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Case report

First identification of eggs of the Asian fish tapeworm *Bothriocephalus acheilognathi* (Cestoda: Bothriocephalidea) in human stool

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ABSTRACT

We report the first case of egg isolation of the Asian fish tapeworm *Bothriocephalus acheilognathi* (Bothriocephalidea) from human stool. A male patient from Saint Laurent du Maroni (French Guiana) presenting abdominal pain was examined in France for the diagnosis of intestinal parasites. *Diphyllobothrium*-like eggs were observed in his stool. However, molecular phylogenetic analyses based on sequences of rDNA and COI genes showed that the eggs observed belong to a bothriocephalidean cestode *B. acheilognathi*. The adult life stages of *B. acheilognathi* cestodes are known as invasive parasites of a wide spectrum of fish; however, they have not been described to parasitize any mammals. This human infection seems to be accidental and represents a parasite passage through human intestine after the consumption of an infected fish host.

1. Introduction

Tapeworms have been recognized as human parasites since antiquity and remain a cause for concern in many endemic world regions [1]. Only two of the 17 recognized tapeworm orders involve parasites that have an impact on human health [2,3]. Cyclophyllidea, the most evolutionary derived order, accommodate around 30 species of several genera known to ordinarily infect humans (e.g. *Taenia*, *Echinococcus*, *Hymenolepis*, *Dipylidium*). The order Diphyllobothriidea includes up to 23 human infecting species of either so-called broad fish tapeworms (*Diphyllobothrium*, *Diplogonoporus*) or sparganum (*Spirometra*). From the two orders, only the following four species are globally distributed and responsible for the absolute majority of human intestinal infections: beef tapeworm *Taenia saginata*, pork tapeworm *Taenia solium*, dwarf tapeworm *Hymenolepis nana*, and broad fish tapeworm *Diphyllobothrium latum*. Tapeworms from the remaining orders have never been reported from man, with the exception of three cases of "pseudo-parasitic" infections apparently caused by a passage of plerocercoid larvae of cestodes of the order Trypanorhyncha [4,5]. In this paper, we report the first evidence of a bothriocephalidean cestode egg presence in human stool and also describe its identification based on coprological (morphological) and molecular phylogenetic techniques.

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2. Material and methods

2.1. Patient and parasite isolation

A 32 year old male, who lived in Saint Laurent du Maroni (French Guiana), was on holiday in Lyons (France) for two weeks in April 2009. He visited the International Travel Vaccination Centre — tropical medicine department to receive a yellow fever vaccination. As he complained of abdominal pain persisting several weeks, his stool was examined for the presence of parasites. Eggs resembling either diphyllobothriidean or bothriocephalidean cestode eggs were found (Fig. 1) and *Diphyllobothrium* was identified based on these morphological criteria as the causative agent. The patient was treated with a dose of niclosamide after which the abdominal pain disappeared and the stool control was negative. No other recorded sign of parasitic infection was found, except for a blood hypereosinophilia (2000/mm^3) in 2007. Blood cell counts performed in April and June 2009 were normal. The patient described that he regularly ate raw freshwater and brackish fish such as aymara (*Hoplias aimara*), tiger fish (*Pseudoplatystoma* sp.), acoupa (*Plagioscion squamosissimus*), or various machoiron catfish (e.g. *Sciades proops*) in Saint Laurent du Maroni.

2.2. Parasite molecular identification

The patient stool was sent in the Parasitology Department of Cochin Hospital in Paris in order to exactly identify the *Diphyllobothrium*

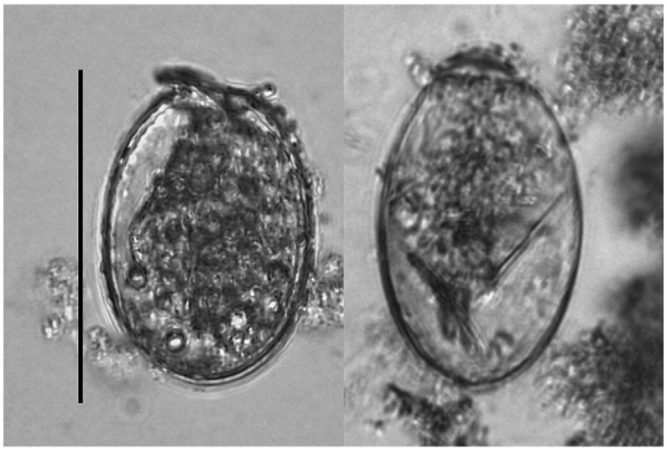

Fig. 1. Eggs of bothriocephalidean cestodes from human stools (×400).

species of the eggs with molecular methods. The eggs were concentrated from the stool using Para-Selles/Kop-Color kit, Fumouze (France), then washed three times in physiological solution. After 3 cycles of freezing at −80 °C and defreezing at 90 °C, the DNA was extracted and used for PCR amplification followed by sequencing nuclear ribosomal small and large subunit rDNA (ssrDNA and lsrDNA), internal transcribed spacers 1 and 2 (ITS1 and ITS2), and mitochondrial cytochrome oxidase subunit 1 gene (COI), as previously described [6,7]. Sequences were submitted to GenBank (accession numbers HM367066, HM367067 and HM439384). BLAST analyses (http://www.ncbi.nlm.nih.gov/Blast.cgi) were performed. Phylogenetic analyses were carried out in the Institute of Parasitology in České Budějovice, Czech Republic under maximum likelihood criteria [8] using all genes characterized. First, ssrDNA data have been analyzed together with representatives of each of the known 17 tapeworm groups. Next, more detailed analyses were run on lsrDNA data to reliably pinpoint the phylogenetic affiliation of the eggs.

3. Results

The eggs obtained from the patient stools (Fig. 1), were oval, 47–52 μm long, 33–36 μm wide, unembryonated, bearing an operculum on one end. As such, they appeared to be smaller than those of *Diphyllobothrium* human parasites such as *D. dendriticum* (52–76 by 38–52 μm), *D. latum* (54–76 by 35–57 μm), *D. nihonkaiense* (53–59 by 35–40 μm), or *D. pacificum* (49–63 by 33–45 μm) [9,10]. According to its size, these eggs would most closely resemble the eggs of either the marine *D. pacificum* reported from South America [11] or the recently resurrected *D. arctocephalinum* (41–56 by 37–44 μm) [12].

Each PCR amplification of the eggs' DNA produced a single amplicon and no multiple signals were observed from the sequences' chromatograms.

BLAST searches based on ssrDNA, lsrDNA and ITS1+ITS2 found bothriocephalidean cestodes of the genus *Bothriocephalus* instead of *Diphyllobothrium* as the closest match. Phylogenetic analysis of the ssrDNA data set confirmed the affiliation of the eggs to a derived lineage of bothriocephalidean cestodes with its closest relative being *Bothriocephalus claviceps* (data not shown). However, complete ssrDNA data of bothriocephalidean cestodes are still relatively scarce and do not suit lower level phylogenetic studies as some other markers. For this reason, D1–D3 region of lsrDNA was phylogenetically analyzed allowing a greater diversity of bothriocephalidean cestode sequences from GenBank to be included. Here our sample clearly formed a monophyletic and strongly statistically supported lineage together with other *Bothriocephalus acheilognathi* tapeworms within a group of bothriocephalidean cestodes from Africa [13,14] (Fig. 2).

Also the results of other analyses support the identification of the eggs as *B. acheilognathi*. BLAST search of the entire ITS1+ITS2 sequence revealed a high sequence similarity of our sample (~95%) with a group of *B. acheilognathi* cestodes (GenBank accession nos. AF362419–24) compared to the pairwise sequence similarity to a distinct closest species of *Bothriocephalus* (~74%, AF362434). However, running a phylogenetic analyses on the ITS data was not feasible thanks to the enormous variability of both ITS1 and ITS2 sequences and the impossibility to align them unambiguously. Alignment of the only available *B. acheilognathi* ssrDNA sequence (AY340106) with our ssrDNA then found only 2 base pairs (bp) difference out of 459 bp long sequence of this V4 variable ssrDNA region. The partial sequence of the COI gene of our *B. acheilognathi* specimen (HM439384) represents the first *B. acheilognathi* COI sequence characterized to be available for future studies and a rare case of bothriocephalidean COI sequence (the only other bothriocephalidean COI sequences deposited in GenBank to this date are the two from *Anchistrocephalus* and *Abothrium*, JQ268539 and JQ280884). If phylogenetic analysis based on COI is carried out with at least one representative of each of the 17 recognized clades of tapeworms, our sequence would create a sister lineage of the bothriocephalidean taxa *Anchistrocephalus* and *Abothrium*.

This molecular identification was in concordance with the morphology of the eggs as contrary to *Diphyllobothrium*, the eggs of *B. acheilognathi* are ovoid in shape and measure approximately 42–62 by 22–40 μm [15]. Variation in the egg size has been shown to change depending on the time of the year as also on the host size and species [16].

4. Discussion

The eggs observed in the patient stool were initially identified as those of *Diphyllobothrium* sp. based on morphological criteria. They could be identified as the eggs of the marine *D. pacificum* reported from South America, usually from the Pacific coast of Chile, Ecuador and Peru with few cases from Argentina [11], but never from tropical part such as French Guiana. French Guiana is close to Brazil, where *D. latum* and *Diphyllobothrium* sp. cases have been described in humans after consumption of raw fish in sushi and sashimi or freshwater fish [11] and *Diphyllobothrium* sp. has been identified in cetaceans off the northeastern coast [17]. However, species identification has never been confirmed by molecular methods [11]. Moreover, our patient regularly ate raw freshwater and brackish fish such as aymara, tiger fish, acoupa, or various machoiron catfish in Saint Laurent du Maroni, that have not been described as intermediate hosts of *Diphyllobothrium* [11,18]. Together, parasite morphology, geographical origin and the mode of contamination were uncharacteristic for *D. pacificum*, thus molecular methods were used to confirm the taxonomic identification.

The members of the genus *Bothriocephalus* are parasites of freshwater and marine fish, with about 100 nominal species [19]. One of the most common species is the Asian fish tapeworm, *B. acheilognathi* Yamaguti, 1934, a widely distributed parasite of freshwater fish. *B. acheilognathi* is not only unique in its extraordinary wide geographical distribution, but also in the extremely wide host spectrum that includes more than 200 species of freshwater fish. This tapeworm is an important pathogen of cultured and feral fish, especially carp fry [13]. It is believed to have disseminated around the world due to an uncontrolled movement of carp, guppies and other fish from Asia to six continents, including isolated oceanic islands and subterranean sinkholes (cenotes) during the last six decades [13]. Nevertheless, *B. acheilognathi* has been rarely described in South America. There are just two reports in Brazil both from cultured hosts including the common carp (*Cyprinus carpio*). Adult worms were occasionally reported also from amphibians, reptiles and birds, but these infections seem to be accidental (i.e. parasite passage after the consumption of an infected fish host) [13]. *B. acheilognathi* has a two-host life cycle. Fish are the final host to the parasite and get infected by eating a parasitized copepod, the intermediate host. Depending on water temperature, the life cycle may be completed between about one