

**A Human Case of *Diphyllobothrium yonagoense* Infection
Found in Okinawa Prefecture, Japan, with Special Reference
to the Scolex Morphology in the Relaxed Worm**

ITARU KIKUYAMA¹⁾, YOSHIHIDE ASATO¹⁾, MEGUMI SHIMABUKURO¹⁾,
NAOYA SHIMABUKURO¹⁾, ISAO SHIMABUKURO²⁾, HIDEO HASEGAWA^{2,3)} AND YOSHIYA SATO^{2,3)}

¹⁾Department of Pediatrics, Okinawa Prefectural Nanbu Hospital, Itoman, Okinawa 901-03, Japan.
²⁾Department of Parasitology and ³⁾Research Center of Comprehensive Medicine, Faculty of Medicine,
University of the Ryukyus, Nishihara, Okinawa 903-01, Japan.
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Abstract

Diphyllobothrium yonagoense infection was observed in a 13-year-old female residing on Okinawa Island, Japan. A complete worm, 407 cm long and composed of ca. 2,700 proglottids, was successfully expelled by Gastrografin administration. The worm was relaxed in tap water with chloroform at 4°C overnight and then fixed with 5% formalin solution for morphological observation. The mature proglottids and eggs were identical with those previously reported for *D. yonagoense*. Meanwhile, the scolex was quite different from those in the former descriptions because it was cordate in lateral view and its bothrial rims were not folded. These discrepancies were considered to have been caused by the difference in fixation methods. It is presumed that an adequately relaxed *D. yonagoense* has a cordate scolex with non-folded bothrial rims. Careful attention should be paid to the morphological changes due to fixation methods on identification of diphyllobothriids expelled from humans.

Key words: *Diphyllobothrium yonagoense*; human infection; Gastrografin; Okinawa; morphology.

Diphyllobothrium yonagoense Yamane *et al.*, 1981 (=so-called *Diphyllobothrium* sp. "Koga-Okamura type") has been considered to have marine mammals as its natural definitive hosts (Yamane *et al.*, 1981; Kamo *et al.*, 1977, 1982a). Fourteen cases of human infection with this cestode have been recorded from Kyushu, Shikoku, Sanin, Kinki and Tokai districts of Japanese mainland (Koga, 1967; Okamura *et al.*, 1972; Yamane *et al.*, 1981; Kagei *et al.*, 1987; Hirai *et al.*, 1988; Kamo *et al.*, 1988; Fukumoto *et al.*, 1992; Noda *et al.*, 1994). In the Ryukyu Archipelago, two cases have been reported from Yoron and Okinawa Islands (Hasegawa *et al.*, 1984, 1989). Moreover, one of the two cases reported by Sunagawa (1965) is also surmised to be due to *D. yonagoense* based on the specimens pre-

served (Hasegawa, unpublished; the other case was caused by *Diphyllobothrium pacificum* (Nybelin, 1931): Kamo *et al.*, 1982b). In addition, one human case of *D. yonagoense* infection was reported in Korea (Lee *et al.*, 1988). Most of the strobilae hitherto expelled from humans lacked scolex, and the scolex morphology was examined only in two cases (Yamane *et al.*, 1981; Noda *et al.*, 1994). Because cestodes remarkably change their morphology according to the methods of fixation and preservation, it has been recommended to relax the worm in tap water with chloroform before fixation (Kamo, 1978). In *D. yonagoense*, morphological comparison has been already made between the mature proglottids fixed with and without relaxation (Hasegawa *et al.*, 1989). However, the morphology of scolex in a completely relaxed specimen of *D. yonagoense* has not been studied. Recently we had an opportunity to relax a complete strobila of *D. yonagoense* expelled from a woman in Okinawa Prefecture. This paper describes the case and mor-

Correspondence: Hideo Hasegawa

喜久山 至¹⁾, 安里義秀¹⁾, 島袋 恵¹⁾, 島袋直哉¹⁾,
島袋 勲²⁾, 長谷川英男^{2,3)}, 佐藤良也^{2,3)} (1)沖縄県立
南部病院小児科, 2)琉球大学医学部寄生虫学教室,
3)同地域医療研究センター

phology of the relaxed worm with special reference to its scolex.

The Case

The patient: 13-year-old female residing in Itoman, Okinawa Prefecture, Japan.

Family and past history: Not remarkable. She was born on Okinawa Island and has not been abroad. She often took raw marine fishes caught around Okinawa Island. She also took raw goat meat last August. However, she denied to have ingested salmon or masou salmon raw.

Present illness: On February 26, 1995, she noticed that a tape-like worm dangled from her anus on defecation after taking a laxative. When she pulled with fingers, it snapped leaving a 70cm-long portion. She admitted to the Okinawa Prefectural Nanbu Hospital with the worm. On admission she was well nourished with body weight of 48 kg. She had no subjective symptoms such as abdominal pain, nausea or loss of weight. Physical and biochemical tests revealed no abnormality. The worm was identified as a diphyllbothriid cestode. Fecal examination also revealed numerous diphyllbothriid eggs. Under the diagnosis of diphyllbothriasis, treatment with Gastrografin (Meglumine sodium amidotrizoate, Schering AG, Germany) was attempted on March 7, 1995. When 400 ml of Gastrografin was administered through a tube inserted into the duodenal lumen, a motile slender worm was observed in the small intestine roentgenographically. However, migration of the worm to the large intestine was not ascertained. After the administration of a laxative, a portion of the worm appeared from the anus. By pulling the worm with fingers, a strobila of 1 m long without scolex was obtained. No additional portion of the worm was pulled out or expelled on the day and the subsequent days. On March 28, fecal examination demonstrated numerous diphyllbothriid eggs indicating the cestode still remained in the intestine. Then, treatment with Gastrografin was performed again. On roentgenography, a long motile worm was observed migrating downwards, and then disappeared from the small intestine. Although the worm was not discernible within the colon, it was judged that the worm had completely entered it. Then, a laxative and an enema were given, and subsequently

a large strobila with a scolex was discharged. Fecal examination on April 4, 1995 demonstrated no diphyllbothriid egg.

The Worm

The worm expelled on March 28, 1995 was relaxed in 1l of tap water with a few drops of chloroform at 4°C overnight, and then fixed in 5% formalin solution. For morphological observation and measurement, the worm was cleared in glycerol aqueous solutions of ascending concentration. Mature segments were embedded in paraffin, serially sectioned longitudinally and transversely, and stained with trichrome as in usual procedure.

The strobila is thick, 407 cm in length and is composed of ca. 2,700 proglottids (Fig. 1). The dorsal and ventral sides have 7–10 deep furrows in each lateral field running longitudinally through proglottids (Figs. 4 and 5). The worm width increases gradually from anterior end, attaining to a maximum of 21.0 mm at midlength of the strobila and somewhat narrows posteriorly. The length of proglottid is 2.2 mm in the middle of the strobila, and it attains to 2.8 mm in posterior portion. The scolex is cordate and much thicker than the neck width in lateral view (Fig. 2), but in dorsoventral view, it is approximately oblong and thinner than the neck width (Fig. 3). The scolex has deep bothria dorsoventrally that are widened anteriorly (Fig. 3). The bothrial rims are not folded (Fig. 3). The scolex is 1.26 mm long (distance from the anterior apex to the posterior end of bothrium), and its maximum breadth and height are 0.42 mm and 1.11 mm, respectively. The neck is 2.37 mm long, and its breadth and height immediately posterior to the bothria are 0.52 mm and 0.32 mm, respectively. Genital primordium becomes discernible at ca. 460th proglottid and eggs become visible posterior to ca. 1240th proglottid. In mature proglottids, the anterior 1/3 of midventral area is elevated hemispherically, and is provided with numerous papillae (Fig. 5). The genital atrium opens at the center of this elevation, and the uterine pore is situated at posterior edge of this elevation (Figs. 5, 6). The cirrus sac is spindle-shaped, situated slightly obliquely against the ventral surface (Fig. 6). The seminal vesicle is spherical, thick-walled, and is

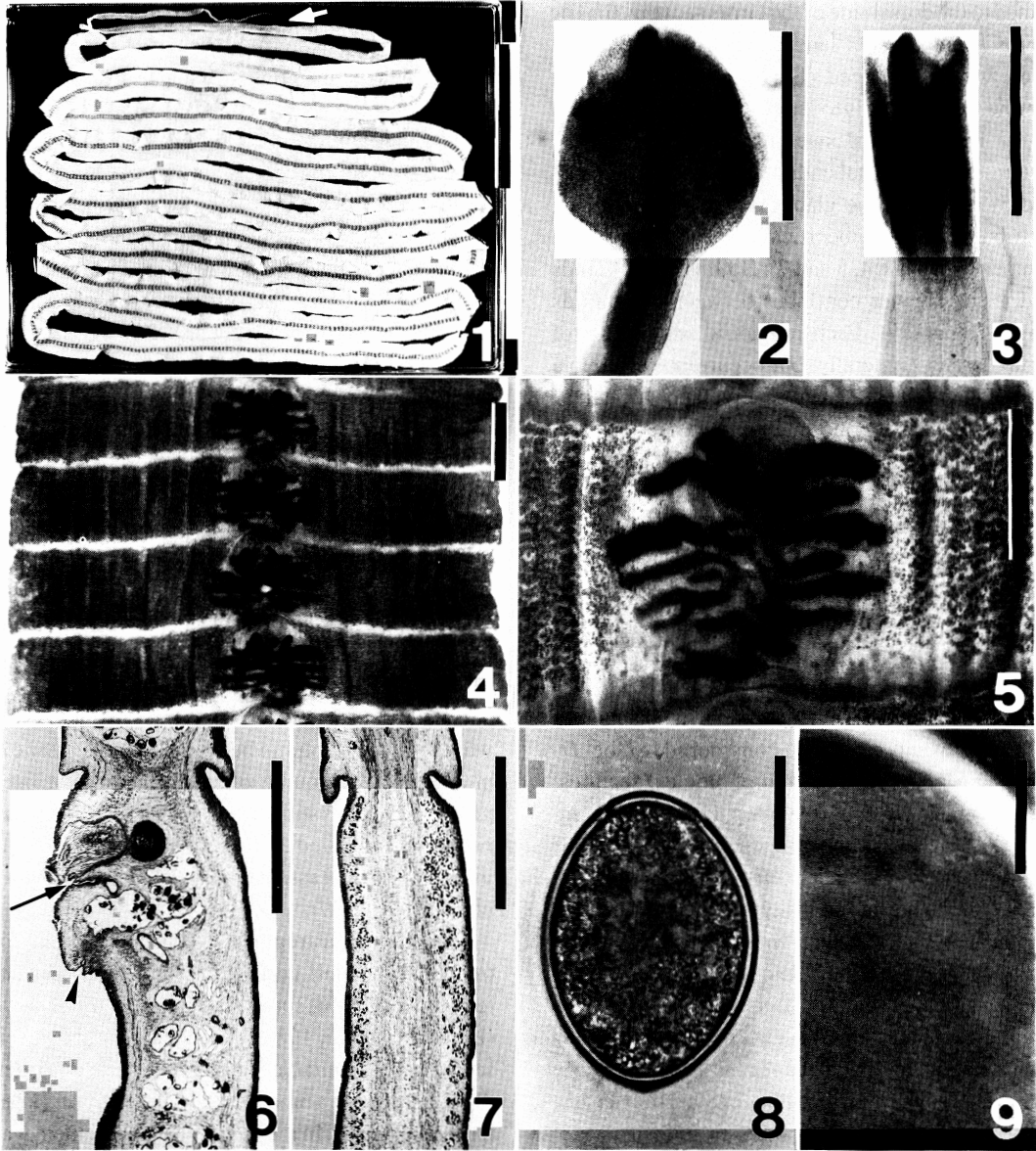


Fig. 1 Whole strobila of *Diphylobothrium yonagoense* expelled by Gastrografin administration. Arrow indicates scolex. (Scale: 10 cm)

Fig. 2 Scolex, lateral view. (Scale: 1 mm)

Fig. 3 Scolex, dorsal view. (Scale: 1 mm)

Fig. 4 Mature proglottids, ventral view. (Scale: 2 mm)

Fig. 5 Central part of mature proglottid, ventral view. (Scale: 1 mm)

Fig. 6 Midsagittal section of mature proglottid. Arrow and arrowhead indicate vulva and uterine pore, respectively. (Scale: 0.5 mm)

Fig. 7 Parasagittal section of mature proglottid. (Scale: 0.5 mm)

Fig. 8 Egg. (Scale: 20 μm)

Fig. 9 Surface of eggshell by Nomarski interference microscopy. (Scale: 10 μm)

joined to the dorsal side of the cirrus sac (Fig. 6). The uterus forms parallel lateral loops of which distal ends are not enlarged (Figs. 4 and 5). Anterior uterine loops extends to lateral to the cirrus sac and to the hemispherical swelling around the genital atrium, but their distal ends are not swollen (Figs. 4 and 5). The vitelline glands are numerous, being distributed in the subcortical layers except in the central field (Figs. 4, 5 and 7). Both vitelline glands and testes are not confluent between proglottids (Figs. 4, 5 and 7). Each proglottid has one set of genital organs generally, but occurrence of double sets of genital organs is observed in 25 out of posterior 500 proglottids. The eggs are ellipsoidal, yellow in color and $64.0\text{--}70.0 \times 1.2\text{--}54.4 \mu\text{m}$ (mean $67.9 \pm \text{SD } 1.4 \times 52.8 \pm 1.0 \mu\text{m}$). The egg shell is relatively thick, about $3 \mu\text{m}$ in thickness, and its surface is provided with numerous deep pits (Figs. 8 and 9). The tubercle of eggshell at opposite side of the operculum is sometimes absent.

Discussion

The present worm is considered to be *D. yonagoense* based on the morphological features, i.e., the thick strobila, the deep furrows running longitudinally, laterally extending uterine loops of which distal ends are not enlarged, the large hemispherical swelling around the genital atrium, the egg size and the thick egg shell with deep pits (Yamane *et al.*, 1981; Kagei *et al.*, 1987; Hirai *et al.*, 1988; Kamo *et al.*, 1988). The frequent occurrence of double sets of the genital organs in a proglottid has been also known in *D. yonagoense* (Yamane *et al.*, 1981; Hasegawa *et al.*, 1984, 1989; Hirai *et al.*, 1988).

Meanwhile, the scolices of *D. yonagoense* hitherto reported were hammer-shaped with folded bothrial rims one of which overlapped on the other (Yamane *et al.*, 1981; Yamane *et al.*, 1989; Noda *et al.*, 1994), differing from that of the present worm. The type specimen of *D. yonagoense* was fixed in 5% formalin solution and was described to be well relaxed (Yamane *et al.*, 1981). However, the scolex of the type material is identical in morphology with that of the worm reported by Noda *et al.* (1994). Because the worm of Noda *et al.* (1994) was spontaneously expelled after ingestion of polyethylene

glycol and then fixed in 5% formalin solution without relaxation, it is strongly probable that the relaxation of the type material was not enough. The scolices of *D. yonagoense* collected from the Risso's dolphin, *Grampus griseus*, were also hammer-shaped with folded bothrial rims although they were relaxed in cold tap water with chloroform before fixation (Yamane *et al.*, 1989; Yamane, personal communication). Nevertheless, the relaxation period for these worms was shorter than usual because they were planned to be subjected for SEM observation (Yamane, personal communication). Thus, the relaxation of them seemed to be incomplete. The present worm was sufficiently relaxed with the standard method recommended for morphological observation of the diphyllbothriids (Andersen, 1971; Kamo, 1978). Therefore, it is highly probable that the present scolex represents the proper shape of fully-relaxed *D. yonagoense*. Further trial of relaxation should be attempted in order to establish the proper morphology of the scolex of *D. yonagoense*.

The life history of *D. yonagoense* has not been adequately elucidated and the only known final host was the Risso's dolphin besides human (Yamane *et al.*, 1989). The source of infection has not been identified yet. It is strongly suggested that the present patient had acquired the infection by ingesting some inshore fishes raw as in the previous cases (Hasegawa *et al.*, 1984, 1989).

The use of Gastrografin, a contrast medium, for treatment of adult cestode infection has been attempted for various species including *D. yonagoense* (Hasegawa *et al.*, 1989). This treatment, however, seems to be unsuitable for infant because it is often difficult to insert a tube into the duodenum and keep it for long period. In the present case, the patient was only 13 years old, but with the physique comparable to adult woman in having 48 kg body weight. Thus, the treatment was applied with the same amount of Gastrografin used for an adult. She might be the youngest patient who was treated with Gastrografin for cestode infection.

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