

Ultrastructure of *Capillaria hepatica* (Syn. *Calodium hepatica*) Isolated from the Liver of Mouse Infected with Artificially Embryonated Eggs Collected from House Rats (*Rattus norvegicus*)

Byoung-Hoon Min^{1,3}, Haeng-Sook Lee¹, Soo-Jin Kim^{2,*}, Kyoung-Hwan Joo^{1,*}

¹Department of Parasitology and the Institute of Travel Medicine, Korea University College of Medicine, Seoul 136-705, Korea

²Department of Life Science, College of Natural Science, Hallym University, Chuncheon 200-702, Korea

³Xenotransplantation Research Center, Seoul National University College of Medicine, Seoul 110-799, Korea

Capillaria hepatica (syn. *Calodium hepatica*) is a parasite found mainly in rodent liver. But, it has also been found in a wide variety of mammals, including humans. This worm is unique as it is the only nematode parasite that is embedded in the liver parenchyma of the host even during the adult stage of the life cycle. They produce eggs that elicit a marked granulomatous reaction that eventually destroys the worms. Fibrosis and lymphoplasmacytic inflammatory infiltration are often observed around adult nematodes embedded in the liver parenchyma of the host. For this reason, complete isolation of this slender worm and observation of the intact ultrastructure is very difficult. In this study, 10 intact whole worms (*C. hepatica*) were isolated from the liver of 3-week-old mouse after inoculation of artificially embryonated eggs collected from house rats (*Rattus norvegicus*). Their external structure was observed with light and scanning electron microscopy. The length of the isolated female and male *C. hepatica* was approximately 69.60 mm and 36.92 mm, respectively. More detailed ultrastructure, including bacillary band, eggs and vulva in female and spicule and spicule sheath in male *C. hepatica* was also described.

Key Words: *Capillaria hepatica*, Genital organ, Spicule, Scanning electron microscopy

*Correspondence to:
Kim SJ,
Tel: +82-33-248-2091
Fax: +82-33-256-3420
E-mail: sjkim@hallym.ac.kr

Joo KH,
Tel: +82-2-920-6161
Fax: +82-2-924-4905
E-mail: kyhwjoo@korea.ac.kr

Received November 19, 2013
Revised December 13, 2013
Accepted December 23, 2013

INTRODUCTION

Capillaria hepatica (syn. *Calodium hepatica*; Bancroft, 1893) is a parasitic nematode living in the liver of rodents and other mammals such as rabbits, dogs, cats, cattle, and humans (Singleton et al., 1991; Roberts et al., 1996; Anderson, 2000; Nabi et al., 2007). About 30 cases of human infections have been reported from Asia, Africa, Europe, North and South America, and Oceania including United States of America, Japan, and Korea (McQuown, 1950; Choe et al., 1993; Kohatsu et al., 1995; Fuehrer et al., 2011). Rat species of the genus *Rattus* are main primary host of *C.*

hepatica and infection rates of up to 100% have been reported (Farhang-Azad, 1977; Singleton et al., 1991; Ceruti et al., 2001; Claveria et al., 2005). In addition to the principal hosts, rodents, other mammals can be infected through ingestion of water or food contaminated by infectious embryonated eggs. *C. hepatica* does not require an intermediate host in their life cycle and thus death of the host is only way to release the embedded eggs. When the embryonated eggs are ingested by a new host, they develop into the adult worm in the liver of the host within approximately 20 days. The natural lifespan of the adult female worm is approximately 60 days (Spratt & Singleton, 2001; Kim et al., 2007) and that of male

This work was supported by a Korea University grant.

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worm is approximately 40 days (Luttermoser, 1938; Wright, 1961). To date, complete isolation of the adult worm and observation of intact ultrastructure has been very difficult, because the slender and hair-like worms are embedded in the liver parenchyma of the host during the adult stage. For these reasons, reports on the morphological study of external structure of *C. hepatica* are limited.

In this study, 10 intact whole worms were isolated from the liver of 3-week-old mouse after inoculation of artificially embryonated eggs collected from house rats (*Rattus norvegicus*). The external and fine structures of genital organ were examined with light and scanning electron microscopy (SEM).

MATERIALS AND METHODS

Experimental Animals and Collection of *Capillaria hepatica* Eggs

House rats (*R. norvegicus*) were taken from an urban marketplace in Seoul, Korea. The liver of house rats infected with *C. hepatica* was homogenized with a sterilized slide glass. Then, the eggs were collected with a micropipette. Collected eggs were embryonated by incubating at 37°C for 5 weeks in 0.5% formalin solution. Individual 8-week-old male BALB/c mouse was inoculated with 1,000 embryonated eggs (n=10, Samtako, Osan, Korea) through oral zonde and mice were bred for 3-weeks.

Isolation of *Capillaria hepatica*

Whole worms were isolated from the mouse liver after inoculation of the embryonated eggs using enzyme digestion methods. Briefly, liver tissue was homogenized with a sterilized

slide glass and incubated for 4 to 5 hours in phosphate buffer solution containing type IV collagenase and antibiotics (pH 7.4) at 37°C. The homogenized livers were washed with normal saline solution including antibiotics at 37°C and released worms were isolated using a pair of tweezers.

Microscopic Observation

Isolated *C. hepatica* was fixed with 10% buffered neutral formalin, dehydrated, and then mounted on the slide glass for light microscope observation. External structures of the worm were observed with a light microscope and the length and width of the isolated worm was measured with MetaMorph software (Universal Imaging Co., West Chester, PA, USA). For H&E staining, liver was fixed in 10% neutral formalin and embedded in paraffin according to standard protocol. Four µm-thick liver section was deparaffinized and then stained with H&E. For SEM observation, the isolated *C. hepatica* were fixed with 1% paraformaldehyde-1% glutaraldehyde (pH 7.4) and 2% osmium tetroxide. Fixed samples were dehydrated in a graded ethanol series and treated with isoamyl acetate. Pre-treated samples were dried with a critical point dryer (HCP-2; Hitachi, Tokyo, Japan) and the surface was coated with gold in a vacuum using an ion coater (IB-III; Eiko, Tokyo, Japan). Prepared samples were observed with a SEM (S-2500; Hitachi).

RESULTS

When the extracted liver harboring *C. hepatica* was examined under a light microscope, several white clusters consisting of *C. hepatica* and eggs were easily observed and the hepatomegaly around the worms was noticeable. Clusters, measured 2 to 4

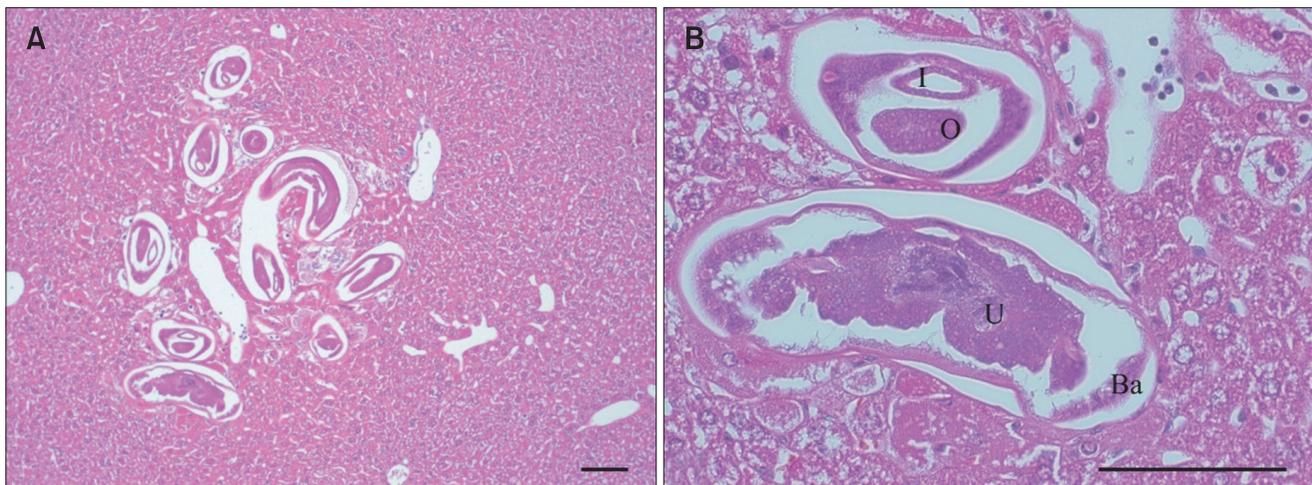


Fig. 1. Light micrograph of mouse liver at 3 weeks after embryonated *Capillaria hepatica* egg infection. (A) *C. hepatica* was embedded in the liver parenchyma surrounded by fibroblasts and macrophages. (B) The intestine (I), uterus (U), ovary (O), and two bacillary bands (Ba) were observed. Scale bars=100 µm.

mm in diameter, were scattered on the surface of the mouse liver. Numerous fibroblasts and macrophages were observed around the clusters in histological sections of infected mouse liver (Fig. 1A and B).

The length of the isolated female and male *C. hepatica* was approximately 69.60 mm and 36.92 mm, respectively (Fig. 2). Female *C. hepatica* had a thin and pointed anterior end (Fig. 3A). Many oval shaped eggs (54 μm long and 31 μm wide)

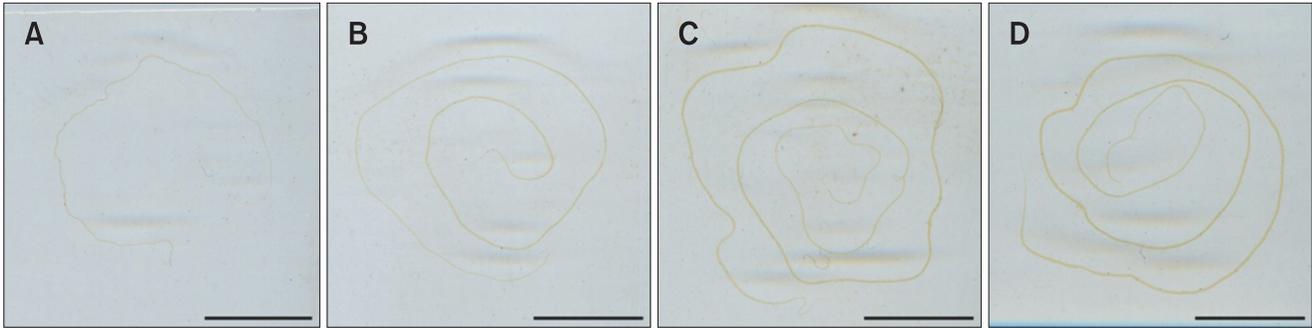


Fig. 2. Whole *Capillaria hepatica* isolated from the liver of mouse. Ten mice were experimentally infected with embryonated eggs of *C. hepatica* and livers were extracted at 3 weeks after infection. (A, B) Male worm. (C, D) Female worm. Scale bars=5 mm.

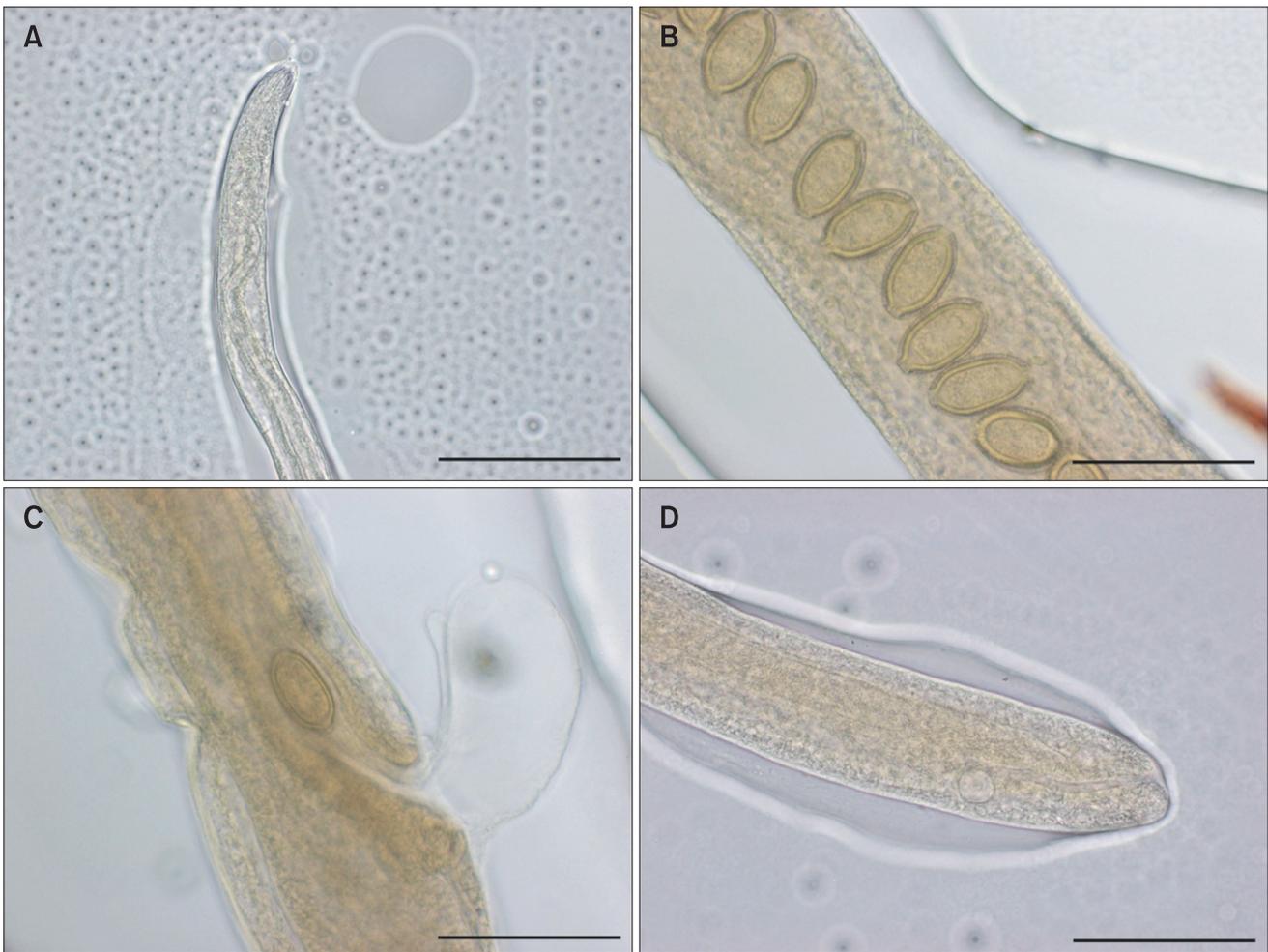


Fig. 3. Light micrograph of female *Capillaria hepatica*. (A) Anterior region. (B) Mid region of the worm containing unembryonated eggs in uterus. Vulva region of the female worm showing postvulval uterine sac (egg sac) projected from the worm (C) and posterior region (D). Scale bars=100 μm .

were observed in the uterus (Fig. 3B) and a pore like vulva was opened to outside in the posterior ventral region of the worm (Fig. 3C). The posterior end of female worms had a bluntly conical structure and was about 2.5 times wider than

the anterior region (Fig. 3D).

The shape of anterior region of the male worm was similar to that of the female and had a thin and pointed end (Fig. 4A). A funnel-shaped spicular sheath was protruded from

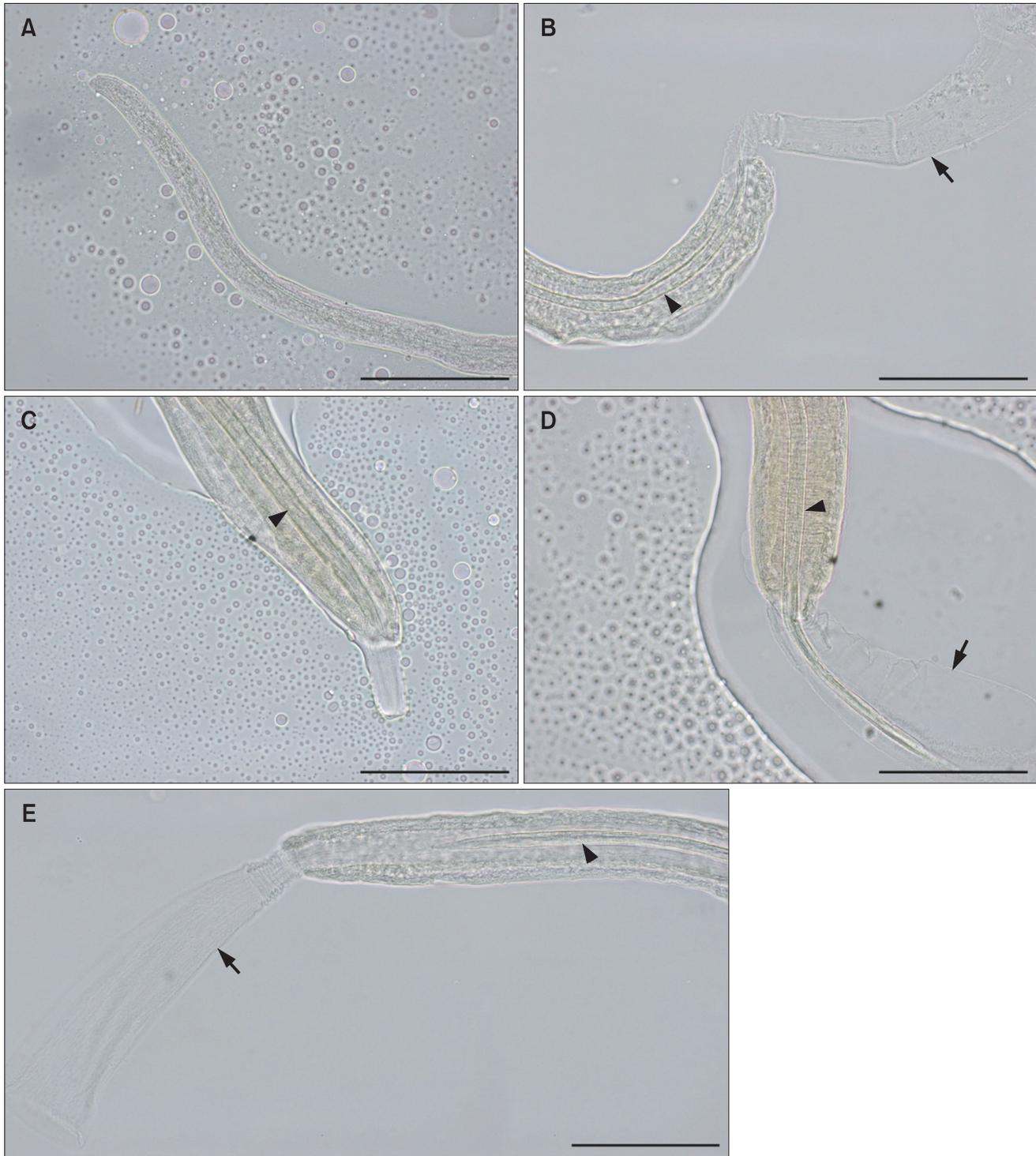


Fig. 4. Light micrograph of male *Capillaria hepatica*. (A) Anterior region. (B-E) Posterior region and genital organ. Spicule (arrowheads) and spicular sheath (arrows). Scale bars=100 μm.

the posterior end (Fig. 4B). A projected spicule (246.49 μm long and 10.59 μm wide) was observed at the end of the male worm. Several interesting patterns were observed in the morphological characteristics of the spicule and spicular sheath. First, a spicule protruded about 40 μm from the posterior end (Fig. 4C). Second, a spicule of about 318 μm in length was wrapped by the spicular sheath (Fig. 4D). Third, spicular sheath was funnel-shaped and protruded about 230 μm from the posterior end. Spicule was also occasionally located in the inner-cavity of the worm (Fig. 4E).

When the worm was examined by SEM, the anterior end

was mostly thin (less than 10 μm) (Fig. 5A), and the width of the anterior and posterior regions was approximately 24.68 μm and 71.23 μm , respectively in the female *C. hepatica*. The anterior region has transverse striations at the interval of about 0.7 μm . The posterior end was tapered to a rounded end with an excretory pore (Fig. 5B). Mid region of the female worm was about 84.26 μm thick and had a pair of bacillary bands with the bacillary pore located on the surface of the worm (Fig. 5C). A pore-like vulva (about 8 μm in diameter) was observed in the posterior ventral region of the worm (Fig. 5D). In addition to the vulva, the bacillary pores were

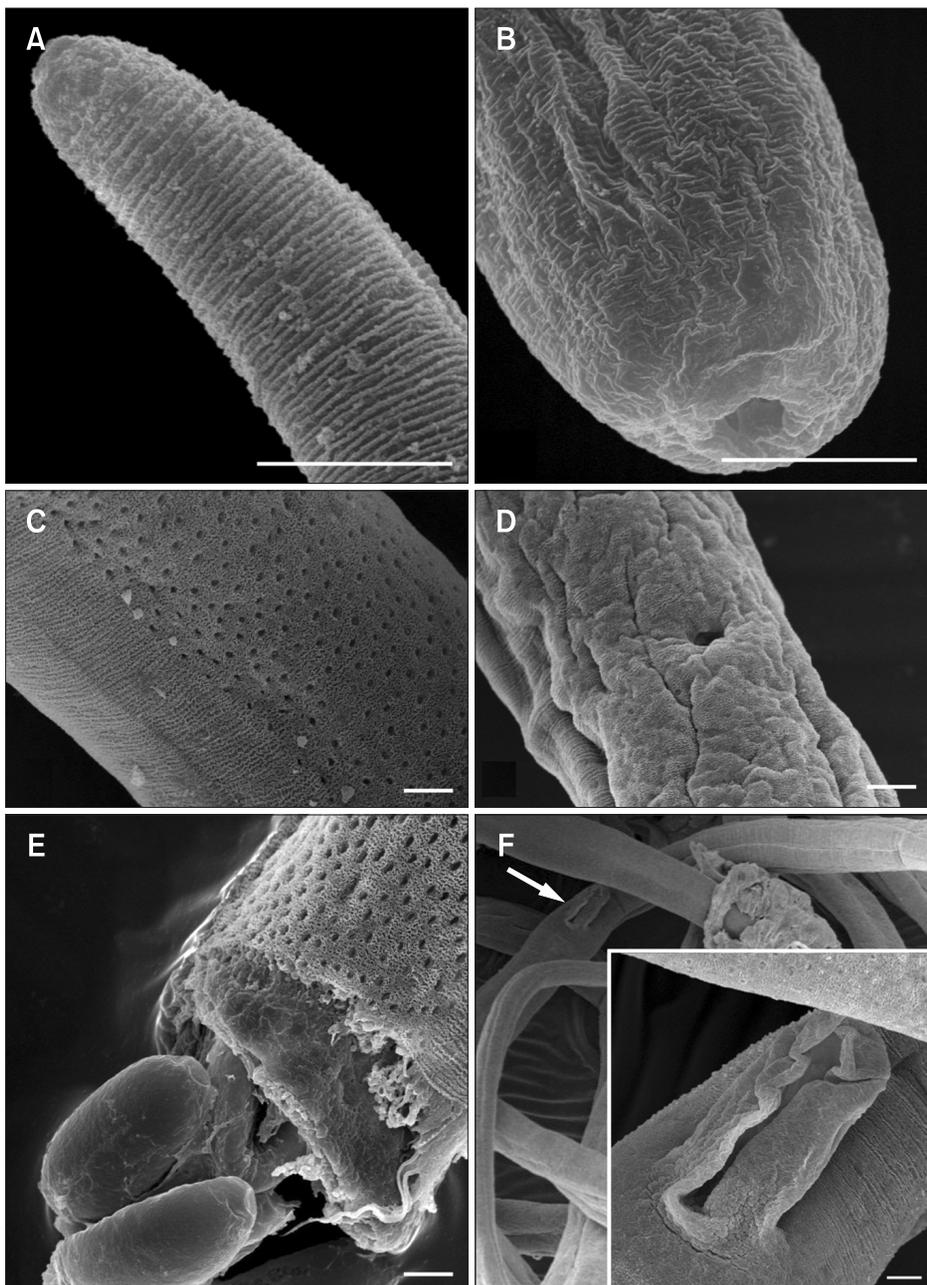


Fig. 5. Scanning electron micrograph of female *Capillaria hepatica*. It showed anterior (A), posterior (B), and mid region (C-F) of isolated female *C. hepatica*. (A) Anterior region with transverse striations was most thin in the worm. (B) Excretory pore (anus) observed in the end of the worm. (C) A pair of bacillary bands situated in the mid region of the worm. (D) Vulva was a pore-like structure situated on the ventral region. (E) Eggs, epithelial tissue and muscle fibers observed in the mid region of fragmented worm. (F) Postvulval uterine sac (egg sac) projected from the worm (arrow and enlarged inset). Scale bars=10 μm .

distributed on the ventral part of the worm (Fig. 5D). Occasionally, the fragment of *C. hepatica* was observed. In this section, the internal organs such as cuticle layers, uterus containing eggs were observed. Epithelial tissues and muscle fibers were included in the surface of the cuticle (Fig. 5E). An ovipositor was seen to project out from the worm (Fig. 5F). In male *C. hepatica*, the anterior region was similar to that of female counterpart, which was tapered to a rounded anterior end (Fig. 6A). The width of anterior region, posterior, and mid region was 31.13 μm (Fig. 6A), 50.16 μm (Fig. 6B), and 60.74 μm (Fig. 6C), respectively. Mid region of the male worm

had a pair of bacillary bands with bacillary pore located on the surface of the worm (Fig. 6C). Several different characteristics were observed in the spicule and spicular sheath. The spicular sheath had a tubule with each different length and the surface was corrugated with transverse striations. Spicular sheath was folded like the wrinkled straw (Fig. 6D) and was protruded about 100 μm from the posterior end (Fig. 6E). In other case, the spicular sheath had approximately 46 μm in length and spicule projected approximately 6 μm from the end of sheath (Fig. 6F). In addition, a particular morphological feature of spicular sheath was noted. When we observed the lateral side,

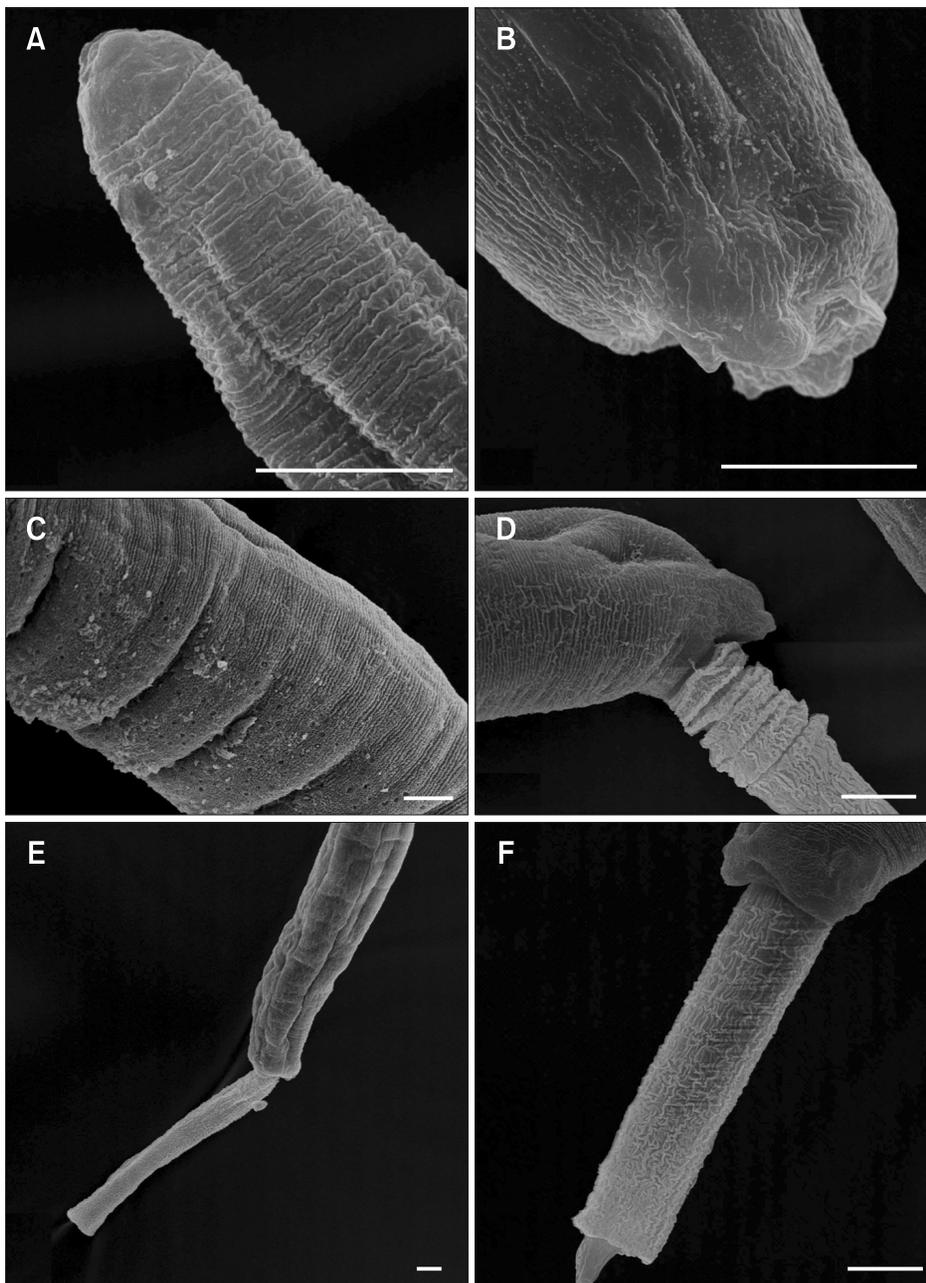


Fig. 6. Scanning electron micrograph of male *Capillaria hepatica*. It showed anterior (A), posterior (B), mid region (C) and spicule of isolated male *C. hepatica* (D-F). (A) Anterior region with transverse striations was most thin of the worm. (B) Posterior region of the male worm which was not exist spicule and spicular sheath. (C) A pair of bacillary bands was situated on the mid region. Spicule and spicular sheath had several form in posterior end of male worm. (D) Partially wrinkled spicular sheath was adjacent to the posterior end. (E, F) Spicular sheath had tubular structure and transverse striations. Scale bars=10 μm .

the angle of the posterior end from the region with a spicule was approximately 110 to 120 degrees (Fig. 6B-F).

DISCUSSION

C. hepatica is a parasitic nematode living in the liver of rodents and other mammals, such as lagomorphs, dogs, cattle, and humans. Rat species of the genus *Rattus* are the primary host and natural reservoir of *C. hepatica*. However, infection to other mammals occurs due to ingestion of water or food contaminated with the embryonated *C. hepatica* eggs (Roberts et al., 1996; Spratt & Singleton, 2001). The Norway rat, *R. norvegicus*, and the black rat, *Rattus rattus*, are common and widely distributed rodent species. In Korea, almost all reports of *C. hepatica* infection have been in Norway rat *R. norvegicus* (Lee, 1964; Seo et al., 1964; Min, 1979; Seong et al., 1995; Jeong et al., 2008).

The genus *Capillaria* includes approximately 300 species of which four of them (*C. philippinensis*, *C. aerophila*, *C. plica*, and *C. hepatica*) has been found in humans (Spratt & Singleton, 2001; Lloyd et al., 2002; Ruas et al., 2003; Nakamura, 2005; Mowat et al., 2009). Although the width and length of each nematode varies, gross biological structures and shape are similar among the worms of the same genus. However, the differences of size, structures, and distribution are noted among the worms of different species.

Previous reports showed that the length of male and female *C. hepatica* was about 15 to 37 mm and 52 to 104 mm, respectively (Luttermoser, 1938; Neva & Brown, 1994; Sun, 1999). Adult *C. hepatica*, measured 88 to 154 μm in the diameter has a genital tube, an intestinal tract, and other external structure of the class *Enoplea* (*adenophorea*, *aphasmidea*) such as stichosomes and two bacillary bands (Kim et al., 2009; Resendes et al., 2009).

In this study, the male and female worms were measured about 36.92 mm long and 23 to 59 μm wide and 69.6 mm long and 28 to 124 μm wide, respectively (Table 1). The values

were similar to those in other reports. This worm also showed the typical characteristics of *C. hepatica* such as several genital organs including a genital tube containing eggs in the female worms.

C. hepatica has a capillary-like (slender-shaped) structure with a narrow anterior part (7 to 10 μm), and the posterior part is gradually thicker. Female worms have a length of 27 to 100 mm and a width of 0.1 to 0.89 mm whereas male worms are smaller with 15 to 50 \times 0.04 to 0.1 mm (Schmidt, 2001).

The anterior region of *C. hepatica* was less than 10 μm and gradually thicker from the anterior end. Mid region of the female worm was about 84.26 μm thick and had a pair of bacillary bands with bacillary pore located on the surface of the worm. Mid region of male worm was 60.74 μm thick and had a pair of bacillary bands with bacillary pore located on the surface of the worm.

C. hepatica eggs found in the host liver are barrel-shaped and unembryonated, with typical bipolar plugs and prominent radial striations in the outer layer of the shell, and also have a dark middle layer of shell between two colorless layers (Beaver et al., 1984). They were measured 21 to 31 μm wide and 40 to 54 μm long (Wright, 1961; Zaman & Keong, 1995; Resendes et al., 2009). When we observed the isolated *C. hepatica* using SEM, we can find several genital organs and eggs in the female worm such as vulva and several shapes of spicule and spicular sheath in the male worm. In the female worm, vulva was observed as posterior ventral part of the worm. Eggs measured 55 μm long and 31 μm wide were contained in the genital tube, but they did not exist in liver parenchyma of the mouse.

In the male genital organ, a single spicule and spicular sheath were seen. A single spicule was projected from the posterior end of the worm. However, it was occasionally located in the inner-cavity of the worm. The spicule was thin, long, and string-like in shape and was 446 μm long and 6 μm wide. The spicular sheath was tubular with a corrugated surface and transverse striations. Male nematodes usually have one or two spicules for reproduction (Zaman & Keong, 1995), although Order Trichurida have one spicule (Roberts et al., 1996) and Order Spirurida have two spicules (Mutafchiev & Georgiev, 2008; Smales et al., 2009). Order Trichurida *Capillaria philippinensis* from the human intestine have smooth and well-sclerotized spicules of 411 to 468 μm in length and 3 to 12 μm in width (Moravec, 2001).

These results show that the external structure of *C. hepatica* was similar to that of other species in genus *Capillaria*. By contrast, the body length and spicule size was longer than that of other species in genus *Capillaria*. The body length of male *C. hepatica* was 19 to 27 times longer than that of male *C. philippinensis* and the body length of female *C. hepatica* was 10 to 17 times longer than female *C. philippinensis*. Spicule length was approximately 1.6 times longer than that of *C.*

Table 1. Body length and width of *Capillaria hepatica* isolated from mouse livers at 3 weeks after inoculation of embryonated eggs

Data set	Region	Male (n=5)	Female (n=5)
Length	Total (mm)	36.92 \pm 11.94	69.60 \pm 10.81
	Spicule (μm)	446.80 \pm 77.97	-
	Spicular sheath (μm)	246.49 \pm 39.43	-
Width	Anterior part (μm)	31.13 \pm 2.97	24.68 \pm 1.67
	Mid part (μm)	60.74 \pm 2.29	84.26 \pm 2.21
	Posterior part (μm)	50.16 \pm 2.32	71.23 \pm 3.27
	spicule (μm)	6.08 \pm 0.62	-
	Spicular sheath (μm)	10.59 \pm 0.88	-

Values are presented as mean \pm standard deviation.

philippinensis. The length and width of the isolated *C. hepatica* were approximately 10 to 20 times longer than those of other species in the genus *Capillaria* (Chitwood et al., 1968).

C. hepatica has a short life cycle within approximately 40 to 60 days (Luttermoser, 1938; Wright, 1961). Adult *C. hepatica* sexually matures in the liver parenchyma and is able to mate approximately 15 to 20 days post-infection. In case of mouse, sexually matured males are found in the livers at 15 to 18 days post-infection and the females are found at 15 to 20 days post-infection. Eggs are produced from the fertilized female worm at 18 to 33 days post-infection (Luttermoser, 1938; Wright, 1961; Sun, 1999). *C. hepatica* eggs become infective in about 45 to 60 days (Campbell, 1991). In particular, those eggs are not released from the liver and develop to the eight-cell stage, until the death of the host (Juncker-Voss et al., 2000; Schmidt, 2001). This is why we have conducted an experiment at 3 weeks after infection, because this time is appropriate to examine genital organs such as spicule, spicule sheath, vulva and eggs.

Our results suggest that spicule and egg formation may occur about 3 weeks after experimental infection. These results are consistent with previous reports that *C. hepatica* may mature within 18 to 29 days after infection (Spratt & Singleton, 2001). This study was designed to observe the fine structure of *C. hepatica* through isolation of undamaged whole worms. Female *C. hepatica* dies immediately after laying eggs in the liver. Autopsy specimens show a histologically different pattern of disease. Early, immature worms, granulomas, and eosinophilic infiltration are evident. Later, the adult worms can be identified with numerous mixed eggs. The latter stages

are characterized by the lack of adult worms, the presence of viable eggs, and marked fibrosis (Gutierrez, 2006). Because the worms are “walled off” after laying eggs and death, most reports are limited to observation of embedded *C. hepatica* or pathological findings as case reports. Although understanding of the inner structure and the hepatic symptoms caused by *C. hepatica* was relatively well known, it is difficult to find the external structure and special organ structure because of this parasitic life cycle. Therefore, we have isolated 10 whole *C. hepatica* worms without damage from the liver of laboratory mouse at 3 weeks after inoculation of artificially embryonated eggs collected from house rats (*R. norvegicus*). We were able to report the characteristics and fine structures of the undamaged *C. hepatica* through light and SEM. These results can be useful for further studies on the morphology of nematodes including *C. hepatica*.

SUMMARY

C. hepatica was isolated without damage from the liver of the laboratory mice at 3 weeks after inoculation of artificially embryonated eggs collected from house rats (*R. norvegicus*). Detailed characteristics and fine structures of the undamaged *C. hepatica* were presented. The length of the isolated female and male *C. hepatica* was approximately 69.60 mm and 36.92 mm, respectively. In addition, the ultrastructure of the *C. hepatica*, including bacillary band, eggs and vulva in female and spicule and spicule sheath in male *C. hepatica* was also described.

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