Parasitology International 68 (2019) 14–16

Contents lists available at ScienceDirect

Parasitology International

journal homepage: www.elsevier.com/locate/parint

A case of small bowel obstruction due to infection with *Bolbosoma* sp. (Acanthocephala: Polymorphidae)

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ARTICLE INFO

Keywords:
Bolbosoma
Ileus
DNA barcoding
Zoonosis

ABSTRACT

A case of small bowel obstruction caused by *Bolbosoma* sp. infection is reported. A 27-year-old woman admitted with abdominal pain was diagnosed as small bowel obstruction. Laparoscopic surgery revealed induration in jejunum at ca. 120 cm distal to the ligament of Treiz, attributed to a band connecting the serosa to the ascending mesocolon. Resected band contained an acanthocephalan accompanying foreign body reaction with abscess formation. The parasite belonged to the genus *Bolbosoma*, of which identification was made by DNA sequence analysis. This is the eighth case of *Bolbosoma* infection in humans, and the first one causing an ileus.

1. Introduction

Acanthocephalans are very rare parasites of humans [1]. *Macracanthorhynchus hirudinaceus* (Pallas, 1781) of swine and *Moniliformis moniliformis* (Bremser, 1811) of rats, both utilize dung insects as intermediate host, have been known to infect humans. Besides these terrestrial species, some marine species belonging to the genera *Bolbosoma* and *Corynosoma*, both being parasites of cetaceans and pinnipeds in adult stage, have been known to cause zoonotic infections in humans [e.g. 2–6]. We recently experienced a case of small bowel obstruction caused by *Bolbosoma* infection. Identification of the worm was made by DNA sequence analysis. The case is reported herein as the eighth human infection.

2. Case

The patient was 27-year-old woman who was from northern Kyoto with unremarkable family and past history except abdominal pain she had a few years ago. At that time, the symptoms improved spontaneously though the cause was not determined by endoscopic examination. On 9th August 2017, she was admitted to the Kyoto Medical Center with complaints of severe abdominal pain and nausea. On admission, obstruction of gastrointestinal tract was not observed at computer tomography (CT), ileus tube was inserted but the symptoms did not improved. On third day of admission, contrast enhanced computer tomography (CECT) revealed closed loop obstruction of the jejunum suggesting strangulated ileus (Fig. 1a). Laboratory study revealed moderate leukocytosis without sign of eosinophilia and lightly elevated CRP level.

Under clinical diagnosis of small bowel obstruction, laparoscopic surgery to release the intestinal obstruction was performed. At the operation, an induration was noticed in the small intestine at the ileus, ca. 120 cm distal to the ligament of Treiz. This indulation was due to a band, ca. 1.5 cm long by 8 mm wide, connecting the serosa to the ascending mesocolon. After detaching this band from the mesocolon, partial jejunum of ca. 3 cm long was resected with the band (Fig. 1b), and end-to-end anastomosis was made for the remaining jejunum. After the operation, the symptoms subsided. The postoperative course was uneventful, and the patient was discharged on 17th August 2017. Thereafter, she had relapses twice on 20th August 2017 and 8th October 2017, and was admitted to the hospital. CT revealed a partial dilation of intestine near the anastomosed site of the jejunum but obstruction was not observed. Conservative therapy was applied and her symptom was relieved successfully.

The resected intestine along with the band were fixed in buffered 10% formalin solution. For histopathological examination, the band

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https://doi.org/10.1016/j.parint.2018.09.007
Received 8 April 2018; Received in revised form 3 September 2018; Accepted 30 September 2018
Available online 02 October 2018
1383-5769/

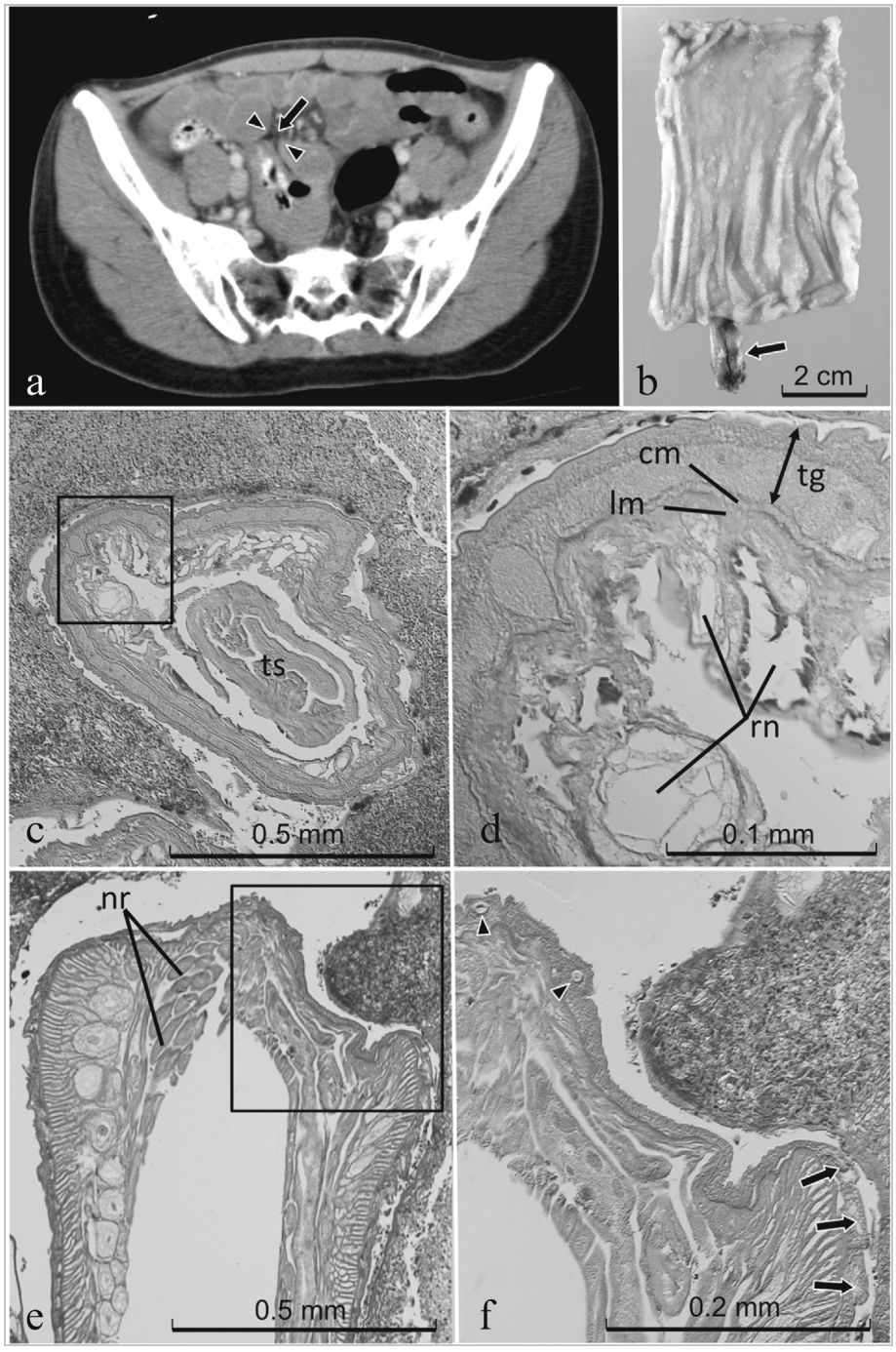

Fig. 1. (a) Contrast enhanced computer tomography (CECT) showing small bowel obstruction with closed loop of the jejunum. Arrowheads and arrow indicating ends of the closed loop and obstruction site, respectively. (b) The portion of jejunum resected by laparoscopic surgery. No malignant lesion was seen on the mucosa but a band (arrow) extending from the serosa was noticed. (c–f) Sections of acanthocephalan appeared in the cord formed between the serosa of jejunum and ascending mesocolon. c, d. Cross section found in the basal part of the cord (c) and enlarged view of the surrounded part (d). e, f. Longitudinal section through anterior body (e) and enlarged view of the surrounded part (f) showing sclerotized spines on the trunk (arrows) and hooklets of proboscis (darts). Abbreviations: cm. circular muscle; lm. longitudinal muscle; nr. neck retractor muscle; rn. rene retwork; ts. testis (?); tg. tegument.

along with the connecting portion of jejunum was cut in the midline, and one half was embedded in paraffin for longitudinal section observation. Meanwhile, the other half was cut into 12 pieces and then embedded for cross-sectional examination. Hematoxylin and eosin staining was applied for the sections.

Histopathological examination revealed slight edema due to foreign body reaction in the mucous membrane of the jejunum. In the longitudinal section of the band, four sections of a helminth, one long oblique section, ca. 4 mm long by ca. 0.4 mm wide in basal portion, and three cross sections with diameter of ca. 0.5 mm in distal half, were found. Foreign body reaction and abscess with neutrophil accumulation but also with infiltration of lymphocytes and eosinophils were observed around the worm sections. The worm had wrinkled surface, and the body wall was three-layered, corresponding to the outer tegument, middle group of circular muscles and inner group of longitudinal muscles with rete network characteristic to acanthocephalans (Fig. 1c, d) [7,8]. In two of the three cross sections, organs, presumably corresponding to testes, and associated tissues were seen in pseudocoelom, (Fig. 1d). In the remaining one cross section, thick membraneous tissues forming folded layers were observed. Spermatozoa were not found, indicating immature condition of the worm. Among 12 sections cut at different levels of the other half of the band, one contained longitudinal section of through anterior body, ca. 1 mm long by 0.5 mm wide (Fig. 1e). Sections of proboscis hooklets and trunk spines were observed (Fig. 1f). These intact structures indicated that the parasite was still fresh when the human tissue was resected. It is surmised that the inflammatory reaction around the worm formed a firm band connecting the jejunum with the ascending mesocolon, causing strangulation.

Because the morphological observation proved that the causative agent was an acanthocephalan, further identification was attempted using DNA sequence analysis. The parasite tissue was manually cut off from a thick section of the paraffin-embedded human tissue under a stereomicroscope, and then dewaxed with DEXPAT® DNA purification kit (TaKaRa, Japan) to prepare a template DNA for PCR. The Tks Gflex® DNA polymerase (TaKaRa) was employed for PCR, together with the manufacturer-supplied reaction buffer. A nuclear DNA including a set of internal transcribed spacers 1 and 2 (ITS1 and ITS2) in ribosomal RNA gene cassette was amplified by previously reported primers [4]. A mitochondrial DNA (mtDNA) of cytochrome *c* oxidase subunit 1 gene (*cox1*) was also targeted by original primers as follows: 5′-GCT TTG CCT CGT TTA AAT AAT TTG AG-3′ and 5′-AAA TCT TGT ACT AAA ATT ACG ATC-3′. The PCR was run for 40 cycles (98 °C for 10 s, 55 °C for 30 s, and 68 °C for 30 s) in a total volume of 25 μl including 0.25 μM of each primer and 1 μl of template DNA. The PCR amplicons were sequenced using BigDye terminator cycle sequencing kit and ABI genetic analyzer 3500 (Applied Biosystems). Each of the PCR primers was used as a sequencing primer. The resultant nucleotide sequences were deposited in DDBJ/ENA/GenBank databases under accession numbers LC375174 (789 bases of a set of ITS1 and ITS2) and LC377776 (328 bases of *cox1*).

3. Discussion

The BLAST homology search (blast.ncbi.nlm.nih.gov) showed that the parasite DNA sequence (a set of ITS1 and ITS2) from the present case was almost identical to that of *Bolbosoma* cf. *capitatum* (Acanthocephala: Polymorphidae) from a Japanese patient reported previously [4] with the level of the identity reached to 99.7%. Whereas, the parasite mtDNA sequence (*cox1*) showed only 81.8% identity with that of *Bolbosoma caenoforme* [9]. Because of the lack of appropriate data of *cox1* in the DNA databases, these molecular results were insufficient to conclusively identify the parasite to species. We, therefore, treate the parasite as an unspecified *Bolbosoma* sp. herein.

Members of the genus *Bolbosoma* are parasitic in whales in adult stage and utilize euphausiids as intermediate hosts, and marine fishes and cephalopods as paratenic hosts [e.g. 10,11]. Actually, most patients in the previous human cases had took raw fish and squids [2,3,12–15]. It is hence surmised that the present patient also had acquired the infection by eating such seafood containing the cystacanth raw. She often took raw fishes also, but could not remember exact species of the fish she ate prior to the onset of the symptoms.

The first and second cases of human infection with *Bolbosoma* were reported in 1983 [2,3]. Since then, five human cases have been added [12–16]. All cases occurred only in Japan. In four cases, the patients had abdominal pain or epigastric pain [2,3,12,13], while in the remaining three cases the worms were incidentally found at surgical operations of cancer or by routine endoscopic examination [14–16]. Hence, the present case is the first one in which an ileus was caused by *Bolbosoma* infection.

In humans, *Bolbosoma* tends to invade the intestinal wall, often causing perforation, and migrate into the peritoneal cavity. Actually, the worms were collected on the serosal surface or abdominal cavity in six cases including the present one ([2,3,12,15,16]. Humans seem to be unsuitable final host for *Bolbosoma* because none of the worms collected from the patients were matured. These features are contrasting with those of human infections by *Corynosoma*, another polymorphid acanthocephalan of marine mammals, in which the worms attain gravid adults in the human intestine, not causing intestinal perforation [5,6].

Acanthocephalans of *Bolbosoma* and *Corynosoma* are distributed widely with their hosts in the oceans and seas worldwide. Although the known cases of human infections have been restricted mainly to Japan, this disease could emerge in any areas where raw marine fishes/squids are consumed. Nowadays, Japanese-style meal is widely accepted as delicacy over the world. Attention should be paid to the zoonotic acanthocephaliasis along with the famous anisakiasis acquired by eating raw fishes/squids. As shown in this report, DNA sequence analysis is applicable to the identification of the causative organisms even in histopathological specimens. The DNA barcoding system based on the highly variable sequence of mitochondrial *cox1* is especially useful for epidemiological studies to specify food sources of the infection. Nevertheless, the sequence data of *cox1* from marine acanthocephalans are very poor in DNA databases at the present time. It is necessary to strengthen the molecular taxonomy of marine acanthocephalans in order to identify the causative organisms to species correctly.

Conflict of interest

The authors declare that they have no conflicts of interest.

Acknowledgments

The authors would like to express sincere thanks to Dr. Tsunehiro Shimizu (Kyoto City Hospital) and Dr. Masahide Yoshikawa (Nara Medical University, Japan) for their helpful discussions. Thanks are also rendered to Dr. Koki Moriyoshi and Dr. Takao Odagaki (Kyoto Medical Center) for their kindness in providing pathological and clinical information.

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