ISSN (Print) 0023-4001
ISSN (Online) 1738-0006

ORIGINAL ARTICLE

Korean J Parasitol Vol. 51, No. 5: 531-536, October 2013
http://dx.doi.org/10.3347/kjp.2013.51.5.531

Tetrathyridia of *Mesocestoides lineatus* in Chinese Snakes and Their Adults Recovered from Experimental Animals

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Abstract: Morphological characteristics of *Mesocestoides lineatus* tetrathyridia collected from Chinese snakes and their adults recovered from experimental animals were studied. The tetrathyridia were detected mainly in the mesentery of 2 snake species, *Agkistrodon saxatilis* (25%) and *Elaphe schrenckii* (20%). They were 1.73 by 1.02 mm in average size and had an invaginated scolex with 4 suckers. Adult tapeworms were recovered from 2 hamsters and 1 dog, which were orally infected with 5-10 larvae each. Adults from hamsters were about 32 cm long and those from a dog were about 58 cm long. The scolex was 0.56 mm in average width with 4 suckers of 0.17 by 0.15 mm in average size. Mature proglottids measured 0.29 by 0.91 mm (av.). Ovaries and vitellaria bilobed and located in the posterior portion of proglottids. The cirrus sac was oval-shaped and located median. Testes were follicular, distributed in both lateral fields of proglottids, and 41-52 in number per proglottid. Gravid proglottids were 1.84 by 1.39 mm (av.) with a characteristic paruterine organ. Eggs were 35 by 27 μm in average size with a hexacanth embryo. These morphological characteristics of adult worms were identical with those of *M. lineatus* reported previously. Therefore, it has been confirmed that the tetrathyridia detected in 2 species of Chinese snakes are the metacestodes of *M. lineatus*, and 2 snake species, *A. saxatilis* and *E. schrenckii*, play the role of intermediate hosts.

Key words: *Mesocestoides lineatus*, tetrathyridium, Chinese snakes, *Agkistrodon saxatilis*, *Elaphe schrenckii*

INTRODUCTION

Mesocestoides spp. are members of the tapeworm order Cyclophyllidea. However, they have some different characters from other groups of cyclophyllideans. In their life cycle, 3 hosts including 2 intermediate and 1 definitive hosts, are required. Adult worms live in the small intestines of carnivorous mammals, i.e., the dog, cat, fox, wolf, coyote, jackal, raccoon, badger, lynx, and some species of wild felines [1-11], and rarely birds and humans [12-17]. The tetrathyridium, the metacestode of *Mesocestoides* spp., is found in the abdominal cavity of a great variety of intermediate hosts, such as amphibia, reptiles, birds, and mammals [18,19]. Meanwhile, the role of coprophagic arthropods as its first intermediate host is presumed as Padgett and Boyce [20] detected *Mesocestoides* sp. DNA from ants. There were experimental trials to infect insects with eggs; however, enough evidence has not yet been obtained [21]. Morphologically, the median ventral position of the genital atrium and bipartite vitelline gland in these tapeworms are unique and differ from other groups of cyclophyllideans [22].

In the Republic of Korea (=Korea), Kobayashi [23] reported adult *Mesocestoides* infection from dogs in Seoul in 1928. Thereafter, Cho et al. [24] in 1982 detected tetrathyridia in a snake, *Elaphe rufodorsata*, from Gangwon-do [24]. Two cases of human infections with adult *Mesocestoides lineatus* were reported by Choi et al. [25] and Eom et al. [16]. However, there have been few studies on the life cycle of *Mesocestoides* tapeworms in Korea. Moreover, there is no information on biological relationships between the tetrathyridia in intermediate hosts and the adults in definitive hosts. In the present study, we succesfully obtained adult tapeworms of *M. lineatus* from 2 hamsters and a dog which were experimentally infected with tetrathyridia collected from Chinese snakes. The morphological characteristics of the tetrathyridia and adult tapeworms are described herein.

• Received 11 June 2013, revised 24 July 2013, accepted 19 August 2013.
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MATERIALS AND METHODS

A substantial number of snakes obtained from the Incheon customs officer were transferred to the Parasitology Laboratory of Gyeongsang National University School of Medicine in November, 2003. Among them, 421 snakes which included *Agkistrodon saxatilis* (n=60), *Elaphe schrenckii* (n=80), *Dinodon rufozonatum* (n=120), *Agkistrodon brevicaudus* (n=111), and *Elaphe davidi* (n=50) were examined to detect parasitic helminths including tetrathyridial larvae (Table 1). The muscle and viscera of each snake were isolated after skinned off and artificially digested with pepsin-HCl solution in an incubator at 36°C for 2-5 hr. The digested material was washed with 0.85% saline until the supernatant became clear, and the sediments were examined under a stereomicroscope. Some collected tetrathyridia were fixed in 10% formalin under a cover glass pressure, and 5-10 larvae per animal were orally infected to 3 rats, 5 hamsters, 2 cats, and 2 dogs to obtain the adult *Mesocestoides* tapeworm. About 1 month later, experimental animals were killed after anesthesia, and their intestines were isolated and longitudinally opened with a pair of scissors in a beaker with 0.85% saline. The intestinal contents of each animal were washed with 0.85% saline until the supernatant cleared, and the sediments were examined with naked eyes and under a stereomicroscope. The adult tapeworms were fixed in 10% formalin under a slide glass pressure. The tetrathyridia and adults fixed with 10% formalin were stained with Semichon's acetocarmine and observed using a light microscope with a micrometer.

In order to observe the surface ultrastructure of the tetrathyridium, some worms were washed several times in 0.2 M cacodylate buffer (pH 7.2) and fixed in 2.5% glutaraldehyde at 4°C. After washing 3 times with the same buffer, they were dehydrated through a graded alcohol series (50%, 70%, 80%, 90%, 95%, and absolute alcohol), dried in a critical point dryer, coated with gold in the JFC-1100E ion sputtering device (JEOL, Tokyo, Japan), and observed using a scanning electron microscope (Philips XL-30S, Amsterdam, Netherlands) with an accelerating voltage of 20 kV.

Table 1. Snakes examined in this study

Species of snake	No. of snakes examined	Length (cm) Range (average)	Weight (g) Range (average)
Agkistrodon saxatilis	60	62-82 (72.4)	93-273 (152.0)
Agkistrodon brevicaudus	111	44-65 (55.2)	26-94 (59.6)
Dinodon rufozonatum	120	84-105 (93.8)	73-253 (143.0)
Elaphe davidi	50	65-87 (79.6)	58-90 (74.5)
Elaphe schrenckii (Y)[a]	40	148-195 (155.8)	688-1,354 (824.0)
Elaphe schrenckii (B/W)[b]	40	122-161 (138.8)	237-633 (436.9)

[a]Yellow Amur ratsnake; [b]Black and white Amur ratsnake.

RESULTS

Infection status of Chinese snakes with tetrathyridia and adult worm recovery

Tetrathyridial larvae were collected from 15 (25%) *A. saxatilis*, 12 (30%) *E. schrenckii* (yellow), and 4 (10%) *E. schrenckii* (black/white). They were mainly detected in the mesentery of snakes, and their infection status was as shown in Table 2. No tetrathyridia were found in *D. rufozonatum*, *A. brevicaudus*, and *E. davidi*. Adult tapeworms were recovered from 2 (40%) hamsters and 1 (50%) dog. No worms were detected from 3 rats, 3 hamsters, 2 cats and 1 dog, which were experimentally infected with tetrathyridia.

Morphology of *Mesocestoides lineatus* tetrathyridia and adults

Tetrathyridia of *M. lineatus* were oval or elongated with a somewhat pointed posterior end, slightly constricted at the anterior portion, longer than wide, 1,000-3,750 (1,730 in average) by 680-2,050 (1,020 in average) µm in size. They had an invaginated scolex with 4 suckers in the anterior constricted portion (Fig. 1).

Adult worms from hamsters were about 32 cm (n=3) and from a dog was 58 cm (n=1) in length respectively. Scolices were transversely oval or slightly quadrigonal in shape and 480-680 (560) µm in width. They had 4 cup-like suckers of 160-180 (173) by 135-165 (150) µm size, without a rostellum (Fig. 2A). Immature proglottids were wider than long and had no genital organs. Mature proglottids were also wider than long and 260-320 (290) by 730-1,210 (910) µm in size. Ovaries and vitellaria were bilobed and located in the posterior portion of the mature proglottid, and were 93-108 (102) by

Table 2. Infection status of Chinese snakes with tetrathyridia of *M. lineatus*

Species of snake	No. of snakes examined	No. (%) of snakes infected	No. of larvae detected: Total	Range	Average
Agkistrodon saxatilis	60	15 (25.0)	547	1-92	36.5
Elaphe schrenckii (Y)	40	12 (30.0)	309	1-87	25.8
Elaphe schrenckii (B/W)	40	4 (10.0)	7	1-3	1.8

45-75 (60) and 70-88 (79) by 60-70 (64) µm in size, respectively. The cirrus sac was oval-shaped, 75-95 (86) by 55-70 (61) µm in size and located median. Testes were follicular, 55-75 (61) by 38-50 (42) µm in size and distributed in both lateral fields of proglottids, and 41-52 in number per proglottid (Fig. 2B, C). Gravid proglottids were longer than wide, 1,550-2,400 (1,840) by 1,280-1,700 (1,390) µm in size, and had a characteristic paruterine organ of 530-600 (578) by 440-550 (481) µm in size (Fig. 2D). Eggs were elliptical with thin shell and a hexacanth embryo, and 33-36 (35) by 25-28 (27) µm in size (Fig. 2E).

Surface ultrastructure of tetrathyridia

Tetrathyridia were elongated with a somewhat pointed posterior end and had an invaginated scolex at the anterior constricted portion. The whole body surface was covered with numerous microtriches (Fig. 3A). The evaginated scolex with 4 cup-like suckers and the neck portion were also covered with microtriches (Fig. 3B). Microtriches on the tegument were somewhat morphologically different according to the body locations. Long filamentous micritriches were distributed at the inner portion of suckers (Fig. 3C). Numerous hair-like microtriches were compactly distributed between suckers in the scolex (Fig. 3D). More or less stouter microtriches were seen on the tegument just below the scolex and neck portion (Fig. 3E). On the surface of the posterior body, except for the scolex and neck, somewhat short microtriches were distributed and their length and density were decreased posteriorly (Fig. 3F-H).

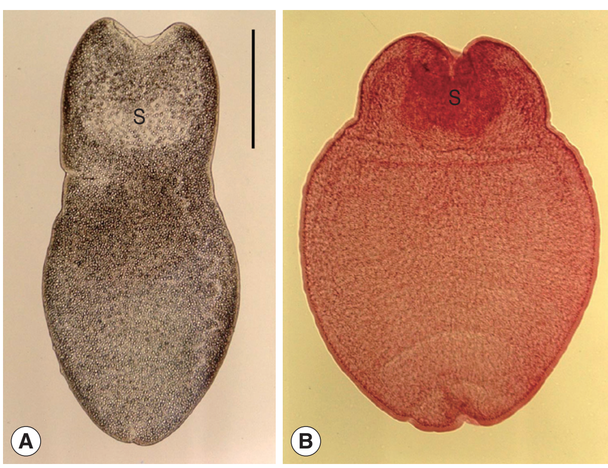

Fig. 1. Tetrathyridia (A: fresh worm; B: Semichon's acetocarmine-stained one) collected in the mesentery of a viper snake, *Agkistrodon saxatilis*, from China. They are slightly constricted in the anterior portion, oval or elongated in shape with a somewhat pointed posterior end, and have an invaginated scolex (S) with 4 suckers at the anterior constricted portion. Scale bar is 500 µm.

DISCUSSION

In the present study, tetrathyridia of *M. lineatus* were detected in 2 species of snakes, *A. saxatilis* and *E. schrenckii*, from China. No tetrathyridia were found in the other 3 snake species, *D. rufozonatum*, *A. brevicaudus*, and *E. davidi*. However, these 3 snake species have been reported as the intermediate hosts for *Gnathostoma hispidum* [26,27]. Generally, foodborne parasite infections are related to the food-chain in the natural ecosystem. Snakes, as the predator or a transport host, are infected with gnathostome larvae or tetrathyridia by preying upon the other intermediate or transport hosts, such as amphibians, reptiles, avians, and small mammals. How are the real intermediate hosts infected with the tetrathyridia of *Mesocestoides* spp. then? It is difficult to answer because of the link between the eggs and tetrathyridia (the first intermediate host necessary?) is obscure in the life cycle of *Mesocestoides* spp. However, tetrathy-

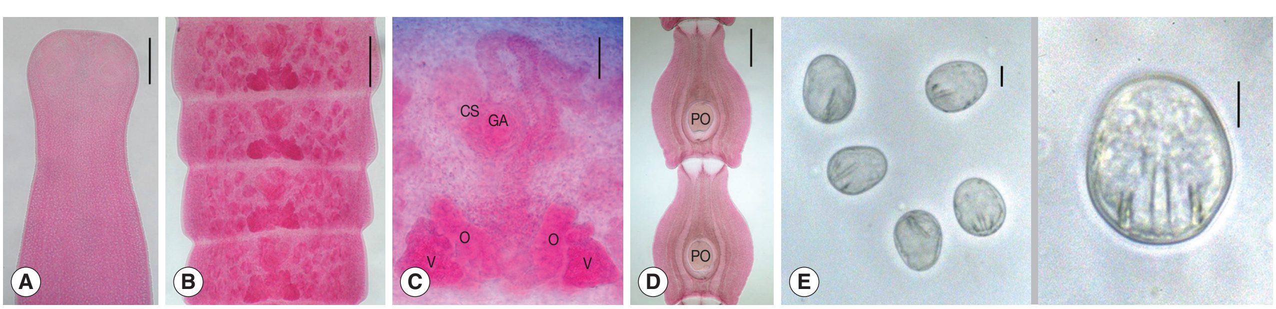

Fig. 2. Adult *Mesocestoides lineatus* recovered in the small intestine of a dog experimentally infected with tetrathyridia. (A) Scolex with 4 cup-like suckers. Scale bar = 250 µm. (B) Four mature proglottids. Scale bar = 200 µm. (C) Magnified view of a mature proglottid with bi-lobed ovaries (O) and vitellaria (V) in the posterior portion, oval-shaped cirrus sac (CS) and genital atrium (GA) in the median portion, and follicular testes in both lateral fields of proglottids. Scale bar = 50 µm. (D) Gravid proglottids with a characteristic paruterine organ (PO). Scale bar = 500 µm). E. Eggs with a hexacanth embryo. Scale bar = 10 µm.

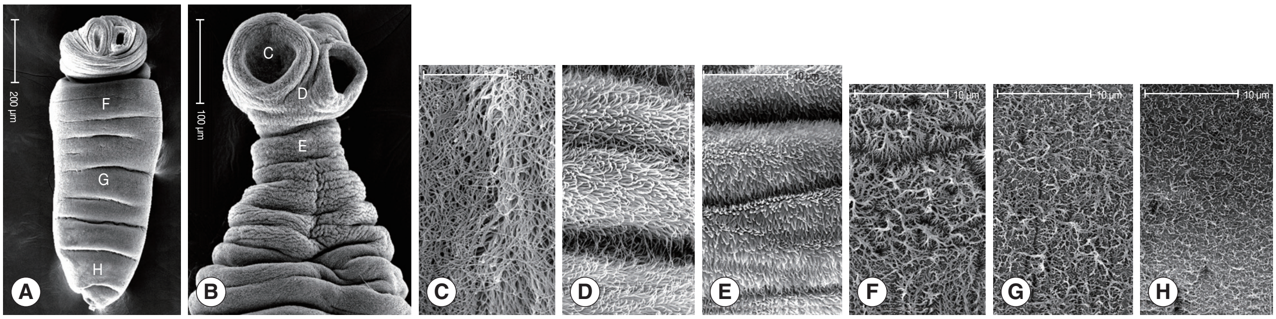

Fig. 3. SEM findings of tetrathyridia collected in the mesentery of a viper snake, *A. saxatilis*. (A) A whole worm elongated in shape with a somewhat pointed posterior end has an invaginated scolex at the anterior constricted portion. The whole body surface is covered with numerous microtriches, of which length and density are different according to the body level; anterior (F), middle (G), and posterior (H) portions of the posterior body. (B) Evaginated scolex with 4 cup-like suckers and neck portion covered with microtriches, of which shapes are different according to the body locations; the inner portion of a sucker (C), between suckers (D), and neck portion (E). (C) Tegument of the inner portion of a sucker (C portion in Fig. 3B) showing numerous long filamentous microtriches. (D) Tegument between suckers (D in Fig. 3B) showing numerous hair-like microtriches. (E) Tegument just below the scolex and neck portion (E in Fig. 3B) showing numerous stouter microtriches. (F) Tegument at the anterior portion of the posterior body (F in Fig. 3A). (G) Tegument on the middle portion of the posterior body (G in Fig. 3A). (H) Tegument in the posterior portion of the posterior body (H in Fig. 3A) showing somewhat short microtriches, of which length and density are decreasing posteriorly.

ridia have been detected in various kinds of animals, i.e., 22 reptilian spp., 15 avian spp., and 20 mammalian spp. [18,19]. Therefore, snakes as an upper-level predator may be easily infected by feeding their prey, i.e., reptiles, birds, and small mammals. As the snake intermediate hosts, *Agkistrodon halys* and *E. rufodorsata* were reported in Japan and Korea [24,28].

So far, about 30 human infections with *Mesocestoides* spp. adults have been documented in Japan, China, Korea, USA, Ruanda-Urundi, and Greenland [14-17]. Human infections are acquired by ingesting raw wild animals containing tetrathyridia. Several species of snakes, such as *A. brevicaudus*, *A. halys*, *Elaphe quadrivirgata* and *E. rufodorsata*, and chickens were reported as the source of human infections in Japan and Korea [14,16,24,25,28].

Adult *Mesocestoides* tapeworms have been found in surveys of carnivorous mammals, such as, dogs, cats, foxes, wolves, coyotes, jackals, raccoons, badgers, lynxes, and some species of wild felines [1-11]. Among the carnivorous mammals, foxes were the most frequently examined at various localities of the world and their infection rate with *Mesocestoides* spp. was relatively high [9,29-31]. Recently, Hrčkova et al. [9] detected *Mesocestoides* tapeworms from 41.9% out of 3,157 red foxes in central Europe. Magi et al. [29] found *M. lineatus* in 45.4% of 129 red fox from central Italy. Saeed et al. [30] also detected *Mesocestoides* sp. tapeworms from 35.6% of 1,040 red foxes in Denmark. In these endemic areas, foxes play a crucial role as the definitive host in maintaining the life cycle. What kinds of wild animals are the natural definitive hosts for *M. lineatus* in Korea? Adult *Mesocestoides* tapeworms were found in dogs and 2 human cases in Korea [16,23,25]; however, no reports are available on wild carnivorous mammals. Hence, keen observations are needed for detection of *Mesocestoides* tapeworms in surveys of wild carnivorous mammals in Korea.

To obtain adult worms, we orally infected 5-10 tetrathyridial larvae to 3 rats, 5 hamsters, 2 cats and 2 dogs. However, no worms were recovered in 3 rats, 3 hamsters, 2 cats, and 1 dog, and the fate of uninfected larvae is uncertain. Most of them may have died and passed out of the digestive tract with feces. However, some survived larvae might have migrated into the other organs and provoked the tetrathyridiasis like the canine peritoneal larval cestodosis (CPLC) [19]. Unfortunately, however, we examined only the small intestine of experimental animals to detect adult worms. It is a new finding in this study that hamsters are an experimental definitive host for *M. lineatus*.

Mesocestoides sp. tapeworms have morphological characteristics, such as the median ventral position of the genital atrium and bipartite vitelline gland in the mature proglottid and a paruterine organ in the gravid proglottid, which differ from those of other cyclophyllidean tapeworms. Especially, the paruterine organ in gravid proglottids is unique and regarded as a diagnostic key in the subfamily Mesocestoidinae [22]. On the other hand, morphological characteristics of adult worms observed in the present study were identical with those of *M. lineatus*, which were previously reported by Kamegai et al. in 1967 [32]

Table 3. Comparison of the current *Mesocestoides lineatus* with previous reports

Item (unit)	Present study (2013)	Kamegai et al. (1967)	Eom et al. (1992)
Scolex width (mm)	0.48-0.68	0.56	-
Cirrus sac (µm)	75-95×55-70	162-253×116-209	120-170×70-100
Testes number	41-52	41-60	42-54
Testis (µm)	55-75×38-50	38	35-89×30-68
Paruterine organ (µm)	530-600×440-550	465-600×468-735	450-590×410-550
Egg (µm)	33-36×25-28	31-34×24-29	28-32×20-24
Host	dog, hamster	dog, cat, fox	human

and Eom et al. in 1992 [16] (Table 3). Morphological characteristics of the metacestode and adult of *M. lineatus* observed in the present study will be helpful in taxonomic studies of *Mesocestoides* tapeworms.

ACKNOWLEDGMENTS

This work was financially supported by Anti-Communicable Disease Control Program of the National Institute of Health (NIH 348-6111-215), National Research and Development Program, Ministry of Health and Welfare, the Republic of Korea. We thank staffs of Incheon Customs Officer for their help to provide the snakes distrained. We also thank Jung-A Kim and Soo-Jung Park, Department of Parasitology, Gyeongsang National University School of Medicine, Jinju, Korea, for her help in recovery of worms from snakes and experimental animals.

Conflict of interest

We have no conflict of interest related with this study.

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