A Case Study of Human Infection with Small-Size Strobilae of Diphyllobothriid Tapeworm Discharged from a Man in Okayama Prefecture, Japan

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ABSTRACT. A rare case of human infection with small-size diphyllobothriid tapeworm found in Okayama Prefecture, Japan, is reported. The patient was a 27-year-old man living in Yakage-Cho, Oda-Gun in Okayama. On April 28th 1995, the patient had spontaneously discharged 5 fragmented strobilae (3 to 33 cm long) without scolex of 64 cm in total length and 2.3 mm in width. Then he brought the specimen to Tsutsui Clinic wrapped with toilet paper. The external appearance of the strobilae resembled to that of *Diphyllobothrium parvus* (Stephes, 1908) Faust, 1929 known as parvum type of *D. latum*, although there was definite difference in arrangement of testes, thickness of longitudinal muscle layer and size of eggs. The specimen of the present case was identified as *Diphyllobothrium latum* (Linnaeus, 1758) Lühe, 1910 by Rausch and Hilliard (1970) based on morphological characteristics of serial sections (transverse, sagittal and horizontal) of proglottides and the eggs. The patient had customarily been eating raw meat of freshwater salmon as well as marine fishes such as common-makerel, yellow-tail, sea bream and flatfish. The route of the infection of the present case is not clarified.

Key words: human diphyllobothriasis — *Diphyllobothrium latum* — Diphyllobothriidae — Cestoda — Okayama Prefecture

Diphyllobothriid tapeworms in adult form infecting man are known in 17 species in the world, of which 8 species of *Diphyllobothrium* are reported infected to man in Japan : these are ; *D. latum* (Linnaeus, 1758) Lühe, 1910, *D. hians* (Diesing, 1850), *D. scoticum* (Rennie *et* Reid, 1912), *D. pacificum* (Nybelin, 1931), *D. cameroni* (Rausch, 1969), *D. yonagoense* Yamane *et al*, 1980, *D. nihonkaiense* Yamane *et al*, 1986 and *D. orcini* Hatsushika *et* Shirouzu, 1990. As *Diphyllobothrium* species-induced human diphyllobothriasis have been increasing in number, taxonomical study on *Diphyllobothrium* species became increasingly difficult.

In this paper, a rare case of human infection with small-size strobilae of diphyllobothriid tapeworm found in Okayama Prefecture, Japan, is described with photomicrographs of light and scanning electron microscopy

CASE NOTES

Patient (K. Y.) was a 27-year-old man, industrial worker, living in

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Yakage-Cho, Oda-Gun in Okayama Prefecture. Around noon April 28th 1995, the patient had spontaneously discharged several number of string-like white strange bodies along with severe abdominal pain which was continued for 10 minites. He collected the strange bodies and wrapped with toilet paper, then immediately brought them to his home doctor at Tsutsui Clinic. Abdominal pain of the patient made a rapid recovery. A total of 5 fragmented strobilae were confirmed. The patient had a habit to eat raw meat of freshwater salmon as well as marine fishes such as common-makerel, yellow-tail, sea bream and flatfish.

DESCRIPTION OF THE WORM

Morphological study was done on the strobilae and the eggs. The proglottides were observed in a whole mount preparation stained with

TABLE 1. Comparison of morphological data of the present specimen and *D. latum*

	Present specimen	*D. latum* (Rausch and Hilliard, 1970)
Strobila		
length	64 cm	156～1140 cm
maximum width	2.3 mm	14 mm
Proglottid		
length	0.8～1.5 mm	0.7～1.2 mm
thickness	0.6～0.7 mm	
Cirrus-sac		
length	460 μm	375～640 μm
width	265 μm	245～390 μm
wall thickness	7.0～14.0 μm	25 μm
Seminal vesicle		
length	237 μm	172～357 μm
width	167 μm	130～233 μm
wall thickness	7.0～21.0 μm	20～57 μm
Arrangement of testes	single layer	single layer
Diameter of testes	94～138×21～45 μm	
Thickeness of muscle layer		
longitudinal	31.2～76.3 μm	97～146 μm
transverse	6.9～17.4 μm	18～48 μm
No. of uterine loops	5～6	
Thickness of		
cortical parenchyma	111～279 μm	
medullary parenchyma	84～237 μm	
Size of eggs		
length	55.5～72.9 μm (av. 64.4 μm)	62～76 μm (av. 67.0 μm)
width	41.6～48.6 μm (av. 46.5 μm)	42～51 μm (av. 46.0 μm)
thickness of egg shells	1.0～1.7 μm (av. 1.1 μm)	

Semicon's carmine. Some proglottides were embedded in paraffin and serially sectioned at 10 μm each in transverse, sagittal and horizontal directions, then stained with Gomori's trichrome solution. The eggs examined were removed from uterine ducts of mature proglottides. Ventral surface of the proglottid and the egg-shell surface were studied with a Hitachi S-570 scanning electron microscope after preparation by the ordinary techniques.

Measurements of each part of the proglottid and the eggs are shown in Table 1. The fragmented strobilae without scolex were already amputated into 5 individuals (3, 9, 9.5, 10 and 33 cm long each). The strobilae were enormously slender as a whole and measured 64 cm in total length and 2.3 mm in maximum width (Fig 1). The proglottides were much wider than long and single set of reproductive organ recognized on the midline of each proglottid. The proglottides measured 0.8 to 1.5 mm in length and 0.6 to 0.7 mm in thickness. The uterus was distributed bilaterally with 5 to 6 loops on each side (Fig 2).

The cortical zone situated in the ventral and dorsal parts was 111 to 279 μm in thickness in the transverse sections, having longitudinal muscle fibers and yolk glands. A layer of longitudinal muscle was relatively thick, measuring 31.2 to 76.3 μm and a layer of transverse muscle was 6.9 to 17.4 μm in thickness (Fig 3, 4). Medullary zone was 84 to 237 μm in thickness in the transverse section. Testes were arranged in a single layer occupying in the medullary zone with a distinct boundary neighboring proglottides (Fig 8). The testicular follicles were elongated with a ventrally to dorsally directed axis,

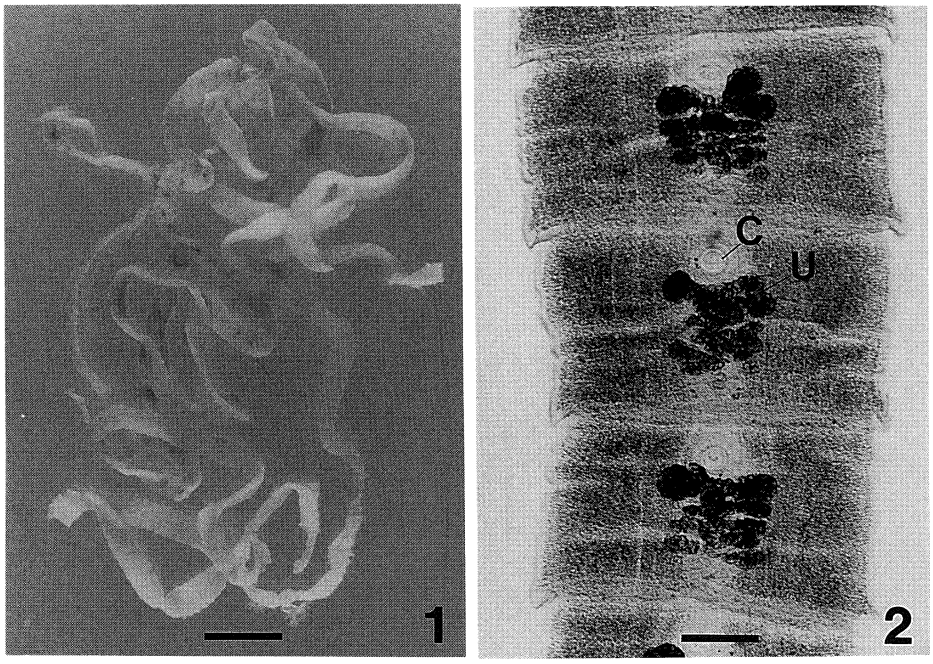
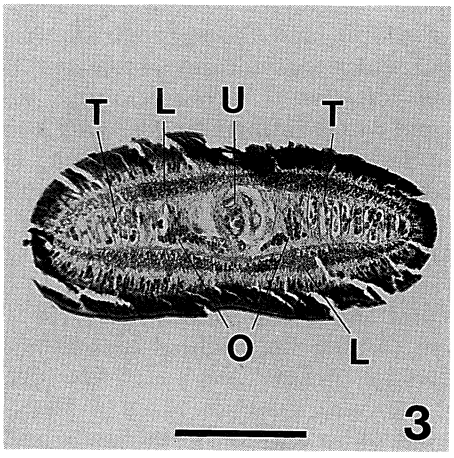


Fig 1. Whole body of vital strobilae obtained (Scale bar=2.0 cm)
Fig 2. Whole mount preparation of proglottides from the posterior part of strobila (Scale bar=0.5 mm)
C: cirrus-sac, U: uterus

measuring 94 to 138 μm dorso-ventrally and 21 to 45 μm laterally in the transverse sections (Fig 4, 6). Longitudinal nerve trunk and excretory canal were not visible. Locations of cirrus and vaginal openings were opened separately (Fig 7, 8).



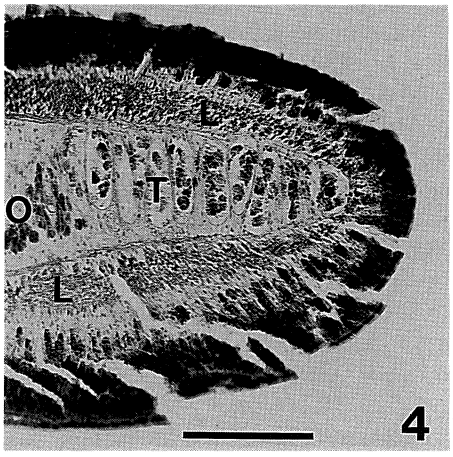
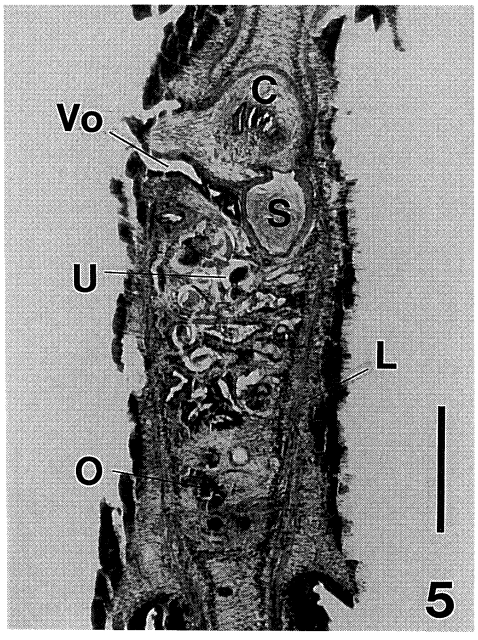


Fig 3. Transverse section of proglottid passing through the level of ovarian lobes (Scale bar=0.5 mm)
Fig 4. A high power view of the lateral field in Fig 3 (Scale bar=0.2 mm)
L : longitudinal muscle layer, O : ovary, T : testis, U : uterus



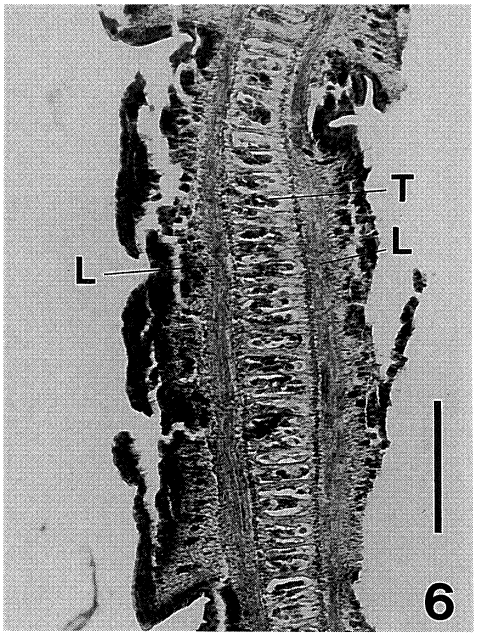


Fig 5. Sagittal section of proglottid passing through the level of the vaginal opening (Scale bar=0.3 mm)
Fig 6. Sagittal section of proglottid passing through the lateral field (Scale bar=0.3 mm)
C : cirrus-sac, L : longitudinal muscle layer, O : ovary, S : seminal vesicle, U : uterus, Vo : vaginal opening

The genital opening was located ventrally on the midline of proglottid, and surrounded by distinct genital papillae (Fig 9). The cirrus opening was recognized 0.25 μm posterior to anterior margin of proglottid which occupies approximately 1/5 of total length of the proglottid (Fig 2, 8). The cirrus-sac was pyrifrom and extended dorsally from the cirrus opening, measuring 460 μm dorso-ventrally and 265 μm in diameter in the sagittal sections. The cirrus-sac wall was 7.0 to 1.4 μm in thickness. The seminal vesicle was antero-posteriorly oval and situated just under the surface of the cirrus-sac, which made each

Fig 7. Horizontal section of proglottid passing through the level of three different kinds of genital openings (Scale bar=0.2 mm)
Fig 8. Horizontal section of proglottid, showing the testes- and vitelline follicles-free area (arrow) exists between neighboring proglottides (Scale bar=0.5 mm)
C: cirrus-sac, Co: cirrus opening, S: seminal vesicle, T: testis, U: uterus, Uo: uterine opening, Vf: vitelline follicle, Vo: vaginal opening

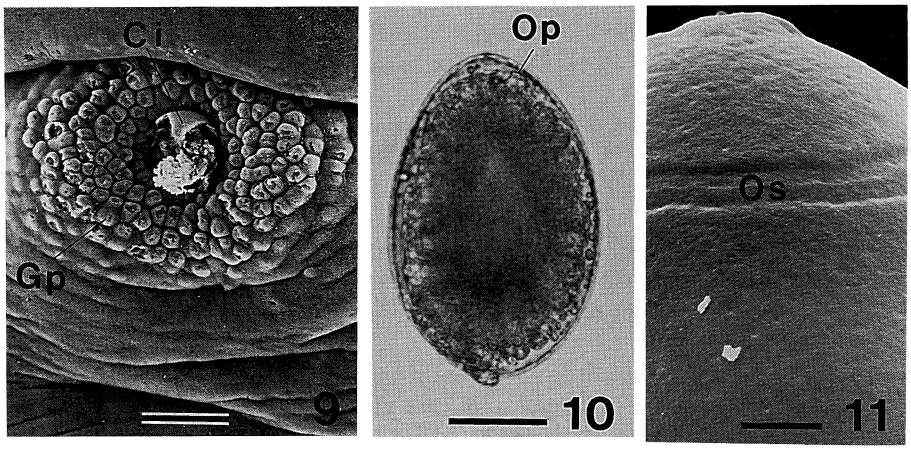


Fig 9. Genital papillae and cirrus of proglottid by scanning electron microscopy (Scale bar=10 μm)
Fig 10. An egg from the uterus (Scale bar=0.02 mm)
Fig 11. Egg-shell surface by scanning electron microscopy (Scale bar=3.0 μm)
Ci: cirrus, Gp: genital papillae, Op: operculum, Os: opercular suture

other almost a right angle. The seminal vesicle can be seen from ventral side of proglottid (Fig 5), measuring 237 μm antero-posteriorly and 167 μm in diameter in the sagittal sections. The seminal vesicle wall measured 7.0 to 21.0 μm in thickness.

The eggs were ovoid with apical knobs situated on opposite side of operculum, measuring 55.5 to 72.9 μm (av. 64.4) in length and 41.6 to 48.6 μm (av. 46.5) in maximum width (Fig 10). The egg-shells were 1.0 to 1.7 μm (av. 1.1) in thickness, without having scattered surface pits (Fig 11).

TABLE 2. Morphological data of *Diphyllobothrium parvus* in the previous studies

	Stephens (1908)	Leon (1915)	Yoshida & Ogata (1924)	Lee *et al* (1994)
Strobila				
length	69~103 cm	20 cm	232 cm	15~120 cm
maximum width	5.0 mm	5.0 mm	7.0 mm	4.0 mm
Proglottid				
length	0.6~4.0 mm		1.0~3.0 mm	1.0~1.2 mm
Muscle layer			weakly developed	
Arrangement of testes			single or two layers	
No. of uterine loops	4~5		4~6	4~6
Size of eggs				
length	59.2 μm		52.7~67.3 μm (av. 58.0 μm)	55.6~59.6 μm (av. 57.5 μm)
width	40.2 μm		36.5~41.0 μm (av. 39.0 μm)	33.1~40.7 μm (av. 37.4 μm)

DISCUSSTION

The present specimen is more comparable to *Diphyllobothrium parvus* reported by Stephens (1908),[1] although it differs from *D. parvus* by means of arrangemest of the testes, thickness of the longitudinal muscle layer and size of the eggs.

The first human case of *D. parvus* infection was reported by Stephens (1908)[1] on a 37-year-old Tasmanian man residing in Australia. Thereafter 2 cases were reported by Leon (1915)[2] on a 45-year-old Rumanian man and also reported by Yoshida and Ogata (1924)[3] on a 9-year-old boy living in Himeji City, Japan. More recently Lee *et al* (1994)[4] has reported additional 2 cases of *D. parvus* infection on a 46-year-old woman and a 22-year-old man residing in Seoul, Korea. The morphological data of *D. pervus* strobila in the previous cases are shown in Table 2.

The external appearance of the present specimen is very similar to that of *D. parvus* except body size, eggs and internal organs. The strobilae of the present case are less wide than those of *D. parvus* as indicated in Table 2. Based on the strobila observations, the specimen is assumed to shrink to about one-third in transverse direction, probably due to desiccation. This fact induced dorso-ventral elongation of the testes located in the medullary zone both in the transverse (Fig 3, 4) and sagittal (Fig 6) sections. Magath (1927)[5]

had reported that incompletely developed *D. latum* removed from an experimentally infected dog was identical to *D. parvus*, thus considered *D. parvus* as young form of *D. latum*. Lee *et al* (1994),[4] on the other hand, have asserted that *D. parvus* was a morphological variant of *D. latum*. According to present day knowledge, *D. parvus* in generally considered as a synonym for *D. latum*.

It is a commonly accepted opinion that the scattered pits can be recognized on the egg-shell surface of marine species of the genus *Diphyllobothrium*.[6] In our present specimen, the typical scattered pits were not present on the egg-shell surface (Fig 11). It is therefore probable that the present specimen is certainly differentiable from marine species such as *D. hians*, *D. scoticum*, *D. pacificum*, *D. cameroni*, *D. yonagoense*, and *D. orcini*. Although *D. nihonkaiense* is recently described as a new species of diphyllobothriid tapeworm by Yamane *et al* (1986),[7] the texonomical data of the paratype of *D. nihonkaiense* noticeably differ from the present specimen by size of the eggs, winding pattern of the uterine loops, structure of the genital papillae and relative position of the cirrus-sac and the seminal vesicle. In addition, the taxonomical data of *D. nihonkaiense* in the original publication are based on the adult worm from experimentally reared golden hamster, for holotype however a strobila expelled from a man is fixed. Nowhere is any description as to be human origin in the paper. Therefore, the authors highly suggest that complete taxonomical data of the holotype of *D. nihonkaiense* should be opened at the earliest chance. If this problem is not solved, taxonomical confusion for comparative morphological study among other diphyllobothriids and *D. nihonkaiense* of human origin will become more pronounced.

As shown in Table 1, internal morphological characteristics of the present specimen are more comparable to that of *D. latum* found from Eskimos by Rausch and Hilliard (1970).[8] Especially the proglottid length, size of the cirrur-sac, the seminal vesicle and the eggs, and arrangement of the testes bear a striking resemblance to those of *D. latum*. Otherwise, relative position of the cirrus-sac and the seminal vesicle (Fig 5), the prominent papillae existed around the genital opening (Fig 9) and the existence of distinct boundary between neighbouring proglottides (Fig 8) are essentially common to the characteristics of *D. latum*. From these findings, the strobilae of the present specimen is identified as *Diphyllobothrium latum* (Linnaeus, 1758) Lühe, 1910 by Rausch and Hilliard (1970).[8] The route of infection of the present patient is not clarified.

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