Larvae invading among the epithelial cells of digestive canal (Magnification of Fig. 1). Bar=100 μm.

Average recovery rates of adult worms in control rats were 12.2% and 8.4% in 4 and 6 week age groups, respectively, and no worm was recovered from the 10 week old group (Table 4).

Discussion

Adult worms obtained from rats colon, coinciding with *H. spumosa* Yamaguti (Yamaguti, 1954) in the morphology and measurements. Larvae of the

Table 3 Comparison of *H. spumosa* larvae in earthworms, *E. foetida*, with those in eggs

Larvae	Length		Width	
	range	ave.±SE	range	ave.±SE
In earthworm	365.2–387.6	370.2±1.2	14.6–22.5	18.8±0.5
In embryonated egg	231.2–274.2	242.4±1.4	11.3–15.1	12.5±0.1

Measurement was made on 25 and 50 larvae obtained from embryonated eggs and earthworms 35 days after feeding of eggs respectively.

Table 4 Growth of *H. spumosa* larvae in rats

Inoculum	Age of rat (in week)	Number of worms recovered	Recovery rate (Ave.±SE)
Larvae from earthworm	4	8,10,13,15,16	12.4±1.5
	6	7, 8, 9,10,17	10.2±1.8
	10	4, 5, 9,11,11	8.0±1.5
Embryonated eggs	4	8, 9,11,16,17	12.2±1.8
	6	5, 6, 8,11,12	8.4±1.4
	10	0, 0, 0, 0, 0	0

Five rats were used in each group of experiment and each rat received 100 larvae from earthworms or 100 embryonated eggs.

gapeworm *Syngamus trachea* in earthworms, the transport host, are highly infective to fowl than those not transmitted by earthworms (Clapham, 1934). *H. spumosa* larvae recovered from earthworms also were more highly infective to rats of advanced age, 10 weeks old, than larvae in eggs. These results show that earthworms play an important role in transmission of the rat cecal worm as well as the gapeworm.

H. spumosa is commonly parasitic in wild Norway rats, *Rattus norvegicus* (Kamiya *et al.*, 1971, Uga *et al.*, 1983), but is also recorded from other species of rat. Food chain must be established between transport and final hosts to complete the life cycle of the parasites, and the infection rate with the nematode varies according to the presence of transport host. Earthworms have been detected from the stomach of Norway rats in Atsugi city and Miyake Island of the Izu islands where rats live on earthworms, but the relation between the food habit of rats and the infection rate of rat cecal worms was not mentioned in the report (Yabe, 1979). In the Ogasawara islands where environmental conditions are favorable for roof rats, *Rattus rattus*, and rats live on trees or on both ground and trees, no earthworm was detected from the stomach and the infection rate with the cecal worm was only 1% (Yabe and Matsumoto, 1982). On the other hand, in Torishima Island with hard environmental conditions where roof rats live not on trees but on the ground, earthworms were recovered from the stomach of rat and the infection rate of cecal worms reached 54% (Yabe, 1982). These findings show that cecal worms are found at higher infection rate in rats which live on earthworms without respect to the species of rat.

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