Case Study of Human Infection with a Marine Species Diphyllobothriid Tapeworm: Morphological Variations of Strobila due to Fixation Procedures

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ABSTRACT. A case of human infection with a marine species diphyllobothriid found in Hiroshima Prefecture, Japan is reported. The infected patient was a 49-year-old man living in Fukuyama City, Hiroshima Prefecture, Japan. On July 4th, 1994, the patient had spontaneously discharged a diphyllobothriid strobila with no scolex about 50 cm in length and 22 mm in maximum width. After the treatment with praziquantel on November 12th, 1994, the patient had discharged again a remaining strobila without scolex of 85 cm in length and 21 mm in maximum width. Morphological characteristics of the strobila and eggs were as follows. 1) Segments 3.5 to 4.0 mm in length, 22 mm in maximum width and 0.7 mm in thickness. 2) Single set of reproductive organs per segment. 3) Uterus with 6 to 8 loops on each side, not much extended laterally. 4) Single layer testes about 2000 testes per segment. 5) Very prominent genital papillae. 6) Pyriform cirrus-sac oblique and thick-walled seminal vesicle connected dorso-caudally in sagittal section. 7) Eggs 72.4 μm by 54.1 μm in average and egg-shells with scattered pits on the surface. From these findings, the strobila in the present case was identified as of *Diphyllobothrium yonagoense* Yamane *et al*, 1981.

To our best knowledge, the present report deals with 15th finding of human infection with *D. yonagoense* in Japan. Morphological variations of the strobila due to the fixation procedures are also discussed.

Key words: human diphyllobothriasis — *Diphyllobothrium yonagoense* — marine species — Diphyllobothriidae — Cestoda

Recently, human infection with marine species of the genus *Diphyllobothrium* is gradually increasing in Japan. Although the marine diphyllobothriid tapeworms are primarily endoparasites of marine mammals such as numerous members of cetaceans and pinnipeds, they are consequently parasitic of humans by ingestion of marine fishes which are common food resources to marine mammals and humans. About 40 human infection cases with marine *Diphyllobothrium* species have so far been reported in Japan, although reliable sources of human infection are not well understood. A majority of patients infected with the diphyllobothriid tapeworms in past have had common habits to consume marine fishes such as sardine, salmon, trout, sea bream, horse-mackerel, bonito etc. in the form of raw meat Sashimi, known as Japan's delicacy.

So far at present, 9 species of marine diphyllobothriid tapeworms have

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been reported from the infected patients in the world, of which 6 species are from Japan. In this paper, a case of human infection with a marine diphyllobothriid tapeworm species is described together with morphological variations between the stretched and contracted forms of strobila due to fixation procedures.

CASE NOTE

Patient (T. I.) was a 49-year-old man, an industrial worker, living in Fukuyama City, Hiroshima Prefecture, Japan. On July 1st, 1994, typical eggs of diphyllobothriid tapeworm were found from feces of the patient by stool examination done at his home doctor Kobayashi Clinic. Three days later, the patient had spontaneously discharged a strobila of diphyllobothriid tapeworm without scolex. He then visited the clinic again carrying the removed strobila. The strobila was immediately fixed in a 10% formalin solution and sent to our laboratory through Okayama Medical Laboratory for taxonomical evaluation.

Thereafter, the victim was treated with praziquantel (Biltricide®), 10 mg/kg, on November 11th, and the following day he had discharged a remaining strobila without scolex in his feces. In this instance, the strobila was preserved in normal saline solution and directly sent to our laboratory. The received strobila was fixed in a 5% formalin solution after it was transferred to tap water added with a small amount of chloroform and placed in a refrigerator (approximately 6°C) for 5 hours according to a fixation technique by Andersen (1971).[1)]

There was no trace of the diphyllobothriid eggs from patient's feces after the treatment. The patient has resided in Hiroshima Prefecture for almost all his life and has never left Japan. Moreover, he had a habit to eat raw meats of marine fishes and shell-fishes such as halfbeak, yellow-tail, tunny, squid, shrimp etc. fished from nearby Inland Sea of Japan.

MATERIALS AND METHODS

Morphological examination was made on relaxed form of the strobila discharged from the patient after the medical treatment, contracted form of the strobila spontaneously discharged in the first instance, and the eggs. The segments were observed in a whole mount preparation stained with Semicon's acetic carmine. Some mature segments were embedded in paraffin in a usual manner and serially sectioned at 10 μm in transverse and sagittal directions, then stained with Gomori's trichrome stain solution and mounted in Canada balsam. The eggs examined were removed from uterine ducts of mature segments. Surface of genital area of the mature segment and the egg-shell surface were studied with a Hitachi S-570 scanning electron microscope after preparation by ordinary techniques.

OBSERVATION OF THE WORM

Morphological data of each part of the relaxed and contracted forms of strobila and the eggs are shown in Table 1.

1) Relaxed form of strobila: The strobila was moderately relaxed, and it properly represents morphological characteristics of the present species. The

TABLE 1. Morphological comparison of the present species and *D. yonagoense*

	Present species		*D. yonagoense*
	relaxed form	contracted form	Yamane *et al* (1981)
Mature segment			
length	3.5~4.0 mm	0.862 mm	
maximum width	21 mm	22 mm	13 mm
thickness	0.7 mm	1.7 mm	0.6 mm
length/width	1 : 5	1 : 26	1 : 10-3
Cirrus-sac			
length	630 μm	640 μm	432~515 μm
width	310 μm	280 μm	287~360 μm
wall thickness	5.6 μm	13.9 μm	
Seminal vesicle			
length	330 μm	380 μm	206~463 μm
width	350 μm	330 μm	206~360 μm
wall thickness	av. 69.5 μm	av. 69.5 μm	62~103 μm
Arrangement of testes	single layer	single layer	single layer
No. of testes in			
transverse section	108	112	
sagittal section	18	17	
Diameter of testes	97.2~111.1 μm	125.0~138.9 μm	82~111 μm
Thickness of muscle layer			
longitudinal	av. 92.2 μm	av. 208.4 μm	
transverse	av. 41.7 μm	av. 55.6 μm	
No. of uterine loops	6~8	6~8	8~9
Diameter of nerve trunk	152.8~194.5 μm	138.9~180.6 μm	
Thickness of			
cortical parenchyma	347.3 μm	652.8 μm	
medullary parenchyma	166.7 μm	208.4 μm	
Size of eggs			
length	69.4~76.3 μm (av. 72.40)	65.9~75.6 μm (av. 71.81)	56.5±1.6 μm
width	52.1~58.0 μm (av. 54.10)	50.3~60.7 μm (av. 55.16)	43.4±1.3 μm
thickness of egg shell	2.1~3.1 μm (av. 2.7)	2.1~3.5 μm (av. 2.8)	2.0±0.3 μm

strobila without scolex was relatively thin and measured about 85 cm in length and 21 mm in maximum width. All of the segments were much wider than long and a single set of reproductive organs was recognized on midline of each segment. The segments measured 3.5 to 4.0 mm in length and 0.7 mm in thickness (Fig 1). A length/width ratio of the mature segments was about 1 : 5. The uterus was distributed bilaterally with 6 to 8 loops on each side, but not much extended laterally (Fig 2).

In a transverse section of the segments, cortical parenchyma situated in the ventral and dorsal parts of the segments was 347.3 μm thick, in which there existed longitudinal muscle fibers and vitelline follicles (Fig 3). Many of the vitellaria were oval, measuring 86 μm in diameter. A layer of longitudinal muscle was relatively developed, measuring 92.2 μm thick and a layer of transverse muscle was 41.7 μm in thickness. The medullary parenchyma intervening between 2 transverse muscle layers was 166.7 μm in thickness. The testes were arranged in a single layer occupying in medullary zone (Fig 3), and there was a distinct boundary between neighboring segments (Fig 1). Number of testes was 108 in a transverse section and 18 in a sagittal section, about 2000 in each segment. The testicular follicles were 97.2 to 111.1 μm in diameter. A single pair of nerve trunks situated in the medullary zone was located about halfway between genital opening and lateral margin of the segment. The nerve trunk measured 152.8 to 194.5 μm in diameter in the transverse section (Fig 3).

In a sagittal section of the segments, the cirrus-sac was pyriform and situated somewhat obliquely in the segment, measuring 630 μm dorso-ventrally and 310 μm in diameter (Fig 5). The cirrus-sac wall was about 5.6 μm in thickness, and cirrus opening was located about 0.9 mm posterior to anterior margin of the segment. The seminal vesicle was spherical and situated behind the cirrus-sac, which was dorso-caudally connected each other (Fig 5). The seminal vesicle measured 330 to 350 μm in diameter, and the seminal vesicle wall was considerably thick compared with that of cirrus-sac, measuring 69.5 μm on the average. The vaginal opening was located posterior to the cirrus opening and vaginal duct extended dorsally along under wall of the cirrus-sac. The uterine opening was located about 0.5 mm from the cirrus opening.

The eggs were ellipsoidal with or without apical knobs, measuring 69.4 to 76.3 μm (av. 72.4) in length and 52.1 to 58.0 μm (av. 54.1) in maximum width. The egg-shells were 2.1 to 3.1 μm (av. 2.7) in thickness.

By scanning electron microscopic observation, genital atrium and the uterine opening were surrounded by fairly distinct papillae (Fig 6). There existed densely scattered pits on the egg-shell surface (Fig 7), known as characteristics for marine diphyllobothriid tapeworms.[2,3]

2) Contracted form of strobila: The received strobila was already severely contracted in an antero-posterior direction, probably due to the influence of fixing solution.

The strobila without scolex was about 50 cm in length and in comparison with that of the relaxed form, there was external and internal variations. The segments measured 0.862 mm in length and 22 mm in maximum width (Fig 8), and a length/width ratio of mature segments was about 1 : 26. Number of uterine loops was 6 to 8 on each side, while they extended quite parallel to lateral sides (Fig 9). The mature segment was more than twice as thick as that of the relaxed strobila, measuring 1.7 mm in thickness in transverse and sagittal sections (Fig 10, 11). The tegument (cuticle) was about 10 μm thick and the cortical parenchyma was 652.8 μm in thickness in a transverse section. The vitelline follicles were elongated, measuring about 143 μm in length and 36 μm in width in the transverse section. A layer of longitudinal muscle was well-developed, measuring 208.4 μm thick in a sagittal section and a layer of transverse muscle was 55.6 μm in thickness.

The cirrus-sac was long-ellipsoidal and dorsally extended from genital

TABLE 2. Marine species of *Diphyllobothrium* reported in human infection

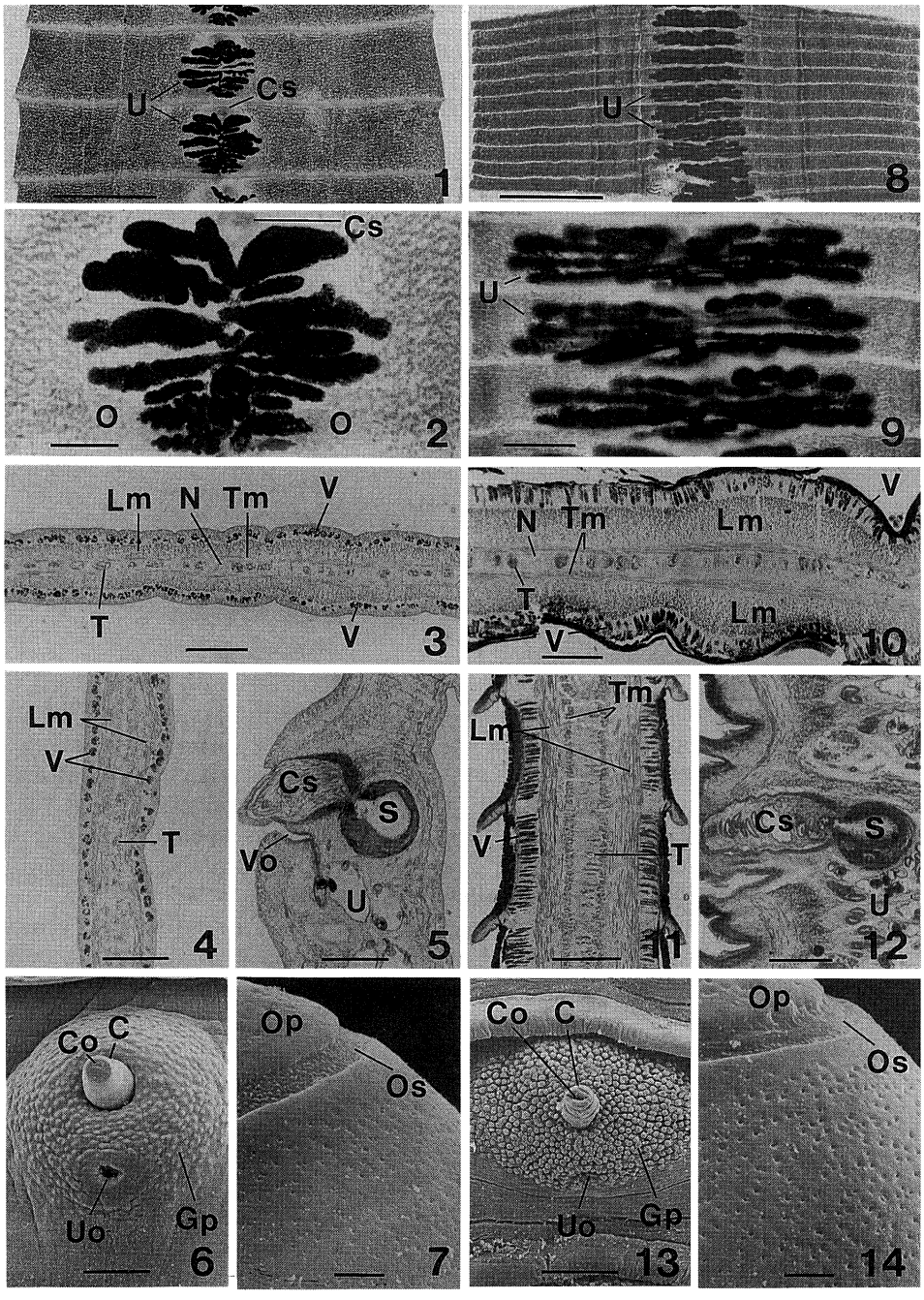
Species	Localities of infection occurred	Final hosts
D. hians	Japan	*Monachus monachus* *M. schauinslandi* *Erignathus barbatus*
D. cordatum	Greenland	*Erignathus barbatus* *Phoca groenlandica* *P. vitulina* *Odebaenus rosmarus*
D. lanceolatum	Greenland Spitsbergen Canada Alaska	*Erignathus barbatus* *Phoca hispida* *Phocaena phocaena*
D. scoticum	Japan	*Hydrurga leptonyx* *Otaria byronia*
D. pacificum	Peru Chili Japan	*Callorhinus ursinus* *Eumetopias jubata*
D. alascense	Alaska	*Canis familiaris*
D. cameroni	Japan	*Monachus schauinslandi*
D. yonagoense	Japan	*Grampus griseus*
D. orcini	Japan	*Orcinus orca*

atrium, measuring 640 μm dorso-ventrally and 280 μm in diameter in a sagittal section, and the wall was 13.9 μm in thickness. The seminal vesicle was spherical and situated behind the cirrus-sac, which was horizontally connected each other (Fig 12). It measured 330 to 380 μm in diameter and the wall was relatively thick 69.5 μm in a sagittal section. The cirrus opening was located about 0.3 mm posterior to anterior margin of the segment, and the uterine opening was situated 0.3 mm from the cirrus opening.

By scanninsg electron microscopy, the papillae surrounding the genital openings were clearly recognizable than those of the relaxed form of strobila (Fig 13). Basic characteristics of the egg-shell surface were exactly identical to those of the eggs from the relaxed form of strobila (Fig 14).

DISCUSSION

The tapeworms of the genus *Diphyllobothrium* Cobbold, 1858 are common parasites of fish-eating birds and mammals including humans. Cobbold (1858)[4] has published a description of *Diphyllobothrium stemmacephalum* sp. nov. from the porpoise *Phocaena phocaena* caught in Scottish waters as type species of this genus. Thereafter, the diphyllobothriid tapeworms have so far been recorded nearly 80 species in the world (Andersen, 1987).[5] Among these, about 35 species are considered to be parasitic in marine mammals. The taxonomical criteria of marine species of *Diphyllobothrium*, however, are still not well defined.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EXPLANATION OF FIGURES

Figs 1-7. Relaxed form of strobila discharged from the patient after the treatment with praziquantel.

Fig 1. Whole mount preparation of a mature segment stained with Semicon's acetic carmine solution. (Scale=5.0 mm)

Fig 2. Uterine loops and ovaries of a mature segment. (Scale=1.0 mm)

Fig 3. Portion of transverse section of a mature segment, showing detailed of lateral field stained with Gomori's trichrome solution. (Scale=0.5 mm)

Fig 4. Sagittal section of a mature segment passing through lateral field. (Scale=0.5 mm)

Fig 5. Sagittal section of a mature segment passing through the level of the genital field. (Scale=0.3 mm)

Fig 6. Genital area of a mature segment, showing genital papillae, cirrus, cirrus and uterine openings by scanning electron microscopy. (Scale=0.3 mm)

Fig 7. Egg-shell surface by scanning electron microscopy. (Scale=3.0 μm)

Figs 8-14. Contracted form of strobila discharged spontaneously from the patient in the first instance.

Fig 8. Whole mount preparation of a mature segment stained with Semicon's acetic carmine solution. (Scale=5.0 mm)

Fig 9. Uterine loops of mature segment. (Scale=1.0 mm)

Fig 10. Portion of transverse section of a mature segment, showing detailed of the lateral field stained with Gomori's trichrome solution. (Scale=0.5 mm)

Fig 11. Sagittal section of a mature segment passing through the lateral field. (Scale=0.5 mm)

Fig 12. Sagittal section of a mature segment passing through the level of the genital field. (Scale=0.3 mm)

Fig 13. Genital area of a mature segment, showing genital papillae, cirrus, cirrus and uterine openings by scanning electron microscopy. (Scale=0.3 mm)

Fig 14. Egg-shell surface by scanning electron microscopy. (Scale=3.0 μm)

Abbreviations used in Figures

C=cirrus, Co=cirrus opening, Cs=cirrus-sac, Gp=genital papillae, Lm=longitudinal muscle layer, N=nerve trunk, O=ovary, Op=operculum, Os=opercular suture, S=seminal vesicle, T=testis, Tm=transverse muscle layer, U=uterus, Uo=uterine opening, V=vitelline follicle, Vo=vaginal opening

As stated above, 6 species of marine diphyllobothriid tapeworm have been recorded from humans in Japan, those are: *Diphyllobothrium yonagoense* Yamane *et al*, 1981[6]; *D. orcini* Hatsushika *et* Shirouzu, 1990[7]; *D. hians* (Diesing, 1850) Meggitt, 1924[8]; *D. scoticum* (Rennie *et* Reid, 1912) Meggitt, 1924[9]; *D. pacificum* (Nybelin, 1931) Margolis 1956[10] and *D. cameroni* Rausch, 1969[11] in order of appearance (Table 2).

Andersen (1987)[5] has reported that marine species of *Diphyllobothrium* can be classified systematically into 4 groups: (I) very large worms with distinct genital papillae, and large muscular thick-walled seminal vesicle, *Diphyllobothrium stemmacephalum* Cobbold, 1858 (syn. *D. yonagoense*) for model species, including *D. macroovatum* Yurakhno, 1973[12] redescribed by Kamo *et al* (1980)[13], *D. polyrugosum* Deljamure *et* Skrjabin, 1966[14] and *D. fuhrmanni* Hsü, 1935[15] sensu Yazaki *et al* (1982)[16] for related species; (II) medium-size to relatively large worms with small cirrus-sac and seminal vesicle, *D. elegans* (Krabbe, 1865) for model species, including *D. lashleyi* (Leiper *et*

Atkinson, 1914), *D. scotti* (Shipley, 1907) and *D. pacificum* sensu Maejima *et al* (1981)[17] for related species; (III) medium-size stout muscular worms with uterine opening closely to genital atrium, *D. cordatum* (Leuckart, 1863) for model species, including *D. cameroni*, *D. lanceolatum* (Krabbe, 1986) and *D. hians* for related species; and (IV) small-size worms with few large testes, *D. wilsoni* (Shipley, 1907) for model species, including *D. minutus* Andersen, 1987 and *D. quadratum* (Linstow, 1892) for related species.

Our present specimen appeared to be a marine diphyllobothriid because there existed densely scattered pits on the egg-shell surface (Hilliard, 1960[2], 1972[3]). Judging from the morphological features, its characteristics resemble very closely to those of group I type *Diphyllobothrium* by Andersen (1987)[5] having large strobila, numerous uterine loops, distinct genital papillae, thick-walled seminal vesicle, well-developed longitudinal muscle layers, relatively small-sized testes, and genital atrium and uterine opening at some distance from each other. Therefore, our specimen is clearly differentiable from group II to IV type of *Diphyllobothrium*. Two species of *D. scoticum* and *D. alascense* Rausch and Williamson, 1958 presented in Table 2 belong to group II type of *Diphyllobothrium*.

D. macroovatum is easily differentiable from our specimen by thickness of seminal vesicle wall and the sizes of the cirrus-sac and the eggs which are elongated and oval, measuring 84 to 96 μm by 40 to 54 μm. *D. polyrugosum* (including *D. orcini*[18]) obviously differs from our specimen in the size of the cirrus-sac, number of testes per segment and uterine loops, and in the relative position of cirrus-sac and seminal vesicle. *D. fuhrmanni* including Yazaki *et al* (1982)[16] and Kamo *et al* (1982)[19] differs from our specimen in a length/width ratio of mature segments and number of uterine loops which is 14 to 16 and/or 18 to 19 on each side.

The original description of *D. stemmacephalum* is woefully incomplete, and its morphology closely resembles that of *D. yonagoense*. According to the re-examinations by Stunkard (1949)[20] and Deljamure (1968),[21] and the redescription by Andersen (1987),[5] *D. stemmacephalum* is conclusively differentiable from our specimen by sizes of cirrus-sac and seminal vesicle, and number of uterine loops. The morphological characteristics of our specimen bear a striking resemblance to those of *D. yonagoense* as shown in Table 1. From these findings, the strobila in the present species was identified as *Diphyllobothrium yonagoense* Yamane *et al* (1981) despite lack of the scolex. The first human case of *D. yonagoense* infection in Japan was reported by Yamane *et al* (1981)[6] of a 41-year-old man residing in Shimane Prefecture, and thereafter 14 cases were hitherto recorded. Hence, this report is the 15th human case infected with *D. yonagoense* in Japan.

Hasegawa *et al* (1989)[22] has reported that morphological appearances of diphyllobothriid tapeworm vary with different fixation. In our specimen, the morphological variations were clearly found between the relaxed and contracted forms of strobila. The most marked differences were recognized in length and a length/width ratio of mature segments, in the thickness of strobila, cirrus-sac wall, and cortical and medullary parenchymal muscle layers (Table 1), in the winding pattern of uterine loops (Fig 2, 9), and in the relative position of cirrus-sac and seminal vesicle (Fig 5, 12). The morphological differences could exert strong influence to taxonomical criteria for diphyllobothriid tapeworms.

Therefore, the authors would like to stress that reliable information in regard to fixation procedures of worm bodies should primary be considered preceding morphological observation.

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